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The journal embraces the so-called "IMRAD" structure (Introduction, Methods, Results, And Discussion). Manuscripts should be divided into:

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- **Results:** address the main findings, giving specific effect sizes and their statistical and clinical significance (if possible).
- **Conclusion:** should emphasize only the new and important aspects of the study and not overinterpret findings.

Keywords: 3-5 keywords for indexing purposes.

Introduction

- It should summarize the motivation (the nature of the problem and its significance) and offers a concise research background, but not a meticulous review.
- States in a single paragraph the aim of the study and specify the study's main and secondary objectives (usually identified as primary and secondary outcomes).
- Do not include any results or conclusions from the work being reported.

Subjects and Methods

- Should provide a comprehensive protocol of the study involving; design type, setting and duration of the study, sample groups definition (eligibility, inclusion and exclusion criteria) interventions and investigations performed throughout the study (however, methodological details previously published are not needed).
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- Specifications (including the manufacturer, city, and the country) should be given for the main drugs, chemicals, and instruments.
- The statistical methods used in analyzing study results should be indicated and statistical significance should be identified. Moreover, define statistical terms, abbreviations, symbols, and specify the statistical software package and version used.
- Questionnaires should not be added to the manuscript; however, the source reference should be cited in the article.
- Clinical trials: the CONSORT flow diagram should be added as a figure. RCTs should be registered, and their registration number should be mentioned.

Results

- Should be presented in logical sequence to reveal the main or most important findings first.
- To diminish the length of the text, comprise your data in tables, and use graphs only as an alternative to tables (do not duplicate data in graphs and tables).
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- Identify your results using appropriate statistical measures of variations (such as standard deviation and standard error of mean), as well as indicators of measurement of error or uncertainty (such as confidence intervals), and specify the statistical significance attached to them, if any.
- The maximum numbers for tables and figures are 5 for each.

Discussion

- Do not repeat in details data or other information given in other parts of the manuscript, such as in the introduction or the results sections.
- It is useful to begin the discussion by briefly summarizing the main new and important outcomes, and then explore possible mechanisms or explanations for these findings.
- Compare and contrast the results with other relevant studies, in the context of the entirely of the best available evidence (evade citing references that do not have a close relationship with the present result).
- State the limitations of the study and explore the implications of the findings for future research and for clinical practice.

Conclusion

- Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not adequately supported by the data (in particular, distinguish between clinical and statistical significance, and avoid making statements on economic benefits and costs unless the manuscript includes the appropriate economic data and analysis).

- Avoid claiming priority or mentioning work that has not been completed.
- Whenever warranted, state new suggestions and recommendations, but make them as simple and clear as possible.

Acknowledgments

- If present should be placed in a separate section after the conclusion.

References

- 20-40 references should be stated for original articles.
- References should be listed at the end of the article in numbered order according to Vancouver style.
- References should be numbered consecutively (by Arabic numerals in parentheses) in the order in which they are first mentioned in the text and should not be placed at the end of a sentence before the punctuation.
- Please use cross-referencing if the same reference is used more than once.
- References to journal articles should include, in order (1) authors (expecting a maximum of 3 author names before 'et al ' , (2) title, (3) journal name (as abbreviated in MEDLINE), (4) year of publication, (5) volume and issue number, (6) page numbers.
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Hameed EK, Al-Ameri LT. Artificial Intelligence in Medical Diagnosis; a Promising Future. Al-Kindy College Medical Journal. 2024 Mar;20(1):1–3. https://doi.org/10.47723/w1ymg293 .

Book chapter:

Herring JA. Limb Deficiencies. In: Herring JA, Tachdjian's Pediatric Orthopeidatrics, 4th ed. Philadelphia (PA): Saunders Elsvier; 2008, p.1983-2023.

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3- Introduction: Provide the impetus for reporting the study.
4- Case presentation: should consist of a full description and information about the case that was available only at presentation.
5- Comprise in a logical sequence all the tests performed as part of the management.
6- Treatment: comprise in a logical sequence all the interventional performed as part of the management.
7- Outcome and Follow - up: should emphasize primary outcomes of the management, as well as the long term Follow - up if possible.
8- compare the results with other relevant studies.
9- Discussion, conclusion, acknowledgment (if applicable), and references, 5-10 references should be stated.
10- Case reports should optimally be accompanied by relevant figures to document findings.

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Al-Kindy College Medical Journal (KCMJ)

Editorial

A Revolutionary Road Map for Obesity Management and Beyond: Tirzepatide as a Dual-Acting Insulinotropic Polypeptide Receptor Agonist

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ABSTRACT

Tirzepatide is a revolutionary and promising medication with a high impact in the treatment of Obesity and T2DM with their complications. Its efficacy was proven through different trials in achieving favorable weight loss and a significant reduction in glycemic index. It also treated a large diversity of related co-morbidities, including fatty liver, cardiovascular disease, dyslipidemia, and more. Tirzepatide is well tolerated, has a good safety profile, and is highly reliable and suitable for use in a population.

It is now widely accepted that Obesity is a long-term, complex, and multifactorial disease rather than a lifestyle and behavioral issue with different negative impacts on health in both personal and community domains (1).

Although debated, Obesity was declared a serious chronic disease by different scientific societies and organizations, and different countries adopted this: Centers for Disease Control and Prevention (CDC), the American Medical Association (AMA) in 2013, and the American Obesity Society in 2008 are examples. However, due to

insufficient data, lacking a clear diagnostic criterion, and acceptable standards and indexing measures that could be applied to everyone, many organizations and countries don't formally recognize Obesity as a disease; the United Kingdom is an example (2-5).

Obesity and being Overweight have multiple, well-recognized adverse effects on health, including but not limited to Type 2 Diabetes Mellitus (T2DM), Cardiovascular events, Hypertension, Lipid disorders, chronic kidney diseases, and Hepatic diseases. Additionally, there is increased evidence linking Obesity as a major etiological factor for malignancy developments, including colorectal, renal, pancreatic, and other cancerous conditions (6-8).

On the other hand, obese persons increasingly experience social stigmatization with possible feelings of depression and even condemnation (9-11).

During the last decades, Obesity has shown a significant increase in prevalence; it has doubled since 1980, with a more ominous figure for adolescents showing a quadrupled increase. About 2% of children and adolescents were diagnosed with Obesity in 1990, compared to a figure of 8% of them in 2022. According to the World Health Organization (WHO), in 2022, 43% of the adult population were obese or overweight, and 16% were obese. In the USA, a higher rate was reported, showing a significant increment in children with a prevalence of 19.7%, according to the CDC. In Iraq, 31.8% of the participants of a national survey were overweight, and 33.9% were obese (5, 12-14).

To achieve favorable health outcomes, different modalities were used for the management of Obesity; lifestyle and nutritional modifications are the initial steps that are widely used; however, failure to achieve a favorable weight with a high relapse rate may be due to multiple factors requires the scientific community to suggest and develop a wide range of medications, however, few of these medications were considered effective and get the required approval. Additionally, different approaches to surgical procedures were tried with variant success rates; however, being an invasive procedure was a major limitation (15-18).

The recent revolution in medical therapy was brought through the emergence of Glucagon-like peptide-1 (GLP-1) receptor agonists targeting GLP-1, a gut-derived hormone belonging to the family of incretin hormones; those agonists were initially used for the treatment of T2DM with a variable efficacy on reducing body weight. Additionally, the application of GLP-1 receptor agonists shows benefits beyond the management of Obesity and T2DM by making favorable modifications, although with variable degrees with different GLP-1 receptor agonists, on other related co-morbidities, including cardiovascular events, Hypertension, lipid disorders, chronic kidney diseases, and nonalcoholic liver diseases (19-20).

The GLP-1 class includes Exantide, Liraglutide, Dulaglutide, and Semaglutide; the Food and Drug Administration approved only Semaglutide and Liraglutide for obesity management (21-22).

Tirzepatide, is a promising, newly released medication with dual-acting insulinotropic polypeptide agonist properties, its dual mechanism synergist efficacy toward T2DM and obesity management with more favorable outcome, this brought through its glucose-dependent insulinotropic polypeptide (GIP) agonist property in addition to its GLP-1 effect, both GLP-1 and GIP are incretin hormones acting through enhancing insulin secretion from pancreatic beta cell, preventing their apoptosis and promoting regeneration, additional actions are through delaying gastric emptying, appetite suppressing, promoting satiety, and inhibiting glucagon secretion. All these effects are more powerful if mediated through dual-acting insulinotropic polypeptide receptor agonists than GLP-1 receptor agonists alone, with increasing evidence of more favorable outcomes regarding managing Obesity, T2DM, and other related co-morbidities (23-26).

Many clinical trials were conducted to assess Tirzepatide benefits, and safety compared to other GLP-1 receptor agonists regarding different variables. The efficacy and safety of Tirzepatide in T2DM management effect were evaluated through the phase three III Global SURPASS program; eight published SURPASS trials were conducted: SURPASS 1-5, SURPASS J-mono, and SURPASS J-combo. Participants for the abovementioned trials were from different countries and patients diagnosed with T2DM. According to the SURPASS trials program, Tirzepatide was superior to other treatment modalities for T2DM management with a greater reduction in HbA1C (27-28).

In the SURPASS J-combo trial, a significant weight loss was noted in addition to glycemic control. Additionally, the recent phase 3 SURMOUMT clinical trial programs (SURMOUNT 1-5) show evidence regarding the efficacy and safety of Tirzepatide in weight reduction through many conducted trials with many of these results published recently, last Study, SURMOUNT 5 is now active aims to evaluate the efficacy and safety of Tirzepatide compared to Semaglutide regarding weight reduction and its related co-morbidities (27-29).

The positive impact on the nonalcohol-fatty liver was noted. The Phase 2 SYNERGY-NASH clinical trial suggests that Tirzepatide was effective in resolving Metabolic dysfunction–associated steatohepatitis without worsening fibrosis (26, 30-31).

Concerning other health impacts, the SUMMIT trial was conducted to evaluate the effect of Tirzepatide on cardiac health. It concludes that Tirzepatide lowers the risk of death from cardiovascular events or heart failure in patients with normal ejection fraction and Obesity (32-33).

Different studies encountered different adverse effects. Gastroinestinal symptoms were the most common when using Tirzepatide like other GLP-1 receptor agonists; these may include nausea, vomiting, diarrhea, dyspepsia, and constipation. Other less-reported adverse effects were cholelithiasis, cholecystitis, and, in rare cases, acute pancreatitis. Generally, most adverse effects were found to be dose-related, which may lead in some instances to discontinue the treatment; a proportion of serious side effects leading to drug discontinuation shows a significant dose-related with an incidence of 7.23%, 8.68%, and 10.39% for the doses of 5 mg, 10 mg, and 15 mg respectively (34-36).

Although rare, yet warrants attention, severe hypoglycemia was reported in some trials. However, these findings were non-significant (34-36).

Psychiatric issues were also reported; however, the incidence was 1.2%, considered as a rare adverse effect; women accounted for two-thirds of the reported cases, and depression was the most common

psychiatric adverse effect, followed by anxiety and suicidal thoughts. Although rare, fatal outcomes of patients who completed suicidal attempts warrant further interest and future investigation (38).

Compared to other GLP-1 receptor agonists, Tirzepatide has a similar profile regarding adverse effects and tolerability (35-36,39).

The main risk for Tirzepatide use was the possibility of developing C cell thyroid cancer, including medullary type. These findings were supported through experimental research on rats; however, this was uncertain and has yet to be proved in humans by different trials. An explanation for this may be due to the less expression of GLP-1 receptors of C cells in humans, which reduces the possibility of developing this type of cancer. Furthermore, C cells in healthy humans have no GLP-1 expression at all. (40-42)

Currently, even though this risk is uncertain, Tirzepatide is contraindicated in patients with a history of medullary thyroid cancer, familial thyroid cancer, and those with genetic susceptibility to thyroid cancer. Although not thyroid cancer, multiple endocrine neoplasia 2 is also contraindicated as a relevant type of malignancy (43).

The expensive cost of Tirzepatide, like other GLP-1 receptor agonists, is the primary limitation for its adoption for many obese patients; more inclusion of Tirzapatide and other GLP-1 receptor agonists in insurance will minimize the healthcare cost for obesity complications and its related co-morbidities. Additional limitations may include the possibility of developing thyroid cancer, although not proven, and the lack of long-term trials to confirm its safety profile rather than efficacy.

In conclusion, Tirzepatide is well established to be considered a revolutionary and promising medication with a high impact in the treatment of Obesity and T2DM with their complications. Its efficacy was proven through different trials in achieving favorable weight loss and a significant reduction in glycemic index. It also treated a large diversity of related co-morbidities, including fatty liver, cardiovascular disease, dyslipidemia, and more. Tirzepatide is well tolerated, has a good safety profile, and is highly reliable and suitable for use in a population. However, ongoing and future long-term trials are still needed to confirm safety and explore more benefits.

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Conflict of Interest

Authors declare no conflict of interest.

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References

[1] Lin X, Li H. Obesity: Epidemiology, Pathophysiology, and Therapeutics. Front Endocrinol (Lausanne). 2021 Sep 6;12:706978. https://doi.org/10.3389/fendo.2021.706978

[2] Rosen H. Is Obesity A Disease or A Behavior Abnormality? Did the AMA Get It Right? Mo Med. 2014 Mar-Apr;111(2):104-108.

[3] Luli M, Yeo G, Farrell E, Ogden J, Parretti H, Frew E, Bevan S, Brown A, Logue J, Menon V, Isack N, Lean M, McEwan C, Gately P, Williams S, Astbury N, Bryant M, Clare K, Dimitriadis GK, Finlayson G, Heslehurst N, Johnson B, Le Brocq S, Roberts A, McGinley P, Mueller J, O'Kane M, Batterham RL, Miras AD. The implications of defining obesity as a disease: a report from the Association for the Study of Obesity 2021 annual conference. EClinicalMedicine. 2023 Apr 6;58:101962. https://doi.org/10.1016/j.eclinm.2023.101962

[4] Von Wachenfeldt, V., & Hofmann, B. M. (2024). The concept of disease in the Norwegian National Insurance Court. *Scandinavian journal of public health*, *52*(1), 89–94. https://doi.org/10.1177/14034948221128868

[5] World Health Organization. Obesity and overweight. [Internet]. 2021 [cited 2024 Oct 1]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

[6] Hameed EK, Al-Ameri LT, Hasan HS, Abdulqahar ZH. The cut-off values of triglycerides-glucose index for metabolic syndrome associated with type 2 diabetes mellitus. Baghdad Science Journal. 2022 Apr 1;19(2):0340. https://doi.org/10.21123/bsj.2022.19.2.0340

[7] Hassan HJ, Mohammad TU, Hameed EK. Assessment of Serum Metalloendopeptidase level in Patients with Double Diabetes. AL-Kindy College Medical Journal. 2023;19(3):21–25. https://doi.org/10.47723/kcmj.v19i3.999

[8] Ansari S, Haboubi H, Haboubi N. Adult obesity complications: challenges and clinical impact. Ther Adv Endocrinol Metab. 2020 Jun 22;11:2042018820934955. https://doi.org/10.1177/2042018820934955

[9] Chu DT, Nguyet NT, Nga VT, Lien NV, Vo DD, Lien N, Ngoc VT, Le DH, Nga VB, Van Tu P, Van To T. An update on obesity: Mental consequences and psychological interventions. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2019 Jan 1;13(1):155-60. https://doi.org/10.1016/j.dsx.2018.07.015

[10] Sarwer DB, Polonsky HM. The Psychosocial Burden of Obesity. Endocrinol Metab Clin North Am. 2016 Sep;45(3):677-88. https://doi.org/10.1016/j.ecl.2016.04.016

[11] Smith NR, Zivich PN, Frerichs L. Social Influences on Obesity: Current Knowledge, Emerging Methods, and Directions for Future Research and Practice. Curr Nutr Rep. 2020 Mar;9(1):31-41. https://doi.org/10.1007/s13668-020-00302-8

[12] Lafta RK, Kadhim MJ. Childhood obesity in Iraq: prevalence and possible risk factors. Ann Saudi Med. 2005 Sep-Oct;25(5):389-93. https://doi.org/10.5144/0256-4947.2005.389

[13] Pengpid S, Peltzer K. Overweight and Obesity among Adults in Iraq: Prevalence and Correlates from a National Survey in 2015. Int J Environ Res Public Health. 2021 Apr 15;18(8):4198. https://doi.org/10.3390/ijerph18084198

[14] LaftaR. Health System in Iraq Post 2003 War. AL-Kindy College Medical Journal. 2023; 19(3):5–11. https://doi.org/10.47723/kcmj.v19i3.1040

[15] Elmaleh-Sachs A, Schwartz JL, Bramante CT, Nicklas JM, Gudzune KA, Jay M. Obesity Management in Adults: A Review. JAMA. 2023 Nov 28;330(20):2000-2015. https://doi.org/10.1001/jama.2023.19897

[16] Contreras F, Al-Najim W, le Roux CW. Health Benefits Beyond the Scale: The Role of Diet and Nutrition During Weight Loss Programmes. Nutrients. 2024 Oct 22;16(21):3585. https://doi.org/10.3390/nu16213585

[17] Roomy MA, Hussain K, Behbehani HM, Abu-Farha J, Al-Harris R, Ambi AM, Abdalla MA, Al-Mulla F, Abu-Farha M, Abubaker J. Therapeutic advances in obesity management: an overview of the therapeutic interventions. Frontiers in Endocrinology. 2024 Apr 23;15:1364503. https://doi.org/10.3389/fendo.2024.1364503

[18] Medical Advisory Secretariat. Bariatric surgery: an evidence-based analysis. Ont Health Technol Assess Ser. 2005;5(1):1-148.

[19] Nauck MA, Quast DR, Wefers J, Meier JJ. GLP-1 receptor agonists in the treatment of type 2 diabetes-state-of-the-art. Mol Metab. 2021; 46: 101102. Go to original source. 2020. https://doi.org/10.1016/j.molmet.2020.101102

[20] Collins L, Costello RA. Glucagon-Like Peptide-1 Receptor Agonists. 2024 Feb 29. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan.

[21] Petrova L, Andreevska K, Parvova I, Petkova V (2024) Systematic review of the efficacy and safety of GLP-1 receptor agonists in the treatment of patients with type 2 diabetes mellitus. Pharmacia 71: 1-17. https://doi.org/10.3897/pharmacia.71.e132148

[22] Hu E-H, Tsai M-L, Lin Y, Chou T-S, Chen T-H. A Review and Meta-Analysis of the Safety and Efficacy of Using Glucagon-like Peptide-1 Receptor Agonists. *Medicina*. 2024; 60(3):357. https://doi.org/10.3390/medicina60030357

[23] Lisco G, Disoteo OE, De Geronimo V, De Tullio A, Giagulli VA, Guastamacchia E, De Pergola G, Jirillo E, Triggiani V. Is Tirzepatide the New Game Changer in Type 2 Diabetes? *Endocrines*. 2024; 5(1):72-86. https://doi.org/10.3390/endocrines5010005

[24] Prasad-Reddy L, Isaacs D. A clinical review of GLP-1 receptor agonists: efficacy and safety in diabetes and beyond. Drugs Context. 2015 Jul 9;4:212283. https://doi.org/10.7573/dic.212283

[25] Scheen AJ. Dual GIP/GLP-1 receptor agonists: New advances for treating type-2 diabetes. InAnnales d'Endocrinologie 2023 Apr 1 (Vol. 84, No. 2, pp. 316-321). Elsevier Masson. https://doi.org/10.1016/j.ando.2022.12.423

[26] Dutta P, Kumar Y, Babu AT, Giri Ravindran S, Salam A, Rai B, Baskar A, Dhawan A, Jomy M. Tirzepatide: A Promising Drug for Type 2 Diabetes and Beyond. Cureus. 2023 May 1;15(5):e38379. https://doi.org/10.7759/cureus.38379

[27] le Roux CW, Zhang S, Aronne LJ, Kushner RF, Chao AM, Machineni S, Dunn J, Chigutsa FB, Ahmad NN, Bunck MC. Tirzepatide for the treatment of obesity: Rationale and design of the SURMOUNT clinical development program. Obesity (Silver Spring). 2023 Jan;31(1):96-110. https://doi.org/10.1002/oby.23612

[28] Mishra R, Raj R, Elshimy G, Zapata I, Kannan L, Majety P, Edem D, Correa R. Adverse Events Related to Tirzepatide. J Endocr Soc. 2023 Jan 26;7(4):bvad016. https://doi.org/10.1210/jendso/bvad016

[29] Aronne LJ, Sattar N, Horn DB, et al. Continued Treatment With Tirzepatide for Maintenance of Weight Reduction in Adults With Obesity: The SURMOUNT-4 Randomized Clinical Trial. *JAMA.* 2024;331(1):38–48. https://doi.org/10.1001/jama.2023.24945

[30] Vuppalanchi R, Loomba R, Sanyal AJ, Nikooie A, Tang Y, Robins DA, Brouwers B, Hartman ML. Randomised clinical trial: Design of the SYNERGY-NASH phase 2b trial to evaluate tirzepatide as a treatment for metabolicdysfunction-associated steatohepatitis and modification of screening strategy to reduce screen failures. Aliment Pharmacol Ther. 2024 Jul;60(1):17-32. https://doi.org/10.1111/apt.18042

[31] Vuppalanchi R, Loomba R, Sanyal AJ, Nikooie A, Tang Y, Robins DA, Brouwers B, Hartman ML. Randomised clinical trial: Design of the SYNERGY-NASH phase 2b trial to evaluate tirzepatide as a treatment for metabolic dysfunction-associated steatohepatitis and modification of screening strategy to reduce screen failures. Alimentary Pharmacology & Therapeutics. 2024 May 20. https://doi.org/10.1111/apt.18042

[32] Packer M, Zile MR, Kramer CM, Baum SJ, Litwin SE, Menon V, Ge J, Weerakkody GJ, Ou Y, Bunck MC, Hurt KC. Tirzepatide for heart failure with preserved ejection fraction and obesity. New England Journal of Medicine. 2024 Nov 16.10.1056/NEJMoa2410027 https://doi.org/10.1056/nejmoa2410027

[33] Borlaug, B.A., Zile, M.R., Kramer, C.M. *et al.* Effects of tirzepatide on circulatory overload and end-organ damage in heart failure with preserved ejection fraction and obesity: a secondary analysis of the SUMMIT trial. *Nat Med* (2024). https://doi.org/10.1038/s41591-024-03374-z

[34] Mishra R, Raj R, Elshimy G, Zapata I, Kannan L, Majety P, Edem D, Correa R. Adverse Events Related to Tirzepatide. J Endocr Soc. 2023 Jan 26;7(4):bvad016. https://doi.org/10.1210/jendso/bvad016

[35] Liu L. A real-world data analysis of tirzepatide in the FDA adverse event reporting system (FAERS) database. Frontiers in Pharmacology. 2024 Jun 7;15:1397029. https://doi.org/10.3389/fphar.2024.1397029

[36] Patel H, Khunti K, Rodbard HW, Bajaj HS, Bray R, Kindracki Z, Rodríguez Á. Gastrointestinal adverse events and weight reduction in people with type 2 diabetes treated with tirzepatide in the SURPASS clinical trials. Diabetes, Obsity and Metabolism. 2024 Feb;26(2):473-81. https://doi.org/10.1111/dom.15333

[37] Tobaiqy M, Elkout H. Psychiatric adverse events associated with semaglutide, liraglutide and tirzepatide: a pharmacovigilance

analysis of individual case safety reports submitted to the EudraVigilance database. Int J Clin Pharm. 2024 Apr;46(2):488-495.
https://doi.org/10.1007/s11096-023-01694-7

[38] Tobaiqy M, Elkout H. Psychiatric adverse events associated with semaglutide, liraglutide and tirzepatide: a pharmacovigilance analysis of individual case safety reports submitted to the EudraVigilance database. Int J Clin Pharm. 2024 Apr;46(2):488-495.
https://doi.org/10.1007/s11096-023-01694-7

[39] Frias JP, Nauck MA, Van J, Benson C, Bray R, Cui X, Milicevic Z, Urva S, Haupt A, Robins DA. Efficacy and tolerability of tirzepatide, a dual glucose-dependent insulinotropic peptide and glucagon-like peptide-1 receptor agonist in patients with type 2 diabetes: A 12-week, randomized, double-blind, placebo-controlled study to evaluate different dose-escalation regimens. Diabetes Obes Metab. 2020 Jun;22(6):938-946.
https://doi.org/10.1111/dom.13979

[40] Waser B, Beetschen K, Pellegata NS, Reubi JC. Incretin receptors in non-neoplastic and neoplastic thyroid C cells in rodents and humans: relevance for incretin-based diabetes therapy. Neuroendocrinology. 2011;94(4):291-301.
https://doi.org/10.1159/000330447

[41] Song Y, Zhou M, Cao Y, Qi J, Geng J, Liu X. Expression of GLP-1 receptor and CD26 in human thyroid C-cells: The association of thyroid C-cell tumorigenesis with incretin-based medicine. Oncol Lett. 2017 Apr;13(4):2684-2690.
https://doi.org/10.3892/ol.2017.5752

[42] Caruso I, Di Gioia L, Di Molfetta S, Caporusso M, Cignarelli A, Sorice GP, Laviola L, Giorgino F. The real-world safety profile of tirzepatide: pharmacovigilance analysis of the FDA Adverse Event Reporting System (FAERS) database. J Endocrinol Invest. 2024 Nov;47(11):2671-2678.
https://doi.org/10.1007/s40618-024-02441-z

[43] Kannan S, Nasr C. Should we be concerned about thyroid cancer in patients taking glucagon-like peptide 1 receptor agonists?. Cleveland Clinic Journal of Medicine. 2015 Mar 1;82(3):142-4.

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Review Article

The Role of Insulin-Like Growth Factor (IGF-1) Signaling During Physical Exercise: A Systematic Review

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ABSTRACT

Exercise is one of the non-pharmacological therapies that functions to improve public health. This study seeks to determine how increased IGF-1 levels during exercise as a hormone can trigger growth. For this study, a number of journal databases were searched, such as PubMed, Web of Science, Embase, and Science Direct. This study considers several aspects, such as research on exercise and IGF-1 published in reputable journals over the past five years. Our analysis only includes items published in reputable international journals. Using databases total of 159 publications were found. In this comprehensive analysis, about ten carefully selected and peer-reviewed papers were included. The standard operating procedure for this investigation was developed using Preferred Reporting Systematics and Meta-analysis (PRISMA). Based on the results of this systemic investigation, exercise has been shown to increase IGF-1 levels, a hormone that promotes growth. Physical exercise as a therapeutic effort and a means of improving public health.

Introduction

Human beings undergo a biological aging phase that is associated with a decrease in muscular mass, brain size, and a reduction in mental functions including memory, and a decrease in fat-free mass commonly referred to as sarcopenia (1). The aging process is a natural physiological mechanism characterized by a decline in body functions both physically and hormonally. In this phase, most of the body's hormones decline in function including testosterone, growth hormone, and estrogen (2). Aging is strongly associated with the emergence of physiological degenerative effects, including body fat storage, skeletal muscle atrophy, and reduced cardiovascular function (3).

Skeletal muscle atrophy is a result of the decline of muscle function with age, which is closely related to the risk of developing metabolic syndrome in elderly people, is the most important health problem that needs to be anticipated (4). Maintaining healthy skeletal muscle is also important to prevent metabolic syndrome. Muscles have a very important role in glucose uptake (5). Physical exercise is a non-pharmacological therapy that has a beneficial impact on improving people's health status. Physical exercise promotes skeletal muscle mass, enhances insulin sensitivity, and boosts mitochondrial

biogenesis (6). Inactivity or sedentary lifestyle worsens the condition of the body by increasing the risk of developing metabolic syndrome (7). Skeletal muscle mass and ability for exercise are influenced by a number of variables, such as hormone levels and metabolic enzyme levels (8).

The function of exercise in improving public health has been well documented. Physical exercise is defined as planned, systematic, and repetitive physical activity that can improve body performance, physical fitness, and motor skills (9). IGF-1 is the most potent mediator that prevents organ failure brought on by illness by promoting cell division and proliferation (10). Furthermore, IGF-1 has a strong correlation with the growth of muscle mass and strength, as well as the control of metabolism and the regeneration (11). Under the direction of growth hormone, the liver produces the majority of the insulin-homologous protein IGF-1 (12). Local production of IGF-1 occurs in many organs, including vascular smooth muscle cells, endothelial cells, and immune cells such as monocytes, macrophages, and lymphocytes (13).

Age-related atrophy can be prevented by exercise by boosting IGF-1 expression, which increases muscle mass and function. This has been demonstrated in previous studies (14). Additional research has demonstrated that by upregulating IGF-1 expression, exercise can decrease oxidative stress, increase neurogenesis, and decrease inflammation (3). However, the mechanism of exercise in increasing IGF-1 expression is still debated and needs to be further explored whether the increase in IGF-1 during exercise can inhibit the mechanism of muscle atrophy or not and how the mechanism of IGF-1 increase during exercise is also still not fully understood. Therefore, this systematic review will discuss the role of physical exercise in increasing the expression of IGF-1.

Subjects and Methods

Study Design

As part of a systematic review process, this study examined and evaluated several journal databases, including Pubmed, Embase, Web of Science, and Science Direct.

Eligibility Criteria

The research on physical activity and the growth factor known as insulin-like (IGF-1) that have been published in the recent five years were the source of the inclusion criteria for this investigation. Among the publications not included in our analysis, papers published in renowned journals met the exclusion criteria for our study analysis.

Procedure

Articles that had been reviewed and verified were uploaded to Mendeley information database with their full text, abstract, and title. Using the databases from Pubmed, Web of Science, Embase, and Science Direct, 159 publications were located and included in the study's initial phase. In the subsequent phase, 87 publications were assessed for inclusion criterion appropriateness based on the abstract and title. 39 paper items were to be verified for additional processing during the third stage. We then applied a filter depending on whether the topic met the inclusion requirements and was appropriate for general discussion. Ten publications that satisfied the inclusion criteria were carefully chosen after thorough evaluation for this systematic review. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) assessment were utilized in this study.

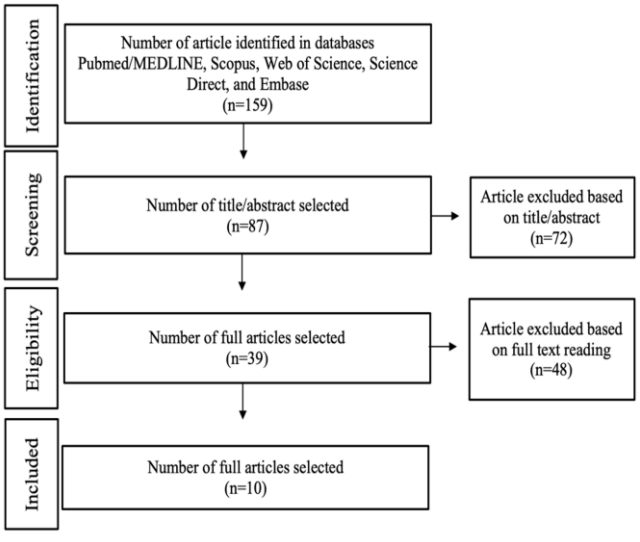

Figure 1. PRISMA flowchart of the article selection process

Results

The effect of the insulin like grawth factors on physical exercise are detailed in Table 1.

Discussion

This systematic review aims to investigate the mechanisms behind physical exercise as a non-pharmacological therapeutic effort in increasing IGF-1 expression. Physical exercise is proven to improve the health status of the community. Studies have demonstrated that engaging in physical activity can elevate IGF-1 expression. In this study, sixty-minute sessions of resistance and aerobic exercise were compared. Five days a week for four weeks. Additionally, compared to aerobic training, resistance training had increased IGF-1 expression, according to the results (3). So, from this study there are differences in IGF-1 expression depending on the kind of activity carried out. The results of another study with the type of resistance exercise intervention with a duration of 60 minutes per session 3x a week for 12 weeks also proved a rise in IGF-1 expression (15).

This also reinforces that resistance training has a beneficial effect on increasing IGF-1. Another factor that affects the high level of IGF-1 is a healthy person without having a disease disorder in the body. In accordance with the results of a study comparing IGF-1 levels in people suffering from dementia and not suffering from dementia, the results showed that people with no dementia had higher IGF-1 levels (16). Research from Pierce et al., 2020 also reinforces that resistance physical exercise provides a significant upregulation of IGF-1 expression (17). We can therefore conclude that exercise positively affects the rise in IGF-1 levels.

Table 1. Results of Physical Exercise Review on Increasing Insulin-Like Growth Factor (IGF-1)

Author	Sample Characteristics	Study Design	Intervention	Results
(Li et al., 2022) (3)	40 male rats participated in this study and were split up into five groups: eight people each for the groups that received electrical stimulation, whole-body vibration, resistance training, aerobic exercise, and control.	Experimental	Aerobic exercise running on a treadmill 60 minutes per day with an intensity of 76% VO2Max, For four weeks, five days a week. Resistance exercise by climbing stairs performed as many as 9 sets, with 3x per set for approximately For four weeks, spend 60 minutes a day, five days a week.	IGF-1 levels increased in the group that exercised. And the group that engaged in resistance exercise saw the biggest rise in IGF-1 levels.
(Son et al., 2020) (15)	Ten women were assigned to the treatment group and ten women to the control group among the twenty women who participated in the study.	Experimental	Resistance training sessions of sixty minutes each, three times a week for a duration of twelve weeks.	In the group that underwent physical activity intervention, IGF-1 levels rose.
(Stein et al., 2021) (16)	74 people participated in this study consisting of (n=34) with Alzheimer's illness and a group (n=40) free of dementia.	Experimental	Submaximal aerobic exercise on a treadmill	The group free of dementia illness had higher levels of IGF-1.
(Pierce et al., 2020) (17)	20 people participated in this study to administer the pretest and posttest.	Experimental	Acute resistance training test with six sets of ten exercises individual maximum repetitions (6 × 10-RM), separated by a 2-minute recovery period between sets.	Shortly after the physical exercise intervention, IGF-1 levels increased.
(Arazi et al., 2021) (1)	30 elderly men participated in this research and were split up into three groups: physical exercise intervention groups for strength (n = 10), endurance (n = 10), and control (n = 10).	Experimental	Participants in the endurance group jogged for thirty minutes at a maximum heart rate of sixty-seven percent, while those in the strength group engaged in two sets of resistance training (6 exercises with ten repetitions at a maximum of sixty-seven percent of one repetition).	IGF-1 levels significantly increased in the resistance and endurance intervention groups.
(Avazpour, 2020) (18)	27 nurses voluntarily divided into 3 groups namely HIIT type 1 (n=9), HIIT type 2 (n=9), and control group (n=9).	Experimental	HIIT (type 1) consists of 12 seconds of active recovery followed by 8 seconds of spring running. HIIT (type 2), which consists of a sprint-only 40-meter shuttle run. For four weeks, there were three sessions per week of instruction.	IGF-1 levels rose in the two groups that underwent physical activity intervention.
(Kang et al., 2020) (19)	The study involved 20 older women who were divided into two groups: the control group (n = 10) and the physical activity intervention treatment group (n = 10).	Experimental	Aquatic exercise was conducted for 60 minutes throughout a 16-week period, three times a week.	IGF-1 levels in the group that had physical activity intervention rose noticeably.
(Cho & Roh, 2019) (20)	In this study, 37 healthy women were split into two groups: regular taekwondo practitioners (n = 19) and control practitioners (n = 18).	Experimental	Taekwondo training for 60 minutes per session at an intensity of 50%-80% HRmax 5x a week for 16 weeks.	There was an increase in IGF-1 levels in the group with taekwondo training intervention.
(Żebrowska et al., 2020) (21)	28 people consisting of 14 people in the group with type 1 diabetes mellitus and 14 people in the healthy group participated in this study to do the pretest and posttest.	Experimental	On a cycle ergometer, perform a 40-minute continuous exercise at a moderate level (50% lactate threshold) in normoxia (Nor) and hyperemia (FiO2 = 15.1%).	There was an increase in IGF-1 levels in both groups but the highest increase occurred in people without diabetes mellitus.
(Birinci et al., 2022) (22)	In this study, 40 participants were split into 4 groups: 10 table tennis players, 10 long distance runners, 10 chess players, and 10 controls.	Experimental	Each sport's recommended training regimen consists of a 10-minute warm-up and 40 minutes of targeted physical activity.	IGF-1 levels increased significantly in the runners and tennis groups.

Improving the quality of human life is influenced by muscle mass and function. As we get older, organ function and performance decline, so regular exercise can increase muscle hypertrophy and metabolic capacity of the human body (23). Atrophy that occurs with age can be prevented by doing regular physical exercise. So this provides basic assurance that exercise is the best effort in improving the health status of the community. Regular physical exercise is very effective in increasing muscle hypertrophy and reducing muscle atrophy caused by pathological factors (24). It has been studied that variables such as increased IGF-1 levels, protein synthesis, angiogenesis, and proliferation of muscle satellite cells could be targets for exercise-induced muscle hypertrophy (25). It has also been demonstrated that IGF-1 enhances the strength and shape of muscle fibers (26).

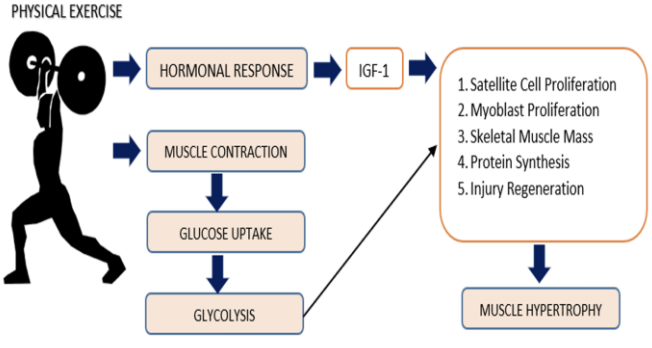

Figure 2. The Mechanisms Physical Exercise Increase Insulin-Like Growth Factor (IGF-1)

Skeletal muscle growth has been shown to be mediated by IGF-1/IGF-1R via a number of mechanisms, including the PI3K/Akt signaling pathway (11). In this animal studies, we discovered that physical exercise greatly increased skeletal muscle composition and performance, which is highly correlated with the IGF-1/IGF-1R-PI3K/Akt signaling pathway's activation (3). Still unknown, though, is whether other elements also contribute to muscle growth or if physical exercise's ability to promote muscle hypertrophy is primarily due to IGF-1 signaling activation (3). In mice with myocardial infarction, previous studies have shown that resistance and aerobic exercise can increase the expression of IGF-1 protein and promote skeletal muscle growth (14). Insulin-like growth factor-1 (IGF-1) controls the proliferation, differentiation, and survival of cells, which is essential in correcting disease-induced organ failure (10). IGF-1 has also been linked to the growth of muscle mass and strength, as well as the control of metabolism and the regeneration of skeletal muscle (11).

The mechanism of IGF-1 increase during physical exercise is still not entirely clear and is debated among researchers. The complex molecular mechanisms that occur in cells make us always want to understand more deeply how the stages that occur in cells during physical exercise. During physical exercise, the need for ATP will increase along with the activity undertaken. The primary regulator of skeletal muscle metabolism is adenosine monophosphate-activated protein kinase (AMPK), an intracellular sensor that controls ATP consumption (11). The primary source of circulating IGF-1 is the liver, and deletion of the IGF-1 gene unique to the liver causes a 70–80% reduction in serum IGF-1 levels (11). Nonetheless, IGF-1 levels in serum rose dramatically following aerobic exercise, according to a study done on aged males (1).

Other research' findings demonstrate that high intensity interval training (HIIT) is proven to increase IGF-1 levels (18). Another study, an aquatic exercise intervention of 60 minutes demonstrated that the IGF-1 levels significantly increased after each session, three times a week, for sixteen weeks (19). The findings of other research support this idea, showing that taekwondo athletes who receive taekwondo training interventions have elevated IGF-1 levels. Five sessions of sixty minutes a week for sixteen weeks (20). So, it has been proven that IGF-1 levels increase during physical exercise. However, this systematic review has the limitation that it only examines the increase in IGF-1 during physical exercise. There are still many other parameters that should be further explored that are related to increasing muscle mass and growth. This is very important because it can provide knowledge to researchers and readers in the wider audience related to how the role of physical exercise provides efforts related to public health benefits and increased muscle mass through IGF-1 secretion.

Conclusion

Research results prove that physical exercise can increase IGF-1 levels. These IGF-1 levels circulate and become a trigger signal for muscle hypertrophy. Regular physical exercise is a non-pharmacological therapy in improving the health status of the community.

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Conflict of Interest

The authors declare no conflict of interest.

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References

[1] Arazi H, Babaei P, Moghimi M, Asadi A. Acute effects of strength and endurance exercise on serum BDNF and IGF-1 levels in older men. BMC Geriatr. 2021;21(1):1-8. https://doi.org/10.1186/s12877-020-01937-6

[2] Sundari LPR, Arsani NLKA. Regular Physical Exercise Increase Of Growth Hormone (GH) And Insulin-Like Growth Factor-1 (IGF-1) Activity in Elderly Improve the Aging Process and Quality of Life: A Mini Review. Biomed Pharmacol J. 2022;15(2):883-890. https://dx.doi.org/10.13005/bpj/2422

[3] Li B, Feng L, Wu X, Cai M, Yu JJ, Tian Z. Effects of different modes of exercise on skeletal muscle mass and function and IGF-1 signaling during early aging in mice. J Exp Biol. 2022;225(21). https://doi.org/10.1242/jeb.244650

[4] Loh DR, Tan RS, Lim WS, Koh AS. Cardio-sarcopenia: A syndrome of concern in aging. Front Med. 2022;9(October):1-8. doi:10.3389/fmed.2022.1027466 https://doi.org/10.3389/fmed.2022.1027466

[5] Sylow L, Tokarz VL, Richter EA, Klip A. The many actions of insulin in skeletal muscle, the paramount tissue determining glycemia. Cell Metab. 2021;33(4):758-780. https://doi.org/10.1016/j.cmet.2021.03.020

[6] Bishop DJ, Botella J, Genders AJ, et al. High-intensity exercise and mitochondrial biogenesis: Current controversies and future research directions. Physiology. 2019;34(1):56-70. https://doi.org/10.1152/physiol.00038.2018

[7] Pillon NJ. Mitochondrial response to inactivity-induced muscle disuse and exercise training. Eur J Appl Physiol. 2023;123(2):243-245. doi:10.1007/s00421-022-05120-0 https://doi.org/10.1007/s00421-022-05120-0

[8] Barone B, Napolitano L, Abate M, et al. The Role of Testosterone in the Elderly: What Do We Know? Int J Mol Sci. 2022;23(7):1-21. https://doi.org/10.3390/ijms23073535

[9] Mahindru A, Patil P, Agrawal V. Role of Physical Activity on Mental Health and Well-Being: A Review. Cureus. 2023;15(1):1-7. https://doi.org/10.7759/cureus.33475

[10] Ahmad SS, Ahmad K, Lee EJ, Lee YH, Choi I. Implications of Insulin-Like Growth Factor-1 in Skeletal Muscle and Various Diseases. Cells. 2020;9(8):1-15. https://doi.org/10.3390/cells9081773

[11] Yoshida T, Delafontaine P. Mechanisms of IGF-1-Mediated Regulation of Skeletal Muscle Hypertrophy and Atrophy. Cells. 2020;9(9):1-25. https://doi.org/10.3390/cells9091970

[12] Wolters TLC, Netea MG, Riksen NP, Hermus ARMM, Netea-Maier RT. Acromegaly, inflammation and cardiovascular disease: a review. Rev Endocr Metab Disord. 2020;21(4):547-568. https://doi.org/10.1007/s11154-020-09560-x

[13] Grzywa-Czuba R, Trojanek JB, Michałkiewicz J, et al. Association between Expression of Insulin-like Growth Factor-1 (IGF-1), IGF-1 Receptor (IGF-1R), and Hypertension-Mediated Organ Damage (HMOD) Parameters in Leukocytes and Plasma of Children/Adolescents with Primary Hypertension. J Pers Med. 2024;14(3). https://doi.org/10.3390/jpm14030255

[14] Feng L, Li B, Xi Y, Cai M, Tian Z. Aerobic exercise and resistance exercise alleviate skeletal muscle atrophy through IGF-1/IGF-1R-PI3K/Akt pathway in mice with myocardial infarction. Am J Physiol - Cell Physiol. 2022;322(2):C164-C176. https://doi.org/10.1152/ajpcell.00344.2021

[15] Son WM, Pekas EJ, Park SY. Twelve weeks of resistance band exercise training improves age-associated hormonal decline, blood pressure, and body composition in postmenopausal women with stage 1 hypertension: a randomized clinical trial. Menopause. 2020;27(2):199-207. https://doi.org/10.1097/GME.0000000000001444

[16] Stein AM, da Silva TMV, Coelho FG de M, Rueda AV, Camarini R, Galduróz RFS. Acute exercise increases circulating IGF-1 in Alzheimer's disease patients, but not in older adults without dementia. Behav Brain Res. 2021;396(August 2020). https://doi.org/10.1016/j.bbr.2020.112903

[17] Pierce JR, Martin BJ, Rarick KR, et al. Growth Hormone and Insulin-like Growth Factor-I Molecular Weight Isoform Responses to Resistance Exercise Are Sex-Dependent. Front Endocrinol (Lausanne). 2020;11(August):1-9. https://doi.org/10.3389/fendo.2020.00571

[18] Avazpour S. The Effect of Two Types of High-Intensity Interval Training on Serum Value of GH and IGF-1 in Overweight Nurses. 2020;11(4):6-11. https://doi.org/10.5812/asjsm.103135.Research

[19] Kang D wang, Bressel E, Kim D yeon. Effects of aquatic exercise on insulin-like growth factor-1, brain-derived neurotrophic factor, vascular endothelial growth factor, and cognitive function in elderly women. Exp Gerontol. 2020;132:110842. https://doi.org/10.1016/j.exger.2020.110842

[20] Cho SY, Roh HT. Taekwondo enhances cognitive function as a result of increased neurotrophic growth factors in elderly women. Int J Environ Res Public Health. 2019;16(6). https://doi.org/10.3390/ijerph16060962

[21] Żebrowska A, Sikora M, Konarska A, et al. Moderate intensity exercise in hypoxia increases IGF-1 bioavailability and serum irisin in individuals with type 1 diabetes. Ther Adv Endocrinol Metab. 2020;11:1-17. https://doi.org/10.1177/2042018820925326

[22] Birinci Y, Sagdilek E, Taymur I, et al. Acute effects of different types of exercises on insulin-like growth factor-1, homocysteine and cortisol levels in veteran athletes. Med Sci | Int Med J. 2022;11(3):968. https://doi.org/10.5455/medscience.2022.02.028

[23] Lewsey SC, Gerstenblith G, Weiss RG, et al. Exercise intolerance and rapid skeletal muscle energetic decline in human age-associated frailty. JCI Insight. 2020;5(20):e141246. https://doi.org/10.1172/jci.insight.141246

[24] Yin L, Lu L, Lin X, Wang X. Crucial role of androgen receptor in resistance and endurance trainings-induced muscle hypertrophy through IGF-1/IGF-1R-PI3K/Akt-mTOR pathway. Nutr Metab. 2020;17(1):1-10. https://doi.org/10.1186/s12986-020-00446-y

[25] Larsson L, Degens H, Li M, et al. Sarcopenia: Aging-related loss of muscle mass and function. Physiol Rev. 2019;99(1):427-511. https://doi.org/10.1152/physrev.00061.2017

[26] Ascenzi F, Barberi L, Dobrowolny G, et al. Effects of IGF-1 isoforms on muscle growth and sarcopenia. Aging Cell. 2019;18(3):1-11. https://doi.org/10.1111/acel.12954

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Review Article

Deciphering Neuro-Ophthalmic Presentations of Carotid Cavernous Fistulas: A Comprehensive Analysis of Venous Flow Dynamics and Clinical Implications

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Keywords: Carotid cavernous fistula; Neuro-ophthalmic presentations; Venous flow dynamics; Cranial nerve pathology; Optic nerve.

ABSTRACT

Background: Carotid cavernous fistulas (CCFs) are abnormal arteriovenous connections between the carotid arterial system and the cavernous sinus, presenting with complex neuro-ophthalmic manifestations due to the redirection of arterial blood into the venous system. This study systematically reviews the diverse neuro-ophthalmic presentations of CCFs, emphasizing the influence of venous flow dynamics.

Subjects and Methods: A comprehensive literature search was conducted in PubMed, Scopus, and Web of Science, focusing on keywords such as "carotid cavernous fistula," "neuro-ophthalmic presentations," and "optic nerve compression." Recent and historical studies were analyzed to assess the neuro-anatomical impacts on neuro-ophthalmic symptoms from CCFs. This review aimed to consolidate knowledge and refine diagnostic and therapeutic strategies for CCF management.

Results: The findings demonstrated that the neuro-ophthalmic presentations were quite distinctive, depending on the direction of venous flow. Anterior flow, overall, was associated with proptosis, conjunctival arterialization, and red, dilated vessels. In contrast, posterior flow was associated with cranial nerve complications, most notably abducens nerve palsy and trigeminal neuralgia, manifesting as double vision and facial pain, respectively. It therefore seems that the symptomatology and severity of CCFs is heavily dependent on the direction of blood flow.

Conclusions: The flow direction through the veins is of significant importance in the clinical presentation and prognosis of CCFs. These dynamics must be appreciated to improve diagnosis and allow for optimal treatment, thus averting the risk of fatal complications. In this review, the need for tailored therapeutic strategies through detailed vascular and neuro-ophthalmologic analysis is outlined.

Introduction

Carotid cavernous fistulas represent an abnormal arteriovenous connection between the carotid arterial system and the cavernous sinus with a host of neuro-ophthalmic manifestations (1,2). In essence, this was an excellent transcript on how this condition significantly impacts ocular and cranial structures through the shunting of arterial blood into the venous system. Symptoms may vary from proptosis and conjunctival arterialization to vision loss and ophthalmoplegia, depending on the flow dynamics and the particular venous drainage (1,3). Precise diagnosis is essential in view of the

numerous presentations of the condition and the critical importance of appropriate management based on diagnosis; often it is necessary to use classifications such as the Barrow system (4). In spite of the great technical and material progress of imaging and intervention methods, there is a continuing critical need that, to our best knowledge, no one else provides: a comprehensive review of the extant literature, which consolidates the current status with regard to the neurophthalmic presentations of this clinical entity: the carotid cavernous fistula. Such a review is pivotal for refining diagnostic accuracy and optimizing treatment strategies, thereby enhancing patient outcomes. This article aims to address this gap by systematically examining and synthesizing the wealth of information available.

Subjects and Methods

A literature search was conducted through PubMed, Scopus, and Web of Science using the relevant keywords "carotid cavernous fistula," "neuro-ophthalmic presentations," and "optic nerve compression". This review will focus on contemporary research and will be substantiated by the relevant key articles of the past, which will act as critical foundational information on carotid cavernous fistulae and their neuro-ophthalmic implications. The selection of articles for this review was based on inclusion criteria for those studies that impact the neuro-anatomical symptoms caused by the carotid cavernous fistulas. Included articles have been qualitatively analyzed to describe and discuss the information they provided on the advances made on both diagnosis and treatment. This review will create a scaffold and will indicate potential areas for further research regarding the neuro-ophthalmic management of carotid cavernous fistulas and the concomitant development of diagnostic criteria and treatment modalities.

Results

According to the identified neuro-ophthalmic manifestations of CCFs and as organized in Table 1 (1-8), the characteristic clinical manifestations are related to the venous flow direction across the anatomical structures of question. For example, anterior flow is mainly the cause of proptosis and the characteristic "red eye" since it leads to arterialization across the superior and inferior ophthalmic veins (1).

In contrast, posterior flow mainly compromises the cranial function and is the chief cause of the double vision that results from the abducens nerve palsy (2) and the trigeminal neuralgia-associated facial pain or headache (3). Therefore, depending on the venous flow direction, the CCF symptoms in the patients will be dependent and varied.

Table 2 presents a comprehensive summary of the clinical characteristics and venous flow dynamics observed in patients with carotid cavernous fistulas (CCFs) across various studies (Table 2) (12-27).

This table includes data from selected case reports and original research studies, highlighting key parameters such as sample size, patient age, sex distribution, study design, clinical manifestations, and venous flow dynamics.

The analysis reveals that the clinical manifestations of CCFs vary significantly based on the direction of venous flow. Anterior venous flow is primarily associated with ocular symptoms such as proptosis, conjunctival arterialization, and red dilated vessels. In contrast, posterior venous flow is more frequently linked to cranial nerve complications, including abducens nerve palsy, resulting in double vision, and trigeminal neuralgia, presenting as facial pain or headache. This differentiation underscores the importance of understanding venous flow dynamics in diagnosing and managing CCFs effectively. Overall, the data emphasize the necessity of tailored diagnostic and therapeutic approaches based on the specific venous flow patterns in CCF patients. This targeted approach can help optimize patient outcomes and mitigate potential complications associated with CCFs (Table 2) (12-27).

Table 1. Neuro-Ophthalmic Presentations of Carotid Cavernous Fistulas by Venous Flow Direction

Flow Direction & Anatomical Impact	Clinical Presentation	Explanation
Anterior Flow: Superior and Inferior Ophthalmic Veins	Proptosis, "red eye," and potential vision loss due to increased venous pressure.	Arterialized blood flows into the ophthalmic veins, causing dilation and congestion, visible as red, dilated vessels.
Anterior Flow: Conjunctiva and Episclera	Arterialization visible as squiggly, dilated, tortuous vessels on the conjunctiva and episclera.	The direct arterial blood into these areas causes distinct redness and vessel tortuosity.
Posterior Flow: Cranial Nerve VI (Abducens)	Double vision due to inability to move the eye laterally, typically presenting as abducens nerve palsy.	Increased pressure or compression within the cavernous sinus impacts the abducens nerve.
Posterior Flow: Cranial Nerve V (Trigeminal)	Facial pain or headache, indicative of trigeminal neuralgia.	The trigeminal nerve is affected by altered venous drainage patterns or direct compression in the cavernous sinus.

Discussion

The literature review related to CCFs underlines the fact that their neuro-ophthalmic presentations must be well understood. Abnormal communications between the carotid arterial system and the cavernous sinus create different signs and symptoms due to unusual arterial blood flow into the venous system. This paper nicely outlines the various clinical presentations, the Barrow classification, which subdivides fistulas according to the source of the blood flow: from the internal carotid artery itself (Type A) and its branches (Types B, C, and D) (4). Understanding this classification and the associated clinical presentations helps to develop an excellent management approach to CCFs.

It is through this pathophysiological understanding that allows CCFs' clinical manifestations to be explained. The pathophysiology is based on a redirection of arterial blood into the venous system through the cavernous sinus, which affects the superior and inferior ophthalmic veins. Clinical symptoms are best described by the flow, either

anterior or posterior. Anterior flow leads to "red eye shunts." These include proptosis of the eyes with a visible arterialization phenomenon of the conjunctiva and episclera, described as squiggly, dilated, and tortuous vessels (1). Presentation may also include symptoms of optic neuropathy or ocular ischemia due to the pressure exerted by the sudden increase in venous return (2,3).

On the other hand, posterior flow affects the cranial nerves within the cavernous sinus. It affects the abducens and trigeminal nerves. When the abducens nerve is affected, there is diplopia and lateral gaze palsy, while involvement of the trigeminal nerve leads causes facial pain or headache sometimes associated with venous congestion or even direct nerve compression (3). The diseases generally present as "red eye shunts," but as it progresses, so does the pressure in the vein. Initially, they can be "white eye shunts," where there are not very many findings on the face, but there is significant cranial nerve involvement.

The management of CCFs relies heavily on understanding the nature of the fistula, with the Barrow classification guiding treatment decisions. For instance, direct high-flow fistulas (Type A) typically require more aggressive and immediate intervention to prevent irreversible damage due to high arterial pressure within the ocular and cranial structures. In contrast, the lower-flow fistulas (Types B, C, and D) might be managed conservatively depending on the symptoms and the risk of progression to cortical venous drainage, which can be life-threatening (4).

Management is further complicated by the possible long-term complications of neglected or poorly managed CCFs. These may lead to chronic ocular symptoms, unrelenting cranial nerve palsy, and even increased susceptibility to stroke because of cortical venous reflux. In this context, this is the exact importance of early and proper intervention, for which detailed angiographic studies and classifications are needed in the mitigation of the risks and the improvement of the outcomes for these patients.

The spread of diagnostic imaging, particularly through computed tomography (CT), magnetic resonance imaging (MRI), and digital subtraction angiography (DSA), has greatly increased the detection and characterization of carotid cavernous fistulas (CCFs). Additionally, such diagnostic tools were not only essential for confirmation but also provided crucial information on the anatomy of the fistula, the course, and flow characteristics thereof, and the neighboring structures in the anticipated therapeutic procedures (1, 4, 5).

The variety of clinical presentations and outcomes of CCFs demonstrates the complexity of the condition. According to Williams (2018) (2), therapy diversified substantially, and case-tailored treatment was being constantly emphasized at different clinical representations. Sunit Das et al. (2006) (5) demonstrated that, following a posterior approach, transarterial coiling led to stabilization of vision of a CCF patient and, if appropriate interventions are taken, significant recovery may be expected. Additionally, E. Klevtsova et al. (2015) (6) presented a case of acquired posttraumatic CCF with seizures and focal neurological findings. Such studies or reviews show the scale of possible neuronal manifestations of CCFs.

Spontaneous resolution without surgery might also occur in CCF. Accordingly, M. Bujak et al. (2010) (7) reported two cases of spontaneous closure of dural carotid-cavernous fistula. Findings suggest that a natural occlusion mechanism may be present in some dural CCFs. On the other hand, post-embolization, N. Bonnin et al. (2013) (8) also displayed changes in signs as well as neuroradiological features that pointed toward recovery. W. Spencer et al. (1973) (9), on the other hand, reported extreme cases as well as those of ischemic ocular necrosis secondary to untreated CCFs, which reiterated the need for management on time and at all levels.

These studies collectively emphasize the necessity of a comprehensive diagnostic and therapeutic approach. A. Eswar et al. (2014) (10) reported on a case of CCF mimicking myasthenia gravis, posing a diagnostic challenge and necessitating advanced diagnostic strategies to differentiate CCF from other neurological disorders. Furthermore, collaborative efforts described by E. Zanaty et al. (2005) (11) between neuro-ophthalmologists and interventional neuroradiologists exemplify the multidisciplinary approach required to effectively manage these complex cases.

The findings of this study align with previous research on CCFs which underscores the significance of understanding venous flow dynamics in managing CCFs. Clinical and angiographic characteristics of dural arteriovenous fistulas, as highlighted by Suh et al. (2013) and Miller et al. (1995), demonstrate the complexity and variability of these conditions. The endovascular treatment of CCFs, particularly through transvenous embolization, has been shown to be effective in numerous studies, including those by Gupta et al. (2006) and Alexander et al. (2010), further validating our approach to patient management (28-31).

Moreover, the pathophysiological understanding of CCFs and their clinical manifestations, as described by Tsai et al. (2016) and Miller et al. (2015), emphasizes the role of detailed imaging and angiographic studies in diagnosis and treatment planning. Studies by Halbach et al. (1987) and Suet al. (2014) have shown that the direction of venous drainage significantly influences clinical outcomes, reinforcing the need for tailored therapeutic strategies based on individual venous flow patterns. The varied clinical presentations and outcomes reported by Biondi et al. (2003) and Ellis et al. (2012) further highlight the importance of a personalized approach to CCF management (32-40).

This critical review on CCF has a number of limitations: the lack of consistency in reporting standards and reliance on historical data mean that potential bias can be introduced into the results; hence, it affects both the final result and generalizability. Besides, rapid improvement of diagnostic and treatment approaches makes data from the past hardly having a clear impact on present practices. In addition, these events are very rare, with difficulty in collection of large and diverse groups, thus limiting the strength of the results. These gaps need prospective research with standard reporting to upgrade our understanding and management of CCFs.

Table 2. Clinical Findings and Venous Flow Dynamics of A number of Case Reports and Original Research Studies Associated with Carotid Cavernous Fistulas

Author	Publication year	Sample size	Mean age±SD	Sex (N,%)	Study Design	Origin of the Study	Clinical Manifestations of patients	Venous Flow Dynamics
Pérez Sempere et al.[12]	1991	2	66.5 ± 3.5	Female (2, 100%)	Case Report	Spain	Case 1 presents with right-sided ptosis, impaired right-eye adduction, a dilated nonreactive right pupil, headache, and diplopia. Case 2 presents with left-sided ptosis, impaired left-eye adduction, headache, and diplopia.	Posteriorly draining dural fistula
Aciernoe t al.[13]	1995	2	63.5 ± 2.5	Female (2, 100%)	Case Report	USA	Case 1 presents with left abduction deficit, periocular pain, and horizontal diplopia. Case 2 exhibits persistent headache, horizontal diplopia, right upper eyelid ptosis, and impaired ocular movement in the right eye.	Posterior drainage into inferior petrosal sinus
Loré et al.[14]	2003	1	67 (Single patient)	Female (1, 100%)	Case Report	Italy	The patient presents with moderate swelling around both eyes, redness of the sclera, swelling of the conjunctiva, slight bulging of the eyes, eye pain, excessive tears, and diplopia.	Dural carotid cavernous fistula draining into the ipsilateral superior ophthalmic vein
Peng et al.[15]	2004	1	42 (Single patient)	Female (1, 100%)	Case Report	Taiwan	The right eye exhibits pain, protrusion, and limited movement. Elevated intraocular pressure of 30 mmHg in the right eye. Proptosis and abduction restriction. The right eye exhibits dilated conjunctival and episcleral vessels, as well as a hyperemic disc.	Using color Doppler ultrasonography, retrograde pulsatile flow in the right superior ophthalmic vein was seen.
Ikeda et al.[16]	2005	1	55 (Single patient)	Female (1, 100%)	Case Report	Japan	The patient is experiencing a severe and persistent headache in the right orbitofrontal region, as well as diplopia caused by paralysis of the right abducens nerve. No symptoms of orbito-ocular involvement were found during the entire clinical course.	In the arterial phase, the dural carotid-cavernous sinus fistula has three directional drainage pathways. significant outflow into the vein of the eyes (SOV), High-rate outflow into the angular facial vein that stops the SOV from being enhanced for an extended period of time during the venous phase
Rooij et al.[17]	2006	11	61.6 ± 15.2 (ranging from 27 to 77 years)	Female (8, 72.7%), Male (3, 27.3%)	Original Research	Netherlands	The presence of a clearly audible pulsatile bruit is observed in 100% of cases. There is a condition called bilateral exophthalmus with ophthalmoplegia, which occured in 63.6% of cases. Reduced visual acuity (8, 72.7%), Hemiplegia with aphasia occured in 9.1% of cases. The occurrence of cerebral bleeding is related with significant cortical venous drainage in a majority of cases (2, 18.2%).	High-flow CCFs: 5 cases, Intermediate-flow CCFs: 3 cases, Low-flow CCFs: 3 cases, Venous drainage to superior ophthalmic veins, minor cortical venous drainage
Das et al., [18]	2006	1	58 (Single patient)	Male (1, 100%)	Case Report	USA	The patient experienced a gradual deterioration of vision in the right eye, leading to total blindness within a period of 5 weeks after undergoing carotid artery angioplasty and stenting. The patient presents with symptoms including right proptosis (bulging of the eye), retro-orbital discomfort (pain behind the eye), right facial numbness, pulsatile proptosis impaired visual acuity, and intermittent diplopia. The individual experiences total loss of vision in the right eye, including the inability to perceive light, and paralysis of the muscles that control eye movement.	Angiography verified high-flow direct CCF, and the cavernous region was reached with dissection of the proximal right ICA. Superior and inferior ophthalmic vein engorgement

Théaudin et al.[19]	2008	1	75 (Single patient)	Female (1, 100%)	Case Report	France	The patient experienced frontal headache of severe extent, temporary and recurrent diplopia, bilateral conjunctival injection, episcleral and conjunctival hyperemia on both eyes, vertical paresis of the left eye, ptosis and complete ophthalmoplegia of the right eye, partial motor seizures, facial palsy on the right side, and aphasia.	DCCF draining into leptomeningeal veins; Outflow veins: right inferior petrosal sinus occluded; left superficial sylvian vein draining into left temporal and parietal lobe cortical veins; left inferior petrosal sinus draining into the internal jugular vein
Kim et al.[20]	2013	1	32 (Single patient)	Female (1, 100%)	Case Report	Republic of Korea	Blowout fractures on both sides, Diplopia characterized by impaired abduction of the left eye, No orbito-ocular indications, including chemosis, ptosis, or exophthalmos.	Posteriorly draining CCF with isolated abducens nerve palsy
Erickson et al.[21]	2014	1	32 (Single patient)	Male (1, 100%)	Case Report	USA	The patient demonstrated proptosis, ocular motility deficits, decreased vision in the right eye, orbital bruit, minor right exotropia, moderate motility deficits in all cardinal gaze directions, dilated conjunctival blood vessels, and 3 mm of proptosis in the right eye.	Profound dilatation of the right superior ophthalmic vein
Leishang them et al.[22]	2017	1	71 (Single patient)	Female (1, 100%)	Case Report	USA	Progressive left-sided monocular diplopia and ptosis were initially diagnosed as monocular myasthenia gravis. The patient also exhibited left-sided proptosis, ocular bruit, partial third and fourth nerve palsies, chemosis of the left eye inferiorly, and corkscrewing of conjunctival blood vessels.	Indirect high-flow left CCF, type D (supply from both ICA/ECA meningeal branches)
Lin et al.[23]	2019	1	32 (Single patient)	Male (1, 100%)	Case Report	Taiwan	The patient demonstrated progressive double vision for four months, right-sided headache and periocular pain, right-side partial ptosis with a mid-dilated right pupil and a poor reaction to light, limited right-side extraocular movement with impaired adduction, no chemosis, proptosis, conjunctival injection, swollen eyelids, or ocular bruits, normal best-corrected visual acuity and intraocular pressure in both eyes, and normal other cranial nerve functions.	Right-sided CCF is mostly fed by the right inferior petrosal sinus via venous drainage from the dural branch of the right middle meningeal artery.
Azzam et al.[24]	2021	1	29 (Single patient)	Male (1, 100%)	Case Report	USA	The patient exhibited progressive proptosis, diplopia, right-sided proptosis, periorbital edema, and conjunctival injection. Generalized ophthalmoplegia of the right eye was most pronounced during abduction. The patient also exhibited tortuous episcleral vessels and blood in Schlemm's canal, as well as dilated, tortuous retinal vessels.	Low-flow indirect carotid-cavernous fistula (CCF) with flow reversal into the right superior ophthalmic vein and Cavernous sinus enhancement on CT
Cavasin et al.[25]	2021	1	68 (Single patient)	Female (1, 100%)	Case Report	Italy	The patient demonstrated lacrimation, visual disturbance, redness and edema of the conjunctiva, spontaneous retrobulbar pain, and pain on horizontal or vertical gaze. Additionally, the patient experienced subjective intermittent diplopia due to eye motility impairment. The patient did not exhibit any signs of optic nerve involvement, such as visual acuity loss, and no significant	Exophthalmos on the left Without a discernible hypertrophic superior or inferior ophthalmic vein, the size of the global left extrinsic ocular muscles increases.

							hypertrophic superior or inferior ophthalmic vein.	
Pellegrini et al.[26]	2022	1	92 (Single patient)	Female (1, 100%)	Case Report	USA	The patient displayed redness in both eyes, swelling of the eyelids, inflammation of the conjunctiva, paralysis of eye movements, drooping of the eyelids, protrusion of both eyes, severe inflammation and congestion of the conjunctiva, almost complete paralysis of eye movements in both eyes, complete drooping of the right upper eyelid, visual acuity of 20/200 with corrective lenses in both eyes, intraocular pressure of 21 mmHg in both eyes, and mild enlargement of retinal veins with no swelling of the optic disc.	Dilation of the superior ophthalmic veins on both sides. High-flow carotid-cavernous fistula in direct alignment with subsequent expansion of the extraocular muscles
Krothapalli et al.[27]	2023	1	56 (Single patient)	Male (1, 100%)	Case Report	USA	Progressive right eye proptosis, congestion, reduced visual acuity (20/40), limited duction, exophthalmos, pulsatile tinnitus, elevated intraocular pressure, and no optic disc swelling were all present in the patient. However, there was a significant amount of inflammation in the right orbit, as well as unusual enhancement of the basal frontal lobe next to the orbit.	Superior ophthalmic vein enlargement enlarged frontal vein Right carotid cavernous fistula (CCF) in indirect form Right internal jugular vein stenosis combined with right sigmoid sinus thrombosis

Conclusion

Neuro-ophthalmological examination of patients with carotid-cavernous fistulas shows that the venous flow direction determines the clinical manifestation. Anterior fistula flow results in the appearance of visible symptoms like proptosis and red eye, due to arterialization, while the posterior fistula flow impinges on the functioning of the cranial nerves with the symptoms that carry the potential of the worst eventualities, like diplopia and facial pain. Appropriate management of CCFs relies on correct classification and understanding of the flow dynamics in a manner that can prevent serious complications and improve outcomes.

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Conflict of Interest

Authors declare no conflict of interest.

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References

[1] Brosnahan D, McFadzean RM, Teasdale E. Neuro-ophthalmic features of carotid cavernous fistulas and their treatment by endoarterial balloon embolisation. J Neurol Neurosurg Psychiatry. 1992;55(7):553-556. https://doi.org/10.1136/jnnp.55.7.553

[2] Williams ZR. Carotid-Cavernous Fistulae: A Review of Clinical Presentation, Therapeutic Options, and Visual Prognosis. Int Ophthalmol Clin. 2018;58(4):103-114. https://doi.org/10.1097/IIO.0000000000000215

[3] Chong Ying Sze, Bakin S, Ismail R. Neuropsychiatric Presentation in Indirect Carotid-Cavernous Fistula: A Case Report. Cureus. 2023;15:e37523. https://doi.org/10.7759/cureus.37523

[4] Barrow DL, Spector RH, Braun IF, Landman JA, Tindall SC, Tindall GT. Classification and treatment of spontaneous carotid-cavernous sinus fistulas. J Neurosurg. 1985 Feb;62(2):248-56. https://doi.org/10.3171/jns.1985.62.2.0248

[5] Das S, et al. Return of vision after transarterial coiling of a carotid cavernous sinus fistula: case report. Surg Neurol. 2006;66(1):82-85. https://doi.org/10.1016/j.surneu.2005.10.008

[6] Klevtsova E, et al. Posttraumatic carotid cavernous fistula presenting with seizure and focal neurological deficits. J Emerg Med. 2015;48(2):186-190. https://doi.org/10.1016/j.jemermed.2014.09.043

[7] Bujak M, et al. Spontaneous resolution of two dural carotid-cavernous fistulas presenting with optic neuropathy and marked congestive ophthalmopathy. J Neuroophthalmol. 2010;30(3):222-227. https://doi.org/10.1097/WNO.0b013e3181ceb483

[8] Bonnin N, et al. Carotid-cavernous fistula: comparison of clinical and neuroradiologic features pre- and post-embolization. J Fr Ophtalmol. 2013;36(10):862-867.

https://doi.org/10.1016/j.jfo.2013.05.011

[9] Spencer W, et al. Ischaemic ocular necrosis from carotid-cavernous fistula. Br J Ophthalmol. 1973;57(3):145-152. https://doi.org/10.1136/bjo.57.3.145

[10] Eswar A, et al. Carotid-cavernous fistula as a mimicker of myasthenia gravis. Surg Neurol Int. 2014;5:141. https://doi.org/10.4103/2152-7806.141888

[11] Zanaty M, Chalouhi N, Tjoumakaris SI, Hasan D, Rosenwasser RH, Jabbour P. Endovascular treatment of carotid-cavernous fistulas. Neurosurgery Clinics. 2014 Jul 1;25(3):551-63. https://doi.org/10.1016/j.nec.2014.04.011

[12] Pérez Sempere A, Martinez Menéndez I, Cabeza Alvarez C, Calandre Hoenigsfeld L. Isolated oculomotor nerve palsy due to dural cavernous sinus fistula. J Neurol Neurosurg Psychiatry. 1991;54(3):276-8. https://doi.org/10.1159/000116675

[13] Acierno MD, Trobe JD, Cornblath WT, Gebarski SS. Painful oculomotor palsy caused by posterior-draining dural carotid cavernous fistula. Arch Ophthalmol. 1995;113(4):471-5. https://doi.org/10.1001/archopht.1995.01100080097035

[14] Loré F, Polito E, Cerase A, Bracco S, Loffredo L, Pichierri P, Talidis F. Carotid-cavernous fistula in a patient with Graves' ophthalmopathy: an unusual presentation. Eur J Ophthalmol. 2003;13(6):603-6. https://doi.org/10.1210/jc.2003-030272

[15] Peng PH, Liu CJ. Color Doppler imaging of retrobulbar hemodynamics in a case of carotid-cavernous fistula. J Clin Ultrasound. 2004;32(9):462-5. https://doi.org/10.1016/S0929-6441(09)60062-X

[16] Ikeda K, Deguchi K, Tsukaguchi I, Sasaki K, Shimamura N, Urai Y, Touge T, Kawanishi M, Takeuchi H, Kuriyama Y. Painful ophthalmoplegia caused by dural carotid-cavernous sinus fistula: successful treatment with endovascular embolization. Intern Med. 2005;44(12):1281-4. https://doi.org/10.1016/j.jns.2005.03.037

[17] van Rooij WJ, Sluzewski M, Beute GN. Endovascular treatment of ruptured cavernous sinus aneurysms presenting with epistaxis or orbital symptoms. Neuroradiology. 2006;48(1):15-21.

[18] Das S, Bendok BR, Novakovic RL, Parkinson RJ, Rosengart AJ, Macdonald RL, Frank JI. Return of vision after transarterial coiling of a carotid cavernous sinus fistula: case report. Surgical neurology. 2006 Jul 1;66(1):82-5. https://doi.org/10.1016/j.surneu.2005.10.008

[19] . Théaudin M, Chapot R, Vahedi K, Bousser MG. Dural carotid-cavernous fistula: relationship between evolution of clinical symptoms and venous drainage changes. Cerebrovascular Diseases. 2008 Apr 1;25(4). https://doi.org/10.1159/000120691

[20] Kim JW, Kim SJ, Kim MR. Traumatic carotid-cavernous sinus fistula accompanying abducens nerve (VI) palsy in blowout fractures: missed diagnosis of 'white-eyed shunt'. International Journal of Oral and Maxillofacial Surgery. 2013 Apr 1;42(4):470-3. https://doi.org/10.1016/j.ijom.2013.01.007

[21] Erickson BP, Johnson TE. A 32-year-old man with delayed onset post-traumatic proptosis and diplopia. The Journal of emergency medicine. 2014 Apr 1;46(4):475-8. https://doi.org/10.1016/j.jemermed.2013.09.015

[22] Leishangthem L, Satti SR. Indirect carotid cavernous fistula mimicking ocular myasthenia. Case Reports. 2017 Oct 19;2017:bcr-2017. https://doi.org/10.1136/bcr-2017-222048

[23] Lin HL, Hu TT. Isolated third nerve palsy with pupillary involvement resulting from carotid-cavernous sinus fistula: a case report. Medicine. 2019 Feb 1;98(6):e14472. https://doi.org/10.1097/MD.0000000000014472

[24] Azzam DB, Cypen SG, Conger JR, Tao JP. Carotid-cavernous sinus fistula masquerading as thyroid eye disease. Cureus. 2021 Apr;13(4). https://doi.org/10.7759/cureus.14261

[25] Cavasin N, Presotto F, Bellamio M, Cagliari E. Cavernous Sinus Dural Arteriovenous Fistula in a Patient with Thyroid-Associated Ophthalmopathy: Complete Resolution after Medical Treatment. Brain Sciences. 2021 Dec 29;12(1):45. https://doi.org/10.3390/brainsci12010045

[26] Pellegrini S, Zappacosta L, Cirone L, Ciabattoni L, Lee AG. Bilateral direct carotid-cavernous fistula presenting without trauma. Neuroophthalmology. 2022;46(2):86-91. https://doi.org/10.7759/cureus.24634

[27] Krothapalli N, Fayad A, Sussman E, Bruno A, Ollenschleger M, Mehta A. Indirect carotid-cavernous fistula causing severe orbital hypertension: A case report. Neurology. 2023;100(13). https://doi.org/10.4103/bc.bc_64_22

[28] Suh DC, Lee JH, Kim SJ, Chung SJ, Choi CG, Kim HJ, Kim CJ, Kook M, Ahn HS, Kwon SU, Kim JS. New concept in cavernous sinus dural arteriovenous fistula: correlation with presenting symptom and venous drainage patterns. Stroke. 2005 Jun 1;36(6):1134-9. https://doi.org/10.1161/01.STR.0000166194.82027.63

[29] Miller NR, Monsein LH, Debrun GM, Tamargo RJ, Nauta HJ. Treatment of carotid-cavernous sinus fistulas using a superior ophthalmic vein approach. Journal of neurosurgery. 1995 Nov 1;83(5):838-42. https://doi.org/10.3171/jns.1995.83.5.0838

[30] Gupta AK, Purkayastha S, Krishnamoorthy T, Bodhey NK, Kapilamoorthy TR, Kesavadas C, Thomas B. Endovascular treatment of direct carotid cavernous fistulae: a pictorial review. Neuroradiology. 2006 Nov;48:831-9. https://doi.org/10.1007/s00234-006-0132-x

[31] Alexander MD, Halbach VV, Hallam DK, Cooke DL, Ghodke B, Dowd CF, Amans MR, Hetts SW, Higashida RT, Meyers PM. Relationship of clinical presentation and angiographic findings in patients with indirect cavernous carotid fistulae. Journal of NeuroInterventional Surgery. 2019 Sep 1;11(9):937-9. https://doi.org/10.1136/neurintsurg-2018-014421

[32] Tsai LK, Liu HM, Jeng JS. Diagnosis and management of intracranial dural arteriovenous fistulas. Expert Review of Neurotherapeutics. 2016 Mar 3;16(3):307-18. https://doi.org/10.1586/14737175.2016.1149063

[33] Miller TR, Gandhi D. Intracranial dural arteriovenous fistulae: clinical presentation and management strategies. Stroke. 2015 Jul;46(7):2017-25. https://doi.org/10.1161/STROKEAHA.115.008228

[34] Halbach VV, Higashida RT, Hieshima GB, Hardin CW, Dowd CF, Barnwell SL. Dural fistulas involving the cavernous sinus: Results of treatment in 30 patients. Radiology. 1987;163(3):437-442. https://doi.org/10.1148/radiology.163.2.3562823

[35] Su IC, Cruz JP, Krings T. Low-flow direct carotid-cavernous fistula caused by rupture of an intracavernous carotid aneurysm. Interventional Neuroradiology. 2014 Jul;20(4):476-81. https://doi.org/10.15274/INR-2014-10057

[36] Cognard C, Januel AC, Silva NA, Tall P. Endovascular treatment of intracranial dural arteriovenous fistulas with cortical venous drainage: new management using Onyx. American journal of neuroradiology. 2008 Feb 1;29(2):235-41. https://doi.org/10.3174/ajnr.A0817

[37] Ellis JA, Goldstein H, Connolly ES Jr, Meyers PM. Carotid-cavernous fistulas. Neurosurg Focus. 2012;32(5). https://doi.org/10.3171/2012.2.FOCUS1223

[38] Debrun GM, Vinuela F, Fox AJ, Davis KR, Ahn HS. Indications for treatment and classification of 132 carotid-cavernous fistulas. Neurosurgery. 1988;22(2):285-9. https://doi.org/10.1227/00006123-198802000-00001

[39] Lewis AI, Tomsick TA, Tew JM Jr. Management of 100 consecutive direct carotid-cavernous fistulas: Results of treatment with detachable balloons. Neurosurgery. 1995;36(2):239-244. https://doi.org/10.1227/00006123-199502000-00001

[40] Gemmete JJ, Ansari SA, Gandhi DM. Endovascular techniques for treatment of carotid-cavernous fistula. J Neuroophthalmol. 2009;29(1):62-71. https://doi.org/10.1097/WNO.0b013e3181989fc0

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Research Article

Diagnostic Performance of Cytocolposcopy versus Biopsy for Premalignant and Malignant Lesions in a Women's Hospital Dysplasia Clinic

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ABSTRACT

Background: Cervical cancer (CRC) is a public health problem because it is the fourth most common gynecologic neoplasm worldwide. The screening tests used to diagnose this pathology are cervical cytology, which in suspected malignancy or with malignancy requires colposcopy to identify the affected area and thus guide the biopsy, which is the gold standard for diagnosis. Therefore, these tests are complementary, and a high diagnostic concordance is required to make a confident diagnosis.

Subjects and Methods: A retrospective, cross-sectional, observational, and analytical study was performed. A total of 1470 medical records were analyzed, of which 175 patients met the inclusion criteria. The cyto-colposcopic diagnostic yield was compared with the histopathologic yield. The concordance between screening tests and the gold standard was calculated using Cohen's kappa coefficient

Results: The sample comprised 175 subjects who met the selection criteria (11.9%). The mean age was 34.59 + 11.01 years, ranging from 17 to 65 years. The mean sexual debut was 16.6 years, with a mean of 3.1 ± 2 sexual partners. When patients were classified according to lesion type, the highest percentages were found in low-grade squamous intraepithelial lesions (LSIL). With 45.71, 61.14, and 49.14% for cytologic, colposcopic, and histopathologic examination, respectively. The highest concordance between histopathology and cytology was found in the high-grade squamous intraepithelial lesion (HSIL) with 0.41, and the concordance between histopathology and colposcopy in HSIL and cancer was 0.55 and 0.74, respectively.

Conclusions: Papanicolaou tests and colposcopy showed moderate concordance with histopathologic findings; the diagnostic accuracy of colposcopy is superior to that of cytology.

Introduction

Cervical cancer (CRC) is a public health problem as it is the fourth most common gynecologic neoplasm worldwide. In developing countries, it is the second leading cause of cancer-related death among women.(1) It is also considered a preventable disease due to its prolonged pre-invasive stage, which facilitates its early detection by cytology, colposcopy, and histology and the treatment of pre-invasive lesions2. In the United States, even with screening programs in place with appropriate protocols, the odds of developing CRC at some point

in life are estimated to be 1:128, and up to 30% of CRC cases occur in patients who have undergone cervical cytology (Pap smear).(2)
Screening tests include vaginal cytology, which is performed to evaluate cervical cytologic abnormalities or dysplasia. It is recommended to be performed every three years, as annual tests have similar results to those performed every three years.(3) In addition, the incidence of high-grade cytologic abnormalities in the three years following a normal test is very low (10-66 per 10,000).(4) Colposcopy is also used to diagnose CRC and detect precancerous and cancerous lesions. It is used as a secondary test when abnormal cervical cytology is detected.(5)
CRC is one of the easiest gynecologic malignancies to detect and stage early, provided a culture of routine screening allows for early treatment. Worldwide, according to the International Agency for Research on Cancer, it is the fourth most common gynecologic neoplasm, with a prevalence of 5.8% in 2020, surpassed by breast (30.3%), colorectal (9.3%), and thyroid (6%). Likewise, in our country, the estimated prevalence rate is 560.8-1321.5 per 100,000 inhabitants, being the second cause of death in women in Mexico, surpassed only by breast cancer (6).
The 5-year survival rate, according to the American Cancer Society, is related to the initial staging, which is why screening tests are vital, as mentioned above, since CRC is one of the most common neoplasms and is highly preventable and curable in its early stages.(6-8)
In Sinaloa, Mexico, according to the last CRC report obtained from epidemiological week 18, 2 new cases were reported, with a cumulative total of 60 cases from epidemiological week 1 to 18 of 2022. It is more prevalent in women of reproductive age in 76% of the cases between 20 and 49 years but less frequent in women over 65 years.(9) Our study aimed to determine the diagnostic yield of cyto-colposcopy versus biopsy for premalignant and malignant lesions in a dysplasia clinic of a women's hospital in northwestern Mexico.

Subjects and Methods

A retrospective, cross-sectional, observational, and analytical study was conducted in which 1470 files of women between 15 and 65 years of age were reviewed, registered, and attended in the Gynecology and Obstetrics Outpatient Clinic and referred to the Dysplasia Clinic of the Women's Hospital from January 1, 2020, to September 30, 2022.
The inclusion criteria were compliance with the epidemiologic clinical follow-up protocol, suspicious cervical cytology results, having undergone colposcopy and having the diagnostic conclusion by histopathology. All patients who did not meet the above inclusion criteria were excluded from the study.
Operative Definitions.
The Bethesda system was used for cervical cytology reporting.[10] This is the World Health Organisation (WHO) recommended classification for cytology reporting and is as follows (Figure 1); Normal: Any result that does not fall within the range of epithelial abnormalities or abnormal cytology: according to Bethesda System terminology,[10] cytology with abnormalities were those with results of atypical squamous cells of uncertain significance (ASC-US), Atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H), low grade squamous intraepithelial lesion (LSIL), High grade squamous intraepithelial lesion (HSIL), invasive carcinoma, atypical glandular cells (AGC), adenocarcinoma in situ, or adenocarcinoma. Indeterminate: Atypical squamous cell carcinoma of undetermined significance and/or atypical squamous cell carcinoma cannot exclude HSIL. Low-grade squamous intraepithelial lesion (LSIL): Includes cellular changes associated with the cytopathic effect of human papillomavirus (HPV) infection (known as pilocytic atypia), usually confined to the superficial layers. High-grade squamous intraepithelial lesion (HSIL): Cellular changes involving two-thirds or more of the thickness of the squamous epithelium. This type of lesion corresponds to those classified above as moderate and severe dysplasia and cancer in situ. Cancer: Malignant tumor caused by loss of control of cell growth that may invade adjacent structures or spread to distant sites, resulting in death.
Statistical Analysis
Cyto-colposcopic diagnostic yield was compared with histopathologic yield. Cohen's kappa coefficient calculated the agreement between screening tests and the gold standard. Data were analyzed using Stata Intercooled version 13.1, College Station, Texas. Sensitivity and specificity estimates for colposcopic diagnosis were calculated by cross-tabulation. Forest plots with corresponding 95% confidence intervals (95% CI) were generated for each test. Pooled estimates of test accuracy are presented graphically with summary receiver operating characteristic (SROC) curves.
Variables assessed
Chronological age, sexual partners, cervical cytology, colposcopy, and histopathology.
Patient and public involvement
Participants were not directly involved in designing or implementing the study**.**

Results

A review of 1,470 medical records of patients seen between January 1, 2020, and September 30, 2022, was performed, of which 175 subjects (11.9%) met the selection criteria. The mean age was 34.59 + 11.01 years, with a range of 17 to 65 years, and the median age was 33. The mean age of sexual debut was 16.6 years, with a mean of 3.1 ± 2 sexual partners.
When patients were classified according to lesion type, the highest percentages were found in LSIL, with 45.71, 61.14, and 49.14% for cytology, colposcopy, and histopathology, respectively (Table 1, Figure 1)

Table 1. Percentage distribution of cytology, colposcopy, and histopathology results in patients in the dysplasia clinic.

Diagnosis	Normal	Indeterminate	LSIL	HSIL	Cancer	n
Cytology	6(3.43)	41(23.43)	80(45.71)	48(27.43)	0(0.00)	175
Colposcopy	14(8)	6(3.43)	107(61.14)	38(21.71)	10(5.71)	175
Histopathology	26(14.86)	0(0.00)	86(49.14)	52(29.71)	11(6.29)	175

Low-grade squamous intraepithelial lesion (LSIL). High-grade squamous intraepithelial lesion (HSIL); **n:** sample

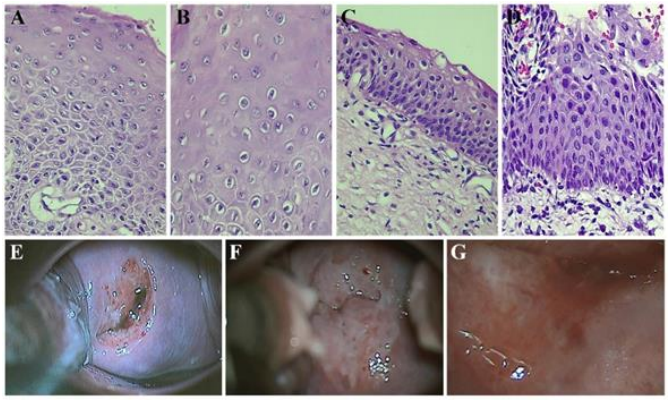

Figure 1. Representative images of cervical tissue stained with haematoxylin and eosin (A to D) and colposcopic examination of the cervix (E to G). Hematoxylin and eosin staining, A) Squamous epithelium with changes associated with a low-grade squamous epithelial lesion (LSIL), B) Squamous epithelium with cytopathic changes (koilocytes), C) Squamous epithelium with LSIL, borderline changes towards a low-grade squamous epithelial lesion (HSIL), D) Squamous epithelium with HSIL-related changes; Colposcopy, E) Eutrophic cervix with type I vascular pattern, type 1 transformation zone, visible squamocolumnar junction, mature squamous epithelium, negative Hiselman test, no lesions, F) Hypertrophic cervix with type I vascular pattern, type 1 transformation zone, visible squamocolumnar junction, mature squamous epithelium, positive Hiselman's test for dense acetowhite epithelium with glandular infiltration at 10 to 12 o'clock and 5 to 8 o'clock, well defined and raised borders, high-grade lesion, G) Eutrophic cervix with type II vascular pattern, type 3 transformation zone, invisible squamocolumnar junction, mature squamous epithelium and positive Hiselman's test for dense acetowhite epithelium. Hemorrhagic endocervical neoformation is observed, with high-grade HPV-associated lesions.

Table 2 shows that cytopathology reports have low sensitivity compared with histopathology reports. However, specificity and negative predictive values (NPV) were higher for HSIL; colposcopy was better in specificity, positive predictive values (PPV), and negative predictive values for LSIL and cancer. A high NPV indicates the probability that the patient is healthy. Similarly, a high PPV suggests that there is an actual probability that she has the disease.

For indeterminate lesions (ASC-US, ASC-H) by cytology and colposcopy, the false positive rate was 21.47% and 1.34%, respectively, and the false negative rate was 12.68% and 14.96%, respectively. The diagnostic accuracy for this type of lesion was 72% and 86.2% for cytology and colposcopy, respectively.

Cytology showed a diagnostic accuracy of 76% and 62.28% for high- and low-grade lesions, respectively; colposcopy showed an accuracy of 82.85%, 76.57%, and 97.14% for LSIL, HSIL, and CANCER, respectively. The likelihood ratio found for colposcopy in LSIL and cancer is high, as is the area under the curve, which was 0.76 and 0.85, respectively (Table 2).

The highest concordance between histopathology and cytology was in HSIL, with 0.41, and the concordance between histopathology and colposcopy in HSIL and cancer was 0.55 and 0.74, respectively, see Table 3.

Table 2. Diagnostic yield of cytology and colposcopy in the dysplasia clinic**.**

Diagnostic test	S	E	VPP	VPN	LR+	LR-	ABC
Cytology for LSIL	62.5	62.1	58.1	66.2	1.72	0.63	0.6
Cytology for HSIL	55.7	84.5	60	81.8	3.59	0.18	0.70
Colposcopy for LSIL	57.69	93.49	78.94	83.94	8.86	0.45	0.76
Colposcopy for HSIL	88.37	65.16	71.02	50	2.53	0.17	0.75
Colposcopy for Cancer	72.72	98.78	80	98.18	59.6	0.27	0.85

LSIL: low-grade intraepithelial lesions; HSIL: high-grade intraepithelial lesions; S: sensitivity, E: specificity, PPV and NPV: positive and negative predictive values; LR+: positive likelihood ratio; LR-: negative likelihood ratio; ABC: area under the curve.
The positive and negative predictive values (PPV and NPV, respectively)

Table 3. Diagnostic concordance between cytology and colposcopy compared to histopathology.

Diagnostic test	Normal	indeterminate	LSIL	HSIL	Cáncer
Cytology	0.14	0.00	0.24	0.41	0
Colposcopy	0.49	0.00	0.53	0.55	0.74

LSIL: low-grade intraepithelial lesions; **HSIL:** high-grade intraepithelial lesions.

Discussion

Knowing the concordance between different screening tests and the gold standard is essential to reduce unnecessary procedures and make timely decisions when needed, thus optimizing the resources available in public institutions. This is the first study performed at the Women's Hospital to determine the performance and concordance of diagnostic tests with histopathologic results.

Our results indicate that cytology was more specific and had an adequate negative predictive value for HSIL but inferior to colposcopy. That colposcopy was more sensitive and had a proper positive predictive value for HSIL compared to cytology.

We can say that our hospital diagnostic screening for premalignant and malignant lesions demonstrates and confirms that colposcopy has better accuracy than cytology, as already established by other authors, although according to the guidelines updated by the International Federation for Colposcopy and Cervical Pathology in its recently published meta-analysis of 15 articles with 22. 000 participants reported a sensitivity of 92% and a specificity of 51% for detecting LSIL, a sensitivity of 68%, and a specificity of 93% for LSIL, which are very high figures. Like those found in the present study.(11) The concordance was moderate according to the Landis J scale for assessing the degree of concordance.(12)

A study conducted by Barut et al. in 2015 (13) aimed to correlate diagnostic tests in detecting malignant and premalignant lesions of the cervix, given the significant variability in the sensitivity of cytology reported by other authors and the cost of it. In the analysis of abnormal cervical lesions, colposcopy, and biopsy invasiveness found in their research at the tertiary level of care in women with a low

socioeconomic level, they reported a cytology sensitivity of 57%, which is much lower than the result obtained in our study; a specificity of 76%, much lower than that reported in our study, as well as a PPV of 26% and NPV of 92%.(13)

In the study conducted by Singhal et al. in 2019, where they compared the diagnostic concordance, the diagnostic accuracy of cytology and colposcopy for HSIL was 100% and 91.3%, respectively, where the mean age was 34 years. However, the highest percentage was centered in the group from 26 to 35 years, unlike our study, where we found an age range from 16 to 65 years, although the accuracy of our tests was 72% for cytology and 86% for colposcopy.(14)

According to the authors, the poor results obtained are most likely because the specific diagnosis in these techniques is highly operator-dependent and subjective. Therefore, the severity of the lesions tends to be underdiagnosed.(15)

Scales are recommended to reduce intra-operator error, as proposed by the International Federation of Cervical Pathology (IFCPC 2011).(16) In a study by Rema Prabhakaran Nair et al. in 2020, comparing several colposcopic visual scales, they concluded that the best one proposed by the IFCPC 2011 compared with histopathologic findings, reporting a correlation of 65. 7% for squamous intraepithelial lesions regardless of grade, compared to our study where the colposcopic-histopathologic correlation was demonstrated for HSIL, LSIL, and CANCER with an accuracy of 82.85%, 76.57%, and 97.14%, respectively.(17, 18)

Fadi W. Abdul-Karim et al., published 2017 the results of a study that aimed to compare the discrepancy between diagnostic tests for CRC (19). They reported a histopathologic prevalence of 29% for LSIL in the control group and 2.2% in the group in which colposcopy was used. Compared to our study, the percentage was 21.7% for colposcopy and 29.7% for histopathologic results, with a sensitivity of 88.37 and specificity of 65.1, reporting a lower diagnostic discrepancy in our study.(19)

In a study published by Juan Li Wei Wang et al., whose aim was to analyze the agreement between colposcopic impression and histopathological diagnosis of cervical biopsy, they reported a perfect agreement between colposcopy and histopathology of 46.9%, with a kappa concordance of 0.23. With a PPV of 93.1%, NPV of 57.8%, and sensitivity and specificity of 80.9% and 93.9%, respectively, for LSIL or higher.(20) Our study reported higher concordance for both LSIL and HSIL, as previously discussed; in terms of sensitivity, specificity, PPV, and NPV, the results were very similar (Table 2), the diagnostic performance of cytology and colposcopy in the dysplasia clinic. They also conclude by pointing out that the experience of the colposcopist is an integral part of the underdiagnosis factor of HSIL.(20)

Peng Xuw et al., in 2020, reported that the diagnostic accuracy of colposcopy-guided cervical biopsy for the detection of squamous intraepithelial lesions is relatively low, ranging from 30% to 70%, attributing it to the lack of capacity of colposcopy services in low- and middle-income countries.(21) In comparison, in our study, colposcopy showed a diagnostic accuracy for HSIL, LSIL, and CANCER of 82.85%, 76.57%, and 97.14%, respectively. The training and quality of the teams in our dysplasia center can effectively justify this.(21)

Women with a clinical diagnosis of cervical dysplasia should be evaluated by cytology to detect premalignant or malignant lesions. It has been concluded that cytology, colposcopy, and histopathology should be evaluated to assess cervical findings in low socioeconomic regions.(13) On the other hand, it is essential that when a result is found, whether positive or negative, the patient is referred as soon as possible for timely follow-up and treatment.(22)

The diagnostic tests evaluated in our study are complementary in arriving at a diagnosis of CRC. Currently, the screening method is cervical cytology; however, we must remember that it may be subject to false bias due to the person taking the samples and the patient's condition at the time of screening. If it is positive or suspicious of malignancy, or if the result is equivocal, the ideal is to send the patient to a dysplasia center for colposcopic examination and biopsy to increase our diagnostic certainty since this is the gold standard.(23, 24)

Conclusions

Cytology and colposcopy showed moderate agreement with the histopathology report; however, the sensitivity of the tests was low for cytology. Although the diagnostic accuracy of colposcopy is superior to that of cytology, the high rate of false negatives and positives must be considered due to quality issues, as cytology samples were taken and read by personnel from outside our hospital in different laboratories, so the expertise of each professional plays a key role in the diagnosis. Therefore, diagnosis should be complemented by more effective tests, such as molecular tests (hybrid capture and PCR), which promote early diagnosis and treatment of the disease to prevent HPV infection from progressing to cervical cancer.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request

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References

[1] Marzo-Castillejo M, Bartolome-Moreno C, Bellas-Beceiro B, Melus-Palazon E, Vela-Vallespin C: [PAPPS Expert

Groups. Cancer prevention recommendations: Update 2022]. Aten Primaria 2022, 54 Suppl 1(Suppl 1):102440.

[2] Siegel RL, Miller KD, Fuchs HE, Jemal A: Cancer statistics, 2022. CA Cancer J Clin 2022, 72(1):7-33. https://doi.org/10.3322/caac.21708

[3] Salud Sd: Lineamientos para la vigilancia por laboratorio de cáncer del cuello del útero: laboratorio de citología. Instituto de Diagnostico y Referencia Epidemiologicos Dr Manuel Martinez Daez, Plan nacional de desarrollo Mexico 2016, https://www.gob.mx/cms/uploads/attachment/file/487426/LVLCaCu_Citolog_a_4T.pdf last access: June 7, 2023.

[4] Zhu Y, Feldman S, Leung SOA, Creer MH, Warrick J, Williams N, Mastorides S: AACC Guidance Document on Cervical Cancer Detection: Screening, Surveillance, and Diagnosis. J Appl Lab Med 2023, 8(2):382-406. https://doi.org/10.1093/jalm/jfac142

[5] Hariprasad R, Mittal S, Basu P: Role of colposcopy in the management of women with abnormal cytology. Cytojournal 2022, 19:40. https://doi.org/10.25259/CMAS_03_15_2021

[6] Cancer IAfRo: Mexico Source: Globocan 2020, The Global Cancer Observatory. In.: World Health Organization; 2021: https://gco.iarc.fr/today/data/factsheets/populations/484-mexico-fact-sheets.pdf, Last access: June 487, 2023.

[7] Isla-Ortiz D, Salcedo-Hernandez RA, Leon-Takahashi AM, Estrada-Rivera F, Barquet-Munoz SA, Reynoso-Noveron N: [Resultados quirurgicos de histerectomia radical laparoscopica en pacientes con cancer de cervix en etapa temprana: experiencia inicial en Instituto de Cancer]. Cir Cir 2018, 86(3):220-227. https://doi.org/10.24875/ciru.m18000035

[8] Musunuru HB, Pifer PM, Mohindra P, Albuquerque K, Beriwal S: Advances in management of locally advanced cervical cancer. Indian J Med Res 2021, 154(2):248-261. https://doi.org/10.4103/ijmr.ijmr_1047_20

[9] Salud Sd: Boletín Epidemiológico, Sistema Nacional de Vigilancia Epidemiológica, Sistema Único de Información. In., vol. 40: Sinave Mexico City, https://www.gob.mx/cms/uploads/attachment/file/825120/sem18.pdf, last access: June 7, 2023; 2023: 18.

[10] Pangarkar MA: The Bethesda System for reporting cervical cytology. Cytojournal 2022, 19:28. https://doi.org/10.25259/cmas_03_07_2021

[11] Qin D, Bai A, Xue P, Seery S, Wang J, Mendez MJG, Li Q, Jiang Y, Qiao Y: Colposcopic accuracy in diagnosing squamous intraepithelial lesions: a systematic review and meta-analysis of the International Federation of Cervical Pathology and Colposcopy 2011 terminology. BMC Cancer 2023, 23(1):187. https://doi.org/10.1186/s12885-023-10648-1

[12] Landis JR, Koch GG: The measurement of observer agreement for categorical data. Biometrics 1977, 33(1):159-174. https://doi.org/10.2307/2529310

[13] Barut MU, Kale A, Kuyumcuoglu U, Bozkurt M, Agacayak E, Ozekinci S, Gul T: Analysis of Sensitivity, Specificity, and Positive and Negative Predictive Values of Smear and Colposcopy in Diagnosis of Premalignant and Malignant Cervical Lesions. Med Sci Monit 2015, 21:3860-3867. https://doi.org/10.12659/MSM.895227

[14] Singhal A, Kaul Raina R, Verma S, Verma AJCJoH, Research: Predictive Accuracy of Cervical Cytology and Colposcopy in Diagnosing Premalignant and Malignant Cervical Lesions: A Hospital-based Study from the Sub-Himalayan Region of Indian Subcontinent. 2019, 6(1). http://dx.doi.org/10.4103/cjhr.cjhr_51_18

[15] Ruan Y, Liu M, Guo J, Zhao J, Niu S, Li F: Evaluation of the accuracy of colposcopy in detecting high-grade squamous intraepithelial lesion and cervical cancer. Arch Gynecol Obstet 2020, 302(6):1529-1538. https://doi.org/10.1007/s00404-020-05740-x

[16] Bornstein J, Bentley J, Bosze P, Girardi F, Haefner H, Menton M, Perrotta M, Prendiville W, Russell P, Sideri M et al: 2011 colposcopic terminology of the International Federation for Cervical Pathology and Colposcopy. Obstet Gynecol 2012, 120(1):166-172. https://doi.org/10.1097/aog.0b013e318254f90c

[17] Rema PN, Mathew A, Thomas S: Performance of colposcopic scoring by modified International Federation of Cervical Pathology and Colposcopy terminology for diagnosing cervical intraepithelial neoplasia in a low-resource setting. South Asian J Cancer 2019, 8(4):218-220. https://doi.org/10.4103/sajc.sajc_302_18

[18] Zhang B, Hong S, Zhang G, Rong F: Clinical application of the 2011 IFCPC colposcope terminology. BMC Womens Health 2021, 21(1):257. https://doi.org/10.1186/s12905-021-01395-1

[19] Abdul-Karim FW, Yang B: Cytologic-Histologic Discrepancies in Pathology of the Uterine Cervix: Analysis of the Clinical and Pathologic Factors. Adv Anat Pathol 2017, 24(5):304-309. https://doi.org/10.1097/pap.0000000000000165

[20] Li J, Wang W, Yang P, Chen J, Dai Q, Hua P, Liu D: Analysis of the agreement between colposcopic impression and histopathological diagnosis of cervical biopsy in a single tertiary center of Chengdu. Arch Gynecol Obstet 2021, 304(4):1033-1041. https://doi.org/10.1007/s00404-021-06012-y

[21] Xue P, Ng MTA, Qiao Y: The challenges of colposcopy for cervical cancer screening in LMICs and solutions by artificial intelligence. BMC Med 2020, 18(1):169. https://doi.org/10.1186/s12916-020-01613-x

[22] Mayeaux EJ, Jr., Novetsky AP, Chelmow D, Choma K, Garcia F, Liu AH, Papasozomenos T, Einstein MH: Systematic Review of International Colposcopy Quality Improvement Guidelines. J Low Genit Tract Dis 2017, 21(4):249-257. https://doi.org/10.1097/lgt.0000000000000344

[23] Brown BH, Tidy JA: The diagnostic accuracy of colposcopy - A review of research methodology and impact on the outcomes of quality assurance. Eur J Obstet Gynecol Reprod Biol 2019, 240:182-186. https://doi.org/10.1016/j.ejogrb.2019.07.003

[24] Wei B, Zhang B, Xue P, Seery S, Wang J, Li Q, Jiang Y, Qiao Y: Improving colposcopic accuracy for cervical precancer detection: a retrospective multicenter study in China. BMC Cancer 2022, 22(1):388. https://doi.org/10.1186/s12885-022-09498-0

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Al-Kindy College Medical Journal (KCMJ)

Research Article

Expression of the Hopeful Therapeutic Target CD15 in Women with Breast Cancer

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Keywords: CD15, Invasive Mammary Carcinoma, Target Therapy.

ABSTRACT

Background: CD15 is emerging as a recent prognostic marker with potential as a future therapeutic target in human malignancies. Its biological role in human cancer involves facilitating tumor cell adhesion through interaction with E-, L-, and P-selectins. This interaction allows adhesion to endothelial cells, enhancing metastatic potential.
Objective: to study CD15 expression and its relationship to some pathologic parameters to provide the first Iraqi data-driven analysis of this growing concept in women with breast cancer.
***Subjects and Methods*:** A retrospective study was carried out utilizing tissue samples obtained from 85 patients with invasive breast cancer. Materials included one formalin-fixed paraffin-embedded tissue block, one hematoxylin and eosin-stained histological section, and two immunohistochemical-stained ER and HER2/NEU sections for each patient. The samples were obtained through core needle biopsy under ultrasound guidance. Data review and immunohistochemical staining with CD15 were conducted, followed by statistical analysis using the chi-square test to explore correlations.
Results: CD15 expression was observed in 55% of breast cancer samples. There was a highly significant statistical relationship between CD15 expression and the presence of axillary lymph node metastasis (proven by fine needle aspiration cytology). However, there was no significant statistical relationship between CD15 expression and the histological grade, ER receptor, HER2-NEU status, and tissue calcification.
Conclusion: CD15 expression was detected in breast cancer cells, with a highly significant association observed between CD15 expression and axillary lymph node metastasis at presentation. CD15 could serve as a prognostic marker as association with axillary lymph nodes reflects negative impact on prognosis and at the same time a promising therapeutic target for patients with breast cancer as blockage of CD15 antigen's function will result in reduction of the metastatic potential and it could be an immunotherapeutic target.

Introduction

Breast cancer is the most prevalent malignant tumor in women; it is the main cause of cancer-related deaths (accounts for ~22%) in women globally (1-2). Breast cancer is not a single disease but rather a heterogeneous entity at both the histological and molecular levels, characterized by multiple subtypes with variable clinical outcomes (3). Although histological classification offers some insights, it has limited predictive value and clinical utility (4). Recently, molecular types, particularly those using microarrays for gene expression analysis, have gained significant attention. Despite their high prognostic and predictive value, molecular testing is expensive and not widely applied, especially in developing countries (5).
Immunohistochemistry-based classification, focusing on estrogen (ER)/ progesterone (PR) receptor and HER2 status, offers a less expensive alternative with good prognostic and therapeutic information (6). Hormone receptors on the surface of breast cancer

cells, such as ER and PR, facilitate tumor growth. However, the independent prognostic and therapeutic role of PR receptor status, irrespective of ER, remains highly debated in oncology. Consequently, the "Royal College of Pathologists" considers testing for Progesterone receptor status in breast cancer non manditory (7). Testing all invasive mammary cancers for ER and HER2 status, either on tru cut or excisional biopsy, has become standard care for patients with breast cancer (8).

CD15 (X-Hapten) is a clustered form of glycoproteins and glycolipids. Initially discovered on the cell surface of terminally differentiated myeloid cells but not in hematopoietic progenitors, CD15 is present in both sialylated and unsialylated forms (9). Its physiological roles include phagocytosis, bactericidal activity, and chemotaxis (10). Aberrant expression of CD15 in epithelial cells of the breast, kidney, lung, and intestinal tract has been noted, as well as its constant presence in astrocytes (10). the most common application of CD 15 immunohistochemical test is in the diagnosis of classical Hodgkin lymphoma.

Literature-based data suggest CD15's role in neoplasia involves facilitating tumor cell adhesion through interaction with E-, L-, and P-selectins, enhancing metastatic potential and promoting changes in cancer-associated membrane proteins (12,13). CD15 expression is often associated with lymphovascular invasion, lymph node metastasis, and distant metastasis in human malignancies (14).

Given CD15's crucial role in cancer metastasis, it is now considered an interesting target for cancer immunotherapy. Chemical preparations are under extensive investigation to modulate CD15 and E-selectin expression to block endothelial cell adhesion and, thereby, metastatic potential (13, 14). CD15 expression has been identified in various human malignancies, including thyroid papillary carcinoma, Hodgkin lymphoma, glial tumors, non-small cell lung cancer, invasive mammary carcinoma, and oral carcinoma (15). However, significant heterogeneity in antigen expression and prognostic value of CD15 among these cancer types has been reported, suggesting the need for individual tumor characterization (16).

This study investigates the expression of CD15 in breast cancer, mainly its association with axillary lymph node metastasis, histological grade, tissue calcification and ER and HER2 expression, to provide the first Iraqi data-driven analysis of this growing concept among females with breast cancer.

Subjects and Methods

This retrospective study collected histological materials of 85 patients with invasive breast cancer from the Pathology Department of Al Massa Center, a private breast center in Baghdad, from "January" 2023 to "December" 2023. Diagnostic histological materials were obtained through core needle biopsy under ultrasound guidance. For each patient with invasive breast cancer included in this study, the following were retrieved from the laboratory archive of the Pathology Department: one formalin-fixed paraffin-embedded tissue block, one hematoxylin and eosin-stained histological section, and two immunohistochemically stained sections of ER and HER2/NEU. Axillary lymph node metastasis (proven by fine needle aspiration cytology done for patients showing positive ultrasound finding) was reported. The only clinical information extracted from the patient data was age.

Sample selection

The inclusion criterion was all histological materials with complete radiological, histopathological, and cytological data in a single center within one year period. The exclusion criteria included histological materials with deficient relevant data and those with equivocal (2+) HER2/NEU immunohistochemically results.

Immunohistochemistry

A 4-micron thick tissue section was prepared from each paraffin block using "Leica Biosystems Microtome" , a charged slide "JSHD Jiangsu", China was used, after drying for 30 minutes at 62°C, the processing was performed by using an autostainer, "Agilent Link 48", "Dako A/S -Denmark-Glostrup" was performed , the samples underwent "Standard Heat Epitope Retrieval" at pH 8.0 for 30 minutes in "ethylene diamine tetraacetic acid" (Unilong Industry Co., Ltd.). Incubation with primary antibody monoclonal mouse CD15 "(clone Carb, code number M3631)" provided by "(Dako A/S -Denmark-Glostrup)". Followed by biotinylated anti-mouse immunoglobulin and peroxidase-labeled streptavidin "(LSAB Kit, Dako A/S -Denmark-Glostrup)", was conducted. Harris hematoxylin "PathnSitu Biotechnologies"; cat. no. PS021 was used for counterstaining. Optimal incubation times and concentrations for the primary antibody were determined via the instructions provided by the manufacturer of the products "(Dako A/S -Denmark-Glostrup)". The required dilution was 1:50 1:200 and the time for incubation

At room temperature was 30 60 min. Positive (renal tissue) and negative external controls were included in each run.

1. Samples obtained in our study included histological material obtained by true cut needle biopsy under ultrasound guidance, so, histological classification of the invasive mammary carcinoma may not be achieved on small tissue sample as it requires full histological evaluation of the entire tumor by excisional biopsy for definitive histological categorization. Reviewing the histological grade according to the "Nottingham modification of the Bloom-Richardson system" and scoring of ER according to "Allred score" (table 1).

"The final Allred scores are the summation of both intensity and proportion scores. Accordingly, "scores 0 and 2" are regarded negative for ER, while "scores of 3 to 8" are regarded as positive). Regarding HER2\NEU scoring on invasive cancer cells:

IHC 3+ was considered (strong positive) if strong complete, membrane staining pattern was observed in more than 10% of cancer cells.

IHC 2+ was considered (Equivocal) if weak to moderate complete membrane staining pattern was observed in more than 10% of the cancer cells.

IHC 1+ was considered (Negative) if weak incomplete membrane staining pattern was observed in more than 10% of the cancer cells.

IHC 0 was considered (Negative) if no stain or incomplete faint / barely perceptible membrane staining pattern was observed in less than or equal to 10% of the invasive tumor cells (17-19)

Table 1: Allred score "for estrogen and progestrone receptor evaluation".

ER Status (Positive Cells %)	Proportion Score	Intensity	Intensity Score
0	0	None	0
<1	1	Weak	1
1 to 10	2	Intermediate	2
11 to 33	3	Strong	3
34 to 66	4		
≥67	5		

2. Interpreting CD15 expressions.

The interpretation of staining results was done by immunohistochemecal evaluation of the intensity and proportion of the staining tumor cells (membranous or cytoplasmic patterns). The immunohistochemical staining intensity was regarded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The final score was calculated by multiplication of the proportion and intensity scores of the stained tumor cells, with results considered positive if the final score exceeded 10(19). A light microscope (Leica Microsystem GmbH) was used in interpretation of our results. Leica ICC 50E camera was used in photographing our results.

Ethical approval

The study was approved by the ethical committee of Gazi Al Hariri teaching hospital (reference number 0011-012-19 INT23) at 19\12\ 2023 and conducted by its institutional policy. As it was a retrospective study using data without violating patient privacy, consent to participate was deemed not applicable.

Statistical analysis:

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 25. The participant's age range was presented as means ± standard deviation (SD). Frequencies of various variables were tabulated for easy reference and comparison. The chi-square test was employed to compare between different variables. In this analysis, a p value of less than 0.05 was considered statistically significant.

Results

Current study analyzed 85 histological samples from female patients with invasive breast cancer (Figure 1).

The age range of these patients was from 30 to 89 years, with a mean age of 66.8235 +- 14.803SD. Among the 85 histological samples of invasive breast cancer, 47(55%) were CD15 positive (to (Figure 2).

Among the 85 histological samples of invasive breast cancer, 47(55%) were CD15 positive and 38 (45%) were negative (refer to Figure 2 and 3).

The correlation between CD15 expression and histological grade, ER status (Figure 4), HER2\NEU status (Figure 5), tissue calcification revealed a non-significant statistical relationship with p value > 0.05

However a highly significant statistical relationship was achieved between CD15 expression and the presence of axillary lymph node metastasis with p value < 0.01 (table 2).

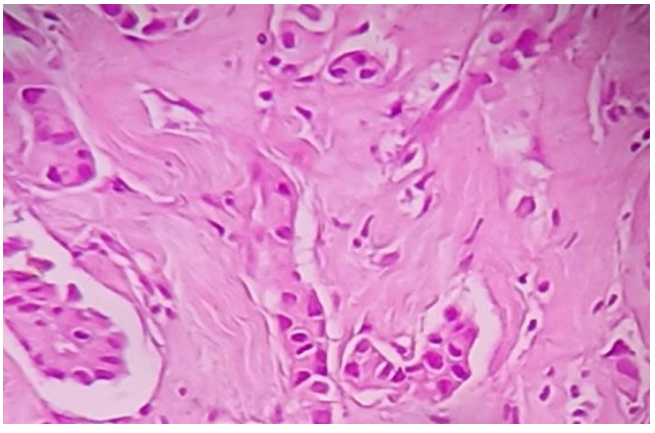

Figure 1: Histological section of invasive breast cancer with a desmoplastic stroma (Hematoxylin and eosin, X100**)**

The age range of these patients was from 30 to 89 years, with a mean age of 66.8235 +- 14.803SD. Among the 85 histological samples of invasive breast cancer, 47(55%) were CD15 positive (refer to Figure 2).

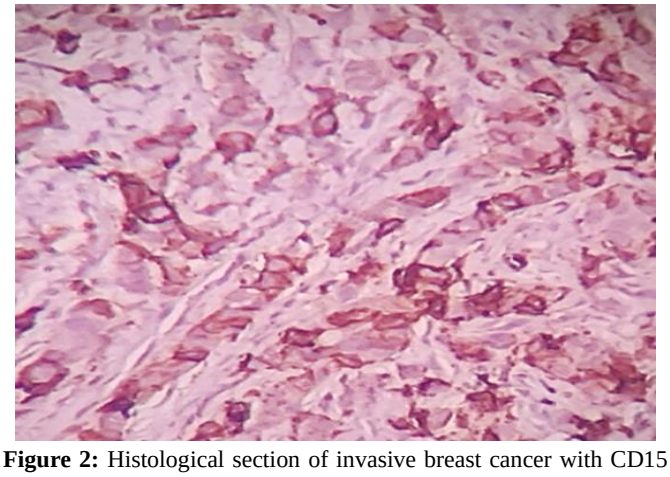

Figure 2: Histological section of invasive breast cancer with CD15 showed positive membranous and cytoplasmic staining pattern (arrow) (Immunohistochemically stained, X100**)**

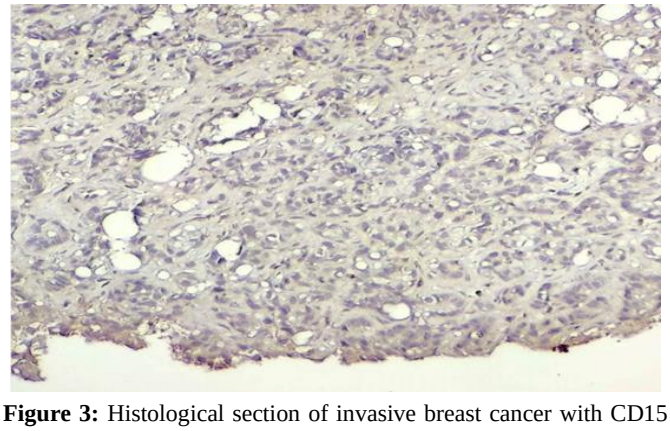

Figure 3: Histological section of invasive breast cancer with CD15 showed negative results (Immunohistochemically stained, X40)

The correlation between CD15 expression and histological grade, ER status (Figure 4), HER2\NEU status (Figure 5), tissue calcification revealed a non-significant statistical relationship with p value > 0.05.

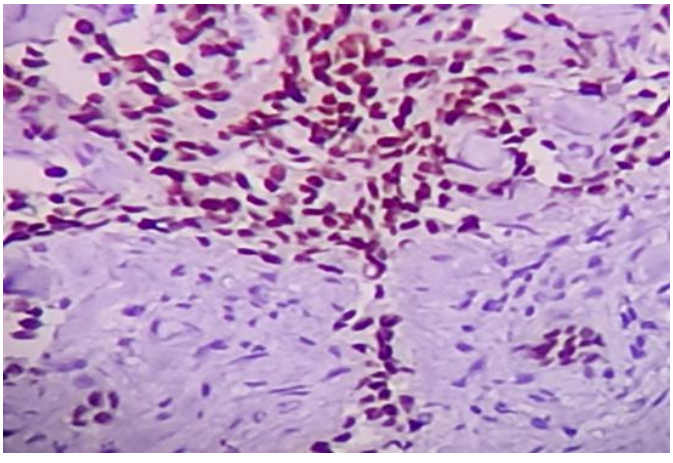

Figure 4: Histological section of invasive breast cancer with ER showed positive nuclear staining pattern (arrow) (Immunohistochemically stained, X100)

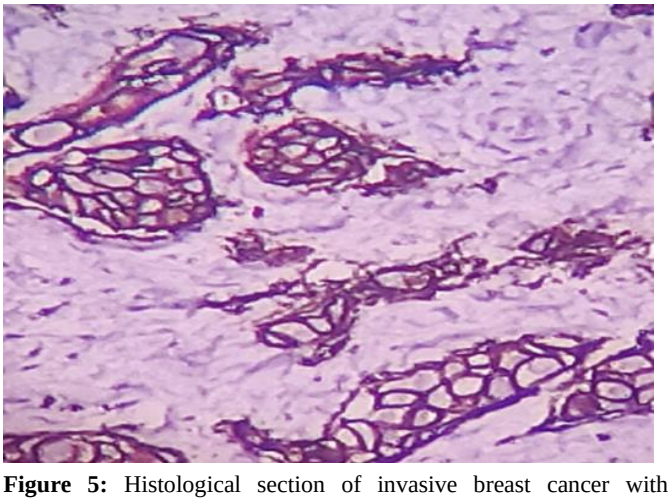

Figure 5: Histological section of invasive breast cancer with HER2\NEU showed positive cell membranous staining pattern (arrow) (Immunohistochemically stained, X100)

Discussion

CD15 is emerging as a recent prognostic marker with potential as a future therapeutic target in human malignancies (12,14). This study found that, out of 85 histological samples of invasive breast cancer, 47 (55.2%) were CD15 positive. There are conflicting data on CD15 expressions in the literature. For instance, a review by Wojciech Szlasa et al (14), which included two older studies, reported varied findings in this regard: 100% (expression in 30 out of 30 samples) and 34% (expression in 33 out of 98 samples) . More recently, Sozzani et al. (21) investigated the prognostic significance of CD15s in a prospective study with 127 primary mammary cancer patients and found CD15 antigen expression in 21% (37 out of 127 samples). The discrepancy with our results could be attributed to variations in sample sizes, differences in the manufacturers of immunohistochemical markers, and detection systems used in these studies.

Table 2: Association between CD15 expression and histological grade, ER receptor status, HER2/NEU status, tissue calcification, axillary lymph node status

Study variable	CD 15 status		Total	P value
	CD15 positive	CD15 negative		
Histological grade				
Grade I	**13 (15.3%)**	**13 (15.3%)**	**26 (30.6%)**	
Grade II	**25 (29.4%)**	**13 (15.3%)**	**38 (44.7%)**	
Grade III	**9 (10.5%)**	**12 (14.1%)**	**21 (24.6%)**	**0.192***
Total	**47 (55.2%)**	**38 (44.7%)**	**85 (100%)**	
ER receptor status				
ER-positive	**30 (35.2%)**	**22 (25.8%)**	**52 (61.2%)**	
ER-negative	**17 (20%)**	**16 (18.8%)**	**33 (38.8%)**	**0.577***
Total	**47 (55.2%)**	**38 (44.7%)**	**85 (100%)**	
HER2-NEU				
HER2-NEU positive	**20 (23.5%)**	**14 (16.5%)**	**34 (40%)**	
HER2-NEU negative	**27 (31.7%)**	**24 (28.2%)**	**51 (60%)**	**0.593***
Total	**47 (55.2%)**	**38 (44.7%)**	**85 (100%)**	
Tissue calcification				
Presence	**20 (23.5%)**	**12 (14.1%)**	**32 (37.6%)**	
Absence	**27 (31.7%)**	**26 (30.6%)**	**53 (62.3%)**	**0. 299***
Total	**47 (55.2%)**	**38 (44.7%)**	**85 (100%)**	
Axillary lymph node status				
Positive for metastasis	**32 (37.6%)**	**6 (7.1%)**	**38 (44.7%)**	
Negative for metastasis	**15 (17.6%)**	**32 (37.6%)**	**47 (55.2%)**	**0.000†**
Total	**47 (55.2%)**	**38 (44.7%)**	**85 (100%)**	

* Non-significant with p value > 0.05
† Highly significant with p value < 0.01

In current study, the correlation between CD15 expression and histological grade, ER and HER2/NEU receptor status, and calcification revealed a non-significant relationship. The only significant correlation was observed between positive CD15 expression and the presence of axillary lymph node metastasis (confirmed by ultrasound-guided fine needle aspiration cytology) at presentation. This finding aligns with CD15's well-documented biological effect in enhancing metastatic potential (12). Unfortunately, there are no similar studies discussing the correlation of CD15 expression in breast cancer with ER and HER2/NEU status, either Iraqi or worldwide, for further comparison and extensive discussion.

The expression CD15 in human malignancies was first described many years ago based on limited published data (14). Recently, there has been increasing interest in blocking CD15's function to mitigate metastatic potential, suggesting it may be a promising immunotherapeutic target. Several clinical trials have explored biological therapies targeting CD15 in various malignancy, including metastatic renal cell carcinoma (RCC), non-small cell lung cancer, leukaemia, melanoma, and colon cancer (14). Notably, two drugs, nivolumab and pembrolizumab, have been described; their therapeutic effects are either direct, targeting cancer cells, or indirect, monitoring CD15 expression on myeloid stem cells. This monitoring is crucial for understanding cancer initiation and progression (16, 22-24). Current research efforts should focus on enhancing the clinical application of anti-CD15 target therapy, particularly regarding its safety. This study aims to serve as a starting point for more extensive, prospective clinical trials, focusing on the significance of testing CD15 expression in breast cancer and providing reference data for future comparison and extensive discussion.

The main limitation of the present study was that it was carried in a single center with a limited small number of cases, many of them lacking relative clinical information

Conclusion

CD15 expression was detected in breast cancer cells. CD15 could serve as a prognostic marker as association with axillary lymph nodes reflects negative impact on prognosis and at the same time a promising therapeutic target for patients with breast cancer as blockage the antigen's function of CD15 will result in reduction of the metastatic potential and it could be an immunotherapeutic target. A prospective study, using a larger sample size is hopefully recommended with a special concern on enhancing the clinical application of anti-CD 15 target therapy.

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Conflict of Interest

The authors declare no conflict of interest.

Data availability

Data are available upon reasonable request

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References

[1] Lafta R. Health System in Iraq Post-2003 War. Al-Kindy College Medical Journal. 2023; 19(3):5–11. https://doi.org/10.47723/kcmj.v19i3.1040

[2] Sahu N, Agrahari AK, Parida B, Das S. Radiological Significance of Shear-Wave Elastography Technique for Evaluation of Solid Breast Masses with Histopathological Correlation. AL-Kindy College Medical Journal. 2023;19(3):26–30. https://doi.org/10.47723/kcmj.v19i3.1218

[3] Liantao G, Deguang K, Jianhua L, Ling Z , Lan L, Weijie Z, Qingyuan Z, Chuang Ch and Shengrong S. Breast cancer heterogeneity and its implication in personalized precision therapy. Exp Hematol Oncol 2023; 12(3):1-27. https://doi.org/10.1186/s40164-022-00363-1

[4] Raj-Kumar PK, Liu J, Hooke JA , Kovatich AJ , Kvecher L , Shriver CD, Hu H. PCA-PAM50 improves consistency between breast cancer intrinsic and clinical subtyping reclassifying a subset of luminal A tumors as luminal B. Sci. Rep 2019 ;9(1):7956. https://doi.org/10.1038/s41598-019-44339-4

[5] McDonald KA, Kawaguchi T, Qi Q, Peng X, Asaoka M, Young J, et al. Tumor Heterogeneity Correlates with Less Immune Response and Worse Survival in Breast Cancer Patients. Annals of surgical oncology 2019; 26(7):2191–9. https://doi.org/10.1245/s10434-019-07338-3

[6] Hussein IA, Ahmed ST, Hameedi AD, et al: Immunohistochemical expression of BRCA1 protein, ER, PR and HER2 in breast cancer: A clinicopathological study. Asian Pac J Cancer Prev 2020; 21:1025-29. https://doi.org/10.31557/apjcp.2020.21.4.1025

[7] Allison KH, Hammond ME, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, et al. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update.J Clin Oncol 2020 ;38(12):1346-1366. https://doi.org/10.1200/jco.19.02309

[8] Li Z, Wei H, Li S, Wu P, Mao X. The role of progesterone receptors in breast cancer. Drug Des Devel Ther 2022; 16: 305-314. https://doi.org/10.2147/dddt.s336643

[9] Gadhoum SZ, Sackstein R. CD15 expression in human myeloid cell differentiation is regulated by sialidase activity. Nat. Chem. Bio 2008; 4:751–757. https://doi.org/10.1038/nchembio.116

[10] Miyara M, Chader D, Sage E, Sugiyama D, Nishikawa H, Bouvry D, et al. Sialyl Lewis x (CD15s) identifies highly differentiated and most suppressive FOXP3high regulatory T cells in humans . PNAS 2015; 112(23):7225-30. https://doi.org/10.1073/pnas.1508224112

[11] Zhao H, Li W, Zhou X, Pan L, Feng Y, Gao P, et al. CXC motif chemokine ligand 1 promotes colitis by modulating the gut microbiota. J Innate Immun 2023; 16(1):33-44. https://doi.org/10.1159/000535637

[12] Liang J X , Liang Y , Gao W. Clinicopathological and prognostic significance of sialyl Lewis X overexpression in patients with cancer: A meta-analysis. Onco. Targets. Ther. 2016; 9:3113–3125. https://doi.org/10.2147/ott.s102389

[13] An Introduction to the Relationship Between Lewis x and Malignancy Mainly Related to Breast Cancer and Head Neck Squamous Cell Carcinoma (HNSCC) Cancer Investig 2021; 40:173–83. https://doi.org/10.1080/07357907.2021.2016800

[14] Szlasa W, Wilk K, Knecht-Gurwin K, Gurwin A, Froń A, Sauer Net, al. Prognostic and therapeutic role of CD15 and CD15s in cancer. Cancers. 2022 Apr 28; 14(9):2203. 10.3390/cancers14092203 https://doi.org/10.3390/cancers14092203

[15] Elola M , Capurro M I , Barrio MM , Coombs PJ , Taylor ME, Drickamer K and Mordoh J. Lewis x Antigen Mediates Adhesion of Human Breast Carcinoma Cells to Activated Endothelium. Possible Involvement of the Endothelial Scavenger Receptor C-type Lectin. Breast Cancer Res. Treat 2007; 101:161–74. https://doi.org/10.1007/s10549-006-9286-9

[16] Wu CY, Huo JP, Zhang XK, Zhang YJ, Hu WM, Yang P, Lu JB, Zhang ZL, Cao Y. Loss of CD15 expression in clear cell renal cell carcinoma is correlated with worse prognosis in Chinese patients. Jpn J Clin Oncol. 2017 Dec 1;47(12):1182-1188. https://doi.org/10.1093/jjco/hyx139

[17] Goetz MP, Gradishar WJ, Anderson BO. NCCN Guidelines Insights: Breast Cancer, Version 3.2018. 2019. J Natl Compr Canc Netw 2019; 17(2):118–126. https://doi.org/10.6004/jnccn.2019.0009

[18] Rabe K, Snir OL, Bossuyt V, Harigopal M, Celli R, Reisenbichler ES. Interobserver variability in breast carcinoma grading results in prognostic stage differences. Human pathology; 2019, 1(94) :51-7 https://doi.org/10.1016/j.humpath.2019.09.006

[19] Mohammed DR, Alwan NA, Hussein R. Immunohistochemical assessment of estrogen, progesterone receptors and HER2/neu overexpression by core needle biopsy in a sample of Iraqi breast cancer patients. J Contemp Med Sci 2019; 4(4):214-7. doi: https://doi.org/10.22317/jcms.v5i4.659

[20] Kim HM, Koo JS. Immunohistochemical analysis of cancer stem cell marker expression in papillary thyroid cancer. Front. Endocrinol 2019; 2(10:523. https://doi.org/10.3389/fendo.2019.00523

[21] Sozzani P, Arisio R, Porpiglia M, Benedetto C. Is Sialyl Lewis x antigen expression a prognostic factor in patients with breast cancer? IJSP 2008; 16(4):365-74. https://doi.org/10.1177/1066896908324668

[22] Brooks SA, Leathem AJ. Expression of the CD15 antigen (Lewis x) in breast cancer. Histochem J. 1995 Sep;27(9):689-93.

[23] Lee J, Han Y, Wang W, Jo H, Kim H, Kim S, Yang, et al. Phytochemicals in cancer immune checkpoint inhibitor therapy. Biomolecules 2021; 11(8):1107. https://doi.org/10.3390/biom11081107

[24] Lanier OL, Pérez-Herrero E, Andrea APD', Bahrami K, Lee E, Ward DM, Ayala-Suárez N, Rodríguez-Méndez SM, Peppas NA. Immunotherapy approaches for hematological cancers. iScience. 2022 Oct 10;25(11):105326. 10.1016/j.isci.2022.105326. https://doi.org/10.1016/j.isci.2022.105326

[25] Cui P, Li R, Huang Z, Wu Z, Tao H, Zhang S, Hu Y. Comparative effectiveness of pembrolizumab vs. nivolumab in patients with recurrent or advanced NSCLC. Sci Rep 2020; 4;10(1):13160. https://doi.org/10.1038/s41598-020-70207-7

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Al-Kindy College Medical Journal (KCMJ)

Research Article

Moderate Ischemic Mitral Insufficiency at the Time of Coronary Artery Bypass Graft; Repair or Spare?

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Keywords: Ischemic Mitral Insufficiency, Coronary Artery Bypass Graft, Mitral Valve Repair.

ABSTRACT

Background: Approximately 13-59% of myocardial infarction patients develop ischemic mitral regurgitation, impacting left ventricular function and increasing mortality risk. Optimal management of moderate ischemic mitral regurgitation remains controversial, raising the question of whether adding mitral valve surgery to coronary artery bypass graft (CABG) has an overall advantage over revascularization or not.

***Objective*:** To investigate the early and mid-term comparison between the two techniques.

Subjects and Methods: This randomized clinical trial was conducted at Assuit University Heart Hospital, on 50 patients randomized into two groups: Group A: 25 patients underwent CABG and Group B: 25 patients had CABG and mitral valve repair. Inclusion criteria was multi-vessel coronary artery disease, moderate ischemic mitral regurge (MR). All patients were subjected to full history taking, routine physical, laboratory investigations and transthoracic echocardiography. Intraoperative data was collected. Early outcomes included MR degree, and left ventricular (LV) diameters and function, ICU stay duration, and in-hospital mortality. Mid-term outcomes included MR degree, LV diameter and function.

Results: Groups had similar age and gender distribution. Repair procedures showed longer ischemic and operative times than CABG alone (P≤0.0001 and P=0.0012). Early post-operative, repair reduced MR significantly (P≤0.0001). At six months follow-up, CABG group had more rate of improvement than repair group (P≤0.0001).

Conclusions: In moderate ischemic mitral regurgitation with multi-vessel ischemic heart disease, adding mitral valve repair to CABG may reduce mitral regurgitation severity early and at six months compared to CABG alone. However, CABG alone offers shorter ischemic times and operative durations.

Introduction

Approximately 13-59% of myocardial infarction patients develop ischemic mitral regurgitation (IMR). About one-third have mild mitral regurgitation (MR) (1).

IMR has a complex mechanism. It results from left ventricular deformation and remodeling after myocardial infarction, which displaces papillary muscles of the annular plane. This displacement, annular flatness, expansion, and decreased contraction pull on chordae tendineae induces secondary MR by mal-coaptating the otherwise normal mitral valve (1-3).

Secondary MR leaflet tethering can be asymmetric or symmetric (1). Asymmetric tethering occurs when regional LV remodeling displaces the posterior papillary muscle laterally. In contrast, global LV remodeling causes symmetric tethering, which binds anterior and posterior papillary muscles apically. Research shows that revascularization alone seldom resolves severe IMR, and residual MR increases mortality risk (4).

Coronary artery bypass grafting with the addition of mitral valve surgery is recommended for severe IMR. Surgical correction of moderate IMR during coronary revascularization is controversial (5-7). CABG alone may reduce MR at follow-up, but it rarely eliminates it (8).

According to some studies, mitral valve annuloplasty may remove MR after CABG surgery. However, CABG + mitral valve annuloplasty can cause recurrent MR without improving long-term survival (9-10). CABG + mitral valve operations may increase morbidity and death in high-risk moderate IMR patients compared to CABG alone (11).

By the most recent recommendations issued by the American Association for Thoracic Surgery (AATS), patients who have moderate ischemic mitral regurgitation (IMR) may be eligible for coronary artery bypass grafting (CABG) procedures that involve the replacement of the mitral valve with an undersized complete rigid ring annuloplasty. On the other hand, this method is not necessarily advised in comparison to revascularization on its own. There is currently a lack of clarity on the possible advantages of adding mitral valve surgery with CABG for patients with IMR (12-13).

This study compared CABG alone vs CABG plus mitral valve surgery in patients with multi-vessel ischemic heart disease and mild mitral regurgitation. The examination covered early postoperative and six-month follow-up. Ischemic time, early and mid-term postoperative mitral regurgitation severity, and mortality rates were the main outcomes. Secondary outcomes were left ventricular diameter, function, and ICU stay**.**

Subjects and Methods

This randomized clinical trial, conducted between 2019 and 2021 at Assuit University Heart Hospital, on 50 consecutive patients with ischemic heart disease and moderate ischemic mitral regurge who were admitted to Cardiothoracic surgery department. The patients were randomized into two groups: Group A (25 patients) underwent CABG only, while Group B (25 patients) had CABG along with mitral valve repair. Inclusion criteria encompassed patients with multi-vessel coronary artery disease, moderate ischemic (not rheumatic) mitral regurge, and those undergoing elective surgery with cardiopulmonary bypass. Exclusion criteria included off-pump CABG, incomplete revascularization candidates, other valvular affection, and emergency cases.

In this study, data from eligible patients were collected in a data sheet for later analysis without altering their treatment or follow-up. All patients underwent complete history taking (personal details, complaints, drug sensitivity, past medical and surgical history), physical examinations (vital signs and checks for systemic diseases), and investigational studies, including routine laboratory tests (CBC, ESR, C-reactive protein, liver and kidney functions, PT, PTT, and INR).

Radiological investigations included pre-operative transthoracic echocardiography using a Vivid E9 (GE Healthcare, Chicago, US) or Philips iE33 (Royal Philips, Eindhoven, the Netherlands) ultrasound system. The degree of mitral regurgitation (MR) was estimated using the vena contracta method, with values between 3mm and 7mm indicating moderate MR. Other measured parameters included left ventricular diameters and left ventricular function. Operative variables recorded were the type of surgical procedure (CABG only or CABG concomitant with mitral valve repair), ischemic time, and total operative time.

In all cases, a coronary artery bypass grafting (CABG) procedure was performed. This involved making a median sternotomy incision and using hypothermic cardiopulmonary bypass along with intermittent antegrade cardioplegia. The saphenous vein and radial arteries were harvested to serve as secondary conduits, while the left internal mammary artery was used for grafting the left anterior descending coronary artery. In this context, mitral valve surgery and CABG were carried out simultaneously on the patients.

Left atriotomy accessed the mitral valve, and rigid complete ring annuloplasty was performed using 28mm or 30mm rings, the left atrium was accessed by exposing the Sondergaard groove, and proper ring size was

determined using manufacturer-supplied sizers. Ethibond Excel™ (Ethicon, Cincinnati, Ohio, US) sutures tightened the annulus and placed in an interrupted pattern, and the repair was tested with pressurized cold saline before closing the left atrium with polypropylene sutures.

Early outcomes, assessed on the 7thpost-operative day, involved grading the severity of mitral regurgitation (MR) using the vena contracta technique, along with left ventricular (LV) diameters and function, ICU stay duration, and in-hospital mortality. Mid-term outcomes, evaluated in 6th month, included the use of TTE to assess the degree of MR by vena contracta, LV diameters (end-systolic and end-diastolic), and LV function (ejection fraction).

Ethical considerations: The study was approved by the ethical committee of Faculty of Medicine, Assiut University by IRB no:17200405. Every participant was informed about the aim of the study, its benefit to him and to the community. Written consent was taken from all participants. Every participant had the right to withdraw from the study.

The statistical analysis was done utilizing SPSS software (version 26.0). Data were presented as frequencies, medians with ranges, or means ± SD, as appropriate. Key outcome variables included postoperative left ventricular function, the degree of mitral regurgitation (MR), and mortality rates. The prevalence of these outcomes was estimated using non-parametric statistical methods, including the Kaplan-Meier method. Risk factors for these outcomes were identified through multivariable regression analysis, examining demographic and morphological factors. Pre- and post-operative variables were compared using paired t-tests, with a p-value < 0.05 was statistically significant.

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Results

Demographic data

There was no significant difference in age between the CABG group (56.28 ± 9.6 years) and the repair group (58.04 ± 8.37 years), with a p-value of 0.7224. Gender distribution showed no significant difference between groups, with 56% males and 44% females in the CABG group, and 60% males and 40% females in the repair group. The p-value for gender distribution was 0.7799. (Table 1).

Table 1: Demographic data among included subjects in both study groups

	CABG group (N = 25)	Repair group (N = 25)	P. Value
Age (Years)	56.28 ± 9.6	58.04 ± 8.37	0.7224[s.t]
Gender			
Male	14 (56%)	15 (60%)	0.7799[X]
Female	11 (44%)	10 (40%)	

s.t: Skipped T-test, X: Chi square Test

Intra-operative results:

During cardiopulmonary bypass (CPB), ischemic time was significantly longer in the repair group (130.48 ± 13.77 minutes) compared to the CABG group (93.12 ± 17.35 minutes), with a p-value of <0.0001. Similarly, operative time was significantly longer in the repair group (285.16 ± 23.9 minutes) compared to the CABG group (250.84 ± 35.95 minutes), with a p-value of 0.0012. (Table 2)

Table 2: intraoperative data among included subjects in both study groups

	CABG group (N = 25)	Repair group (N = 25)	P. Value
CPB			
Ischemic Time (Minutes)	93.12 ± 17.35	130.48 ± 13.77	<0.0001*[MWU]
Operative time (Minutes)	250.84 ± 35.95	285.16 ± 23.9	0.0012*[MWU]
ICU stay (Day)	3.36 ± 2.06	3.32 ± 3.11	0.4765[MWU]

s.t: Skipped T-test, MWU: Mann-Whitney U Test

In-hospital mortality:

In the CABG group, 8% (2 out of 25) of individuals experienced death, while in the repair group, 4% (1 out of 25) experienced death. However, this difference was not statistically significant, with a p-value of 0.5609 (Chi-square test). (Table 3)

Table 3: In-hospital mortality among included subjects in both study groups

	CABG group (N = 25)	Repair group (N = 25)	P. Value
Death	2 (8%)	1 (4%)	0.5609[X]

X: Chi square Test

Early postoperative results:

Significant differences were observed for the degree of MR (Vena Contracta), with the CABG group showing a mean change of -0.08 ± 0.11 and the Repair group showing a significant decrease of -4.1 ± 0.91 (P < 0.0001). (Table 4)

For LV dimensions, both groups had similar changes in End Systolic Diameter (ESD), with a mean change of -0.06 ± 0.08 in the CABG group and -0.06 ± 0.1 in the Repair group (P = 0.99). There was no change in End Diastolic Diameter (EDD) in either group. (Table 4)

LV function, measured by Ejection Fraction (EF), showed a change of -0.72 ± 2.34 in the CABG group and -0.52 ± 1.55 in the Repair group, with no significant difference between groups (P = 0.9685). (Table 4)

The percentage change from baseline in the degree of MR (Vena Contracta) was -1.43 ± 2.06 in the CABG group and a significant decrease of -74.46 ± 12.25 in the Repair group (P < 0.0001). The percentage change in ESD was -1.35 ± 1.95 in the CABG group and -1.46 ± 2.32 in the Repair group, with no significant difference (P = 0.8994). There was no change in the percentage of EDD in either group. The percentage change in EF was -1.24 ± 4.38 in the CABG group and -0.94 ± 2.99 in the Repair group, with no significant difference (P = 0.7359). (Table 4)

The change in the degree of MR (Vena Contracta) was -1.88 ± 0.36 in the CABG group and significantly decreased to -4.6 ± 0.75 in the Repair group (P < 0.0001). (Table 4)

Table 4: Early Postoperative echocardiographic changes and the percentage change from baseline in both study groups

	CAGB group (N = 25)	Repair group (N = 25)	P. Value
Postoperative Echo change from baseline			
Degree of MR (Vena Contracta)	-0.08 ± 0.11	-4.1 ± 0.91	<0.0001* [MWU]
LV Dimension			
End Systolic Diameter (ESD)	-0.06 ± 0.08	-0.06 ± 0.1	0.99 [MWU]
End Diastolic Diameter (EDD)	0	0	
LV Function (EF)	-0.72 ± 2.34	-0.52 ± 1.55	0.9685 [MWU]
Postoperative Echo percentage of change from baseline			
Degree of MR (Vena Contracta)	-1.43 ± 2.06	-74.46 ± 12.25	<0.000*[MWU]
LV Dimension			
End Systolic Diameter (ESD)	-1.35 ± 1.95	-1.46 ± 2.32	0.8994 [MWU]
End Diastolic Diameter (EDD)	0	0	
LV Function (EF)	-1.24 ± 4.38	-0.94 ± 2.99	0.7359 [t]

t: T-test, MWU: Mann-Whitney U Test

LV dimensions showed an End Systolic Diameter (ESD) change of -0.28 ± 0.22 in the CABG group and -0.37 ± 0.16 in the Repair group, with no significant difference (P = 0.0658). End Diastolic Diameter (EDD) change was -0.57 ± 1.34 in the CABG group and -0.42 ± 1.05 in the Repair group, also showing no significant difference (P = 0.852). (Table 4)

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LV function, measured by Ejection Fraction (EF), increased by 2.74 ± 2.11 in the CABG group and 3.83 ± 2.41 in the Repair group, with no significant difference between groups (P = 0.1118). (Table 4)

Mid-term postoperative results:

The percentage change from baseline in the degree of MR (Vena Contracta) was -0.35 ± 0.05 in the CABG group and significantly decreased to -0.84 ± 0.08 in the Repair group (P < 0.0001). The percentage change in ESD was -0.07 ± 0.05 in the CABG group and -0.08 ± 0.04 in the Repair group, with no significant difference (P = 0.1537). The percentage change in EDD was -0.11 ± 0.26 in the CABG group and -0.07 ± 0.19 in the Repair group, with no significant difference (P = 0.9223). The percentage change in EF was 0.06 ± 0.04 in the CABG group and 0.08 ± 0.05 in the Repair group, with no significant difference (P = 0.1805). (Table 5).

Difference between early and mid-term results:

The change in the degree of MR (Vena Contracta) at 6 months was significantly greater in the CABG group (-1.79 ± 0.36) compared to the Repair group (-0.53 ± 0.48), with a P value < 0.0001. (Table 6).

For LV dimensions, the End Systolic Diameter (ESD) change was -0.22 ± 0.19 in the CABG group and -0.3 ± 0.12 in the Repair group, showing a significant difference (P = 0.0393). The End Diastolic Diameter (EDD) change was -0.57 ± 1.34 in the CABG group and -0.42 ± 1.05 in the Repair group, with no significant difference (P = 0.852). (Table 6)

LV function, measured by Ejection Fraction (EF), increased significantly in both groups, with a change of 2.96 ± 1.94 in the CABG group and 4.29 ± 1.88 in the Repair group (P = 0.0133). (Table 6)

Table 5: Echocardiographic changes at 6 months and the percentage change from baseline in both study groups

	CAGB group (N = 25)	Repair group (N = 25)	P. Value
6 Month Echo change from baseline			
Degree of MR (Vena Contracta)	-1.88 ± 0.36	-4.6 ± 0.75	<0.0001* [w.t]
LV Dimension			
End Systolic Diameter (ESD)	-0.28 ± 0.22	-0.37 ± 0.16	0.0658 [MWU]
End Diastolic Diameter (EDD)	-0.57 ± 1.34	-0.42 ± 1.05	0.852 [MWU]
LV Function (EF)	2.74 ± 2.11	3.83 ± 2.41	0.1118 [s.t]
6 Month Echo percentage of change from baseline			
Degree of MR (Vena Contracta)	-0.35 ± 0.05	-0.84 ± 0.08	<0.0001* [MWU]
LV Dimension			
End Systolic Diameter (ESD)	-0.07 ± 0.05	-0.08 ± 0.04	0.1537 [MWU]
End Diastolic Diameter (EDD)	-0.11 ± 0.26	-0.07 ± 0.19	0.9223 [MWU]
LV Function (EF)	0.06 ± 0.04	0.08 ± 0.05	0.1805 [s.t]

w.t: Wilches T-test, MWU: Mann-Whitney U Test

Table 6: Echocardiographic changes at 6 months and the percentage change from early postoperative echocardiographic data in both study groups

	CAGB group (N = 25)	Repair group (N = 25)	P. Value
6 Month Echo change from Postoperative Echo			
Degree of MR (Vena Contracta)	-1.79 ± 0.36	-0.53 ± 0.48	<0.0001* [MWU]
LV Dimension			
End Systolic Diameter (ESD)	-0.22 ± 0.19	-0.3 ± 0.12	0.0393* [MWU]
End Diastolic Diameter (EDD)	-0.57 ± 1.34	-0.42 ± 1.05	0.852 [MWU]
LV Function (EF)	2.96 ± 1.94	4.29 ± 1.88	0.0133* [MWU]
6 Month Echo percentage of change from Postoperative Echo			
Degree of MR (Vena Contracta)	-30.78 ± 10.26	-34.68 ± 19.9	0.7718 [MWU]
LV Dimension			
End Systolic Diameter (ESD)	-5.11 ± 4.84	-6.89 ± 2.7	0.0557 [MWU]
End Diastolic Diameter (EDD)	-11.26 ± 26.45	-7.48 ± 19.07	0.9223 [MWU]
LV Function (EF)	5.52 ± 5.04	8.36 ± 4.13	0.0093* [MWU]

MWU: Mann-Whitney U Test

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Discussion

Concerning patients who have multi-vessel ischemic heart disease and a moderate degree of mitral regurgitation, the treatment approach that is considered to be the most effective is still up for debate, however complete revascularization appears to have satisfactory outcomes in such patients in most clinical studies, evolution of competent techniques of mitral valve repair adds another option for these patients. We conducted this study trying to find the best possible strategy for our patients and to build our center experience

In our study, pre-operative echocardiographic data indicated that the degree of mitral regurgitation (MR), as measured by Vena Contracta, did not significantly differ between the CABG group (P = 0.7277). This finding aligns with El-Hag-Aly et al. (14), who reported similar mean VC values of 5.2 ± 0.96 for the CABG group and 5.3 ± 0.93 for the CABG + MV repair group in their study.

Comparatively, ByungJin Kim et al. (15) found that the dimensions of the left ventricle (LV) were substantially bigger in patients who underwent CABG + MV surgery than those who underwent CABG alone. This was evidenced by the fact that the LV end-systolic diameter (LVESD) and end-diastolic diameter (LVEDD) were both significantly greater in the former group. In our study, we found significant differences in LV dimensions between groups: ESD was higher in the repair group (4.39 ± 0.25 cm) versus the CABG group (4.05 ± 0.22 cm), p-value = 0.0012; and EDD was larger in the repair group (5.54 ± 0.34 cm) compared to the CABG group (5.12 ± 0.34 cm), p-value = 0.0054 (15). These results underscore the distinct LV dimensional profiles observed in our cohort undergoing different surgical approaches for ischemic heart disease with moderate MR.

Operatively, our study found significantly longer operative and ischemic times in the CABG plus MV repair group compared to the CABG alone group (P=0.0012). Similarly, the mean ischemic time was longer in the repair group compared to the CABG group ($p < 0.0001$). These findings are consistent with those reported by Michler et al. (16), who also noted longer ischemic times in the combined procedure group compared to CABG alone

Early post-operative echocardiographic follow-up in our study revealed a significant change in the degree of mitral regurgitation (MR) between the two groups. The CABG plus MV repair group showed a mean change of vena contracta of -4.1 ± 0.91, significantly more change than the CABG group with a mean change of vena contracta of -0.08 ± 0.11 ($p < 0.0001$). This aligns with findings from El-Hag-Aly et al. (14), where the CABG plus MV repair group had a markedly lower mean vena contracta compared to CABG alone.

Early postoperative echocardiographic data also demonstrated that the degree and percentage of change of the left ventricular (LV) dimensions did not differ significantly in both groups from the baseline data.

Regarding left ventricular function, our study found no significant improvement in early post-operative echocardiographic follow-up compared to pre-operative values. Moreover, there was no significant change in LV function post-operatively (P=0.9685), consistent with findings reported by El-Hag-Aly et al. (14), (P=0.75).

In terms of ICU stay, our findings align with those of Khallaf et al. (17), where no significant difference between the CABG group (3.36 ± 2.06 days) and the CABG plus MV repair group (3.32 ± 3.11 days) (P=0.4765). This suggests comparable post-operative recovery times between the two surgical approaches in our study.

Post-operative mortality was a crucial comparison in our study of surgical strategies. ByungJin Kim et al. (15), reported early deaths in 22 (3.7%) patients in the CABG-only group and 13 (11.2%) in the CABG + MVS group (P=0.001), attributing these findings to prolonged ischemic time and increased incidence of low cardiac output syndrome Conversely, other authors found no significant difference in in-hospital mortality between the strategies (17). Our study recorded two cases of in-hospital mortality in the CABG alone group and one in the CABG + MV repair group, with no significant difference observed (P=0.5609). This may be explained by the good preoperative clinical profiles of our patients, including preserved EF and shorter operative times.

Over six months of follow-up, our study evaluated multiple echocardiographic parameters. We observed a significant change in the degree of MR in the CABG + MV repair group compared to CABG alone in comparison to the baseline data, with mean vena contracta change values of -4.6 ± 0.75versus -1.88 ± 0.36, respectively ($p < 0.0001$). This finding is supported by Khallaf et al., (14), El-Hag-Aly et al., (17), and Chan et al., (18). Regarding LV function, no significant change was found between groups, with a mean change of EF of 2.74 ± 2.11 for CABG alone and 3.83 ± 2.41 for CABG + MV repair (P=not significant). Similar results were reported by Michler et al. (16).

When comparing the results of both techniques in early postoperative terms versus after 6 months, the rate of change of degree of MR estimated by vena contracta was found to be greater in CABG only group with a mean change of -1.79 ± 0.36 than that of CABG in addition to MV repair which had a mean of change of -0.53 ± 0.48, This indicates that complete revascularization alone takes longer time to achieve a significant effect on decreasing the degree of MR

Conclusion

Mitral valve repair may have an advantage when added to CABG in patients with moderate ischemic mitral regurge in terms of less degree of MR when compared to CABG only in both early and midterm follow-up, while having only CABG in such patients is associated with less operative time and less ischemic time, Thus, no overall advantage of any technique over the other had been found.

Limitations: only six months follow-up is the main limitations in our study.

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This research did not receive any specific fund.

Conflict of Interest

Authors declare no conflict of interest.

Data availability

Data are available upon reasonable request.

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References

[1] Magne J, Sénéchal M, Dumesnil JG,Pibarot P. Ischemic mitral regurgitation: a complex multifaceted disease. Cardiology, 2009; 112(4): 244-259. https://doi.org/10.1159/000151693

[2] Loperfido F, Biasucci LM, Laurenzi F, Gimigliano F, Vigna C, Rossi E, Manzoli U. Pulsed Doppler echocardiographic analysis of mitral regurgitation after myocardial infarction. The American journal of cardiology. 1986; 58(9): 692-697. https://doi.org/10.1016/0002-9149(86)90339-5

[3] Otsuji Y, Kumanohoso T, Yoshifuku S, Matsukida K, Koriyama C, Kisanuki A, Tei C. Isolated annular dilation does not usually cause important functional mitral regurgitation: comparison between patients with lone atrial fibrillation and those with idiopathic or ischemic cardiomyopathy. Journal of the American College of Cardiology, 2002; 39(10): 1651-1656. https://doi.org/10.1016/s0735-1097(02)01838-7

[4] Lam BK, GillinovAM, Blackstone EH, Rajeswaran J, Yuh B, Bhudia SK, Cosgrove DM. Importance of moderate ischemic mitral regurgitation. The Annals of Thoracic Surgery. 2005; 79(2): 462-470. https://doi.org/10.1016/j.athoracsur.2004.07.040

[5] Sandoval Y, Sorajja P, HarrisKM. Contemporary management of ischemic mitral regurgitation: a review. The American journal of medicine. 2018; 131(8): 887-895. https://doi.org/10.1016/j.amjmed.2018.01.048

[6] Bax JJ, Braun J, Somer ST, KlautzR, Holman ER, VersteeghMI, DionRA. Restrictive annuloplasty and coronary revascularization in ischemic mitral regurgitation results in reverse left ventricular remodeling. 2004;11(1): 20-103. https://doi.org/10.1161/01.cir.0000138196.06772.4e

[7] Malhotra A, Ananthanarayanan C, WadhawaV, Siddiqui S, SharmaP, Patel K, Shah P. OPCABG for moderate CIMR in elderly patients: a superior option?. Brazilian Journal of Cardiovascular Surgery. 2018; 33(1): 15-22. https://doi.org/10.21470/1678-9741-2017-0114

[8] Salmasi MY, Harky A, Chowdhury MF, Abdelnour A, Benjafield A, Suker F, Vohra HA. Should the mitral valve be repaired for moderate ischemic mitral regurgitation at the time of revascularization surgery?. Journal of Cardiac Surgery. 2018; 33(7): 374-384. https://doi.org/10.1111/jocs.13722

[9] ChanKJ, Punjabi PP, Flather M, Wage R, Symmonds K, Roussin I, Pepper JR. Coronary artery bypass surgery with or without mitral valve annuloplasty in moderate functional ischemic mitral regurgitation: final results of the Randomized Ischemic Mitral Evaluation (RIME) trial. Circulation. 2012; 126(21): 2502-2510. https://doi.org/10.1161/circulationaha.112.143818

[10] Sun X, Huang J, Shi M, Huang G, Pang L, Wang Y. Predictors of moderate ischemic mitral regurgitation improvement after off-pump coronary artery bypass. The Journal of Thoracic and Cardiovascular Surgery, 2015; 149(6): 1606-1612. https://doi.org/10.1016/j.jtcvs.2015.02.047

[11] Rabbah JPM, Siefert AW, Bolling SF, Yoganathan AP. Mitral valve annuloplasty and anterior leaflet augmentation for functional ischemic mitral regurgitation: quantitative comparison of coaptation and subvalvular tethering. The Journal of thoracic and cardiovascular surgery. 2014; 148(4): 1688-1693. https://doi.org/10.1016/j.jtcvs.2014.04.008

[12] SmithPK, Puskas JD, Ascheim DD, Voisine P, GelijnsAC, Moskowitz AJ, Michler RE. Surgical treatment of moderate ischemic mitral regurgitation. New England Journal of Medicine. 2014; 371(23): 2178-2188. https://doi.org/10.1056/nejmoa1410490

[13] KronIL, LaPar DJ, Acker MA, Adams DH, Ailawadi G, Bolling SF, Puskas JD. 2016 update to the American Association for Thoracic Surgery (AATS) consensus guidelines: ischemic mitral valve regurgitation. The Journal of thoracic and Cardiovascular Surgery. 2017; 153(5): 97-114. https://doi.org/10.1016/j.jtcvs.2017.01.031

[14] El-Hag-AlyMA, El Swaf YF, ElkassasMH, Hagag MG, Allam HK. Moderate ischemic mitral incompetence: does it worth more ischemic time?. General Thoracic and Cardiovascular Surgery. 2020; 68(1): 492-498. https://doi.org/10.1007/s11748-019-01212-5

[15] Kim BJ, KimYS, Kim HJ, Ju MH, Kim JB, Jung SH, Chung CH. Concomitant mitral valve surgery in patients with moderate ischemic mitral regurgitation undergoing coronary artery bypass grafting. Journal of thoracic disease. 2018; 10(6): 32-36. https://doi.org/10.21037/jtd.2018.05.148

[16] Michler RE, Smith PK, Parides MK, Ailawadi G, Thourani V, Moskowitz AJ, Gelijns AC. Two-year outcomes of surgical treatment of moderate ischemic mitral regurgitation. New England Journal of Medicine. 2016; 374(20): 1932-1941. https://doi.org/10.1056/nejmoa1602003

[17] Khallaf A, Elzayadi M, AlkadyH, El Naggar A. Results of coronary artery bypass grafting alone versus combined surgical revascularization and mitral repair in patients with moderate ischemic mitral regurgitation. In Heart Surg Forum. 2020; 23(1): 270-275. https://doi.org/10.1532/hsf.2773

[18] Chan KJ, Punjabi PP, Flather M, Wage R, Symmonds K, Roussin I, Pepper JR. Coronary artery bypass surgery with or without mitral valve annuloplasty in moderate functional ischemic mitral regurgitation: final results of the Randomized Ischemic Mitral Evaluation (RIME) trial. Circulation. 2012; 126(21): 2502-2510. https://doi.org/10.1161/circulationaha.112.143818

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Research Article

Role of microRNA-499-5p in Early Diagnosis of Acute Coronary Syndrome and its Subtypes Compared with Highly Sensitive Cardiac Troponin –I

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Keywords: Acute coronary syndrome, hscT-I, miRNA-499-5P, electrocardiogram

ABSTRACT

Background: Acute coronary syndrome (ACS) is still one of the main causes of morbidity and mortality worldwide, to decrease mortality rates if diagnosed early. The ACS classification into three subtypes depends on electrocardiogram. miRNA-499-5p was found to be substantially elevated in AMI patients as compared with the non-AMI group and healthy control and it was already detectable in the plasma 1h after the onset of chest pain in AMI patients
Objectives: To evaluate the role of measuring serum miRNA-499-5p value in early diagnosis of ACS and compare with hscT-I
Subjects and Methods: A total of 120 patients (72 males and 48 females) aged≥30 years were consecutively selected from those who were admitted and diagnosed with ACS. The healthy subjects as controls for this study were recruited from those who had no current illness. The serum miRNA-499-5P and hscT-I were measured.
Results: It was shown that miRNA-499-5p levels had an overall significant difference among study groups ($p<0.001$), also the miRNA-499-5p mean level was significantly different between each ACS subgroup and controls ($P<0.001$)
miRNA-499-5p showed a significant positive correlation with hscT-I 0hrs in STEMI and NSTEMI but UA showed a non-significant correlation. miRNA-499-5p level had perfect AUC and high sensitivity and specificity for discrimination between subgroups of ACS patients.
Conclusions: The present study found the values of serum miRNA-499-5p expression in patients with ACS patients are significantly higher compared with controls after 1h of onset of chest pain**,** additionally, miRNA-499-5p expression have high sensitivity and specificity for differentiation between subgroups of ACS and from controls.

Introduction

An inadequate supply of blood to the myocardium was the result of coronary heart disease (CHD). It was primarily caused by the formation of atherosclerotic plaques within the intima of coronary arteries. (1) The plaque may erode or rupture, initially resulting in thrombosis and then a closure of the vessel that impedes blood flow and leads to ischemia or myocardial infarction (MI) (2).
Myocardium doesn't receive adequate blood supply in CHD, either acutely as it does in MI or chronically as it does in unstable angina pectoris. Angina at rest can only be caused by stenosed lesions at least 90%. Thrombosis is caused by tissue factor being exposed during a plaque rupture. (3) three subtypes of ACS may result from this type of thrombosis, which could result in subtotal or total lumen occlusion (4).4 The three types of ACS include MI with the electrocardiogram (ECG) showing ST-segment elevation (STEMI), the other MI with the ECG showing no ST-segment elevation (NSTEMI) and the third type is unstable angina (UA) (5).

Early diagnosis of MI can decrease the mortality rate for MI patients, as ACS is still one of the most prevalent causalities of morbidity and mortality around the world. Measurement of the blood level of cardiac-specific troponins (cTn-I and cTn-T) has been regarded as the preferred or the standard biomarker for diagnosis of myocardial infarction. An acute myocardial infarction begins with an elevation of serum troponin level that peaks four to ten days after onset and stays heightened for four to ten days afterward (6). Since high-sensitivity cardiac troponins detect very low levels of serum troponin, the newer generation of these cardiac troponins can detect cardiac injury more instantly than conventional cardiac troponins (7).

The management of the three ACS subtypes was not fixed so obtaining unexplored cardiac biomarkers for early diagnosis and differentiation of its subtypes would be of great importance. Recently, MI patients have been reported to have a significantly higher serum expression of a micro RNA (miRNA) of the type miRNA-499-5p which was positively correlated with serum levels of cTn-I and creatine kinase isoenzymes – MB (CK-MB) (8). The miRNA-499-5p was detectable in the plasma 1 h after onset of chest pain in MI patients while not in chest pain patients with no MI or a healthy control group (9). As miRNAs may be a promising method of diagnosing cardiovascular disease, particularly when there is diagnostic uncertainty, their integration with or without currently available biomarkers is a promising tool (10). miRNA-499-5p was highly expressed in the heart and was produced almost exclusively in the heart. Also, it was that found miRNA-499-5p may be involved in myocardial injury and remodeling and miRNA-499-5p was shown to be involved in cardiomyocyte differentiation(11). This study aimed to explore the role of miRNA-499-5p in the common clinical environment for early diagnosis and differentiation of subtypes of ACS compared with cTn-I.

Subjects and Methods

Study patients were recruited from the coronary care unit at Al-Yarmouk Teaching Hospital during the period between the 1st of November 2022 to the 1st of September 2023. One hundred twenty patients (72 males and 48 females), aged ≥ 30 years were consecutively selected from those who were admitted and diagnosed as ACS by specialist cardiologists. The diagnosis of ACS was based on the presence of two out of three criteria:

- Clinical presentation of the patient
- ECG changes
- A positive troponin test

Based on the same adopted criteria, ACS patients comprised three subgroups; namely, STEMI, NSTEMI, and UA. The healthy subjects as a control group were recruited from those who had no current illness with consideration of age and sex matching with the ACS patients. They had no history of CHD or other systemic diseases and have had normal ECG recordings.

Blood samples were collected from patients and controls. The serum was separated, divided into aliquots, and used for measurement of the level of hscT-I and the value of miRNA-499-5p ¯3 UUUGUAGUGACGUUCAGAAUU ¯5). The assay of hscT-I was done by using enzyme-linked immunosorbent assay (ELISA) kits that were supplied by MyBioSource Company, USA, and followed the manufacturer's instructions. The miRNA-499-5P value was estimated by SaCycler-96 Real-Time PCR system

Total RNA was extracted from serum samples of all patients and healthy controls(. All samples of RNA were converted to cDNA (EasyScript *First-Strand cDNA Synthesis SuperMix AE301-02)* by Reverse transcription PCR (Italy). Which was then submitted to qRT-PCR for micro-RNA-499-5P *level* expression and was normalized to the reference gene (GAPDH) by Amplification Kit Use Sybr. Fold change data was calculated using the relative comparative method 2-(ΔΔCt) (called the Livak method calculator) (12).

Table 1: Cycles And Thermal Profile Of qPCR

qPCR Steps	Temp.	Time	Cycle(s)
Initial activation	94°C	30sec	1
Denaturation	94°C	5 sec	45
Annealing	60°C	35 sec	
Melt curve	60-90°C	15 sec	1

The study was conducted after obtaining approval from the scientific committees in the Karkh Health Department in Baghdad. The objectives of the study were explained to the patients and their consent was obtained before starting the blood draw procedure and completing the study.

Data were analyzed using the statistical package of SPSS-24. After assuring that the data was normally distributed, data presentation was done by simple measures like mean, standard error or standard deviation of the mean, and percentage. ANOVA test was used to detect the presence of difference in means among more than two groups while the LSD test was used for the difference between two means. Pearson's correlation analysis was performed to determine the correlation between micRNA-499-5p and the other parameters. It was considered statistically consequential if the P value<0.05. In all study groups, Hanley and McNeil's method was used to analyze the ROC curve and measure the area under the curve (AUC). (13)

Results

The clinical characteristics of the study subjects, which the patients were 64.1% males and 35.8% females, and the control subjects had a similar sex distribution (64 % males, 35.1% females). The age range of study patients who were ≤ 50 years in age constituted 33.3% and those who were > 50 years old constituted 66.66%. Regarding BMI, 20% of patients were normal weight, 32.5% were obese, and 47.5% were overweight. Patients with ACS included those with STEMI (40 patients), NSTEMI (40 patients), and UA (40 patients).

Micro-RNA-499-5P gene expression fold change

Gene expression of micro-RNA-499-5P was detected in samples of all ACS and healthy controls. The expression of micro-RNA-499-5P in different patient subgroups is shown in Figure 1**.** The mean relative value of micro-RNA-499-5P *expression* was 39.98 ±11.23 in the STEMI group, 33.01 ±7.88 in NSTEMI, and 17.98 ±2.37 in UA group while in the control group, it was 1.00 ±0.00 and the difference among subgroups of ACS patients and healthy controls was statistically significant (P ≤ 0.01).

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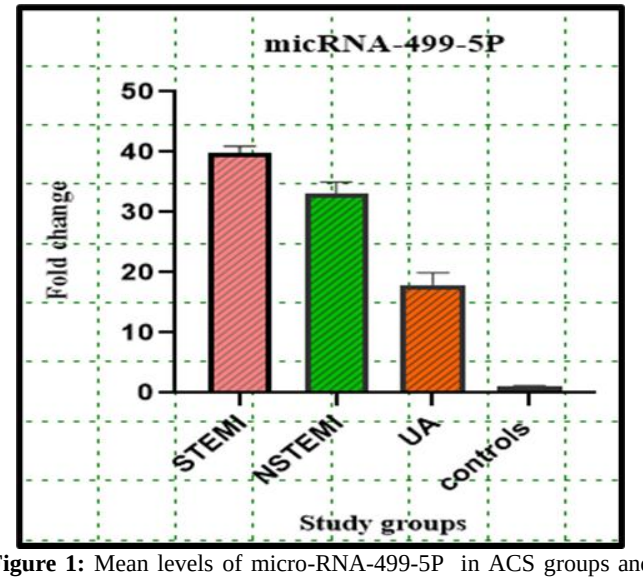

Figure 1: Mean levels of micro-RNA-499-5P in ACS groups and controls

Comparison of micRNA-499-5p among subtypes of acute coronary Syndrome

A comparison was made about the levels of micRNA-499-5p by using ANOVA analysis. It was revealed that study groups had extremely different levels of micRNA-499-5p ($P < 0.001$).

Further analysis by LSD test revealed that the micRNA-499-5p mean level was significantly different between each ACS subgroup and control group ($P < 0.001$). Additionally, STEMI levels were higher than NSTEMI ($P<0.01$) and UA ($P < 0.001$) levels. Also, a significant difference in micRNA-499-5p level was detected between NSTEMI and UA subgroups ($P = 0.028$).

Comparison of hscT-I levels among subgroups of acute coronary syndrome on admission and controls

A comparison was made in regard to the levels of hscT-I on admission among groups of study using the ANOVA test Figure 2. The ANOVA test revealed a significant difference in hscT-I levels among groups of study ($P< 0.05$).

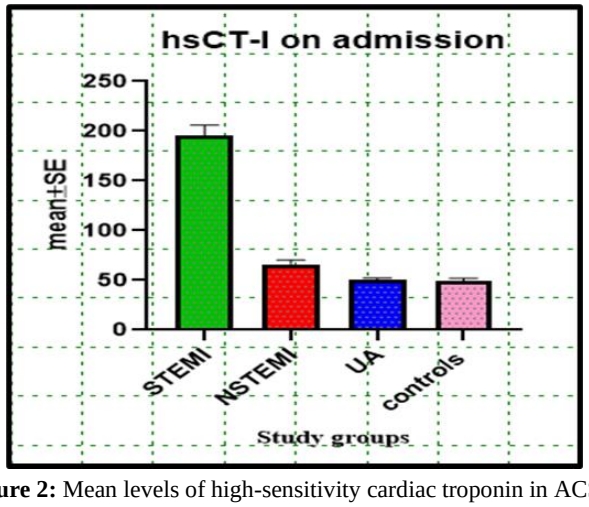

Figure 2: Mean levels of high-sensitivity cardiac troponin in ACS groups on admission and controls

Further analysis by LSD test revealed that hscT-I mean level on admission was significantly higher in STEMI and in NSTEMI subgroups compared with controls ($P = 0.033$ and $P = 0.047$, respectively) while no significant difference was detected among controls and UA. STEMI had an increased mean level of hscT-I than NSTEMI and UA ($P = 0.032$ and $P = 0.041$, respectively), while the NSTEMI and UA subgroups did not show any significant difference in hscT-I levels.

Comparison of hscT-I level three hours after admission among subgroups of acute coronary syndrome and controls

The hscT-I levels were compared in Figure 3. Among study group (ACS) and control groups exhibited overall significant differences in levels of hscT-I by an ANOVA test ($P< 0.001$)

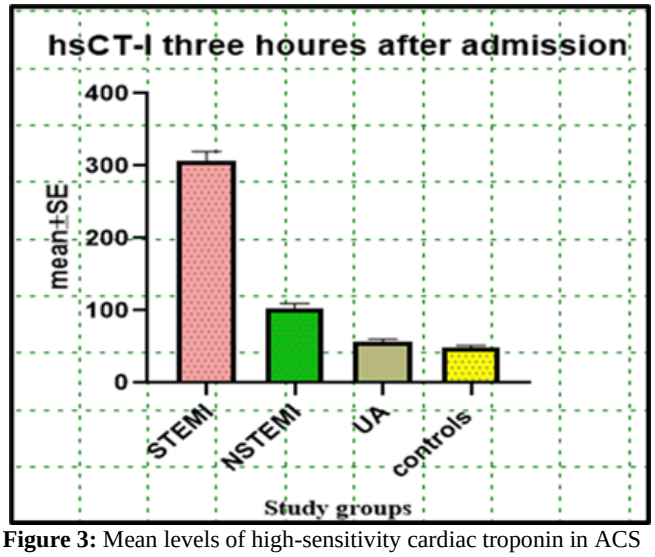

Figure 3: Mean levels of high-sensitivity cardiac troponin in ACS groups three hours after admission and controls

Further analysis by LSD test revealed that hscT-I mean levels were notably greater in both NSTEMI and STEMI subgroups than in the control group ($P = 0.000$) with no significant difference among controls and UA subgroups. The mean level of hscT-I was also significantly higher in STEMI than in both NSTEMI and UA ($P = 0.001$) but at this time a significantly higher mean level of hscT-I was detected between NSTEMI and UA subgroups ($P = 0.001$).

The correlation analysis of research biomarkers in patients with ACS. The micRNA-499-5p levels in the STEMI group showed a significant positive correlation with hscTn-I at admission ($r = 0.382$, $p = 0.041$) and also at three hours after admission ($r = 0.582$, $p = 0.021$). In NSTEMI subgroup, the micRNA-499-5p levels showed also significant positive correlation with hscTn-I levels at admission ($r = 0.127$, $p = 0.035$) and three hours after admission ($r = 0.323$, $p = 0.039$) while in UA subgroup, the micRNA-499-5p levels showed no significant correlation with hscTn-I levels whether at admission ($r = 0.223$, $p = 0.061$) or three hours after admission ($r = 0.324$, $p = 0.053$).

To test the diagnostic and differential discrimination power of study biomarkers among study groups, then ROC analysis was conducted. The ROC analysis between STEMI versus NSTEMI subgroups of ACS patients is presented in Table 2 and figure 4. For micRNA-499-

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5p levels, the results showed that the AUC was 1.00 and at a cut-off value of 38.65, the sensitivity was 97.1 % and the specificity was 100 % with a diagnostic accuracy of 99.5 %. For hscT-I levels at admission, the AUC was 0.791, and at 76.8 ng/ml cut-off value, 75.8 % was test sensitivity while 78.9 % was specificity with a diagnostic accuracy of 78.3 %. For hscT-I levels three hours after admission, the AUC was 0.971 and at 132.58 ng/ml cut-off value, 97.5 % test sensitivity, and 83 % specificity with a diagnostic accuracy of 89.2 %.

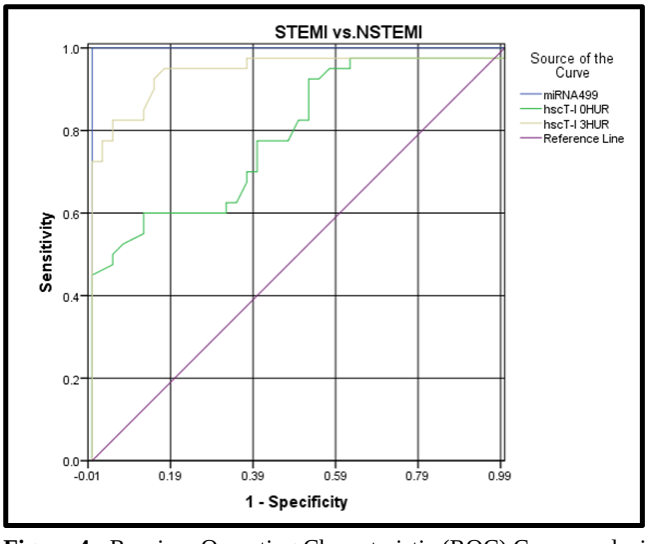

Figure 4: Receiver Operating Characteristic (ROC) Curve analysis of core study biomarkers in the STEMI subgroup of acute coronary syndrome patients versus NSTEMI

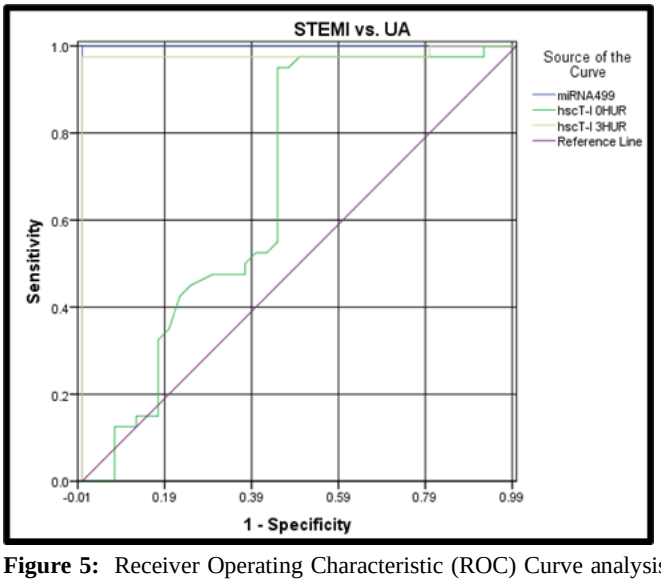

Figure 5: Receiver Operating Characteristic (ROC) Curve analysis of core study biomarkers in STEMI subgroup of acute coronary syndrome patients versus UA

The ROC analysis between STEMI versus UA subgroups of ACS patients was presented in Table 3 and figure 5. For micRNA-499-5p levels, the results showed that the AUC was 1.00 and at a cut-off value of 38.65, the sensitivity of the test was 97.5 % and the specificity was 100 % with a diagnostic accuracy of 99.5 %. For hscT-I levels at admission, the AUC was 0.678 and with an 87.7 ng/ml cut-off value, the test was 76.5% sensitive, 70.8% specific, with 78.3% diagnostic accuracy. For hscT-I levels at three hours after admission, the AUC was 0.981 and with a cut-off value of 123.44 ng/ml, the sensitivity of the test was 97.5% and the specificity was 74.2% with a diagnostic accuracy of 82.6%.

The ROC analysis between NSTEMI versus UA subgroups of ACS patients was presented in Table 4 and figure 6. For micRNA-499-5p levels, the results showed that the AUC was 0.977, and at a 30.10 ng/ml cut-off value, the test was 90% sensitive, 96 % specific with 92.5 % diagnostic accuracy. For hscT-I levels at admission, the AUC was 0.760 and had 43.1 ng/ml as a cut-off value, and the test was 71.5% sensitive, 68.7% specific, and 77.1% diagnostic accuracy. For hscT-I levels at three hours after admission, the AUC was 0.885 with 56.25 ng/ml cut-off value, 85 % test sensitivity, and 81% specificity with a diagnostic accuracy of 86.2%.

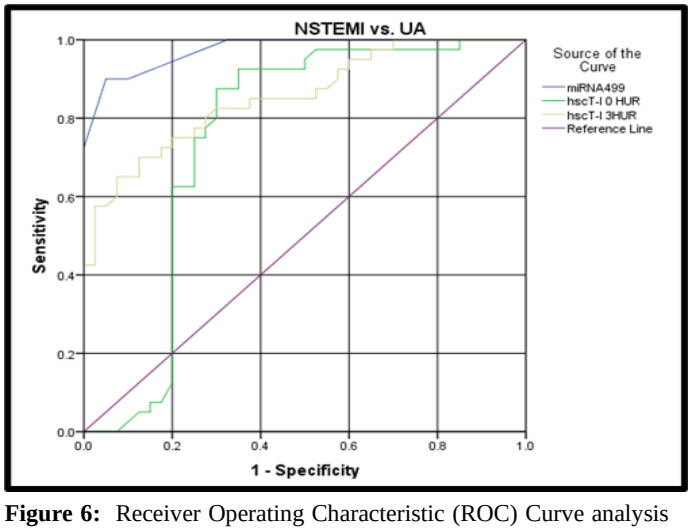

Figure 6: Receiver Operating Characteristic (ROC) Curve analysis of core study biomarkers in the NSTEMI subgroup of acute coronary syndrome patients versus UA

Discussion

Laboratory investigation of certain biomarkers, principally the cardiac troponins, was complementary to the clinical evaluation and ECG recording during the steps of diagnosis, triage, and management of patients with suspected ACS. The interval of 1–2 hours that allows early diagnosis or ruling out of acute MI is currently the most significant clinical hurdle to overcome. The release of cardiac troponin I (cTn-I) is relatively delayed after the onset of MI. The ruling out of acute MI by using ECG and troponins was time-consuming owing to the need for serial blood sampling to determine changes in troponin concentrations, especially in patients with non-ST elevation ACS. It was crucial to conduct successive assessments during the downtime for acute MI diagnosis and such extended monitoring of patients in emergency medical and/or cardiology centers drives a need for the development of fresh rule-in and rule-out tactics for the timely identification of acute MI

(11). Accordingly, an early diagnosis and clinical subtyping of the heterogeneous patients who present with suspected ACS may be improved by the finding of other biomarkers that their levels may change faster and so could be used for earlier diagnosis of patients with chest pain.

In a search for newer biomarkers that may aid in the early diagnosis of ACS and may have importance in the understanding of its progression, this study has evaluated a certain novel biomarker, which is micRNA 499-5P, whose levels are changed in the first few hours after onset of chest pain (often after 1 hour of chest pain) this agrees with (9).

Table 2: Discriminative ability of micRNA-499-5P and hscTn-I between STEMI vs. NSTEMI subgroups of ACS patients

Parameter	AUC	Cut-off value	P-value	Sensitivity	Specificity	Diagnostic accuracy %	discrimination	95 CI	
								Lower	Upper
micRNA-499-5p	1.000	38.65	0.001	97.1	100	99.5	perfect	1.00	1.00
hscT-I (pg/ml)/ 0hr.	0.791	76.8	0.001	75.8	78.9	78.3	fair	0.693	0.889
hscT-I (pg/ml)/ 3hr.	0.971	132.58	0.001	97.5	83.0	89.2	perfect	0.992	1.00

Table 3: Discriminative ability of micRNA-499-5P and hscTn-I between STEMI vs. UA subgroups of ACS patients

Parameter	AUC	Cut-off value	P-value	Sensitivity	Specificity	Diagnostic accuracy %	discrimination	95% CI	
								Lower	upper
micRNA-499-5p	1.000	38.65	0.001	97.5	100	99.5	Perfect	1.00	1.00
hscT-I (pg/ml) /0hr.	0.678	87.7	0.006	76.5	70.8	78.9	Poor	0.554	0.809
hscT-I (pg/ml) /3hr.	0.981	123.445	0.001	97.5	74.2	82.6	Perfect	0.943	1.00

Table 4: Discriminative ability of micRNA-499-5P and hscTn-I between NSTEMI versus UA subgroups of ACS patients

Parameter	AUC	Cut-off value	P-value	Sensitivity	Specificity	Diagnostic accuracy %	discrimination	95 CI	
								Lower	Upper
micRNA-499-5p	0.977	30.10	0.001	90.0	96.0	92.5	Perfect	0.977	1.00
hscT-I (pg/ml) / 0hr.	0.760	43.1	0.038	71.5	68.7	77.1	Fair	0.505	0.973
hscT-I (pg/ml) / 3hr.	0.885	56.25	0.001	85.0	81.0	86.2	Good	0.766	0.930

Discussion

This study revealed that the miRNA-499-5p expression mean level was significantly higher in ACS patients than in apparently healthy subjects. This finding is consistent with previous studies that reported an elevated miRNA-499-5p expression level in patients with acute myocardial infarction and in symptomatic patients referred for coronary angiography or in symptomatic patients referred for echocardiographic characteristics of atherosclerosis that agree with (8) due to suggesting that they could be used as biomarkers for heart injury. miRNA-499-5p is a newly identified member of the myosin gene family's miRNAs, located in an intron of the Myh7b gene. It was highly conserved across species, inhibits cardiomyocyte progenitor cell proliferation, and promotes cell differentiation and its expression in plasma was shown to be higher in patients with AMI

This study showed a highly significant positive correlation between miRNA-499-5p expression and the levels of the established

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biomarker of MI which is the high sensitivity troponin-I in all three subgroups of ACS patients, this agrees with (14) due to suggest miRNA-499-5p was expressed at higher levels in myocardial infarction patients compared to those with other traditional AMI biomarkers, and that these levels were related positively with circulating CKMB and cTnI. As a result, miRNA-499-5p can be considered an early and specific biomarker that is nearly recognized in the blood 1 hour after heart muscle damage.

The mean levels of serum miRNA-499-5p expression in all subgroups of ACS patients were found to be significantly higher in ACS subgroups than in the control group. Such finding is consistent with many previous studies. The miRNA-499-5p level was reported to be significantly and positively correlated with serum cTn-I as well as creatine kinase (CK-MB) levels in acute MI patients (8). In one recent study, the miRNA-499-5p was found substantially elevated in acute MI patients as compared with no acute MI subgroup of ACS or with a healthy controls group (15). In another study, the miRNA-499-5p was detectable in the plasma one hour after the onset of chest pain in acute MI patients (9).

All these findings are consistent with the previous reports that showed that miRNA-499-5p was highly expressed in the heart and is produced almost exclusively in the heart due to this miRNAs are important for the development and proper functioning of the myocardium, and therefore their dysregulation was associated with the occurrence and progression of heart disease. Specifically, miRNA-499-5p was released into the bloodstream in patients with AMI due to cardiomyocyte damage, among other factors, and therefore up-regulation of their plasma levels can be observed. (16)

Also was consistent with the idea that miRNA-499-5p may be involved in myocardial injury and remodeling and that miRNA-499-5p was involved in cardiomyocyte differentiation (11).

The enhanced diagnostic capability of miRNAs can be attributed to their short, noncoding nature that regulates gene expression post-transcriptionally and to their remarkable stability in circulation. A lot of research has been conducted on the diagnostic efficiency of these molecules, and they have been suggested as biomarkers for diagnosing various diseases such as aortic dissection where the plasma of these patients has exhibited human cytomegalovirus-encoded miRNA expression profile (11).

ROC analysis revealed that the highest AUC value for the level of miRNA-499-5p expression was in discrimination between subgroups of ACS (STEMI, NSTEMI, and UA) and in differentiation of ACS subgroups from controls with a high sensitivity and specificity for differentiation between members of ACS. These findings suggest that miRNA-499-5p expression may be useful in undiagnosed acute chest pain with non-diagnostic ECG and normal troponin level and so may support an earlier diagnosis and management of ACS.(17)

ROC analysis revealed the fair to poor AUC for high sensitivity cardiac troponin level on admission and low sensitivity and specificity for differentiation between all subgroups of ACS (STEMI, NSTEMI, and UA) and in the differentiation of ACS from controls. On the other hand, the level of hscT-I, three hours after admission had a good AUC and a high sensitivity and specificity for differentiation between all subgroups of ACS (STEMI, NSTEMI, and UA) and in differentiation of ACS from controls in patients with acute coronary syndrome compared with the healthy control subjects. (18) Besides, ECG, cardiac biomarkers, and in particular troponins were used to diagnose acute MI. However, ruling out AMI with ECG and troponins is time-consuming owing to the need for serial blood sampling to determine the changes in troponin concentrations, especially in patients with non-ST-elevation ACS. The release of high-sensitivity cardiac troponin I (hscTn-I) was relatively delayed in comparison with myocardial infarction onset. However, the HSCT I was very sensitive which to a high number of false-positive results (19).

Circulating miRNA-499-5p a family member of miRNA-499 was shown to have the highest increase in NSTEMI patients by ~80-fold, the diagnostic accuracy of miRNA-499-5p was evaluated with ROC analysis and was comparable to that of cTn-T. In elderly patients with acute NSTEMI, a study showed that circulating miRNA-499-5p had better accuracy in the diagnosis compared with cTn-T. (20)

Conclusion

In the present study, the values of serum miRNA-499-5p expression are significantly higher in patients with ACS patients when compared with controls in early diagnosis ACS patients after 1 h of onset of chest pain that means diagnose the small amount of miRNA-499-5P, also showed a complete miRNA-499-5p expression AUC and a high sensitivity and specificity for differentiation between members of subgroups of ACS

Funding

This research did not receive any specific fund.

Conflict of Interest

Authors declare no conflict of interest.

Data availability

Data are available upon reasonable request

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References

[1] Hussein MF, Hussein HJ. Association of Echocardiographic Epicardial Fat Thickness and Carotid Intima Media Thickness with the Severity of Coronary Artery Disease. AL-Kindy College Medical Journal. 2020 Sep 4;16(1):24-9. https://doi.org/10.47723/kcmj.v16i1.185

[2] Frąk W, Wojtasińska A, Lisińska W, Młynarska E, Franczyk B, Rysz J. Pathophysiology of cardiovascular diseases: new insights into molecular mechanisms of atherosclerosis, arterial hypertension, and coronary artery disease. Biomedicines. 2022 Aug 10;10(8):1938. https://doi.org/10.3390/biomedicines10081938

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[3] Rawi NA, Waheb AM. Association between periodontitis and acquired coronary heart disease. Al-Kindy College Medical Journal. 2016 Jun 30;12(1):10-5.

[4] Nematisouldaragh D. Autophagy gene regulation in cardiac myocytes and cardiac fibroblasts.

[5] Aparicio HJ. Heart disease and stroke Statistics-2021 update a report from the American Heart Association. Circulation. 2021;143:e254.

[6] Neyestanaki MH, Kalantar M. Elevated Troponin: A Comprehensive Review. Mathews Journal of Cardiology. 2023 Dec 8;7(2):1-7. https://doi.org/10.30654/MJC.10031

[7] Hussein AA, Al-bayati AA, Issa AH. Evaluation of the diagnostic value and differentiation efficacy of high sensitivity cardiac troponin T2 (hscTnT2) for STEMI and NSTEMI Iraqi patients with acute coronary syndrome. Biomedicine. 2023 Jul 1;43(3):850-4. https://doi.org/10.51248/.v43i3.2507

[8] Halem EN, Ramadan A, Abo-El-Matty D. MicroRNA-499-5p and chemokine (CC motif) ligand18 as new diagnostic markers of Acute Myocardial Infarction. Records of Pharmaceutical and Biomedical Sciences. 2023 Jan 1;7(1):167-72. https://dx.doi.org/10.21608/rpbs.2023.219577.1237

[9] Abdel-Hamed AR, Nasser E, Abo-Elmatty DM, Nasr GM, Salem AS. Serum microRNA-499-5p Expression and Its Correlation with Chemokine (CC motif) Ligand 18 in Acute Myocardial Infarction. The Egyptian Journal of Hospital Medicine. 2023 Jan 1;90(2):2839-46. https://dx.doi.org/10.21608/ejhm.2023.287338

[10] Bobusoglu O, Balci S, Gundes A, Camsari A, Tamer L. Investigation into miRNA profile in patient groups with and without ST elevation. Turkish Journal of Biochemistry. 2023 Apr 25;48(2):203-8. http://dx.doi.org/10.1515/tjb-2021-0282

[11] Nappi F, Avtaar Singh SS, Jitendra V, Alzamil A, Schoell T. The roles of microRNAs in the cardiovascular system. International Journal of Molecular Sciences. 2023;24(18):14277. https://doi.org/10.3390/ijms241814277

[12] Rao X, Huang X, Zhou Z, Lin X. An improvement of the 2ˆ (–delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. Biostatistics, bioinformatics and biomathematics. 2013 Aug;3(3):71.

[13] Hajian-Tilaki K. Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. Caspian journal of internal medicine. 2013;4(2):627.

[14] Halem EN, Ramadan A, Abo-El-Matty D. MicroRNA-499-5p and chemokine (CC motif) ligand18 as new diagnostic markers of Acute Myocardial Infarction. Records of Pharmaceutical and Biomedical Sciences. 2023 Jan 1;7(1):167-72. https://dx.doi.org/10.21608/rpbs.2023.219577.1237

[15] Abdou DM, Aziz EE, Kazem YS, Elgabrty S, Taha HS. The diagnostic value of circulating microRNA-499 versus high-sensitivity cardiac troponin T in early diagnosis of ST segment elevation myocardial infarction. Comparative Clinical Pathology. 2021 Aug 1:1-6. https://doi.org/10.1007/s00580-021-03204-5

[16] Zhao Q, Yang W, Li X, Yuan H, Guo J, Wang Y, Shan Z. MicroRNA-499-5p inhibits transforming growth factor-β1-induced Smad2 signaling pathway and suppresses fibroblast proliferation and collagen synthesis in rat by targeting TGFβ-R1. Molecular Biology Reports. 2023;50(12):9757-67. https://doi.org/10.1007/s11033-023-08755-0

[17] Katsioupa M, Kourampi I, Oikonomou E, Tsigkou V, Theofilis P, Charalambous G, Marinos G, Gialamas I, Zisimos K, Anastasiou A, Katsianos E. Novel biomarkers and their role in the diagnosis and prognosis of acute coronary syndrome. Life. 2023 Sep 29;13(10):1992. https://doi.org/10.3390/life13101992

[18] Wereski R, Adamson P, Shek Daud NS, McDermott M, Taggart C, Bularga A, Kimenai DM, Lowry MT, Tuck C, Anand A, Lowe DJ. High-sensitivity cardiac troponin for risk assessment in patients with chronic coronary artery disease. Journal of the American College of Cardiology. 2023 Aug 8;82(6):473-85. https://doi.org/10.1016/j.jacc.2023.05.046

[19] Mu D, Ma C, Cheng J, Zou Y, Qiu L, Cheng X. Copeptin in fluid disorders and stress. Clinica Chimica Acta. 2022 1;529:46-60. https://doi.org/10.1016/j.cca.2022.02.002

[20] Gaber MA, Omar OH, El-Deek SE, Hassan AK, Mahmoud MS, Meki AR. Copeptin, mirna-208, and mirna-499 as new biomarkers for early detection of acute coronary syndrome. Applied biochemistry and biotechnology. 2022 Mar 1:1-3. https://doi.org/10.1007/s12010-021-03695-6

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Research Article

Salivary Statherin as a Dental Caries Biomarker among a Sample of Adolescents in Baghdad City

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ABSTRACT

Background: Dental caries is a multifaceted disease that impacts teenagers worldwide. A combination of factors, including fermentable carbohydrates, acid-producing bacteria, saliva, and host-related characteristics, influences its development. Salivary Statherin controls microorganisms by aggregating them and preventing them from adhering to hard tissue and epithelium. Salivary Statherin may maintain tooth integrity.
Objective: This research investigated the potential of salivary Statherin as a biomarker for dental caries.
Subjects and Methods: This comparative (observational) research included 90 adolescents of both sexes aged 15 years old. Participants were divided into two groups: 30 individuals without caries, serving as the control group, and 60 individuals with caries, referred to as the study group. Following the World Health Organization guidelines, adolescents with varying caries experiences were further categorized. Thirty exhibited a moderate level of caries, with 1 to 3 affected teeth, while the remaining thirty were classified as having severe caries, with a Decay-Missing-Filled Teeth (DMFT) score greater than 10. The DMFT Index measured caries experience—salivary Statherin analysis from unstimulated saliva. Height and weight were measured, and body mass index was calculated. classifying adolescents as normal weight, overweight, or obese.
Results: Statherin levels decreased with the severity of caries. There was a significant difference in the severe caries group, Statherin levels varies with different BMI group (P value ≤ 0.05).
Conclusions: Caries severity reduces salivary Statherin. In severe caries patients, Dietary status and dental caries severity affected Statherin levels. No association was found between Statherin levels and salivary pH or flow rate across different BMI group or caries severity groups. However, salivary Statherin may play a role in maintaining tooth integrity.

Introduction

Caries is a biofilm-mediated, sugar-driven, complex, dynamic illness distinguished by phasic demineralization and remineralization of tooth hard tissues (1,2).
Several variables can influence the progression of tooth decay. Caries develop when pathogenic bacteria and a substrate coexist on a vulnerable tooth surface. Bacteria transform the substrate into acid in this environment, leading to tooth decalcification (3-5).

Adolescence is a multi-system shift from childhood immaturity and social dependence to adulthood, with the objective and expectation of reaching one's developmental potential, establishing personal agency, and adopting societal responsibility (6). One-third of a person's total growth occurs throughout adolescence, which begins with the onset of puberty. At this age, cultural and individual variations are pronounced (7). Everyone is at risk for acquiring dental caries, but adolescents are at a higher risk. There is a correlation between the

sugar intake of adolescents and dental caries (8). Studies have shown that teens had a higher prevalence of dental caries than other age groups (9,10). However, the causes linked with dental caries, particularly among adolescents, are poorly understood. This investigation sought to investigate parameters linked with dental caries in connection to teenagers' nutritional status.

Diet and nutrition can influence the formation and integrity of the oral cavity, as well as the advancement of oral disorders (11-13). The relationship between body weight and dental caries remains a subject of debate (14). While a connection between obesity and dental decay has been established in adults, the association in adolescent populations is less clear. This study explored the link between these two significant factors in adolescent samples, with the aim of supporting the planning and promotion of adolescent healthcare.

Saliva is a viscous, aqueous, electrolyte- and protein-rich fluid that plays a role in regulating oral microbiota, protecting tooth enamel, and other oral tissue defenses. Salivary proteins serve as precursors for acquired enamel pellicles (AEP), playing a vital role in protecting oral surfaces. Given its noninvasive nature and ease of collection, saliva has garnered attention from researchers as a potential diagnostic tool. Additionally, saliva contains specific biomarkers that are related to health or disease (16). Plaque is eliminated by salivary proteins, which also delay demineralization, promote remineralization, neutralize acids, and prevent infection (17,18). Lysozyme, lactoperoxidase, immunoglobulins, lactoferrins, mucins, albumin, histatins, defensins, Statherin, and cathelicidin are saliva proteins that protect oral tissue (19).

Early pellicle proteins, proline-rich proteins, and Statherin may produce a protective layer for the oral cavity. By attracting calcium ions and delaying demineralization, pellicle proteins aid salivary calcium and phosphate ions in remineralizing enamel. Salivary (glyco) proteins inhibit the attachment and growth of oral bacteria to the enamel pellicle (20,21).

Rich in tyrosine and dephosphorylated, Statherin is an asymmetric salivary peptide with 43 residues that is released by the acinar cells of the parotid and submandibular salivary glands. The usual range of Statherin concentrations in human saliva is 10–40 μM. (22). Hydroxyapatite (HA) adsorption is the primary function mediated by the prime amino acid sequences, which helps with in situ enamel biomimetic remineralization (22). Therefore, Statherin is important for controlling enamel mineralization, and further study is necessary to determine whether it may be regarded as a powerful salivary indicator of dental cavities (22). Salivary Statherin remineralizes and captures calcium ions to protect tooth surfaces (23). The aggregation of microorganisms restricts bacterial and fungal colonization by limiting adhesion to hard tissue and epithelium (24).

No research has linked Statherin to teenage tooth decay. Therefore, Statherin cannot predict the risk of caries in adolescents without more studies. Along with offering personalized dental care, salivary proteins like Statherin hold potential for identifying associated risk factors, estimating the possibility of dental cavities, and facilitating dental screenings. This study is designed to explore the potential of Statherin as a biomarker for dental caries, particularly in relation to the BMI status of adolescents.

Subjects and Methods

This observational research compared both genders of adolescents aged 15 years recruited from the Teaching Hospital / University of Baghdad, College of Dentistry, and various governmental and private intermediate schools in varying Baghdad areas.

Biochemical analyses were carried out within the study's designated time frame. The University of Baghdad's College of Dentistry's ethical approval committee authorized it. (The ethics committee gave its approval to this study, Ref. number: 482, Date: 19-1-2022). Prior to data collection, legal permission was obtained from Ministry of Education. In order to get authorization to participate in this research, a distinct consent form was also created and sent to every participant. This research was conducted over the period from 12 Dec 2021 to 30 Apr 2022.

The software "G power 3.1.9.7" was created by Franz Faul, a professor at the University of Kiel in Germany, to determine the required sample size. With a big effect size of 0.40 across six groups and an alpha error of 0.05 for two-sided testing, the research showed that a sample of 82 individuals was required to reach a study power of 90%. The overall sample size increased to 90 people when a 10% margin of error was included. There were three categories for the effective size (F): small (0.1), medium (0.25), and big (0.4).

Following a thorough examination of 380 adolescents aged 15 from both genders, 90 individuals were selected and categorized into two groups based on the 2013 WHO regulations. Among them, 60 participants had a history of caries, forming the study group, while 30 had no caries experience, serving as the control. The adolescents with differing experiences of caries were further divided, with thirty having moderate carious teeth (Decay-Missing-Filled Teeth score of 1-3) and thirty exhibiting severe carious teeth (Decay-Missing-Filled Teeth score exceeding 10).

The participants were divided into three groups based on their BMI: those with Normal BMI, overweight, and Obese.

Qualification criteria:

- Adolescents who do not have systemic impacts on salivary secretion or local illnesses.
- Patients expressed their willingness to give consent for participation in the study.

Exclusion criteria:

- Adolescents suffering from systemic illnesses.
- Individuals on a restricted diet.
- Patients who received fluoride as their teeth were growing.
- An adolescent who has structural dental abnormalities and has started orthodontic treatment.

The dental health assessment involved diagnosing and reporting dental caries experience using the DMFT index, following the criteria established by WHO in 2013. A WHO probe, specifically the CPI "Community Periodontal Index" probe, along with a flat mouth mirror, was utilized for examining patients' teeth.

The assessment of Body Mass Index (BMI) for adolescents involves calculating a value based on weight and height, utilizing the formula:

$$BMI = \frac{weight\ (kg)}{height^2\ (m^2)}$$

This BMI measurement is represented in various charts that are specific to age and gender, known as CDC growth charts.

Sample collection will involve gathering morning saliva samples from each adolescent between 9 and 12 A.M. Unstimulated saliva will be collected under standardized conditions, adhering to the established guidelines (27). Salivary Statherin levels were determined using enzyme-linked immunosorbent assay (ELISA) kits for analysis and detection.

Salivary flow rate

The flow rate of salivary was calculated by dividing the collected saliva volume, measured in millilitres (ml), by the duration of the collection period, expressed in minutes. (28).

$$Salivary\ flow\ rate\ \left(\frac{mL}{min}\right) = \frac{Volume\ (mL)}{Time\ (minute)}$$

Salivary pH

A digital pH meter was used to measure the pH of resting saliva. The pH meter was calibrated in accordance with the manufacturer's guidelines.

Statistical analysis

One-way analysis of Variance (ANOVA) and the Games-Howell post hoc test were employed to describe, analyze, and present the data, utilizing the Statistical Package for Social Science (SPSS version 22, Chicago, Illinois, USA) for cases involving unequal variance.

Results

Table (1) shows that salivary Statherin is decreased with increasing caries severity. There is a significant result in normal BMI, obese groups, and the total sample among caries severity group while in overweight is not significant.

The level of salivary Sthatherin among BMI groups rises, and there is a significant difference only in the total sample and in the severe caries group among nutritional status. In contrast, in other caries groups, it is not significant.

Table (2) presents multiple pairwise comparisons of salivary statherin levels concerning caries and nutritional status, utilizing the GamesHowell post hoc test. The findings reveal a significant difference in salivary Statherin levels between individuals of normal weight and those who are obese. Additionally, a significant difference is noted between the groups with no caries and those with severe caries, while the remaining comparisons do not show significant results.

The results indicate that salivary flow rate (SFR) and salivary pH exhibit a weak negative correlation with salivary Statherin, without any significant relationships, except in the severe caries group, where a strong negative correlation is observed between salivary Statherin and SFR. In both the free and severe caries groups, salivary Statherin demonstrates a weak negative correlation with salivary pH, as illustrated in Table (3).

Additionally, findings indicate a weak negative and non-significant correlation between salivary Statherin, pH, and flow rate across various BMI groups. However, a weak but significant negative correlation with salivary pH was observed within the normal weight group. Similarly, in the obese group, salivary Statherin showed a weak significant negative correlation with salivary flow rate, as presented in Table (4).

Table 1: Descriptive analysis and statistical testing of Statherin levels across BMI status and caries severity.

Caries severity groups	BMI	Mean	±Standard Error	F	Pvalue
Free of caries group				1.324	0.283
	Normal BMI	27.054	1.612		
	Overweight	28.157	2.655		
	Obese	30.741	1.512		
Mild caries (1-3) group				1.211	0.314
	Normal BMI	25.114	1.388		
	Overweight	26.151	3.183		
	Obese	30.280	1.713		
Severe caries (Decay < 10) group				5.246*	0.010*
	Normal BMI	20.853	0.669		
	Overweight	22.867	0.567		
	Obese	25.251	0.302		
Total among BMI groups				8.224*	0.001*
	Normal BMI	23.749	0.741		
	Overweight	24.976	1.201		
	Obese	29.528	1.030		
Total among caries severity groups				15.327*	0.000002*
	Free of caries	28.5901	1.045		
	Mild caries	26.2517	1.200		
	Severe caries	21.8970	0.489		

BMI	Caries	F	Pvalue
Normal BMI	Free-mild-severe	8.063*	0.001*
Overweight		1.685	0.208
Obese		4.079	0.045*

* A p-value of less than or equal to 0.05 indicates a significant difference

Table 2: Statherin's Pairwise Comparisons by Caries and Nutritional Status (Games-Howell).

Caries severity category	Group	Mean difference	P value
Severe caries (Decay < 10) group	Normal weight X Overweight	-2.014	0.139
	Normal BMI X Obese	-4.398	0.018*
	Overweight X Obese	-2.383	0.379
Total among caries severity groups	Normal BMI X Overweight	-1.227	0.662
	Normal BMI X Obese	-5.779	0.000141*
	Overweight X Obese	-4.552	0.05102
BMI Categories	Groups	Mean difference	Pvalue
Total among BMI groups	Free X Mild	2.338	0.313
	Free X severe	6.693	0.00000*
	Mild X severe	4.355	0.052023
Normal BMI	Free X Mild	1.940	0.638
	Free X severe	6.201	0.006*
	Mild X severe	4.261	0.05121
Obese	Free X Mild	0.462	0.978
	Free X severe	5.491	0.012*
	MildXsevere	5.029	0.087

*P value <= 0.05 significant

Table 3: Correlation of Salivary pH, Flow Rate, and Statherin with Caries Severity Groups.

Groups of caries severity		Salivary pH		Salivary flow rate	
		relation	Pvalue	relation	P value
Free of caries group	Statherin	-0.363	0.049*	-0.215	0.183
Mild caries (1-3) group	Statherin	-0.235	0.211	-0.197	0.297
Severe caries (Decay < 10) group	Statherin	-0.400	0.011*	-0.390	0.033*

*P value <= 0.05 significant difference

Discussion

According to the current study, salivary Statherin levels decreased with increasing caries severity. There is a significant result in normal weight, obese groups, and the total sample among caries severity groups. Because Statherin helps build the acquired enamel pellicle, which may affect tooth bacteria colonization. Statherin's basic amino acids inhibit sugar metabolism by bacteria, affecting dental plaque (29). Statherin has beneficial antimicrobial effects. It appears to limit microbial colonization of the mouth and modulate salivary calcium phosphate chemistry. This effect, in turn, maintains salivary supersaturation and tooth mineralization. Dental caries is prevented by these actions (30). This finding was consistent with the findings of

Table 4: Correlation between salivary PH, salivary flow rate, and salivary Statherin in different BMI groups

BMI groups		Salivary PH		Salivary flow rate	
		relation	P value	relation	P value
Normal BMI	Statherin	-0.340	**0.011***	-0.236	0.083
Overweight	Statherin	-0.304	0.140	-0.041	0.845
Obese	Statherin	-0.070	0.770	-0.421	**0.036***

*P value <= 0.05 significant difference

Angarita-Daz et al. (31), who discovered that Statherin expression was significantly higher in saliva samples taken from healthy children than from children with moderate or severe caries (ICDAS > 3). Furthermore, a study conducted among preschool children in Iraq discovered that protein levels were higher in caries-free children than in caries-active children (32). Vitorino et al. (33) found a strong link between a child's high level of Statherin and their lack of tooth decay. This finding contrasts with the study of Preethi et al. (34), who discovered that children with caries had significantly higher salivary protein levels than children without caries. Ahmadi-Motamayel et al. (35) conducted a study among healthy 15- to 17-year-old students. They discovered higher levels of total protein but statistically insignificant differences between the caries-active and caries-free groups.

There is a correlation between the BMI and salivary sthatherin levels; however, no such correlation exists between the entire sample and the severe caries group; moreover, no such correlation exists between the other caries groups and nutritional status. This could be attributed to the salivary Statherin's defense against the free diffusion of highly concentrated acids on tooth surfaces (36) due to frequent snacking, sugary drinks, and foods, and snacking between meals can cause dental caries and obesity (37). These findings are consistent with an Iraqi study, which found that obese children had higher amounts of total salivary protein than their normal-weighted counterparts (38). However, this finding was supported by another study by Acevedo, which found that overweight and obese children, compared with the control group, had higher protein content (39). According to the literature, there are no previous studies concerning especially salivary Statherin and BMI.

The data reveals a significant weak negative correlation between salivary Statherin and pH across the caries severity groups. Additionally, in the severe caries group, a strong significant negative correlation exists between salivary Statherin and salivary flow rate (SFR). A decrease in salivary flow rate and pH is observed as both nutritional status and caries severity worsen. Consistent with these findings, research by Bakkal and Kargul identified a weak correlation between unstimulated whole saliva pH and caries activity (40). In both groups, an increase in salivary flow rate was observed, while protein concentration showed a decrease. It seems that the concentration of proteins is inversely related to the salivary flow rate, which makes sense, given that proteins in saliva are diluted.

Conversely, a lower pH of salivary was associated with increased total protein concentrations. These results align with the findings of Vitorino et al., who reported higher total concentrations of protein in the caries-active groups (41). It is possible that lower anti-proteolytic

mechanisms or enhanced proteolytic activity are connected with the greater protein levels seen in the caries-active group. Therefore, it might be interpreted as a protective response when children with caries have saliva with a lower pH and a higher protein content. Additionally, there is an inverse relationship between protein content and salivary flow rate. These observations align with Bhalla et al.'s assertion that an increase in salivary flow rates leads to the dilution of salivary proteins (42).

The study's limitations include the fact that dental caries were evaluated using only visual and tactile responses and no radiographic data. Consequently, the prevalence of dental caries was likely underestimated. The study found it difficult to establish a connection between frequent sugar consumption and dental caries. This may have been due to the study's cross-sectional design; the association could have been demonstrated through a case-control or cohort study.

Conclusion

Adolescents are affected by dental caries. The current research concluded that salivary Statherin declines with caries severity due to its protective function in contradiction to the free diffusion of highly concentrated acids on the tooth surface. It is hypothesized that salivary Statherin may contribute to protecting the teeth from decay caused by acidic bacteria secretions on the surface of the enamel. A weak negative correlation, which is not statistically significant, is present between salivary Statherin and both pH of salivary and salivary flow rate within the different caries severity groups.

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Conflict of Interest

Authors declare no conflict of interest.

Data availability

Data are available upon reasonable request

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References

[1] Al-Moosawi RI, Qasim AA. The impact of dental environment stress on dentition status, salivary nitric oxide and flow rate. Journal of International Society of Preventive and Community Dentistry. 2020 Mar 1;10(2):163-70. https://doi.org/10.4103/jispcd.JISPCD_427_19

[2] Jaddoa MF, Qasim AA. Assessment of the Correlation between the Salivary Flow Rate and Dental Caries Experience among Children with?-Thalassemia Major. Indian Journal of Forensic Medicine & Toxicology. 2020 Jan 16;14(1):1122-7. https://doi.org/10.37506/v14/i1/2020/ijfmt/193058

[3] Flayyih AS, Hassani HH, Wali MH. Identification of Streptococcus mutans from human dental plaque and dental caries using 16srrna gene. Iraqi Journal of Science. 2016:552-7.

[4] Ismail MM, Al Haidar AH. Evaluation of the efficacy of caries removal using papain gel (Brix 3000) and smart preparation bur (in vivo comparative study). Journal of Pharmaceutical Sciences and Research. 2019 Feb 1;11(2):444-9.

[5] Guracho TT, Atomssa EM, Megersa OA, Tolossa T. Determinants of dental caries among adolescent patients attending Hospitals in West Wollega Zone, Western Ethiopia: A case-control study. PLoS One. 2021 Dec 2;16(12):e0260427. https://doi.org/10.1371/journal.pone.0260427

[6] Al-Khayoun JD, Diab BS. Dental caries, Mutans Streptococci, Lactobacilli and salivary status of type1 diabetic mellitus patients aged 18-22 years in relation to Glycated Haemoglobin. Journal of Baghdad College of Dentistry. 2013 Mar;325(2204):1-6.

[7] Mohammed AI, Diab BS. Caries experience and salivary physicochemical characteristics among overweight intermediate school females aged 13-15 years in Babylon–Iraq. Journal of Baghdad College of Dentistry. 2013;25(3):130-3.

[8] World Health Organization. Sugars and dental caries. World Health Organization; 2017.

[9] Kazeminia M, Abdi A, Shohaimi S, Jalali R, Vaisi-Raygani A, Salari N, Mohammadi M. Dental caries in primary and permanent teeth in children's worldwide, 1995 to 2019: a systematic review and meta-analysis. Head & face medicine. 2020 Dec;16:1-21. https://doi.org/10.1186/s13005-020-00237-z

[10] Moca AE, Vaida LL, Negruțiu BM, Moca RT, Todor BI. The influence of age on the development of dental caries in children. A radiographic study. Journal of Clinical Medicine. 2021 Apr 15;10(8):1702. https://doi.org/10.3390/jcm10081702

[11] Diab BS, El Samarrai SK, Al-Alousi WS, Al-Radha AS. PS24 Nutritional Status in Relation to Caries Experience Among Primary School Iraqi Children in the Middle Region of Iraq. J Epidemiol Community Health. 2012 Sep 1;66(Suppl 1):A47-8. https://doi.org/10.1136/jech-2012-201753.123

[12] Al-Talqani JM, Al Haidar AH. Association of Enamel Defects with Nutritional Status among Primary Schools Students in Al-Najaf City. International Medical Journal. 2021 Jun 2;28.

[13] Moustafa MM, Al-Janabi RD. Association of age, parity and body mass index with hemoglobin and serum ferritin levels in pregnant women in Baghdad city. Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512). 2021 Dec 11;30(2):153-7. https://doi.org/10.31351/vol30iss2pp153-157

[14] Alasadi ZA, Qasim AA. Impact of fixed orthodontic therapy on salivary characteristics in relation to weight status. Biomedical and Pharmacology Journal. 2018 Sep 21;11(3):1463-70. https://dx.doi.org/10.13005/bpj/1512

[15] Gao X, Jiang S, Koh D, Hsu CY. Salivary biomarkers for dental caries. Periodontology 2000. 2016 Feb;70(1):128-41. https://doi.org/10.1111/prd.12100

[16] Javaid MA, Ahmed AS, Durand R, Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. Journal of oral biology and craniofacial research. 2016 Jan 1;6(1):67-76. https://doi.org/10.1016/j.jobcr.2015.08.006

[17] Pedersen AM, Belstrøm D. The role of natural salivary defences in maintaining a healthy oral microbiota. J Dent. 2019;80:S3-12. https://doi.org/10.1016/j.jdent.2018.08.010

[18] Piekoszewska-Ziętek P, Turska-Szybka A, Olczak-Kowalczyk D. Salivary proteins and peptides in the aetiology of caries in children: Systematic literature review. Oral Diseases. 2019 May;25(4):1048-56. https://doi.org/10.1111/odi.12953

[19] Silveira EG, Prato LS, Pilati SF, Arthur RA. Comparison of oral cavity protein abundance among caries-free and caries-affected individuals—a systematic review and meta-analysis. Frontiers in Oral Health. 2023 Sep 15;4:1265817. https://doi.org/10.3389/froh.2023.1265817

[20] Al-Ali GM, Jafar ZJ, AL-Ghurabi BH. The relation of salivary cathelicidin and beta-defensin with dental caries of schoolchildren. J Res Med Dent Sci. 2021 Apr;9(4):30-5.

[21] Wang CC, Alderman B, Wu CH, Chi L, Chen SR, Chu IH, Chang YK. Effects of acute aerobic and resistance exercise on cognitive function and salivary cortisol responses. Journal of Sport and Exercise Psychology. 2019 Apr 1;41(2):73-81. https://doi.org/10.1123/jsep.2018-0244

[22] Pateel DG, Gunjal S, Dutta S. Association of Salivary Statherin, Calcium, and Proline-Rich proteins: a potential predictive marker of Dental Caries. Contemporary clinical dentistry. 2022 Jan 1;13(1):84-9. https://doi.org/10.4103/ccd.ccd_859_20

[23] Chen W, Jiang Q, Yan G, Yang D. The oral microbiome and salivary proteins influence caries in children aged 6 to 8 years. BMC Oral Health. 2020 Dec;20:1-6. https://doi.org/10.1186/s12903-020-01262-9

[24] Lv X, Yang Y, Han S, Li D, Tu H, Li W, Zhou X, Zhang L. Potential of an amelogenin based peptide in promoting reminerlization of initial enamel caries. Archives of oral biology. 2015 Oct 1;60(10):1482-7. https://doi.org/10.1016/j.archoralbio.2015.07.010

[25] Petersen, Poul Erik, Baez, Ramon J & World Health Organization. (2013). Oral health surveys: basic methods, 5th ed. World Health Organization. https://iris.who.int/handle/10665/97035

[26] World Health Organization. (2000). The World health report : 2000 : health systems : improving performance. World Health Organization. https://iris.who.int/handle/10665/42281

[27] Navazesh M, Kumar SK. Measuring salivary flow: challenges and opportunities. The Journal of the American Dental Association. 2008 May 1;139:35S-40S. https://doi.org/10.14219/jada.archive.2008.0353

[28] de Carvalho FK, de Queiroz AM, da Silva RA, Sawamura R, Bachmann L, da Silva LA, Nelson-Filho P. Oral aspects in celiac disease children: clinical and dental enamel chemical evaluation. Oral surgery, oral medicine, oral pathology and oral radiology. 2015 Jun 1;119(6):636-43. https://doi.org/10.1016/j.oooo.2015.02.483

[29] Chawhuaveang DD, Yu OY, Yin IX, Lam WY, Mei ML, Chu CH. Acquired salivary pellicle and oral diseases: A literature review. Journal of Dental Sciences. 2021 Jan 1;16(1):523-9. https://doi.org/10.1016/j.jds.2020.10.007

[30] Pateel DG, Gunjal S, Dutta S. Association of Salivary Statherin, Calcium, and Proline-Rich proteins: a potential predictive marker of Dental Caries. Contemporary clinical dentistry. 2022 Jan 1;13(1):84-9. 10.4103/ccd.ccd_859_20. https://doi.org/10.4103/ccd.ccd_859_20

[31] Angarita-Díaz MP, Simon-Soro A, Forero D, Balcázar F, Sarmiento L, Romero E, Mira A. Evaluation of possible biomarkers for caries risk in children 6 to 12 years of age. Journal of Oral Microbiology. 2021 Jan 1;13(1):1956219. https://doi.org/10.1080/20002297.2021.1956219

[32] Kadoum NA, Salih BA. Selected salivary constituents, physical properties and nutritional status in relation to dental caries among 4-5 yearâ€™ s old children (Comparative study). Journal of Baghdad College of Dentistry. 2014;26(2):150-6.

[33] Vitorino R, Lobo MJ, Duarte JR, Ferrer-Correia AJ, Domingues PM, Amado FM. The role of salivary peptides in dental caries. Biomedical Chromatography. 2005 Apr;19(3):214-22. https://doi.org/10.1002/bmc.438

[34] Preethi BP, Reshma D, Anand P. Evaluation of flow rate, pH, buffering capacity, calcium, total proteins and total antioxidant capacity levels of saliva in caries free and caries active children: an in vivo study. Indian Journal of Clinical Biochemistry. 2010 Oct;25(4):425-8. https://doi.org/10.1007/s12291-010-0062-6

[35] Ahmadi-Motamayel F, Goodarzi MT, Hendi SS, Abdolsamadi H, Rafieian N. Evaluation of salivary flow rate, pH, buffering capacity, calcium and total protein levels in caries free and caries active adolescence. J Dent Oral Hyg. 2013 Apr 30;5(4):35-9. https://doi.org/10.5897/JDOH12.011

[36] Taira EA, Ventura TM, Cassiano LP, Silva CM, Martini T, Leite AL, Rios D, Magalhães AC, Buzalaf MA. Changes in the proteomic profile of acquired enamel pellicles as a function of their time of formation and hydrochloric acid exposure. Caries research. 2018 Mar 6;52(5):367-77. https://doi.org/10.1159/000486969

[37] Aziz AR, Mohammed AT. The salivary inflammatory biomarkers (Interleukin-6, C-reactive protein) in relation with caries-experience among a group of 12 year old obese boys. Journal of Baghdad College of Dentistry. 2016;28(1):138-42. https://doi.org/10.12816/0024723

[38] YakubMajid A, Diab BS, Alsaadi AA. Caries experience and salivary constituents among overweight children aged 6-11 years in Baghdad, Iraq. Journal of baghdad college of dentistry. 2010;22(2).

[39] Acevedo AC. Saliva and oral health. Rev Assoc Med Bras (1992). 2010;56(1):2. https://doi.org/10.1590/S0104-42302010000100001

[40] Bakkal M, Kargul B. The differential electrophoretic patterns of Statherin and histatins in caries-active and caries-free children. Int J Appl Dent Sci. 2018;4(3):365-70. https://doi.org/10.22271/oral.2018.v4.i3f.05

[41] Vitorino R, de Morais Guedes S, Ferreira R, Lobo MJ, Duarte J, Ferrer-Correia AJ, Tomer KB, Domingues PM, Amado FM. Two-dimensional electrophoresis study of in vitro pellicle formation and dental caries susceptibility. European journal of oral sciences. 2006 Apr;114(2):147-53. https://doi.org/10.1111/j.1600-0722.2006.00328.x

[42] Bhalla S, Tandon S, Satyamoorthy K. Salivary proteins and early childhood caries: A gel electrophoretic analysis. Contemporary clinical dentistry. 2010 Jan 1;1(1):17-22. https://doi.org/10.4103/0976-237X.62515

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Research Article

Prevalence and characteristics of the Solitary Rectal Ulcer Syndrome among a Cohort of Iraqi Patients with Lower Gastrointestinal Bleeding

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ABSTRACT

Background: Solitary rectal ulcer syndrome (SRUS) is a benign, uncommon rectal disorder characterized by combination of symptoms, clinical findings, and histological abnormalities. It has wide range of presentations and variable endoscopic findings.
***Objectives*:** to study the prevalence of SRUS in patients presenting with lower gastrointestinal bleeding and to further evaluate this syndrome in the affected patients.
Subjects and Methods: A retro-prospective descriptive study conducted in Basrah Gastroenterology and Hepatology Hospital involved revision of 350 colonoscopic reports performed for the period from January 2022–June 2023 for patients presented with bleeding per rectum. Fifteen patients fulfilled the diagnostic criteria of SRUS were reviewed and followed up.
Results: the prevalence rate was 4.28%; nine male (60%), seven female (40%), mean age (22 ±9.3) years (12-45years). Mean duration of symptoms until diagnosis (8.22 ±4) weeks (3-17) weeks, females had shorter diagnosis time compared to males (5.5±2.3) (8.2±5.3) weeks respectively. The most frequent single associated symptom with the bleeding per rectum was constipation (96.3%), 20% had multiple associated symptoms.
On endoscopic examination:11 (73.4%) had ulcerated lesions,4 (26.6%) non ulcer lesions [3(20%) had polypoidal lesion,1(6.6%) had only hyperemic mucosa] Majority of rectal lesions located anteriorly 13 (86.6%). At follow up; symptoms improved in 7 (46.6%), endoscopic improvement in 8 (53.3%) and histological improvement in 11(73.4%), 2(13.3%) underwent endoscopic treatment and 3(20%) ended with surgery.
Conclusions: SRUS is chronic, benign disorder related to straining or abnormal defecation. It has variable clinical presentations and variable endoscopic findings rather than solitary ulcer as the name imply with different therapeutic options are available.

Introduction

Solitary rectal ulcer syndrome (SRUS) is an uncommon type of rectal disease that is defined by a mix of symptoms, clinical findings, and histological abnormalities. Patients may exhibit lower gastrointestinal hemorrhage, mucous passing, straining during defecation, and a feeling of incomplete evacuation (1).

The name of the syndrome is misleading, since patients can often present with lesions that are neither solitary nor ulcerated. The lesions are located in the anterior rectal wall within 10 cm of the anal verge in the majority of patients (2).

Endoscopic and radiologic findings can vary and include mucosal ulcerations, polypoidal or mass-like lesions that mimic rectal cancer, or just hyperemic mucosa (3). Because of this, misinterpretation is

frequent; in one study, up to 26% of patients had received an inaccurate initial diagnosis, which was most frequently a nonspecific ulcer, inflammatory bowel disease, or adenomatous changes (4).

Symptoms are variable or may be absent. In an interpretive series, the most common symptoms were rectal bleeding (56%), straining (28%), and pelvic fullness (23%) while mucous discharge, incontinence, tenesmus and pain were less frequently described (5).

The pathogenesis of the solitary rectal ulcer is incompletely understood, however; a number of factors appeared to have a causative role. It is possible that different etiologies may contribute to the development of the final lesion.

A common observation in a number of reports is rectal prolapse and paradoxical contraction of the puborectalis muscle, which can result in rectal trauma by two different mechanisms (6):

1. The pressures produced by the rectum during defecation force the prolapsed rectal mucosa downward. The opposing force of the puborectalis muscle's paradoxical contraction can create high pressures inside the rectum and cause mucosal ischemia, which makes ulceration more likely.

2. The contraction of the puborectalis muscle results in shear stresses on the rectal mucosa.

However, not all SRUS patients have excessive puborectalis contraction. Additionally, it is unclear whether prolapse leads to ulceration or if they are both different symptoms of the same disease process. Research in which surgical repair of rectal prolapse had no appreciable effect on patients' symptoms supports the concept that prolapse is an associated condition rather than a causal one (7).

In comparison to control groups, patients with solitary rectal ulcer syndrome more commonly experienced paradoxical puborectalis contraction, prolapse of the inner circular smooth muscle of the rectum, and rising anal pressure at strain. They also experienced less complete rectal emptying. Mean resting and compressing anal pressures were substantially higher in these patients compared to those with overt rectal prolapse (6). Direct digital trauma has also been implicated, since many patients have a history of constipation and report attempts at manual disimpaction. However, a number of lesions have been described that were beyond the reach of an inserted finger (8).

A possible hormonal cause has also been proposed. A case report documented a woman with solitary rectal ulcer syndrome that resolved during two pregnancies but recurred when she was not pregnant (9).

Histologically, the pathognomonic triad of fibrous obliteration of lamina propria, Disorientation of muscularis mucosa, extension of muscle fiber into the lamina propria must be present to confirm the diagnosis (10).

Various treatment strategies have been advocated, ranging from conservative management, medical therapy with sucralfate enema, sulfasalazine enema or simply with xylocaine gel, endoscopic therapy with argon plasma coagulation (APC) to a variety of surgical procedures, but the optimal treatment for the condition remained unclear (5).

This study aims to study the prevalence of the solitary rectal ulcer syndrome in patients presenting with bleeding per rectum and referred to the endoscopy unit in this hospital for sigmoidoscopic/ colonoscopic examinations and to study the characteristics findings of this syndrome in the affected patients.

Subjects and Methods

This study was a retro-prospective descriptive, single center study conducted in the Basrah Gastroenterology and Hepatology hospital /southern Iraq. In this study lower endoscopic reports for patients presented with lower gastrointestinal bleeding had been reviewed.

During the period of the study 1578 lower endoscopy (sigmoidoscopy, colonoscopy) had been performed in this hospital , from this number , 350 lower endoscopy had been performed for complain of bleeding per rectum , the diagnosis of solitary rectal ulcer syndrome was in 15 patients out of those 350 patients that complain from bleeding per rectum , those 15 patients were enrolled in this study , full history had been taken, full physical examination had been done , type of treatment offered for them had been reviewed with follow up response of six months for each patient had been considered.

The detailed history taken from the patients included: duration of symptoms before the diagnosis, disorder of defecation (constipation, diarrhea, straining at defecation, digital evacuation, incontinence), perianal and abdominal pain, rectal bleeding, mucus discharge, rectal prolapse, weight loss, use of medications, tenesmus, and other points in the history include backache, skin rash and mouth ulcer.

All patients were submitted to abdominal and rectal examination looking for evidence of blood or prolapse.

After explaining and taking informed written consent from the patient for lower endoscopy and to enrolled in the study, Sigmoidoscopic or total colonoscopic examination were performed using (variable stiffness EC760R-V/I, Fujifilm, Tokyo, Japan) endoscope. The examination was performed under conscious sedation or under deep sedation for paediatric patients (using: propofol with midazolam or fentanyl) and under the supervision of an anesthetist specialist.

These endoscopic procedures were carried out after full bowel preparation by use of the preparation protocol adopted in our endoscopy unit (using split doses of osmotic laxatives with Coloclean (polyethylene glycol) sachets, dietary advice and sometimes with use of add on stimulant laxative (bisacodyl tablets) and normal saline enemas for presumed difficult preparation individuals.

These endoscopic procedures were carried out by different endoscopists at the endoscopy unit of this hospital. During the endoscopic procedure, multiple biopsies (4-6-4) were taken from the edge of lesion, center of the lesion and from the surrounding mucosa respectively and put in three different diluted formalin test tubes, the tubes were labeled for patient's name and site of biopsy taken before subjected to preparation and final examination ,these samples were prepared, stained with eosin and hematoxylin staining ,then were studied and reviewed by one and sometimes by more than one expert gastrointestinal pathologists .

The diagnosis of SRUS was established according to the histological criteria which described by Madigan and Morison [10], which necessitate the presence of the following three findings:

1- Fibrous obliteration of lamina propria.
2- Disorientation of muscularis mucosa.

3- Extension of muscle fibers into the lamina propria.

In addition, all involved patients had general stool examination, biochemical and hematological investigations.

Regarding the treatment of the affected individuals, patients with constipation were advised to avoid straining at defecation, avoid the habit of rectal digitation and they were advised to take a high roughage diet or fiber supplementation.

All patients were subjected to different medications that are appropriate according to the recommended guidelines and include: Sucralfate enema (2gm/day for 6 weeks), Sulfasalazine enema (1-2gm/day for 3-6 months) and/or xylocaine gel.

The clinical, endoscopic and histological state was assessed at time of presentation and during follow up at six months, results were graded as no symptoms ,partially improved ,unchanged or worse according to the patients' assessment of symptoms.

All the fifteen patients continued and completed the follow up period, so complete medical records and follow up data were available for the fifteen patients.

As a Statistical analysis, prevalence rate had been calculated for the studied condition. The studied variables were arranged as numbers and percentages for categorical data while numerical data were represented as mean and standard deviation.

Independent samples t-test was used to compare the means of two samples, with the P-value of < 0.05 was the criterion of statistical significance.

The data were coded and analysed using the Statistical Package for the Social Sciences (SPSS) version 26.

Results

The prevalence rate of the SRUS among patients attending the Basrah Gastroenterology and Hepatology hospital and complained of bleeding per rectum was 4.28% .15 (4.28%) patients out of 350 patients presented with bleeding per rectum and underwent lower endoscopy in this hospital had fulfilled the diagnosis of SRUS. 9(60%) were male and 6(40%) were female (1.5:1 ratio). The mean age at the time of presentation was (22 ±9.3) years, with a range from (12-45years).

The mean duration of symptoms until confirmed the diagnosis in our hospital was (8.22 ±4) weeks, ranging from (3-17) weeks, with the female patients diagnosed earlier than male with the mean duration of (5.5±2.3) weeks and (8.2±5.3) weeks respectively and this was statistically significant (P value= 0.03). Table 1.

Despite all the patients had bleeding per rectum (100%) , but this study also categorized patients according to the associated symptoms with the bleeding per rectum and was as the following : associated constipation 14 (93.3%), associated mucous discharge 13 (86.6%), associated straining at defecation 12 (80%), associated anorectal pain 11 (73.3), associated tenesmus 9 (60 %), associated digital evacuation 7 (46.6%), associated altered bowel habits 5 (33.3%),%),associated lower abdominal pain 4 (26.6%) , associated diarrhea 3 (20%) and associated rectal prolapse 2 (13.3 %) , this study also demonstrated that 12 patients(80%) had multiple associated symptoms in addition to the bleeding per rectum while only 3 patients (20%) had only one associated symptom . Table2.

Table 1: Study characteristics of the patients

Number of Patients(n.)	15
Prevalence rate	4.28%
Age (year)	
Mean ± SD	22±9.3
Range	12-45
Sex (n., %)	
Male	9(60%)
Female	6(40%)
Male to Female ratio	1.5:1
Duration of symptoms (weeks)	
Mean ± SD	8.22±4
Range	3-17
Sex difference in duration of symptoms(weeks)	8.2±5.3
Male (Mean ± SD)	5.5±2.3
Female (Mean ± SD)	0.03
p-value	

Table2: Study of the presenting symptoms

Symptoms	**Number (n.)**	**Percentage (%)**
Bleeding per rectum	15	100%
Constipation	14	93.3%
Mucous discharge	13	86.6%
Straining at defecation	12	80%
Anorectal pain	11	73.3%
Tenesmus	9	60%
Normal bowel habit	8	53.3
Digital evacuation	7	46.6%
Altered bowel habits	5	33.3
Abdominal pain	4	26.6%
Diarrhea	3	20%
Rectal prolapse	2	13.3%
Multiple associated symptoms	12	80%
Single associated symptom	3	20%

From the 15 patients, 7(46.6%) patients underwent imaging study before underwent lower endoscopy in form of dynamic computed tomography (CT scan) of the abdomen and the results interpreted as: suspicious for carcinoma in 4 patients, ulcerative colitis in one patient and nonspecific finding in the remaining 2 patients. While the hematological investigations of the involved patients revealed 7 (46.6 %) patients had iron deficiency anemia with haemoglobin less than 10 g/dl and serum ferritin less than 30 ng/ml, 4(26.6%) patients required oral iron therapy, 2(13.3%) patients required parenteral iron and only 1 (6.6%) patient required blood transfusion. A general stool examination had been performed for all fifteen patients: 1 (6.6%) patient had an Entamoeba histolytic trophozoite, 3 (20%) had an Entamoeba histolytic cyst only and 11(73.4%) had normal general stool examination. Table3.

Table 3: Study investigations of the patients

Number of patients (n.)	15
Imaging study (n., %):	7(46.6%)
Suspicious of carcinoma (n., %)	4(26.6%)
Ulcerative colitis like (n., %)	1(6.6%)
Nonspecific findings (n., %)	2(13.4%)
Iron deficiency anemia (n., %):	7(46.6%)
Received oral iron therapy (n., %)	4(26.6%)
Received parenteral iron (n., %)	2(13.4%)
Received blood transfusion (n., %)	1(6.6%)
General stool examination (n., %):	15(100%)
Normal (n., %)	11(73.4%)
Entamoeba histolytica trophozoite (n., %)	1(6.6%)
Entamoeba histolytica cyst (n., %)	3(20%)

Endoscopic examination demonstrated that 11 (73.4 %) of patients had ulcerated lesions (range from one large ulcer to multiple small ulcers), the remaining 4(26.6%) patients had non ulcer lesions {3(20%) had polypoidal lesion and 1 (6.6%) patient had just erythematous mucosa}.

The majority of the rectal lesions were located anteriorly 12/15(80 %) , circumferential lesion in 2(13.4%)patients ,while only 1(6.6%) patient had posteriorly located lesion.

The median distance of the rectal lesion was (7.6±1.78 SD) cm from anal verge (range 6-12 cm). During endoscopic examination, 12 (80%) of the examined patients had prolapse of anterior rectal wall. Tabl e4

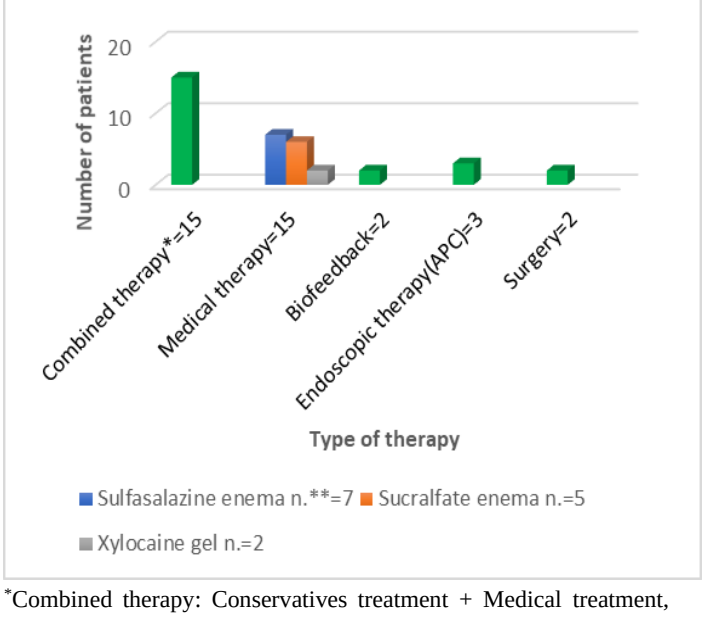
Table 4 :Endoscopic findings of the patients

Endoscopic finding	**Number(n.)**	**Percentage(%)**
Ulcerated lesion	11	73.4%
Polypoidal lesion	3	20%
Hyperemic mucosa	1	6.6%
One ulcer	6	54.5%
Multiple ulcers	5	45.5%
Distance of lesion from anal verge:		
Mean±SD (cm)	7.6±1.78	
Range (cm)	6-12	
Location of rectal wall ulcer:		
Anterior	12	80%
Circumferential	2	13.4%
Posterior	1	6.6%
Anterior rectal wall prolapses	12	80%
Other colonoscopic finding:		
Hemorrhoids	3	20%
Sigmoid diverticulosis	1	6.6%
Fissure in Ano	1	6.6%

According to the histological abnormalities, this study showed that all the patients 15/15(100%) had fibrous obliteration of lamina propria ,hypertrophy of muscularis mucosa and regenerative changes in crypts, while associated granulation tissue were seen in 12 (80%) of the affected patients and 9 (60%) showed associated ulcerations and /or erosions, cystic changes of mucous glands in 3(20%) patients and 4(26.6%) showed neutrophilic infiltration. Table5

Table 5: Histological findings of the patients

Histological finding	**Number(n.)**	**Percentage (%)**
Fibrous obliteration of lamina propria	15	100%
Hypertrophy of muscularis mucosa	15	100%
Regenerative crypts changes	15	100%
Granulation tissue	12	80%
Ulcerations and/or erosions	9	60%
Cystic changes of mucous glands	3	20%
Neutrophilic infiltration	4	26.6%



*Combined therapy: Conservatives treatment + Medical treatment, n.**=Number of patients

Figure1: Study of the patients according to the type of therapy

According to the modalities of therapies offered for the affected patients, this study demonstrated that all the 15(100%) patients were subjected to combined treatment modalities at time of confirmed diagnosis that consist of conservative treatment (i.e. reassurance of the patient that the lesion is benign, encouragement of a high-fiber diet, avoidance of straining, regulation of toilet habits, and attempt to

discuss any psychosocial factors, diet and bulking agents) combined with medical therapy in form of Sulfasalazine enema (1-2gm/day for 3-6 months) used by 7(46.6%) patients ,sucralfate enema (2gm/day for 6weeks) used by 6 (40%) patients and /or xylocaine gel used by 2 (13.4%) patients. 2(13.3%) patients underwent biofeedback therapy, 3(20%) underwent endoscopic therapy in form of Argon Plasma Coagulation (APC) while only 2(13.3) patients underwent surgery in form of rectopexy. Figure1

The average follows up period for the studied patients with SRUS in this study were about 6 months duration and accordingly this study showed that 7 (46.6) patients demonstrated improved symptoms, disappearance of symptoms in 5 (33.4%) while persist in 3(20%).

At the end of the follow up period all the studied patients subjected to sigmoidoscopic examination and biopsy were taken. The endoscopic examination showed that endoscopic healing of the lesion was documented in 5 (33.3%) patients, improved in 8(53.3%) while persisted in 2 (13.4%) patients, while the histopathological results showed that were no changes in histological examination in 2 (13.3%), improved in 11 (73.4%) while disappearance of changes (normal histopathology) in 2 (13.3%) patients. Table6

Table 6: Follow up study of the patients

Follow up (6 months)	Number(n.)	Percentage (%)
Symptom:		
Disappear of symptoms	5	33.4%
Improving of symptoms	7	46.6%
Persistence of symptoms	3	20%
Sigmoidoscopy:		
Disappear of lesion	5	33.4%
Improvement of lesion	8	53.3%
No change in endoscopy	2	13.3%
Histopathology:		
Normal histopathology	2	13.3%
Improving histopathology	11	73.4%
No change in histopathology	2	13.3%

Discussion

Although there is no so much studies to compare the prevalence of the SRUS with it, the Morio O et al showed that the incidence of SRUS is 1/100000/year(11), this study showed increase in the prevalence of the SRUS as this is a single center study , this can be explained by that the endoscopies were done in specialized tertiary hospital and done by expert endoscopists that had increased awareness to this syndrome, and the specimens had been examined by expert pathologists with high awareness to this uncommon syndrome.

There was male predominance of this syndrome in this study (male: female ratio is 1.5:1) , this is similar to a study done by Abusharifah O et al , that showed male predominance for this syndrome(12) , although AlGhulayqah AI et al showed female predominance(13) , while Forootan M, Darvishi M reported in their study equal prevalence in men and women (14).

This study showed that the mean age of the affected patients was (22 ±9.3) years, with range from (12-45years) , this is younger than the age presentation in other studies like that done by Abid S et al in Pakistan and Behera MK et al in India (15,16) , and this can be explained by the increase awareness to this syndrome in our hospital.

Regarding the mean duration for the diagnosis of SRUS in Basrah Gastroenterology and Hepatology(from the onset of symptoms and presentation to the diagnosis in this hospital), this study demonstrated that the mean duration was (8.22 ±4) weeks, ranging from (3-17)weeks and this is shorter than the mean duration in Zubair E et al, that show that the mean duration was (11.5 ± 4.3) weeks with a range from (1-23 weeks) (5) , and this is explained by the adoption of the strategy in our hospital that bleeding per rectum is considered an urgent indication for endoscopy that result in short waiting list in addition to the increase the experience to this syndrome among endoscopists , radiologists and pathologists in this hospital.

The female patients in this study diagnosed earlier than the male, this can be explained by the attitude of the patients in our country; in that women usually seek medical help earlier than the men as with other diseases. Although no study elsewhere is available to compare this result with it.

In this study the association of bleeding per rectum , constipation and mucous discharge were the most common associated presented symptoms , this is similar to the finding by Urganci N et al (17) , while Dehghani SM et al showed that than combination of bleeding per rectum, straining during defecation or forceful defecation and Sense of incomplete evacuation(98.2%, 90.9% and 61.8%)respectively were the most common presenting symptoms(18) ,and this can be explained by the horror of bleeding per rectum for the patient and the neglect of the other associated symptoms in presence of bleeding per rectum.

This study showed that the imaging study in case of SRUS can be confusing as what demonstrated by the finding of suspicious rectal carcinoma, ulcerative colitis and nonspecific finding , similar to that; Bhusal U founded SRUS mimicking rectal tumor on CT scan(19),Powell CR et al founded SRUS mimicking Perianal Crohn's Disease(20) , this is can be explained by the characteristic similarity of the SRUS with the neoplasm and the inflammatory bowel disease on imaging study and the difficulty of differentiation between them based on imaging study only.

Regarding the laboratory investigation , this study show that anemia is a relatively common finding in the affected patients(46.6%) and this can be explained by the ongoing blood loss that sometimes can be massive and by the relatively long duration till reaching the diagnosis , Abusharifah O et al showed similar results in their study that showed iron deficiency anemia in (42.1%) of the affected patients(12).

In this study most of the studied patients had ulcerated lesions ranging from one to multiple ulcers of varying sizes , three patients had polypoidal like lesion and only one patient had hyperemic mucosal lesion , these results similar to the results demonstrated in other studied like that done by Behera MK et al in India and showed that ulcerative lesions were seen in 83% of the patients , Polypoidal

lesions in 17.4% ,erythematous mucosa in 2.2% and rectal polyps in 5.4%(16) , other study conducted in Baghdad by Lafta KB et al showed comparable results as ulcerated lesions (87.5%) were the most common endoscopic findings(21).

This study as other studies conducted for similar reason like that performed by Waniczek D et al in Poland, Kumagai H et al in Japan and showed that most of the rectal lesions located anteriorly, very less commonly the lesions were located circumferentially or posteriorly (22,23).

The histological abnormalities of the studied affected patients showed that all the patients 15 (100%) had fibrous obliteration of lamina propria with hypertrophy of muscularis mucosa and regenerative changes in crypts and this is the typical histopathological diagnostic finding in SRUS ,while associated granulation tissue were seen in 12 (80%) of the affected patients and 9 (60%) showed associated ulcerations and /or erosions, this is identical to the finding in the similar studies like that done by Al-Brahim N et al in Kuwait and that conducted by Suresh N et al in India (24,25).

Different modalities of therapy are available for the SRUS ranging from the conservatives' therapy (dietary modifications, toilet education, avoidance of digitation), single or combined medical treatment with sucralfate enema, sulfasalazine enema and xylocaine gel application, there is also endoscopic treatment with argon plasma coagulation and finally there are different surgical modalities. There is no agreement about the management of SRUS, numerous modalities have not been proved successfully. Patient education and behavioral modifications are the first steps in the treatment of SRUS. In this study all patients received combined conservatives and medical therapy ,in addition there were two patients that received biofeedback therapy, three patients received endoscopic therapy and two patients underwent surgical therapy with rectopexy , these treatment approaches also had been adopted by Forootan M et al that showed the effective role of biofeedback in SRUS, Gouriou C et al in France that showed the effectiveness of the conservative and the medical treatment and Bulut T et al that explored the treatment options available for the SRUS (26,27,28).

On follow up of the patients , this study showed that symptoms improved or disappeared in most of the affected patients (80%) , while symptoms persist in (20%) of patients , with most of the affected patients showed endoscopic improvement or healing of the lesion(86.6%) and only (13.4%) showed persistent lesion on follow up sigmoidoscopic examination , while (86.7%) of the affected patients showed histological improvement and/or healing of the lesion and only (13.3%) showed no histological changes , and as most of those patients received combined conservatives and medical treatment , so the high percentage of symptoms , endoscopic and histological improvement and/or healing can be explained by the effectiveness of the management strategy adopted in this hospital which included the encouragement of a high-fiber diet, avoidance of straining, regulation of toilet habits, and attempt to discuss any psychosocial factors, in combination with stool softeners and bulking laxatives, along with the effect of the topical agents(sucralfate and sulfasalazine enema), in which the sucralfate enema contains aluminum complex salts which coat the rectal ulcer and form a barrier against irritants, allowing the ulcer to heal, while the sulfasalazine enemas has anti-inflammatory properties that help ulcer healing by reducing the inflammatory responses, these results supported by studies done by Blackburn C et al , El-hemaly M et al and Gouriou C et al for similar purposes(29,30,31).

Anorectal physiological testing and defecography are investigative tools that may provide further insight into the pathogenesis of this condition were not performed in this study due to unavailability of these modalities in this hospital, in addition this is a single center study, and these can be considered as limitations for this study.

Conclusion

SRUS is an uncommon benign defecation disorder with diverse clinical presentations, with rectal bleeding being the most common presenting symptom. The endoscopic findings can be variable with the histological examination being the gold standard for establishing the diagnosis.

Different treatment options are available and the majority of patients in our study respond well to non-surgical therapy.

Confusion with other conditions like rectal cancer and inflammatory bowel diseases is not uncommon. Physicians, surgeons and pathologists should be aware of the features of SRUS so that it is not confused with other conditions.

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Conflict of Interest

Authors have declared that no competing interests exist

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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References

[1] Felt-Bersma RJ. Solitary rectal ulcer syndrome. Current Opinion in Gastroenterology. 2021;37(1):59-65. https://doi.org/10.1097/MOG.0000000000000689

[2] Sadeghi A, Biglari M, Forootan M, Adibi P. Solitary rectal ulcer syndrome: A narrative review. Middle East Journal of Digestive Diseases. 2019;11(3):129.

[3] https://doi.org/10.15171%2Fmejdd.2019.138

[4] Anilir E. A Rare Rectosigmoid Mass Mimicking Cancer; Solitary Rectal Ulcer. Nigerian Journal of Clinical Practice. 2021;24(8):1255-8. 10.4103/njcp.njcp_396_20

[5] Liu Y, Chen Z, Dou L, Yang Z, Wang G. Solitary Rectal Ulcer Syndrome Is Not Always Ulcerated: A Case Report. Medicina. 2022;58(8):1136. https://doi.org/10.3390/medicina58081136

[6] Ejaz Z, Khan SU, Rehman RU, Jibran MS. Solitary rectal ulcer syndrome in patients presenting with lower gastrointestinal bleeding: a tertiary-care hospital experience. Cureus. 2023;15(2). https://doi.org/10.7759%2Fcureus.35247

[7] Nkurunziza L, El Bacha H, Malki MC, Gharbi T, Benzzoubeir N, Errabih I. Solitary Rectal Ulcer Syndrome about 26 Cases and Literature Review. Saudi J Med Pharm Sci. 2022;8(6):313-8 https://doi.org/10.36348/sjmps.2022.v08i06.008

[8] Gouriou C, Chambaz M, Ropert A, Bouguen G, Venara A, Meurette G, et al. Management of solitary rectal ulcer syndome: results of a french national survey: Societe Nationale Française de Coloproctologie SNFCP, France. Digestive and Liver Disease. 2020;52(8):885-8 https://doi.org/10.1016/j.dld.2020.05.013

[9] Poddar U, Yachha SK, Krishnani N, Kumari N, Srivastava A, Sarma MS. Solitary rectal ulcer syndrome in children: a report of 140 cases. Journal of pediatric gastroenterology and nutrition. 2020;71(1):29-33. https://doi.org/10.1097/mpg.0000000000002680

[10] Sood SK, Garner JP, Amin SN. Spontaneous resolution of solitary rectal ulcer syndrome during pregnancy: report of a case. Diseases of the colon & rectum. 2008;51(7):1149-52. https://doi.org/10.1007/s10350-008-9268-6

[11] .Madigan M, Morson B. Solitary ulcer of the rectum. Gut. 1969;10(11):871-81. https://doi.org/10.1136/gut.10.11.871

[12] Morio O, Meurette G, Desfourneaux V, D'Halluin PN, Bretagne J-F, Siproudhis L. Anorectal physiology in solitary ulcer syndrome: a case-matched series. Diseases of the colon & rectum. 2005;48(10):1917-22. https://doi.org/10.1007/s10350-005-0105-x

[13] .Abusharifah O, Bokhary RY, Mosli MH, Saadah OI. Solitary rectal ulcer syndrome in children and adolescents: a descriptive clinicopathologic study. International Journal of Clinical and Experimental Pathology. 2021;14(4):399.

[14] AlGhulayqah AI, Abu-Farhaneh EH, AlSohaibani FI, Almadi MA, AlMana HM. Solitary rectal ulcer syndrome: A single-center case series. Saudi Journal of Gastroenterology. 2016;22(6):456-60 https://doi.org/10.4103/1319-3767.195555

[15] Forootan M, Darvishi M. Solitary rectal ulcer syndrome: a systematic review and meta-analysis study protocol. Medicine. 2018;97(18):e0565. https://doi.org/10.1097/MD.0000000000010565

[16] Abid S, Khawaja A, Bhimani SA, Ahmad Z, Hamid S, Jafri W. The clinical, endoscopic and histological spectrum of the solitary rectal ulcer syndrome: a single-center experience of 116 cases. BMC gastroenterology. 2012;12:1-6 https://doi.org/10.1186/1471-230x-12-72

[17] Behera M, Dixit V, Shukla S, Ghosh J, Abhilash V, Asati P, et al. Solitary rectal ulcer syndrome: clinical, endoscopic, histological and anorectal manometry findings in north Indian patients. Tropical Gastroenterology. 2016;36(4):244-50. https://doi.org/10.7869/tg.298

[18] Urgancı N, Kalyoncu D, Eken KG. Solitary rectal ulcer syndrome in children: a report of six cases. Gut and liver. 2013;7(6):752. https://doi.org/10.5009%2Fgnl.2013.7.6.752

[19] Dehghani SM, Bahmanyar M, Geramizadeh B, Alizadeh A, Haghighat M. Solitary rectal ulcer syndrome: Is it really a rare condition in children? World Journal of Clinical Pediatrics. 2016;5(3):343. https://doi.org/10.5409%2Fwjcp.v5.i3.343

[20] .Bhusal U, Basukala S, Tamang A, Dhakal S, Sharma S, Karki A. Solitary rectal ulcer syndrome in a young adult–A surgeon's dilemma with rectal carcinoma. Clinical Case Reports. 2022;10(9):e6316. https://doi.org/10.1002/ccr3.6316

[21] Powell CR, Lawson JA, Lindholm PC. Solitary Rectal Ulcer Syndrome Mimicking Perianal Crohn's Disease. Cureus. 2022;14(4). https://doi.org/10.7759%2Fcureus.23733

[22] Lafta KB, Abdullah MA. Jassim Muhsen kathban (2023) Evaluation of Solitary Rectal Ulcer Syndrome from Twenty-Four Iraqi Patients. Journal of Internal Medicine Research & Reports SRC/JIMRR-123. http://dx.doi.org/10.47363/JIMRR/2023(2)123

[23] Waniczek D, Rubicz N, Rudzki M, Buda K, Arendt J. Solitary rectal ulcer syndrome. Wiadomosci Lekarskie (Warsaw, Poland: 1960). 2012;65(4):220-4.

[24] Kumagai H, Yokoyama K, Sunada K, Yamagata T. Solitary rectal ulcer syndrome: A Misleading term. Pediatrics International. 2021;63(6):739-40. https://doi.org/10.1111/ped.14587

[25] Al-Brahim N, Al-Awadhi N, Al-Enezi S, Alsurayei S, Ahmad M. Solitary rectal ulcer syndrome: a clinicopathological study of 13 cases. Saudi Journal of Gastroenterology. 2009;15(3):188-92. https://doi.org/10.4103/1319-3767.54749

[26] Suresh N, Ganesh R, Sathiyasekaran M. Solitary rectal ulcer syndrome: a case series. Indian pediatrics. 2010;47:1059-61. https://doi.org/10.1007/s13312-010-0177-0

[27] Forootan M, Shekarchizadeh M, Farmanara H, Esfahani ARS, Esfahani MS. Biofeedback efficacy to improve clinical symptoms and endoscopic signs of solitary rectal ulcer syndrome. European Journal of Translational Myology. 2018;28(1). https://doi.org/10.4081/ejtm.2018.7327

[28] Gouriou C, Chambaz M, Ropert A, Bouguen G, Venara A, Meurette G, et al. Management of solitary rectal ulcer syndome: results of a french national survey: Societe Nationale Française de Coloproctologie SNFCP, France. Digestive and Liver Disease. 2020;52(8):885-8. https://doi.org/10.1016/j.dld.2020.05.013

[29] Bulut T, Canbay E, Yamaner S, Gulluoglu M, Bugra D. Solitary rectal ulcer syndrome: exploring possible

management options. International surgery. 2011;96(1):45-50. https://doi.org/10.9738/1376.1

[30] Blackburn C, McDermott M, Bourke B. Clinical presentation of and outcome for solitary rectal ulcer syndrome in children. Journal of pediatric gastroenterology and nutrition. 2012;54(2):263-5. https://doi.org/10.1097/MPG.0b013e31823014c0

[31] El-Hemaly M, El Geidie A, Hamdy E, El-Nakeeb A, El Hanafy E, Atef E, et al. Solitary rectal ulcer syndrome: new hope for the patients. The Egyptian Journal of Surgery. 2012;31(1):36-41.

[32] Gouriou C, Siproudhis L, Chambaz M, Ropert A, Wallenhorst T, Merlini-l'Héritier A, et al. Solitary rectal ulcer syndrome in 102 patients: Do different phenotypes make sense? Digestive and Liver Disease. 2021;53(2):190-5. https://doi.org/10.1016/j.dld.2020.05.013

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Research Article

Iraqi Registry Data Proves Safety and Efficacy of Switching to Adalimumab Biosimilar in Treating Rheumatoid Arthritis

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ABSTRACT

Background: Adalimumab is approved for Rheumatoid Arthritis (RA). In 2021, A biosimilar (ABP501; Amgevita®) was licensed in Iraq. The current study aimed to ensure the safety and Efficacy of Amgevita-Adalimumab biosimilar- in RA Patients in Iraq.

Subjects and Methods: A Prospective Observational Study Started on 69 RA Records Receiving Amgevita. Data collected from the local registry was then examined for disease activity and adverse reactions for 9 months follow-up.

Results: Thirty patients completed the 9 months of the study: aged (49±14) years; 77.5% females. After 3, 6, and 9 months of follow-up, patients' mean ± (SD) Clinical Disease Activity Index (CDAI) was 27.8 (13.60) which was statistically lower (19.80) (6.96), 17.70 (2.790), and 19 (1.040), $p<0.001$. With a mean change of CDAI: 8 ($p<0.001$), 10.1 ($p<0.001$), and 8.78 ($p<0.001$) after 3, 6, and 9 months. The cumulative percentage of responders was 55.00% and the Cumulative percentage of probability of change of disease activity was 86.00%, No patient achieved remission and no significant Side effects were recorded.

Conclusion: the findings suggest that Amgevita holds promise as an effective and safe option for managing rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA), An autoimmune condition with a natural chronic history, is characterized by stiffness, especially in the morning, symmetrical polyarthritis, and extra-articular manifestations leading to disability if left untreated, with higher female susceptibility (1).

The discovery of biological agents, particularly tumor necrosis factor (TNFi) inhibitors, resulted in a qualitative advance in RA treatment (2). Adalimumab is a human-derived monoclonal antibody with the

capacity to neutralize TNF-α molecule made up of two light and two heavy chains (kappa and immunoglobulin G1 (IgG1) respectively) with one N-glycosylation site on each of the heavy chains. In The United States, The Food and Drug Administration (FDA) gave its approval for RA management in 2002 (3). Nevertheless, the pricing of biologic therapies continues to be an issue despite their potential health benefits, particularly in countries such as Iraq, where the Iraqi Ministry of Health (MOH) aims to improve health care access (4).

Biosimilars are a novel group of medications designed to be as safe and effective as the original biologics (5). ABP-501- [USA: named accordingly AMJEVITA™ (adalimumab-atto); in European Union regions (EU): AMGEVITA® (adalimumabis the first biosimilar to adalimumab (HUMIRA), that has been authorized (6). Amgevita was recently licensed in Iraq, however, the literature lacks data on its utilization in Iraqi patients diagnosed with RA. Thus, this multi-center study aimed to ensure the efficacy and safety of Amgevita adalimumab biosimilar (bADA) in Iraqi patients with RA**.**

Subjects and Methods

A Prospective multi-center cohort observational study was undertaken among patients with RA for whom Amgevita was prescribed by consultant rheumatologists. Data were collected from Amgevita registry data that were launched in January 2021 till September 2021 across five centers in Iraq. The Rheumatology Unit at the University of Baghdad approved the study protocol in line with the Helsinki Declaration.

As Inclusion Criteria, The registry includes eligible individuals with moderate to severe RA who met the criteria set by the American College of Rheumatology (ACR) and who had not reacted or not sufficiently responded to Conventional synthetic Disease Modifying Anti-Rheumatic Drugs (csDMARDs) or other biological treatments were given consideration for biologic Adalimumab (bADA) therapy and who freely agreed to receive bADA as their preferred biologic at the participating institutions. According to the center's standard clinical procedure, all of these patients received bADA 40 mg subcutaneously twice a month. Only patients completing the 9-month study period were included in the final analysis to overcome potential bias.

Patients with overlap diseases and those who did not complete 9 months follow-up period were excluded

All patients were required to sign a written consent form and The Ethical Committee at Baghdad Teaching Hospital approved the study according to the Declaration of Helsinki.

Age, gender, BMI, current smoking status, comorbidities, disease duration, current medications, previous biologics use, disease activity assessed by clinical disease activity index (CDAI), complete blood picture (CBP), renal and liver function tests, and any adverse event reported in the period of follow up were recruited from the registry system.

Statistical analysis was conducted using the 26th version of SPSS for Windows (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as numbers and percentages. The normal distribution of the results was assessed using the Shapiro-Wilk test. In cases of missing data, the last-observation-carried-forward (LOCF) method was employed for data analysis to ensure the robustness of the results.

The paired t-test was utilized to determine the statistical significance of the difference in the Clinical Disease Activity Index (CDAI), a reliable tool for assessing disease activity and guiding response to therapy between the follow-up periods and the baseline. This test allows for the comparison of means within the same group of patients at different time points, thus assessing the effectiveness of Amgevita in reducing disease activity over time.

To assess the cumulative percentage of responders and the transition from severe to moderate disease activity, a Kaplan-Meier survival analysis was employed. This analysis estimates the probability of an event occurring over time, such as a response to treatment or a change in disease activity, providing valuable insights into the long-term outcomes of treatment with Amgevita.

Furthermore, a Cox regression analysis was used to evaluate the impact of demographic and clinical characteristics on the response to Amgevita over time, as well as to predict changes in disease activity. This type of regression analysis is suitable for time-to-event data and allows for the examination of multiple predictors simultaneously, thus identifying factors that may influence treatment outcomes.

A significance level of less than 0.05 for the two-tailed p-value was considered as the cutoff for statistical significance in all analyses, ensuring the reliability of the findings.

Results

A total of 69 patients participated in the study who were recorded retrospectively in the registry system of biological therapy in more than one center in Iraq and followed prospectively for nine months. At the end of this period, only 30 patients had completed the 9 months. Follow-up of 39 patients. The last observation was carried out to analyze the missing data (Figure 1).

Baseline characteristics of the patients Extracted from the Registry

The patient's average age was 49 (plus or minus 14) years. The majority of the patients (77.6%) were female, with a mean BMI of 30.66 (±4.25) kg/m2. Only 4 (7.5%) of the patients were smokers. The average duration of the disease and the time taken to start using the adalimumab biosimilar (Amgevita) was 9.90 (±8.99) years. Other baseline characteristics can be found in Table 1.

Based on the data presented in Figure 2A, the average Clinical Disease Activity Index (CDAI) of the patients was significantly lower after three, six, and nine months compared to the baseline CDAI. Specifically, the mean CDAI(±SD) after three months was 19.8 (±6.96) compared to 27.8(±13.6) at baseline ($p<0.001$); after six months it was 17.7 (SD=2.79) compared to 27.8 (SD=13.6) at baseline ($p<0.001$); and after nine months it was 19 (SD=1.04) compared to 27.8 (SD=13.6) at baseline ($p<0.001$). These results are illustrated in Figure 2A.

Furthermore, the mean change in CDAI from baseline was also calculated. The mean change in CDAI after three months was 8 ($p<0.001$), after six months was 10.1 ($p<0.001$), and after nine months was 8.78 ($p<0.001$). These results are shown in Figure 2B.

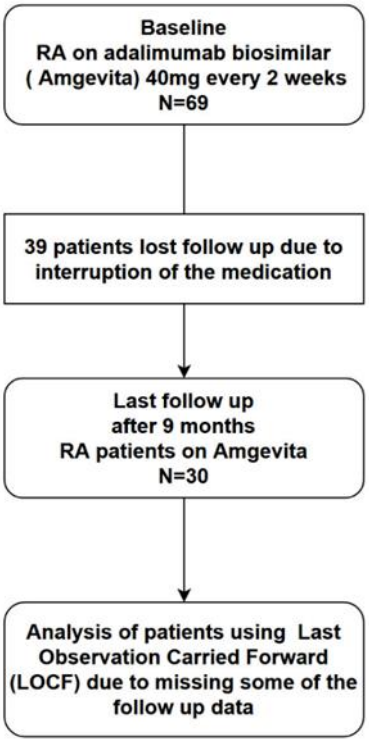

Figure1: Study flow chart

Table 1: Baseline characteristics of the 69 patients

Variables	Value
Age, mean (±SD), years	49 (±14)
Female, N (%)	52 (77.6)
BMI, mean (±SD), kg/m2	30.66 (±4.25)
Smokers, N (%)	4 (7.5)
Disease duration, mean (±SD), years	9.90 (±8.99)
Time to start Amgevita from the onset of the disease	9.90 (8.99)
Positive RF, N (%)	15 (93.8)
Positive ACPA. N (%)	10 (76.9)
Steroid users, N (%)	21 (31.3)
Previous use of other bDMARDs, N (%)	31 (70.5)
csDMARDs users, N (%)	53 (93%)

SD, standard deviation, N, number; BMI, Body Mass Index; RF, Rheumatoid factor; ACPA, Anti-citrullinated-peptide-antibody; bDMARDs, biological disease-modifying antirheumatic drugs, csDMARDs, conventional disease-modifying antirheumatic drugs

Outcome measurement

The cumulative percentage of responses and the transition from severely to moderately active disease

According to the EULAR CDAI response criteria, the cumulative percentage of response among active RA disease patients who were treated with adalimumab biosimilar (Amgevita) was 55%, where a minor response was considered if there was at least a >50% change in CDAI from baseline. Over 9 months, the cumulative percentage of probability of change from high to moderate disease activity was 86%, but none of the patients had changed to a mild or remission state during that time (Figure 3A and B).

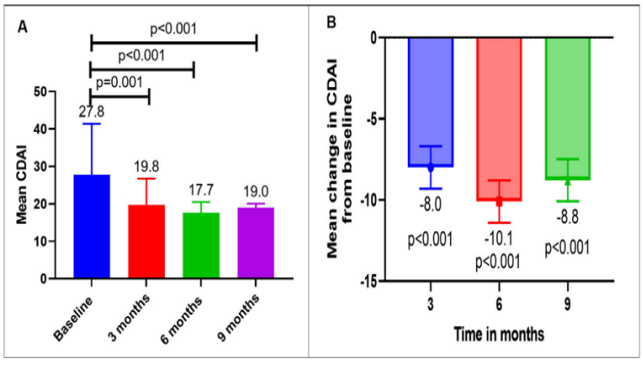

Figure 2: A. Mean CDAI in RA on adalimumab biosimilar (Amgevita) over 9 months. B. Mean change in CDAI over 9 months. CDAI, clinical disease activity index.

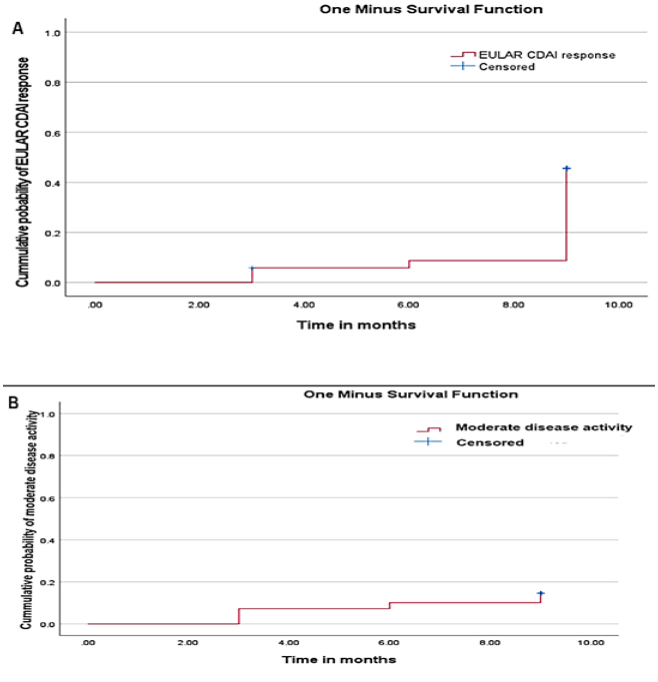

Figure 3: A: Cumulative percentage of EULAR CDAI response criteria in RA patients. B: Cumulative probability of change from severe to moderate disease activity."

Multivariate modeling

We conducted a multivariate Cox regression analysis to determine the factors that may predict response to adalimumab biosimilar (Amgevita) over time or the possibility of transitioning from severe to moderate disease activity. However, we found that baseline demographic and clinical characteristics had no statistically significant impact on the outcome of interest, as shown in Tables 2 and 3.

Table 2: Cox regression analysis to assess predictors of response over time

Variables	HR	P value
Age	0.57	0.99
Female compared to male	0.16	0.54
Smokers compared to non-smokers	0.30	2.04
Disease duration	0.88	1.01
Time to start Amgevita	0.98	0.46
Steroid users compared to non	0.75	0.87
csDMARDs users compared to non	0.86	1.23
Previous bDMARDs users compared to non	0.43	1.75
Presence of comorbidity compared to absence	0.84	0.85

HR, Hazard ratio; csDMARDs, conventional disease-modifying antirheumatic drugs bDMARDs, biological disease-modifying antirheumatic drugs

Table 3: Cox regression analysis to predict moderate disease activity over time

Variables	HR	P value
Age	0.99	0.89
Gender	1.06	0.88
Smokers compared to non-smokers	1.11	0.89
Disease duration	0.99	0.52
Time to start Amgevita	0.99	0.63
Steroid users compared to non	1.00	1.00
csDMARDs users compared to non	1.30	0.73
Previous bDMARDs users compared to non	0.85	0.64
Presence of comorbidity compared to absence	1.00	1.00

HR, Hazard ratio; csDMARDs, conventional disease-modifying antirheumatic drugs bDMARDs, biological disease-modifying antirheumatic drugs

Safety measures

The clinical and hematological effects of adalimumab biosimilar (Amgevita) therapy were monitored and no significant adverse effects or changes were observed (refer to Table 4 for biochemical investigations).

Discussion

Despite the growing evidence supporting the clinical use of biosimilars in the field of autoimmune diseases, many physicians do speculate that this evidence is driven by the big pharmaceutical companies. Hence, they often request more real-world data that can reflect the true characteristics of a given drug; highlighting the need for more experience-based observations (7–10)). To our knowledge, this is the first study attempting to display the results of real registry data of rheumatoid arthritis patients treated by the biosimilar "Amgevita". The study comprised 69 rheumatoid arthritis patients recruited through the local registry system and observed for 9 months. Only 30 patients completed the entire 9-month study period. The results demonstrate that after 9 months, the participants' disease activity (as evaluated by CDAI) was significantly better when compared to the baseline CDAI (figure 2), with the mean CDAI dropping from 27.8 to 19, with a cumulative response of 55%. Figure (3) which reflects clear efficacy of the drug.

Table 4: Laboratory findings during Amgevita therapy over 9 months of treatment

Variables	Baseline	Last, follow up	p
WBC, mean ±SD, 109/L	8.47± 2.71	7.51± 1.93	0.196
HB, mean ±SD, g/dl	12.54± 1.72	12.9± 1.12	0.339
ALT, mean ±SD, U/L	17.976±6.380	18.235±7.704	0.893
AST, mean ±SD, U/L	17.567±8.465	16.472 ± 8.500	0.597
Blood urea, mean ±SD, mg/dl	29.612±11.719	31.206±9.388	0.561
Creatinine, median (IQR), mg/dl	0.7 (0.57 - 0.7)	0.7(0.6 to 0.8)	0.317

These results were comparable with other studies demonstrating clear efficacy of adalimumab biosimilar Amgevita in RA management, such as Cohen et al.'s study, which directly compares the bio-originator Humira with its biosimilar Amgevita (11).

However, the response in the current study is lower compared to the overall change in disease activity because, despite the mean disease activity dropped from high to moderate, none of the participants reached remission or low disease activity by the end of the study period. While in Cohen et al's study, the percentage of active patients who achieved remission was increased over time for both groups (Amgevita and Humira) from weeks 2 to 18 (range: 6.3%–31.1%, Amgevita; 2.8%–27.1%, Humira). At the end of the 24th week, 30.5% (Amgevita) and 35.5% (Humira) of patients had reached remission.

While none of the patients in the current study reached a complete response, this could be attributable to the patients' prior therapies where the majority of the participants in the current study had prior bDMARDs use (70.1%) (Table 1). While in Cohen et al.'s study, the majority were biologic naïve patients (73.1% in the Amgevita arm and 71.8% in the Humira arm), otherwise, both studies carry similar case characteristics.

Another observation by the PREMIER study is that people with RA may achieve a complete response after around 2 years of usage (12), and another Indian registry study showed that 58% of the treated patients at 12 months were in remission or low disease activity (13). So, extended follow-up may give the chance for a few cases to reach remission.

The fact that a large number of people (39 patients) stopped using the drug may also have an impact on the remission criteria, people who felt better after a few months might have escaped further doses.

A similar study on 149 RA patients in India (the ASPIRE registry data) has shown very similar results where, after 6 months, about 58% and 15% of patients were moderate and good EULAR responders.

Although not mentioned directly in the study, no patients reached remission as well from observation of the results (14).
In an attempt to investigate a potential response predictor, some variables were studied as shown in Tables (2 and 3) including age, gender, smoking status, disease duration, drug starting time, steroid use, csDMARDs, previous use of biologics, and comorbidities, but none showed a significant impact on response. This has been observed in other studies, where the clinical predictors of response were incoherent between studies and between groups of patients with high and moderate disease activity, whereas the more expensive biochemical or immunological predictors were not (15,16).

Concerning Amgevita's safety, the side effects seen were quite modest, with no substantial clinical or laboratory issues of concern (table 4). These findings are consistent with earlier trials that showed that both adalimumab and biosimilars are well tolerated (17).
The ASPIRE trial, on the other hand, found a 2% infection rate and 5% tuberculosis activation. However, in addition to RA, their records included individuals with ankylosing spondylitis (AS), psoriatic arthritis (PSA), and juvenile Idiopathic arthritis (JIA) (9).
Limitations of this study are the lost data from the 39 patients who didn't complete the 9 months of follow-up because of the interruption of the medication supply besides the effect of COVID-19 on the patients and their fear of taking the medication. and the lack of direct comparison with the bio-originator "Humira" due to unavailability at the time of collection.

Conclusion

The current study's findings provide valuable insights into Amgevita's potential effectiveness and safety in treating rheumatoid arthritis (RA). However, a larger sample size is necessary for a more comprehensive assessment. Future studies should focus on including more participants by extending the duration of drug registration in related centers. This would enable a better evaluation of Amgevita's efficacy and safety in managing RA within the Iraqi population

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Conflict of Interest
Authors declare no conflict of interest.
Data availability
Data are available upon reasonable request.

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References

[1] Chaudhari K, Rizvi S, Syed BA. Rheumatoid arthritis: current and future trends. Nat Rev Drug Discov. 2016 Apr;15(5):305–306. https://doi.org/10.1038/nrd.2016.21
[2] Rein P, Mueller RB. Treatment with Biologicals in Rheumatoid Arthritis: An Overview. Rheumatol Ther. 2017 Aug;4(2):247–261. https://doi.org/10.1007/s40744-017-0073-3
[3] Azevedo VF, Della Coletta Troiano L, Galli NB, Kleinfelder ADF, Catolino NM, Martins PCU. Adalimumab: a review of the reference product and biosimilars. Biosimilars. 2016 Jul; Volume 6:29–44. https://doi.org/10.2147/bs. s98177
[4] Abdulateef N, et al. Review of biologics, biosimilars, and intended copies in rheumatology, and current practice in Iraq. Open Access Rheumatol Res Rev. 2018 Dec; https://doi.org/10.2147/oarrr. s176965
[5] Kumar R, Singh J. Biosimilar drugs: Current status. Int J Appl Basic Med Res. 2014 Jan;4(2):63. https://doi.org/10.4103/2229-516x.136774
[6] Markus R, et al. A review of the totality of evidence supporting the development of the first Adalimumab biosimilar ABP 501. Adv Ther. 2019 Jun;36(8):1833–1850. https://doi.org/10.1007/s12325-019-00979-6
[7] Sarnola K, Merikoski M, Jyrkkä J, Hämeen-Anttila K. Physicians' perceptions of the uptake of biosimilars: A systematic review. BMJ Open. 2020 May;10(5). https://doi.org/10.1136/bmjopen-2019-034183
[8] Lessons for the United States from Europe's biosimilar experience. [Internet]. Available from: https://getmga.com/wp-content/uploads/2022/04/EuropeBiosimilarsJune2020.pdf [Accessed Aug. 20, 2023].
[9] Humphreys SZ. Real-world evidence of a successful biosimilar adoption program. Future Oncol. 2022 May;18(16):1997–2006. https://doi.org/10.2217/fon-2021-1584
[10] Al-Kinani KK, et al. Iraqi regulatory authority current system and experience with biosimilars. Regul Toxicol Pharmacol. 2020; 117:104768. https://doi.org/10.1016/j.yrtph.2020.104768
[11] Cohen S, et al. Efficacy and safety of the biosimilar ABP 501 compared with adalimumab in patients with moderate to severe rheumatoid arthritis: a randomised, double-blind, phase III equivalence study. Ann Rheum Dis. 2017 Oct;76(10):1679–1687.
[12] Breedveld FC, et al. The Premier Study: A Multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. Arthritis Rheumatism. 2005;54(1):26–37. https://doi.org/10.1002/art.21519

[13] Sharma B. Clinical use of ZRC3197 (adalimumab biosimilar) in patients with inflammatory arthritis: a real-life experience. J Assoc Physicians. 2017; 65:22–25.

[14] Kapoor S, Kaushik V, Jain R, Rao V, Gharia M. Real-Life tolerability and effectiveness of adalimumab biosimilar in rheumatoid arthritis: ASPIRE Registry data. Rheumatol Ther. 2019 Jun; https://doi.org/10.1007/s40744-019-0166-2

[15] Atzeni F, et al. Predictors of response to anti-TNF therapy in RA patients with moderate or high DAS28 scores. Joint Bone Spine. 2014;81(1):37–40. https://doi.org/10.1016/j.jbspin.2013.04.005

[16] Daïen CI, Morel J. Predictive Factors of Response to Biological Disease Modifying Antirheumatic Drugs: Towards Personalized Medicine. Mediators Inflamm. 2014 Jan 12;2014: e386148.

[17] Zhao S, Chadwick L, Mysler E, Moots RJ. Review of biosimilar trials and data on adalimumab in rheumatoid arthritis. Curr Rheumatol Rep. 2018;20(10). https://doi.org/10.1007/s11926-018-0769-6

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Research Article

Pregnant Mothers' Knowledge, Attitude, and Practice Towards Prevention of Iron Deficiency Anemia in Georgia

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ABSTRACT

Background: Anemia in pregnancy is a serious global health challenge, particularly affecting developing countries.
Objectives: This study aimed to explore pregnant women's attitudes and perceptions regarding preventing iron deficiency anemia (IDA).
Subjects and Methods: A qualitative research method was used to survey pregnant women with IDA and physicians through in-depth interviews. The target sample consisted of 9 gynecologists and 26 pregnant women, selected through purposive sampling from three maternity hospitals in Tbilisi (Georgia).
Results: The majority of pregnant women (n=22; 84.6%) had some knowledge about IDA and correctly identified its symptoms. However, despite high awareness, most lacked adequate knowledge about the causes and risk factors of IDA. While pregnant women were aware of healthy eating practices, they did not follow a proper diet. Obstacles to healthy eating included limited financial access to food as well as cultural and religious barriers. Although respondents had some knowledge about iron-rich foods, they were generally unaware of the need to take iron supplements for prevention. The majority of pregnant women (n=21; 80.8%) received little information about anemia from their family physicians, indicating a limited role of family doctors in IDA prevention during pregnancy.
Conclusions: Although pregnant women possess knowledge and positive attitudes towards IDA prevention, their practices remain insufficient. The findings suggest a weak connection between knowledge of IDA prevention and healthy behavior, contributing significantly to anemia prevalence. To address this issue, it is essential to promote proper nutritional counseling for pregnant women during antenatal care, with a focus on strengthening family doctors' involvement. Additionally, awareness should be increased among women of reproductive age and adolescent girls.

Introduction

Iron deficiency anemia (IDA) in pregnancy is a condition in which the hemoglobin (HB) level in a pregnant woman's body decreases. The World Health Organization defines anemia during pregnancy as an HB level below 11.0 g/dL and below 10.0 g/dL in the postpartum period (1). Iron is essential for the production of red blood cells, which transport oxygen throughout the body. When red blood cell counts decrease, organs and tissues do not receive the oxygen they need. There are two types of iron deficiency:

- Hidden or latent iron deficiency: This type is characterized by reduced iron stores in the bone marrow, while red blood cell count and HB levels remain normal.

- IDA is characterized by a decrease of all metabolic funds, and also by a reduction of red blood cells and HB.

It is important to note that foods contain both heme and non-heme iron. Heme iron, found in red meat, poultry, and fish (such as salmon, tuna, and sardines), is a component of HB and myoglobin. Non-heme iron, present in nuts, cereals, spinach, and broccoli, is stored in the body as ferritin and transported throughout the body via transferrin (2).

Despite iron's abundance on earth, iron deficiency is common worldwide, making it the most widespread nutritional deficiency. In developing countries, iron deficiency often results from blood loss due to nutrient deficiencies or parasitic infections such as helminths, while in developed countries, it is often associated with specific dietary behaviors (e.g., vegetarian diets and avoidance of red meat) and pathological conditions (e.g., chronic blood loss or malabsorption) (3,4,5).

Iron deficiency can have serious consequences, particularly for children and pregnant women, as the demand for iron significantly increases during pregnancy (6). If a pregnant woman has insufficient iron stores, IDA may develop, leading to complications such as deterioration of maternal perinatal and postpartum health, growth retardation, and impaired cognitive and motor development in the newborn. IDA also increases the risks of maternal mortality and low birth weight (7).

Studies show that individual iron supplementation and proper nutrition are among the most effective strategies to address IDA (8). According to WHO recommendations, increasing daily iron intake to 15-30 mg/day is essential. Daily intake of iron and folic acid reduces the risk of maternal anemia by 70% and iron deficiency by 57%. WHO recommends daily iron supplementation as part of antenatal care to reduce the risk of IDA, iron deficiency, and low birth weight.

Pregnant women should focus not only on the quantity but also on the quality of their diet. A balanced diet that is low in fats and carbohydrates and rich in protein, calcium, iron, and vitamins is recommended. Iron-rich foods include legumes, leafy green vegetables (especially spinach), bread, dried fruits, eggs, red meat, and fish (9,10).

According to the WHO, 27% of the global population suffers from IDA. Data from 2019 indicate that IDA affects 30-60% of pregnant women worldwide and is responsible for 22% of maternal deaths (11). Globally, IDA-related maternal and neonatal mortality is estimated to reach 2.5-3.5 million cases (12). The WHO has adopted a global program to improve maternal and child nutrition, intending to reduce anemia prevalence among women of reproductive age by 50% by 2030 (13). However, recent findings suggest that the reduction in anemia prevalence is slower than initially anticipated, with particularly high rates observed among pregnant women in middle- and low-income brackets (14).

In Georgia, 2023 data indicate that 40.6% of pregnant women experienced anemia at least once during pregnancy (15) - a rate higher than the global average of 37% ( ). Georgia's State Maternal and Child Health Program includes eight antenatal visits, with free iron supplements for pregnant women diagnosed with IDA (17).

Despite numerous prevention methods, IDA remains a persistent issue, with many women still facing health complications associated with anemia. The risk factors contributing to IDA depend heavily on pregnant women's awareness and attitudes, which are influenced by their primary healthcare providers, including obstetrician-gynecologists and family doctors. Improving the education of pregnant women and encouraging proactive attitudes among healthcare providers may significantly reduce IDA prevalence (18).

The above emphasizes the importance of effective strategies for reducing IDA, particularly in developing countries like Georgia, where the population faces significant social challenges (19).

The knowledge and perceptions of pregnant women about IDA and its risk factors have not yet been studied in Georgia. Addressing gaps in knowledge and perceptions about IDA among pregnant women through targeted educational programs is a crucial step in combating this issue. Therefore, such research is essential for developing effective strategies to reduce IDA.

This study aimed to explore the knowledge, attitudes, and perceptions of pregnant women in Georgia regarding the prevention of IDA. Research findings can inform policy changes and health education programs, ultimately improving maternal health outcomes.

Subjects and Methods

A phenomenological qualitative study was conducted to explore the experiences of pregnant women with IDA through in-depth interviews. A phenomenological qualitative study is a research design used to explore and understand the experiences of individuals regarding a particular phenomenon. The focus is on capturing the essence of participants' experiences as they perceive and interpret them. This approach is rooted in phenomenology, a philosophical tradition that seeks to describe how people make sense of their experiences. The purpose of qualitative study is to gain deep insights into participants' subjective experiences and meanings. Typically involves in-depth interviews. Researchers identify themes, patterns, and shared meanings to describe the essence of the phenomenon. Outcome of the qualitative is A rich, descriptive account that explains how individuals experience and make sense of the phenomenon in their unique contexts.

This design is commonly used in health sciences, education, and social sciences to explore topics like coping with illness, cultural practices, or life transitions.

The study included obstetricians-gynecologists and pregnant patients. The target sample consisted of 9 gynecologists and 26 pregnant women, selected through purposive sampling from three maternity hospitals in Tbilisi (see Table 1).

Pregnant women were initially invited to participate by a nurse. Upon consenting to join the study, a face-to-face meeting was arranged in the polyclinic departments of maternity hospitals during working hours, from 11 am to 3 pm. Women interested in participating were fully informed by the researcher about the study's objectives, process, and ethical considerations. After providing written consent, participants were selected. The inclusion criteria included pregnant women diagnosed with IDA without any complications.

Data were collected through individual in-depth interviews with obstetricians-gynecologists and pregnant patients between March and July 2024. Each interview lasted approximately 35 to 50 minutes and was recorded using a digital voice recorder. The recordings were fully transcribed and verified for accuracy. Transcripts were reviewed

multiple times to gain a deeper understanding of each interview. Codes were then grouped by source category and topic.

Table 1: Demographic characteristics of pregnant women

Characteristics	n=26	%
Age		
18-20	2	7.7
21-33	18	69.2
≥ 34	6	23.1
Number of children		
0	15	57.7
1	11	42.3
> 2		
Employment		
Employed	7	26.9
Self-employed	5	19.2
Housewife	14	53.8
Education		
Secondary education	9	34.6
Bachelor	13	50
Master	4	15.4
Income status		
250 GEL	3	11.5
250-500 GEL	11	42.3
500-800 GEL	9	34.6
800 GEL	3	11.5
Gestational age		
≤12 weeks	15	57.7
≥12 weeks	11	42.3

Ethical Issues

Approval to conduct the research was obtained from the Ethics Council of Caucasus University (CAU No. 012/23). Respondents were informed in advance about the study's objectives, and confidentiality was strictly maintained throughout. Pregnant women participated voluntarily and were free to withdraw from the study at any time without providing a reason. All study data were anonymized using individual codes and presented without personal identifiers.

Research Limitations

The study was conducted in a limited number of randomly selected clinics, outpatient facilities, and maternity hospitals, which limits the generalizability of the results. Additionally, a lack of statistical data and literature on the prevalence and prevention of IDA in Georgia posed another limitation.

Results

Results of a Survey of Obstetrician-Gynecologists

Causes of IDA in Pregnancy

The doctors named the worsening socioeconomic situation as one of the reasons for the increase in the prevalence of inadequate nutrition and anemia. Pregnant women cannot afford many expensive foods, which has become one of the causes of IDA.

"This is the most common situation in our reality. About 80% of my pregnant patients are anemic. Cases of anemia have increased significantly in recent years. It should be noted that in the 80s and 90s of the last century, anemia was less common than now. In my opinion, the reason for this is a change in diet. Previously, pregnant women ate a lot of meat and fat, and meat, as is widely known, prevents IDA, but increases the risk of overweight and hypertension. Now the opposite is true, due to socioeconomic problems, pregnant women receive mainly vegetarian foods, that protects against hypertension but increases the prevalence of anemia".

1st gynecologist.

One of the gynecologists named the short interval between pregnancies and especially multiple pregnancies as the main cause of IDA.

"During pregnancy, conditions conducive to anemia are created. The mother's body supplies the fetus with the substances it needs, including iron. With repeated pregnancies after a while, iron stores in the mother's body cannot be restored, and this is one of the leading causes of IDA".

2nd gynecologist.

According to one of the gynecologists, the increased prevalence of IDA is the result of environmental problems (for example, increased levels of radiation), poor food quality, stress, and unstable economic and marital status.

"Most of the imported products are expired and contain harmful, toxic substances."

3rd gynecologist.

Knowledge of IDA and following its prevention methods

Some doctors (n=7; 77.8%) expressed satisfaction with the general awareness of pregnant women about proper nutrition. In their opinion, pregnant women usually follow the advice as much as possible. However, most of them (n=8; 88.9%) are not satisfied with the adherence of pregnant women to medical recommendations, citing socioeconomic difficulties as the main reason.

"Women read a lot and come to my clinic with basic knowledge about healthy eating."

4th gynecologist.

"When I give my pregnant patients advice on proper nutrition, they are silent and just look at me. How can we afford all these products? "

5th gynecologist.

"The state program for pregnant women does not include free iron supplements, this is why most pregnant women do not want to buy iron supplements for financial reasons."

6th gynecologist.

Preventive methods of IDA

Although doctors prescribe iron supplements and a proper diet to pregnant patients to prevent IDA, the problem still exists. According to most doctors (n=8; 88.9%), it is desirable to distribute free iron supplements and prenatal vitamins to pregnant women in maternity hospitals and women's outpatients. Doctors believe preventive iron replacement therapy is ineffective without properly informing pregnant women.

"If a pregnant woman is unaware of the risk of IDA, she doesn't even want to take vitamins." Therefore, in any case, it is necessary to inform patients about the importance of iron supplements. "

7th gynecologist.

"Sometimes we have a long line of pregnant women waiting for a consultation and we simply don't have time to talk to every pregnant patient about nutritional issues. Brochures about lactation are available, which we printed for our patients. These brochures make it easier for us. Of course, we emphasize the need for breastfeeding when talking to a pregnant woman, but the patient can find more information and answers to questions in this brochure. It would be desirable to create such informative brochures on healthy eating during pregnancy, especially in terms of preventing IDA, as the latter is a serious problem in today's reality."

8th gynecologist

Most doctors (n=8; 88.9%) welcome the opinion, that increasing the level of education of pregnant women and changing their attitude toward iron supplements will help fight IDA and find a way out of the situation. However, they noted that improving the socioeconomic situation was necessary to solve the problem.

The role of the family physician in the prevention of IDA

According to gynecologists (n=9; 100%), it is necessary to involve a family physician and a gynecologist in the process of managing IDA in pregnant women. In this regard, a family doctor's involvement in the process of preventing IDA in Georgia is one of the main tasks.

"There has been a trend in the country that only a gynecologist manages pregnancy as a whole and solves several problems, including IDA. The family doctor is less involved or almost does not participate in this process and does not take on this responsibility."

9th gynecologist

Results of a Survey of Pregnant Women

Knowledge and perceptions of pregnant women about anemia

Most pregnant women (n=22; 84.6%) interpreted anemia as a "lack of blood". Only four respondents (n=4; 15.4%) with higher education knew the medical definition of "anemia". A possible reason for ignorance of medical terminology may be the low level of their general education or the infrequent use of medical terminology by medical personnel when dealing with patients.

"I don't know exactly what the word" anemia "means. I first heard about it from a gynecologist"

A 21-year-old mother in her first pregnancy

Pregnant women named gynecologists, the internet, television, books, family physicians, mothers, and relatives as sources of information about anemia.

"Yes, I have heard that anemia means a lack of blood in the body, which is very common during pregnancy. The doctor advised me to do a blood test as he told me that the number of red blood cells in my blood was reduced, and I had low HB levels."

A 22-year-old mother with one child

Respondents mainly described anemia for its symptoms, such as general weakness, mild fatigue, decreased appetite, nausea and vomiting, aversion to some types of food, pallor of the skin, and conjunctiva, "black circles under the eyes," "dizziness," "fainting," "white lines on the nails."

Twelve respondents (n=12; 46.2%) had mild to moderate weakness during pregnancy, and some experienced dizziness; However, due to the mild course, none of them consulted a doctor. Anemia is not a serious condition, according to some respondents, as anemia-related weakness and dizziness are "normal during pregnancy."

"*Fatigue and weakness are part of pregnancy. It doesn't affect the mother or her baby".*

A 23-year-old mother with two children

"*A pregnant woman carries a different body inside her. It's something new for her body, and she may get tired because of it, or she may have dizziness and other symptoms. "*

A 21-year-old mother with one child

"Feeling nauseous and disgusted with certain foods during pregnancy is natural. Gradually, the pregnant woman adapts to these symptoms".

A 22-year-old mother with two children

Respondent's perception of anemia as a "normal phenomenon of pregnancy" was also supported by the fact that women of reproductive age shared similar experiences in social networks. In their opinion, anemia was a natural part of pregnancy, because at this time changes occurred in the body that did not cause any harm to either the child or the mother.

"If other women, despite these symptoms, can give birth normally and safely, then why take it seriously?"

A 27-year-old-woman with two children

According to the respondents, the symptoms of anemia can be eliminated by resting and eating certain foods.

"I often experience fatigue, dizziness, and weakness during pregnancy. My mother tells me that this is natural during pregnancy. I rest at such times. I feel good after rest."

A 25-year-old mother with two children

However, if pregnant women experienced severe weakness, fever, abdominal pain, white vaginal discharge, or bleeding, they understood, that these symptoms could have a severe impact on the baby and usually consulted a doctor.

"Bleeding can harm the baby, because it won't have enough blood supply, and it can interfere with the development of my son. Bleeding can also cause premature birth or miscarriage."

A 27-year-old mother with two children

"Abdominal pain is a dangerous sign because the baby is growing in my belly and everything that affects me will affect my baby; Therefore, in case of abdominal pain, you should immediately consult a doctor."

A 23-year-old mother with two children

IDA: Causes

The majority of respondents (n=23; 88.5%) noted that the main causes of anemia during pregnancy are insufficient, unbalanced, and low-quality nutrition, sharing blood with the fetus, increased energy consumption, emotional stress, long exposure to the sun, and physiological factors.

Although the respondents mentioned various causes of anemia, almost none of them had the correct knowledge of the etiology of anemia.

"During pregnancy, we need extra nutrition because we are sharing blood with our baby. Therefore, we should eat more vegetables that will increase our HB."

A 24-year-old mother with one child

"When a woman does not follow a proper diet and has emotional problems, does not walk outdoors every day - all this can contribute to the development of anemia."

A 21-year-old mother in her first pregnancy

"Anemia can be caused by the lack of a balanced diet containing essential nutrients that can maintain the necessary volume of blood in the body."

A 20-year-old mother with one child

"Anemia is especially common during pregnancy because a new life is developing inside a woman and therefore, she needs more energy and strength."

A 27-year-old mother with two children

"Prolonged exposure to the sun during working hours and tedious work can lead to a decrease in the amount of blood in the body."

A 21-year-old mother with two children

"Pregnancy can cause anemia because the fetus receives blood from the mother, meaning the blood volume of the fetus depends on the mother's blood. This is how the mother passes on some of her blood to the fetus".

A 19-year-old mother with one child

The role of food in the prevention of IDA

Respondents unanimously stated that the most effective way to prevent anemia in pregnant women was to eat a healthy diet, with medications playing a secondary, supportive role. By their definition, a healthy diet is "nutrient-rich"; "High-calorie," "consisting mainly of dairy products, vegetables and fruits," and "vitamin-rich nutrition."

Most of the study participants (n=23; 88.5%) said pregnant women should eat foods such as fruits, vegetables, meat, milk, dairy and natural juices since healthy food "gives energy and strength to a pregnant woman," which "increases the amount of blood in the body."

"In my opinion, eating properly during pregnancy is more important than ever. We need more fruit and vegetables, we need to eat meat, drink milk and eat more dairy, and natural juices, because healthy food "gives energy and strength to a pregnant woman", and "increases the amount of blood in the body. It is necessary to eat only healthy food every day, which will give the child all the important nutrients. Also, a healthy diet will contribute to the birth of a child with a normal weight. "

A 27-year-old mother with two children

"We should eat foods that can increase blood volume and raise HB levels, such as red meat, beans, honey, walnuts, pomegranate juice, and more. Doctors teach us to eat properly. We must follow their instructions to improve. "

A 22-year-old mother with two children

"I try to make my diet as diverse as possible. A pregnant woman's diet is unimaginable without dairy products as a source of protein and calcium. Also, taking 1-2 tablespoons of red wine, a day improves the HB index".

A 23-year-old mother with two children

When asked if the diet of pregnant women should be different from that of non-pregnant women, the majority of pregnant women (n=24; 92.3%) answered positively, but the answers to what should be the diet of a pregnant woman varied.

"One of the peculiarities of pregnancy is that a pregnant woman may want to eat something different, even strange. Therefore, she must satisfy her desire and eat what she wants. The happier the expectant mother is, the more likely she is to give birth to a healthy child. For example, I have a strong desire to eat pickles and Staphylea, although I know it is not recommended to eat these foods during pregnancy".

A 21-year-old mother in her first pregnancy

"It's important to me to eat the foods I want during pregnancy, even though my doctor recommends other foods to prevent anemia."

A 19-year-old mother in her first pregnancy

Most pregnant women (n=22; 84.6%) were more or less satisfied with their diet. They reported that they increased the amount of meat, dairy, fruit, and vegetables in their diet during pregnancy.

"I am satisfied with my diet. I believe that I have an optimal diet for my condition."

A 22-year-old mother with one child

Although pregnant women had adequate knowledge about healthy eating, they did not follow the necessary, correct diet. Their daily intake of necessary food was unstable. According to several pregnant women (n=7; 26.9%), their diet would be more diverse if they had a better financial situation. One of the obstacles to healthy eating is the high price of food products and less financial access:

"We have to eat a lot of vegetables and fruit during pregnancy, but with food prices rising, it's impossible to eat good food every day."

A 22-year-old woman pregnant with a second child

"If I don't have the money, how can I eat good and healthy food?"

A 29-year-old mother of two children

However, cultural barriers also affect the nutrition of pregnant women. Some women (n=7; 26.9%) relied on a vegetarian diet because of their religious orientation. They also noted that the responsibilities of caring for other family members made it difficult to maintain a healthy diet during pregnancy.

"Being responsible for family members makes it difficult to look after yourself. It's very different when you're in your mother's house,

where you can spend more time on yourself and do whatever you want. "

A 21-year-old mother in her first pregnancy

"I have a mother-in-law and another child at home, and I have to look after them, cook them, and do other things. I get very tired. Sometimes I can't eat in time".'

A 24-year-old mother with one child

According to pregnant women, it is necessary to get more information about "diet and food composition".

"We would like to know more about the foods that are recommended during pregnancy."

A 21-year-old mother in her first pregnancy

Knowledge of pregnant women about foods containing iron and folic acid

Almost every woman (n=24; 92.3%) has heard the words iron and folic acid. They had some idea about foods containing iron, but most of the respondents (n=24; 92.3%) did not know that the cause of anemia is iron deficiency in their bodies. Also, none of the pregnant women knew about foods containing folate.

"Iron is found in fruits, green vegetables, eggs, meat, and fish."

A 21-year-old mother in her first pregnancy

"Foods like fruits and vegetables, especially green vegetables, contain iron."

A 26-year-old mother with one child

"I don't know any food that is high in folate, it can only be taken as a medicine."

A 22 -year-old-mother in her first pregnancy

Knowledge and attitude of pregnant women toward iron-folic acid supplementation

Almost all respondents (n=22; 84.6%) had some idea about iron and folic acid supplements. However, only five women (n=5; 19.2%) identified iron deficiency as a cause of anemia.

The respondents reacted positively to iron preparations for the prevention of IDA. Taking iron and folic acid drugs has a positive effect on women and children's health, "empowers" and is useful for "preventing frailty in women," especially during pregnancy, they said.

"Yes, I know that women should take these supplements during pregnancy- because after taking them, the body feels better, and they help a mother and a growing child to be strong and healthy. "

A 21-year-old woman in her first pregnancy

"Women should take iron and folic acid supplements during pregnancy to stay healthy and strong. It helps you to have a healthy baby."

A 20-year-old woman in her first pregnancy

"I know that iron and folic acid supplements have a positive effect on women's health, especially during pregnancy. It promotes blood production and prevents anemia during pregnancy."

A 20-year-old woman with one child

Some of the interviewed pregnant women (n=12; 46.2%) regularly took iron and folic acid supplements. Respondents explained their regular use of these supplements by the fact that health professionals convinced them of their positive impact on the physical and mental development of the child.

They also believed that supplements should be good for the baby because "doctors know best what is best for pregnant women":

"The doctor explained that these supplements will help my child's mental development. I trust my doctor. By taking these supplements, my child will be born healthy."

A 26-year-old mother with one child

"My dream is to have a healthy child. For my child to be born healthy, I must take supplements regularly. However, you cannot rely on medication alone. There should be a balance between healthy eating and medication."

A 21-year-old woman in her first pregnancy

A small part of the surveyed pregnant women (n=6; 23.1%) did not want to take any medication during pregnancy at all. According to them, if the diet is rich and varied, there is no need to take additional iron preparations to prevent IDA.

"Eating healthy food is more important than taking medicines because nutrition is the body's natural way of development."

A 22-year-old woman with one child

"It is quite possible that iron tablets are more harmful. I can fight anemia with proper nutrition - by taking iron-containing products."

A 21-year-old mother in her first pregnancy

"I prefer to avoid taking any medication during pregnancy as much as possible and iron supplements are no exception."

A 22-year-old woman in her first pregnancy

"Now my son needs good nutrition. So, I will only take the medicine if it has a good effect on my child's growth. "

A 24-year-old mother with one child

These women were unaware of the health problems that could occur if they did not take iron and folic acid supplements before and during pregnancy. However, some pregnant women (n=12; 46.2%) have reported that if they do not take these supplements, the growing child may have physical or mental health problems.

"If women don't take these supplements during pregnancy, they will be physically weak. Not taking supplements harms a child's health, especially their physical or mental health. "

A 22-year-old mother in her first pregnancy

Most pregnant women (n=22; 84.6%) were not aware of the recommended dose of iron and folic acid preparations, which might be due to their low education levels.

Adherence of pregnant women to iron and folic acid supplementation

Some of the pregnant women (n=12; 46.2%) in the study reported not knowing when women should start taking these supplements. This shows that some health professionals do not provide their pregnant patients with adequate information about iron and folic acid supplements.

"I don't know exactly when pregnant women should start taking iron and folic acid supplements, but I know women should start taking them when they get pregnant."

A 19-year-old mother in her first pregnancy

Only a few pregnant women (n=10; 38.5%) knew about the need to start taking these supplements before pregnancy.

"Women should start taking iron and folic acid preparations early in pregnancy, preferably months before pregnancy."

A 20-year-old mother in her first pregnancy

Most participants (n=22; 84.6%) did not know why pregnant women should take iron and folic acid supplements before pregnancy.

"I know why women should start taking these supplements before pregnancy. If women do not take preventive measures before pregnancy, the baby may be born weak mentally and physically."

A 22-year-old mother with two children

Discussion

According to research, the main causes of IDA are inadequate nutrition, poor food quality, environmental issues (such as increased radiation levels), stress, unstable economic conditions, and short intervals between pregnancies. Although doctors report that pregnant women are somewhat informed about proper nutrition, many do not follow medical recommendations due to socioeconomic challenges. Gynecologists noted that family doctors are minimally involved in managing IDA in pregnant women and often do not take responsibility for this issue, largely due to the underdevelopment of primary healthcare in Georgia (20,21,22, 23, 24,25 ,26, 27).

Increasing the involvement of family doctors is considered essential for preventing IDA. Doctors believe that it is necessary to provide free iron supplements and prenatal vitamins to pregnant women in maternity hospitals and outpatient clinics. They also emphasize the importance of raising educational levels among pregnant women and changing attitudes toward iron supplements, though socioeconomic improvements are crucial to effectively addressing the problem.

The study revealed that nearly all respondents had some awareness of IDA, with most correctly identifying its symptoms. This relatively high level of awareness among pregnant women may be due to the socioeconomic characteristics of the study participants, as the research was conducted among urban pregnant women who may have greater access to information about anemia. The study's finding aligns with results from similar studies conducted in various regions (28, 29). For instance: A study in Malaysia reported that 98.3% of respondents recognized iron deficiency anemia as the most common type of anemia (30). Similarly, 92.8% were aware of its symptoms, highlighting high levels of knowledge about IDA among participants who had access to education and health information resources. Research in Ethiopia among urban populations demonstrated that educational attainment and access to antenatal care significantly influenced knowledge levels about IDA. Those living in urban areas, often with better access to healthcare information, showed higher awareness and understanding of anemia symptoms and prevention strategies (31). These findings collectively suggest that urban residency and access to healthcare education play crucial roles in increasing awareness of anemia, aligning with your study's observations. This underscores the importance of targeting rural and less-educated populations to bridge gaps in awareness.

Despite this awareness, however, most respondents did not understand the clinical definition of anemia, its causes, or the risk factors associated with IDA. Many defined anemia as a "lack of blood supply" and considered it a "normal, natural" condition during pregnancy that does not harm the mother or child and does not require medical intervention. These views suggest that, despite educational efforts by governmental and non-governmental organizations, pregnant women's perceptions of anemia have changed little over the years (32). The high prevalence of anemia among pregnant women reinforces the perception that "anemia during pregnancy is normal."

Several studies corroborate the finding that pregnant women often have limited understanding of anemia's clinical aspects, including its causes and risks, while considering it a "normal" condition. Research in Indonesia highlighted that pregnant women frequently viewed anemia as a natural part of pregnancy, perceiving symptoms like fatigue and paleness as typical rather than concerning (33). Many lacked awareness of the significance of hemoglobin levels as a diagnostic marker, reflecting minimal understanding of anemia's clinical basis and potential risks. A study in East Sumba, Indonesia, found that while government efforts to promote iron supplementation were robust, many women underestimated anemia's seriousness, perceiving it as a minor inconvenience rather than a health threat to both mother and child (34). This contributed to low compliance with iron supplementation regimens. Similar findings in other developing regions suggest that cultural beliefs and misconceptions reinforce the notion that anemia is a natural condition during pregnancy, not requiring medical attention, leading to underestimation of its complications (35). These studies underscore the need for culturally tailored educational interventions to improve awareness and reshape perceptions about anemia's risks and prevention strategies during pregnancy.

Respondents consistently noted that a healthy diet is the best preventive method for anemia. However, they did not consistently adhere to proper nutritional practices, with unstable daily intake of essential foods. Although they were somewhat aware of iron-containing foods, most were unaware that iron deficiency is the underlying cause of anemia. Additionally, none of the pregnant women were familiar with foods containing folic acid, often perceiving folic acid solely as a medication. Similar results have been reported in other studies (36,37). Studies have reported findings similar to the observation that while many pregnant women identify healthy diets as key to preventing anemia, their actual dietary practices and knowledge of micronutrients remain inconsistent. For instance, research from Ethiopia indicated that while pregnant women were generally aware of anemia and its prevention, adherence to proper dietary intake and iron-folic acid supplementation was low (38). Many women misunderstood the relationship between specific nutrients like iron and folic acid and their role in anemia prevention, with folic acid often seen as a medication rather than a dietary component. In a study in Indonesia and India, it was observed that nutrition education interventions improved knowledge and adherence

to dietary recommendations, yet significant gaps persisted before the interventions. Women lacked familiarity with food sources rich in iron and folic acid, similar to the findings in our study (39). Additionally, a study in Ethiopia highlighted regional disparities, with low adherence to dietary guidelines despite general awareness of anemia's risks during pregnancy (40). These studies collectively emphasize the need for targeted nutrition education that increases awareness and bridges the gap between knowledge and consistent practice, reinforcing the importance of proper nutrition and supplementation during pregnancy.

While most pregnant women in this study knew about anemia prevention strategies, they did not follow correct dietary patterns. Rising food costs and limited financial resources were major barriers to a healthy diet. A study by Bhutta et al. (2013) emphasized that socioeconomic factors, particularly rising food costs and limited financial resources, significantly affect pregnant women's ability to access iron-rich foods such as red meat, leafy greens, and fortified products (41). These barriers disproportionately impact low-income populations, exacerbating disparities in maternal and neonatal health outcomes.

Cultural factors also influenced dietary habits; some women followed vegetarian diets due to religious or cultural beliefs, reducing iron intake from meat products. Additionally, the responsibility of caring for other family members made it difficult for some women to maintain a balanced diet during pregnancy, highlighting the potential benefits of involving family members in anemia prevention. Other studies, such as one conducted in South Asia (Sharma et al., 2019), explored how cultural practices and dietary restrictions—such as vegetarianism influenced by religious or cultural beliefs—can limit the intake of heme iron (42). Non-heme iron sources, although present, are less bioavailable and require careful meal planning for adequate absorption, a practice not always feasible for all women. Research also suggests that family involvement plays a crucial role in improving maternal nutrition. For instance, a study by Ahmed et al. (2021) demonstrated that engaging family members in nutrition education improved adherence to anemia prevention practices (43). When family responsibilities are shared, women are better able to prioritize their dietary needs.

The findings of our study reinforce the need for multifaceted public health interventions, including:

- Nutrition education campaigns that address cultural sensitivities and promote locally available iron-rich foods.
- Economic support programs like food subsidies to make iron supplements and fortified foods accessible.
- Community-based initiatives that involve family members to foster shared responsibility for maternal nutrition.
- By integrating these approaches, interventions can better address the complex social, economic, and cultural barriers to anemia prevention during pregnancy.

Many pregnant women held misconceptions about anemia prevention methods, believing that proper nutrition alone was sufficient to prevent IDA and that there was no need for iron supplements. They expressed concerns that iron supplements, like other medications, could negatively affect the fetus. Most were unaware of the potential health risks associated with inadequate iron supplementation, which may be due to lower educational levels.

Research has shown that doctors spend limited time counseling pregnant women about IDA. Most women relied on information from the internet, television, or literature, while some received information from gynecologists. Notably, only one of the interviewed women reported receiving counseling on IDA from a family physician, highlighting the limited role of family doctors in IDA prevention.

Conclusion

Despite some knowledge about the causes and prevention of IDA, its prevalence remains high in Georgia. This suggests a lack of connection between knowledge and the healthy behaviors necessary to reduce anemia rates. These findings underscore the need for a multifaceted approach to address IDA, including improved preventive measures, greater involvement of family physicians, and enhanced government recommendations and nutrition programs for pregnant women. Providing free iron supplements to pregnant women in maternity hospitals and outpatient clinics is highly recommended.

While knowledge is essential, it is not sufficient to drive the behavioral changes required to improve health. Increased public engagement and effective policy interventions are crucial in addressing these challenges.

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Conflict of Interest

The authors declare no conflicts of interest.

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References

[1] World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. WHO/NMH/NHD/MNM/111. WHO; 2023: 6. Available from: https://apps.who.int/iris/bitstream/handle/10665/85839/WHO NMH_NHD_MNM_11.1_eng.pdf. (Accessed: 10August2024).

[2] Salman AF, Nori W, Hussein ZA, Kassim MAK, Pantazi AC. Evaluation of Red Cell Distribution Width and Platelet Distribution Width as a Predictor of Iron Stores; A Comparative Cross-Sectional Study. Al-Kindy College Medical Journal. 2024;20(2):111–116. https://doi.org/10.47723/nv4jgv77

[3] Sappani M, Mani T, Asirvatham ES, Joy M, Babu M, Jeyaseelan L. Trends in prevalence and determinants of severe and moderate anaemia among women of reproductive age during the last 15 years in India. PLoS One. 2023;18(6):e0286464. doi: 10.1371/journal.pone.0286464. https://doi.org/10.1371/journal.pone.0286464

[4] Sripaew S, Kongkamol C. Health Problems among Disciplines at a Regional University in Southern Thailand: A

Descriptive Study. J Health Sci Med Res. 2020;38(2):103-114.

[5] Verulava T, Devnozashvili R. Nutrition and academic performance among adolescences. Rom J Diabetes Nutr Metab Dis. 2021;28(3):275–283.

[6] Syarif AL, Ansariadi A, Wahiduddin W, Wijaya E, Amiruddin R, Citrakesumasari C, et al. Awareness and practices in preventing maternal iron deficiency among pregnant women living in urban slum areas in Makassar City, Indonesia. J Educ Health Promot. 2024;12:452. https://doi.org/10.4103/jehp.jehp_551_23

[7] Georgieff MK. Iron deficiency in Pregnancy. Am. J Obstet Gynecol. 2021;223 (4):516-534. https://doi.org/10.1016/j.ajog.2020.03.006

[8] Santhakumar S, Athiyarath R, Cherian AG, Abraham VJ, George B, Lipiński P, et al. Edison ES. Impact of maternal iron deficiency anemia on fetal iron status and placental iron transporters in human pregnancy. Blood Cells Mol Dis. 2023;99:102727. https://doi.org/10.1016/j.bcmd.2023.102727

[9] Safarzadeh S, Banihashemi F, Montazeri F, Roozbeh N, Darsareh F. Maternal and Neonatal Outcomes of Iron Deficiency Anemia: A Retrospective Cohort Study. Cureus. 2023;15(12):e51365. https://doi.org/10.7759/cureus.51365

[10] Wang KW, Ling ZJ, Yuan Z, Zhang J, Yi SJ, Xiong YW, et al. The Long-Term Effect of Maternal Iron Levels in the Second Trimester on Mild Thinness among Preschoolers: The Modifying Effect of Small for Gestational Age. Nutrients. 2023; 15(18):3939. https://doi.org/10.3390/nu15183939

[11] Shand AW, Kidson-Gerber GL. Anaemia in pregnancy: a major global health problem. Lancet. 2023;401: 1550-1551. Epub 20230421. https://doi.org/10.1016/S0140-6736(23)00396-3

[12] Smith C, Teng F, Branch E, Chu S, Joseph KS. Maternal and Perinatal Morbidity and Mortality Associated With Anemia in Pregnancy. Obstet Gynecol. 2019;134(6):1234-1244. https://doi.org/10.1097/AOG.0000000000003557

[13] World Health Organization, Global Nutrition Targets 2025: Anemia Policy Brief (WHO/NMH/NHD/14.4), 2014, https://www.who.int/publications/i/item/WHO-NMH-NHD-14.4

[14] Pasricha SR, Mwangi MN, Moya E, Ataide R, Mzembe G, Harding R, et al. Ferric carboxymaltose versus standard-of-care oral iron to treat second-trimester anaemia in Malawian pregnant women: a randomised controlled trial. Lancet 2023;401(10388):1595-1609. https://doi.org/10.1016/S0140-6736(23)00278-7

[15] Skhvitaridze N, Gamkrelidze A, Manjavidze T, Brenn T, Anda E, Rylander C. Anemia during pregnancy and adverse maternal outcomes in Georgia – a birth registry-based cohort study. medRxiv 2023;11(10):23298382; https://doi.org/10.1101/2023.11.10.23298382

[16] World Bank. Prevalence of anaemia among pregnant women (%). WB; 2023. Available from: https://databank.worldbank.org/reports.aspx?source=2&series=SH.PRG.ANEM&country=

[17] World Health Organization. WHO recommendations on antenatal care for a positive pregnancy experience. Summary. 2018. Available from: https://apps.who.int/iris/bitstream/handle/10665/259947/WHO-RHR-18.02-eng.pdf

[18] Serbesa ML, Iffa MT. Pregnant women's knowledge, attitude and practice regarding the prevention of iron deficiency anemia among Ethiopian pregnant women. Caspian J Reprod Med. 2018;4 (1), 1-7.

[19] Asatiani M, Verulava T. Georgian Welfare State: Preliminary Study Based on Esping-Andersen's Typology. Economics and Sociology. 2017;10 (4):21-28. https://doi.org/10.14254/2071-789X.2017/10-4/2

[20] Verulava T. Barriers to effective communication between family physicians and patients in Georgia. J Family Med Prim Care. 2023; 25(1): 80–85.

[21] Verulava T, Jorbenadze A. Primary health care reforms in Georgia: the experience and challenges. Arch Balk Med Union. 2022;57(4):384-389. https://doi.org/10.31688/ABMU.2022.57.4.07.

[22] Verulava T. Factors influencing medical students' choice of family medicine. J Family Med Prim Care. 2022;24(1):66-70. https://doi.org/10.5114/fmpcr.2022.113017

[23] Verulava T, Jorbenadze R, Karimi L. Patients' perceptions about access to health care and referrals to family physicians in Georgia. Arch Balk Med Union. 2020; 55(4): 642-650. https://doi.org/10.31688/ABMU.2020.55.4.11

[24] Verulava T, Dangadze B, Jorbenadze R, Lordkipanidze A, Karimi L, Eliava E, et al. The Gatekeeper Model: patient's view on the role of the family physician. J Family Med Prim Care. 2020; 22(1): 75–79, https://doi.org/10.5114/fmpcr.2020.92511

[25] Verulava T, Beruashvili D, Jorbenadze R, Eliava E. Evaluation of patient referrals to family physicians in Georgia. J Family Med Prim Care 2019; 21(2):180-183; https://doi.org/10.5114/fmpcr.2019.84555

[26] Verulava T. Health Capital, Primary Health Care and Economic Growth. East J Med 2019; 24 (1): 57 – 62. https://doi.org/10.5505/ejm.2019.19327

[27] Verulava T, Barkalaia T, Chiladze G. Work Motivation and Job Satisfaction among Primary Healthcare Workers in Georgia. Hospital Topics 2024;1–9. https://doi.org/10.1080/00185868.2024.2383909

[28] Al-Sattam Z, Hassan S, Majeed B, Al-Attar Z.Knowledge about anemia in pregnancy among females attending primary health care centers in Baghdad. Maced J Med Sci 2022;10(B):785-792.

[29] Appiah PK, Nkuah D, Bonchel DA.Knowledge of and adherence to anaemia prevention strategies among pregnant

women attending antenatal care facilities in Juaboso district in Western-north region, Ghana. J Pregn. 2020;2020:2139892.

[30] Huong C, Chua JL, Ng RY, Panse DK, Misra S, Sumera A. Knowledge, attitude and practices (KAP) towards anaemia among female university students in Malaysia: A cross-sectional survey. Malaysian Journal of Nutrition 2022;28(2):203-215. https://doi.org/10.31246/mjn-2021-0067

[31] Mengistu GT, Mengistu BK, Gudeta TG. et al. Magnitude and factors associated with iron supplementation among pregnant women in Southern and Eastern Regions of Ethiopia: Further Analysis of mini demographic and health survey 2019. BMC Nutr 2022;8, 66. https://doi.org/10.1186/s40795-022-00562-3.

[32] Klankhajhon S., Pansuwan K., Klayjan K., Thojampa S., Nensat N. Perspectives of Pregnant Women Regarding Iron Deficiency Anemia. Jurnal Ners 2021; 16 (2):119-127.

[33] Darmawati D, Siregar TN, Kamil H. Exploring Indonesian mothers' perspective on anemia during pregnancy: A qualitative approach. Enfermería Clínica 2022; 32(1):S31-S37. https://doi.org/10.21203/rs.3.rs-15771/v1.

[34] Kody MM, Landi M, Gunawan YE, Sukartiningsih MCE, Kambuno NT. Mother's Perception of Anemia and Compliance of Iron Tablet Consumption during Pregnancy. Open Access Maced J Med Sci. 2021;9(E):535-9.

[35] Aden C, Pandin MGR, Nursalam N. Anemia in Pregnant Women as a Cultural Phenomenon: A Literature Review. medRxiv 2023.12.22.23300423; https://doi.org/10.1101/2023.12.22.23300423

[36] Balcha WF, Eteffa T, Arega Tesfu A, Abeje Alemayehu B. Maternal Knowledge of Anemia and Adherence to its Prevention Strategies: A Health Facility-Based Cross-Sectional Study Design. Inquiry. 2023; 60:469580231167731.

[37] Margwe JA, Lupindu AM.Knowledge and attitude of pregnant women in rural Tanzania on prevention of Anaemia. Afr J Reprod Health 2018;22(3):71-79.

[38] Engidaw MT, Lee P, Fekadu G, Mondal P, Ahmed F. Effect of Nutrition Education During Pregnancy on Iron–Folic Acid Supplementation Compliance and Anemia in Low- and Middle-Income Countries: A Systematic Review and Meta-analysis. Nutrition Reviews 2024; nuae170, https://doi.org/10.1093/nutrit/nuae170

[39] Anato A, Reshid M. Effect of Nutrition Education and Iron-folic acid supplementation on anemia among Pregnant Women: A quasi-experimental study, 15 February 2024, PREPRINT available at Research Square https://doi.org/10.21203/rs.3.rs-3943153/v1.

[40] Negesse Y, Temesgen H, Woyraw W, Alie MS, Negesse A. Magnitude and factors associated with iron supplementation among pregnant women in anemia hot spot regions of Ethiopia: Multilevel analysis based on Bayesian approach. PLoS ONE 2024;19(11): e0313116. https://doi.org/10.1371/journal.pone.0313116

[41] Bhutta ZA, Ahmed T, Black RE, et al. What Works? Interventions for Maternal and Child Undernutrition and Survival. The Lancet, 2013;371:417-440. https://doi.org/10.1016/S0140-6736(07)61693-6

[42] Sharma S, Sharma RK, Parashar A. Comparison of the nutritional status and outcome in thermal burn patients receiving vegetarian and non-vegetarian diets. Indian Journal of Plastic Surgery 2014;47(2):236-241.

[43] Lowery CM, Craig HC, Litvin K, Dickin KL, Stein M, Worku B, Martin SL. Experiences Engaging Family Members in Maternal, Child, and Adolescent Nutrition: A Survey of Global Health Professionals. Curr Dev Nutr. 2022;6(2):nzac003. https://doi.org/10.1093/cdn/nzac003Yyyyyy

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Research Article

Assessment of Salivary Neutrophil Gelatinase-Associated Lipocalin Levels in Diseased and Healthy Periodontium

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ABSTRACT

Background: periodontal disease is one of the most frequent human diseases. Depending on the severity of the inflammation, the destructive process may affect both the gingiva (gingivitis) and/or the periodontal ligament and alveolar bone that surround and support the teeth (periodontitis).

Objective: The study aimed to determine and compare the level of salivary Neutrophil gelatinase-associated lipocalin in periodontal health and disease (gingivitis and periodontitis stage I-III).

Subjects and Methods: A total of 90 individuals participated in the research separated into five groups, clinically healthy (n=10), gingivitis (n=20), stage I periodontitis (n=20), stage II periodontitis (n=20) and stage III periodontitis (n=20), periodontal parameters were recorded, and the level of salivary Neutrophil gelatinase-associated lipocalin was assessed by using the enzyme-linked immunosorbent assay.

Results: Neutrophil gelatinase-associated lipocalin salivary level was significantly higher ($p<0.05$) in periodontal disease groups compared to healthy controls. A statistically significant difference was also found between gingivitis and advanced periodontitis (stageII-III) ($p<0.05$). No significant difference ($p>0.05$) was found between different stages of periodontitis.

Conclusions: Neutrophil gelatinase-associated lipocalin is associated with periodontal diseases and my play a role in its pathogenesis

Introduction

Periodontal disease is the most common health issue(1), caused by the microorganisms that adhere to teeth as dental plaque. Bacteria interact with each other and with the host. With time, dysbiotic microbiota and dysregulated host inflammation promote the development of certain microorganisms inside the biofilm, generating elements that increase inflammation, resulting in tissue degradation and tooth loss (2, 3).

Periodontal disease is a collection of inflammatory disorders that damage the teeth's attachment apparatus (4). Unlike gingivitis, in periodontitis, tooth-supporting structures are destroyed, and the junctional epithelium migrates apically. Its main characteristics include impairment of supporting periodontal tissue, as evidenced by clinical attachment loss (CAL), periodontal pockets, gingival bleeding, and radiographic evidence of alveolar bone loss. Early diagnosis of periodontal diseases is important because periodontitis

may result in tooth dysfunction and loss, eventually leading to occlusal and aesthetic problems (5, 6).

One of the important inflammatory modifiers during the onset and development of periodontal disease is the proinflammatory cytokines. Neutrophil gelatinase-associated lipocalin (NGAL) is a novel amino acid adipocytokine, that is predominantly expressed by neutrophils and oral epithelial cells that engage in a range of physiological and pathophysiological processes, including metabolic homeostasis, apoptosis, inflammation, infection, and immune response (7, 8).

Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin family and was originally identified as a glycoprotein in complex with matrix metalloproteinase-9 (MMP-9) in human neutrophils. NGAL is thought to play a role in regulating inflammation and antimicrobial defense (9, 10), having the capability to bind iron, fatty acids, prostaglandins, steroids, and matrix metalloproteinases. By sequestering iron-loaded siderophores, NGAL plays an important role in mediating the innate immune response to bacterial infections; it is a neutrophil chemoattractant that promotes their maturation, adhesion, extravasation, and phagocyte capability. In addition to activating regulatory T cells (11, 12).

Among the first leukocytes to aggregate at the site of inflammation are polymorphonuclear neutrophils. Due to their phagocytotic and microbicidal capabilities, these cells are essential as the first line of defense of the innate immune system. Insufficient resolution and incapacity to restore tissue to equilibrium result in neutrophil-mediated damage and persistent inflammation (13, 14).

In vivo experiments revealed that neutrophils exerted from NGAL-deficient mice were incapable of phagocytosing and killing bacteria, incapable of extravasation to sites of infection, and exhibited poor chemotaxis and adhesion (15, 16).

Both gingival crevicular fluid (GCF) and saliva have been found to contain NGAL (17). NGAL has reportedly been identified as a biomarker for patient monitoring and regulating disease activity (18). However, there hasn't been enough research done on the association between this biomarker and periodontal disease.

Saliva is a very effective biological fluid that contain several interesting salivary biomarkers that correlate with the periodontitis clinical parameters. Saliva collection is a quick, painless, and safe procedure (19, 20).

Since the 1999 workshop, researchers have generated significant new findings that spurred the 2017 workshop to propose a new classification system that included periodontitis which mainly depends on staging and grading. Both the severity of the condition upon assessment and the degree of complexity for management are crucial for determining the stage of periodontitis (21).

This study aimed to evaluate and compare salivary NGAL levels in in periodontal health and disease (gingivitis and periodontitis stage I-III).

Subjects and Methods

The ethical committee of the College of Dentistry/University of Baghdad approved this research, project number 448/448606.

Ninety Iraqi participants with age range 22-58, who were systemically healthy with at least 20 teeth participated voluntarily in this analytical observational case control study after a thorough explanation of the study's objectives and were given informed consent in accordance with the Collage of Dentistry in University of Baghdad to sign. The subjects in this study attended the College of Dentistry University of Kufa and the specialized dental center in Al Najaf city from February 2022 to June 2022.

The sample size were based on a recent study (17), With 80% power and a 5% alpha error of probability, The study subjects were divided into five groups following the "2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions"(21): Control group: clinically healthy periodontium (n=10).

Experimental groups: generalized gingivitis group (n=20), stage I periodontitis group (n=20), stage II periodontitis group(n=20) and stage III periodontitis group (n=20).

The periodontally healthy group is characterized by “no clinical attachment loss (Probing pocket depth ≤3 mm, Bleeding on probing <10%, no Radiological bone loss).” Gingivitis group is characterized by “intact periodontium (no clinical attachment loss), probing pocket depths ≤3 mm, and bleeding on probing >30% (generalized).” Stage I periodontitis is characterized by “Interproximal bone loss <15% or 1-2 mm.” Stage II periodontitis is characterized by “Interproximal bone loss involving coronal third of the root.” Stage III periodontitis is characterized by “Interproximal bone loss extending to the mid-third of the root.”

All Periodontitis groups were determined as "generalized (more than 30% of teeth) with unstable condition PPD ≥ 5mm or PPD at ≥ 4mm and BOP" (22).

The inclusion criteria include systemically healthy participant with healthy periodontium, generalized gingivitis, stage I,II and III periodontitis, while Patients with systemic disease, smokers, alcoholics, patients taking any medication affecting values of periodontal parameters, receiving periodontal or antibiotic treatment within the three months prior to the study, receiving orthodontic treatment, and females who are pregnant or taking contraceptive medication were excluded from the study.

Unstimulated whole saliva samples were collected by passive drooling from all participants into plastic containers (CNWTC, Jangsu,China) between 9 and 11 am, according to Navazesh and Kumar (23). Before sample collection, participants were asked to refrain from eating, drinking, chewing gum, and oral hygiene practices for at least one hour. The samples were immediately put in an ice box (Wanmei, Foshan, China), then centrifuged (80-1 Electric Centrifuge, Shanghai, China), and the clear supernatants were kept in eppendrof tubes (CNWTC/OEM, Jiangsu, China) in a deep freezer at -80 degrees Celsius (Angelantoni Life Science, Perugia, Italy) until further examination (24, 25).

All clinical periodontal parameters for all individuals were assessed by a single calibrated examiner.

The following clinical periodontal parameters were assessed: 1) Bleeding on Probing (BOP), which was noted within 30 seconds of probing . 2) Probing Pocket Depth (PPD) which was assessed "from the gingival margin to the base of the pocket." 3) Clinical Attachment Level (CAL), which was assessed "from the cementoenamel junction to the base of the pocket". These measurements were evaluated using a periodontal probe (UNC 15 probe, Premium Instruments, New

York, USA) at six sites for each tooth (26). Plaque index (PI) was determined using a disclosing agent (Co., Ltd., Etoy, Switzerland) at four sites for each tooth (27).

For the biochemical analysis, Human Specific Enzyme-Linked Immunosorbent Assay (ELISA) kits from (bioassay technology laboratory Zhejiang, china) were utilized to determine salivary NGAL levels per the manufacturer's instructions. Salivary samples were thawed and labeled prior to the procedure. Kit's sensitivity values were 2.01ng/ml, with a detection range of 5-600ng/ml. The optical density was measured at 450 nm.

Shapiro–Wilk test was used for the assessment of normality using SPSS version 26.0 (Statistical Package for Social Science), all data revealed normal distribution ($p>0.05$). Data analyses were performed by one-way analysis of variance (ANOVA) followed by Games-Howell post hoc test, and Pearson correlation coefficient was utilized to determine the correlation between variables. P value is significant at level 0.05.

Results

The demographic data of the study groups are shown in table (1). Ninety participants were included in this study with an age range of 22-58, with 54 males (60%) and 36 females (44%).

The mean age for the study groups increases progressively from to the control group stage III periodontitis group.

As for sex, male percentages were higher in all the studied groups (60%, 75%, 65%, 70%) except for stage I periodontitis group (30%).

Analysis of levels of NGAL using one way ANOVA test showed a statically significant difference ($p<0.05$) amongst study groups; further multiple group comparison revealed a statistically significant difference ($P<0.05$) when comparing the experimental groups with Control and when comparing gingivitis with stage II and III groups (Figure 1).

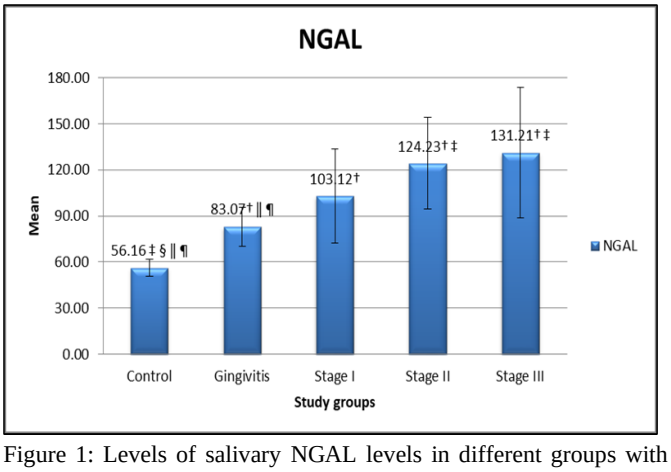

Figure 1: Levels of salivary NGAL levels in different groups with comparison. †: significantly different than Control, ‡: significantly different than Gingivitis, §: significantly different than Stage I, ‖: significantly different than Stage II, ¶ significantly different than Stage III ($p<0.05$).

Regarding the clinical periodontal parameters PI, PPD, BOP and CAL, descriptive statistics are illustrated in table (2).

As for the correlations of periodontal parameter with NGAL, a statistically significant difference was found with a positive correlation between NGAL and CAL in all periodontitis groups($p<0.05$), stage III group also showed a statistically significant difference with a positive correlation between NGAL and PPD($p<0.05$). Control group showed a statistically significant positive correlation between NGAL and BOP (Table 3).

Discussion

NGAL is an acute-phase protein that has gained attention as a possible clinical biomarker for inflammatory diseases. The potential use of NGAL as a biomarker for the onset and monitoring of disease progression is supported by the fact that its levels are typically low in biological fluids and yet are elevated in an inflammatory state (28).

In this case-control study, the level of salivary NGAL in healthy and diseased periodontium were evaluated to investigate the association between this marker and periodontal health and disease. The current study demonstrated a significant increase in salivary NGAL levels in gingivitis and periodontitis in comparison to control group, which is confirmed by its positive association with clinical periodontal parameters (CAL and PPD). NGAL was statistically positively correlated with CAL in all experimental groups. Stage III also showed a statistically positive correlation with PPD, which agrees with Nakajima et al., (29).

NGAL may play a role in the pathogenesis of periodontitis. It stabilizes matrix metalloproteinase 9 (a catabolic enzyme that degrades gelatine, fibronectin, elastin, and type IV, V, VII, and X and the denatured type I collagen) (30) by complexing with it, prolong its activity by preventing its autodegradation and enhancing the collagenolytic activity that is a hallmark of periodontitis (29, 31), which might explain the results of the study. Also, NGAL is produced by extravasated neutrophils, participates in a positive inflammatory feedback loop by promoting chemotaxis, migration of neutrophil, and extravasation to inflammation sites (32).

Regarding the differentiation between healthy controls and periodontitis, NGAL revealed a progressive increase from health to periodontitis groups and showed the capacity to differentiate between healthy controls and periodontitis, in addition to differentiating between gingivitis and periodontitis (stage II and III) with a statistically significant difference, which agrees with Morelli et al., (33) Pradeep et al.,(34) ,Tan et al.,(17) and Ceylan et al.,(32).

Regarding the differentiation between gingivitis and stage I periodontitis, the study results disagreed with Tan et al., (17). As for differentiation between gingivitis and stage III periodontitis, the study results were consistent with Tan et al., (17), while disagreeing with Ceylan et al., (32).

Regarding periodontitis stages, the level of NGAL increases from stage I to stage III but with no statistically significant difference among them, which agrees with Nakajima et al., (29) and disagrees with Tan et al., (17).

Table 1: Demographic parameters of the study population

Parameters	Categorization	Control	Gingivitis	Stage I	Stage II	Stage III
Age	Min.-Max.	26-31	22-55	24-52	23-57	22-58
	Mean±SD	28.7±1.42	33.1±7.9	36.2±8.13	39.3±9.44	43.00±10.22
Sex	Male	6 (60%)	15(75%)	6 (30%)	13 (65%)	14 (70%)
	Female	4 (40%)	5(25%)	14 (70%)	7 (35%)	6 (60%)

Table 2: Descriptive statistics of clinical parameters

Parameters	Categorization	Control	Gingivitis	Stage I	Stage II	Stage III
PI	Mean ± SD	38.7 ±15.52‡§ǁ¶	71.65 ±12.31†	70.03 ±9.56†	74.42 ±10.18†	73.89 ±9.24†
BOP	Mean ± SD	4.06 ±2.59‡§ǁ¶	42.74 ±10.13†	47.33 ±13.57†	49.46 ±13.65†	52.36 ±14.08†
PPD (mm)	Mean ± SD	-	-	3.65 ±0.25ǁ,¶	4.24 ±0.32§,¶	4.71 ±0.45§,ǁ
CAL (mm)	Mean ± SD	-	-	1.56 ±0.25ǁ,¶	2.62 ±0.45§,¶	3.30 ±0.60§,ǁ

†: significantly different than Control, ‡: significantly different than Gingivitis, §: significantly different than Stage I, ǁ: significantly different than Stage II, ¶ significantly different than Stage III ($p<0.05$). PI: Plaque Index, BOP: Bleeding on Probing, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss.

Table 3: Correlations of periodontal parameters with NGAL

Groups	Markers	PI		BOP		PPD		CAL	
		r	p-value	r	p-value	r	p-value	r	p-value
Control	NGAL	0.541	0.439	0.709*	0.022	-	-	-	-
Gingivitis	NGAL	0.232	0.324	0.073	0.759	-	-	-	-
Stage I	NGAL	-0.280	0.232	-0.117	0.623	0.047	0.845	0.448*	0.048
Stage II	NGAL	-0.061	0.800	0.044	0.853	0.329	0.157	0.470*	0.037
Stage III	NGAL	0.148	0.534	0.359	0.120	0.552*	0.012	0.471*	0.036

* statistically significant difference ($p<0.05$)

Saliva is an optimal biological fluid, which can be used to evaluate and diagnose in periodontal disease. There are many benefits to using saliva as a biological fluid. In addition, saliva as a "mirror of the human body" can reflect the physiological and pathological condition of the body. Saliva may be utilized to detect biomarkers specific to the physiological characteristics of periodontal disease because saliva collection is quick, cheap, safe, and non-invasive. (35, 36).

Biomarkers serve a key role in light of the fact that biomarkers are reliable indicators of a variety of diseases (1). The identification of biomarkers is useful for the prevention, diagnosis and prognosis of diseases, as well as for monitoring the progression of pathological disorders (20).

ELISA is reliable, sensitive and specific. Compared to other immunoassays, ELISA provides highly reproducible, quantitative data that makes it an advantageous biotechnological tool in scientific research and clinical diagnosis (37).

Staging comprises four groups (stages I-IV) which depend on several factors like the amount of attachment loss, bone loss percentage, existence and degree of angular bony defects, mobility of teeth, and tooth loss caused by periodontitis (38). The main objectives of staging a patient with periodontitis are to categorize their condition's severity and extent based on the extent of destroyed and damaged tissue that can currently be attributed to the disease, and to evaluate a number of factors that may influence the level of complexity of controlling existing illness and managing both the patient's long-term function and aesthetics of their dentition (22).

The study's findings revealed significant variations in the age distribution between the periodontitis group and the control group, The length of time periodontal tissues have been exposed to bacterial plaque is likely connected to the severity of periodontal disease and bone loss as people age, and is thought to indicate a person's overall oral history (39).

As for sex distribution, the results showed that male percentage was highest in all the experimental groups except stage I periodontitis, This may be related to the oral hygiene ignorance, and poorer oral hygiene habits which is usually observed among males (40).

Further future studies needed to evaluate the cut-off values of salivary NGAL levels in periodontally healthy and diseased patients and using interventional methods (comparing NGAL levels prior to and following non-surgical periodontal treatment) to evaluate its clinical significance in the diagnosis of periodontal disease.

Limitations of the study

The study excluded patients with periodontal diseases associated with risk factors. The study did not include localized gingivitis and stage 4 periodontitis. The usage of saliva does not reflect the specific site of active disease.

Conclusion

Study results suggest that NGAL can be used to differentiate between periodontally healthy and diseased patients, and between gingivitis and moderate to severe periodontitis but failed to distinguish between gingivitis and early state of periodontitis (stage I). CAL was positively correlated with NGAL in all periodontitis groups emphasizing the importance of NGAL's role in the pathogenesis of periodontitis.

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Conflict of Interest

Authors declare no conflict of interest.

Data availability

Data are available upon reasonable request.

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References

[1] Salman ZA, Ghudhaib KK, Fadhil R. Evaluating Osteocalcin and Osteonectin in serum male patients with type 2 Diabetes mellitus and periodontitis. Eurasian Chemical Communications. 2022;4(4):295-302. https://doi.org/10.22034/ecc.2022.325049.1307

[2] Abdulbaqi HR, Himratul-Aznita WH, Baharuddin NA. Anti-plaque effect of a synergistic combination of green tea and Salvadora persica L. against primary colonizers of dental plaque. Archives of oral biology. 2016 Oct 1;70:117-24. https://doi.org/10.1016/j.archoralbio.2016.06.011

[3] Scannapieco FA, Gershovich E. The prevention of periodontal disease—An overview. Periodontology 2000. 2020 Oct;84(1):9-13. https://doi.org/10.1111/prd.12330

[4] Abdulkareem AA. Antibiotics in Periodontal Therapy: To Use or Not To Use?. J Dent Sci. 2018;3(9):000206. http://dx.doi.org/10.23880/oajds-16000206

[5] Ameer LA, Raheem ZJ, Abdulrazaq SS, Ali BG, Nasser MM, Khairi AW. The anti-inflammatory effect of the platelet-rich plasma in the periodontal pocket. European journal of dentistry. 2018 Oct;12(04):528-31. https://doi.org/10.4103/ejd.ejd_49_18

[6] Muhammad EN, Saliem SS, Naaom ER. Serum tumor necrosis factor alpha and high sensitive C-reactive protein as biomarkers in periodontitis in Iraqi patients with osteoarthritis. Journal of Baghdad College of Dentistry. 2018 Sep 15;30(3):7-12. https://doi.org/10.26477/jbcd.v30i3.2524

[7] An HS, Yoo JW, Jeong JH, Heo M, Hwang SH, Jang HM, Jeong EA, Lee J, Shin HJ, Kim KE, Shin MC. Lipocalin-2 promotes acute lung inflammation and oxidative stress by enhancing macrophage iron accumulation. International journal of biological sciences. 2023;19(4):1163. https://doi.org/10.7150/ijbs.79915

[8] Al Jaberi S, Cohen A, D'Souza C, Abdulrazzaq YM, Ojha S, Bastaki S, Adeghate EA. Lipocalin-2: Structure, function, distribution and role in metabolic disorders. Biomedicine & Pharmacotherapy. 2021 Oct 1;142:112002. https://doi.org/10.1016/j.biopha.2021.112002

[9] Bauvois B, Susin SA. Revisiting neutrophil gelatinase-associated lipocalin (NGAL) in cancer: saint or sinner?. Cancers. 2018 Sep 18;10(9):336. https://doi.org/10.3390/cancers10090336

[10] Deng M, Aberle MR, Van Bijnen AA, Van Der Kroft G, Lenaerts K, Neumann UP, et al. Lipocalin-2 and neutrophil

activation in pancreatic cancer cachexia. Frontiers in Immunology. 2023;14
https://doi.org/10.3390/cancers10090336

[11] Nasioudis D, Witkin SS. Neutrophil gelatinase-associated lipocalin and innate immune responses to bacterial infections. Medical Microbiology and Immunology. 2015 Aug;204:471-9. https://doi.org/10.1007/s00430-015-0394-1

[12] Mohammed-Salih HS, Al-Lami HA, Saloom HF, Abdulkareem AA, Al-Obaidi JR, Razali N. Detection of orthodontically induced inflammatory root resorption-associated biomarkers from the gingival crevicular fluid by proteomics analysis: a randomized-controlled clinical trial. 3 Biotech. 2023 May;13(5):157.
https://doi.org/10.1007/s13205-023-03572-5

[13] Kraus RF, Gruber MA. Neutrophils—from bone marrow to first-line defense of the innate immune system. Frontiers in immunology. 2021 Dec 23;12:767175.
https://doi.org/10.3389/fimmu.2021.767175

[14] Ali OH, Raheem ZJ, Imran NK, Ahmed MA. Evaluation of serum levels Superoxide dismutase in women with polycystic ovarian syndrome and gingivitis. Journal of baghdad college of dentistry. 2018 Jun 15;30(2).

[15] Nguyen CT, Nguyen OP. Increased plasma lipocalin-2 levels correlate with disease severity and may be a marker of acute inflammatory response in patients with psoriasis. Dermatology Reports. 2022 Nov 11;14(4).
https://doi.org/10.4081/dr.2022.9469

[16] Liu Z, Petersen R, Devireddy L. Impaired neutrophil function in 24p3 null mice contributes to enhanced susceptibility to bacterial infections. The Journal of Immunology. 2013 May 1;190(9):4692-706.
https://doi.org/10.4049/jimmunol.1202411

[17] Tan A, Gürbüz N, Özbalci Fİ, Koşkan Ö, Yetkin Ay Z. Increase in serum and salivary neutrophil gelatinase-associated lipocalin levels with increased periodontal inflammation. Journal of Applied Oral Science. 2020 Sep 28;28:e20200276.
https://doi.org/10.1590/1678-7757-2020-0276

[18] Alkayali MF, Badria FA, ElBaiomy AA, Youssef JM. Effect of polycaprolactone nanofibers loaded with oxytetracycline hydrochloride and zinc oxide as an adjunct to SRP on GCF lipocalin-2 levels in periodontitis patients: A clinical and laboratory study. Journal of Advanced Periodontology & Implant Dentistry. 2022;14(2):76.
https://doi.org/10.34172/japid.2022.024

[19] Saliem SS. Assessment of alkaline phosphatase, salivary flow rate and salivary potential of hydrogen in relation to severity of chronic periodontitis. Journal of baghdad college of dentistry. 2016;28(3).
https://doi.org/10.12816/0031119

[20] Risteska N, Poposki B, Ivanovski K, Dirjanska K, Ristoska S, Saveski M. Diagnostic and prognostic markers of periodontal disease. prilozi. 2021 Dec 30;42(3):89-95.
https://doi.org/10.2478/prilozi-2021-0039

[21] Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, Garcia R, Giannobile WV, Graziani F, Greenwell H. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Journal of periodontology. 2018 Jun;89:S173-82.
https://doi.org/10.1002/jper.17-0721

[22] Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. Journal of periodontology. 2018 Jun;89:S159-72.
https://doi.org/10.1002/jper.18-0006

[23] Navazesh M, Kumar SK. Measuring salivary flow: challenges and opportunities. The Journal of the American Dental Association. 2008 May 1;139:35S-40S.
https://doi.org/10.14219/jada.archive.2008.0353

[24] Salman SA. Measurement of salivary Immunoglobulin A of participants with a healthy, gingivitis and chronic periodontitis conditions. Journal of Baghdad College of Dentistry. 2015;27(3):120-3

[25] Salim IK, Diajil AR. Assessment of salivary immunoglobulin A, interleu-kin-6 and C-reactive protein in chronic kidney disease patients on hemodialysis and on conservative treatment. Journal of Baghdad College of Dentistry. 2022 Jun 15;34(2):62-73.
https://doi.org/10.26477/jbcd.v34i2.3146

[26] Kawamoto D, Amado PP, Albuquerque-Souza E, Bueno MR, Vale GC, Saraiva L, Mayer MP. Chemokines and cytokines profile in whole saliva of patients with periodontitis. Cytokine. 2020 Nov 1;135:155197.
https://doi.org/10.1016/j.cyto.2020.155197

[27] O'LEARY TJ. The plaque control record. J. periodontol.. 1972;43:38.
https://doi.org/10.1902/jop.1972.43.1.38

[28] Abella V, Scotece M, Conde J, Gómez R, Lois A, Pino J, Gómez-Reino JJ, Lago F, Mobasheri A, Gualillo O. The potential of lipocalin-2/NGAL as biomarker for inflammatory and metabolic diseases. Biomarkers. 2015 Nov 17;20(8):565-71.
https://doi.org/10.3109/1354750x.2015.1123354

[29] Nakajima M, Hosojima M, Tabeta K, Miyauchi S, Yamada-Hara M, Takahashi N, Miyazawa H, Matsuda-Matsukawa Y, Sato K, Sugita N, Komatsu Y. β2-Microglobulin and Neutrophil Gelatinase-Associated Lipocalin, Potential Novel Urine Biomarkers in Periodontitis: A Cross-Sectional Study in Japanese. International Journal of Dentistry. 2019;2019(1):1394678.
https://doi.org/10.1155/2019/1394678

[30] Surlin P, Silosi I, Rauten AM, Cojocaru M, Foia L. Involvement of TSP1 and MMP9/NGAL in angiogenesis during orthodontic periodontal remodeling. The Scientific World Journal. 2014;2014(1):421029.
https://doi.org/10.1155/2014/421029

[31] Bondy-Carey JL, Galicia J, Bagaitkar J, Potempa JS, Potempa B, Kinane DF, Veillard F, Scott DA. Neutrophils alter epithelial response to Porphyromonas gingivalis in a gingival crevice model. Molecular oral microbiology. 2013 Apr;28(2):102-13.

https://doi.org/10.1111/omi.12008

[32] Ceylan M, Erbak Yilmaz H, Narin F, Tatakis DN, Saglam M. Gingival crevicular fluid lipocalin-2 and semaphorin3A in stage III periodontitis: non-surgical periodontal treatment effects. Journal of periodontal research. 2022 Aug;57(4):724-32. https://doi.org/10.1111/jre.12995

[33] Morelli T, Stella M, Barros SP, Marchesan JT, Moss KL, Kim SJ, Yu N, Aspiras MB, Ward M, Offenbacher S. Salivary biomarkers in a biofilm overgrowth model. Journal of periodontology. 2014 Dec;85(12):1770-8. https://doi.org/10.1902/jop.2014.140180

[34] Pradeep AR, Nagpal K, Karvekar S, Patnaik K. Levels of lipocalin-2 in crevicular fluid and tear fluid in chronic periodontitis and obesity subjects. Journal of Investigative and Clinical Dentistry. 2016 Nov;7(4):376-82. https://doi.org/10.1111/jicd.12165

[35] Kaczor-Urbanowicz KE, Martin Carreras-Presas C, Aro K, Tu M, Garcia-Godoy F, Wong DT. Saliva diagnostics–Current views and directions. Experimental Biology and Medicine. 2017 Mar;242(5):459-72. https://doi.org/10.1177/1535370216681550

[36] Cui Y, Yang M, Zhu J, Zhang H, Duan Z, Wang S, Liao Z, Liu W. Developments in diagnostic applications of saliva in human organ diseases. Medicine in Novel Technology and Devices. 2022 Mar 1;13:100115. https://doi.org/10.1016/j.medntd.2022.100115

[37] Hosseini S, Vázquez-Villegas P, Rito-Palomares M, Martinez-Chapa SO, Hosseini S, Vázquez-Villegas P, Rito-Palomares M, Martinez-Chapa SO. Advantages, disadvantages and modifications of conventional ELISA. Enzyme-Linked Immunosorbent Assay (ELISA) from A to Z. 2018:67-115. http://dx.doi.org/10.1007/978-981-10-6766-2_5

[38] Caton JG, Armitage G, Berglundh T, Chapple IL, Jepsen S, Kornman KS, Mealey BL, Papapanou PN, Sanz M, Tonetti MS. A new classification scheme for periodontal and peri-implant diseases and conditions–Introduction and key changes from the 1999 classification. Journal of periodontology. 2018 Jun;89:S1-8. https://doi.org/10.1002/jper.18-0157

[39] AlJehani YA. [Retracted] Risk Factors of Periodontal Disease: Review of the Literature. International journal of dentistry. 2014;2014(1):182513. https://doi.org/10.1155/2014/182513

[40] Lipsky MS, Su S, Crespo CJ, Hung M. Men and oral health: a review of sex and gender differences. American journal of men's health. 2021 May; 15(3):15579883211016361. https://doi.org/10.1177/15579883211016361

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Research Article

Free Testosterone, Dihydrotestosterone, and Adiponectin in the Evaluation of Vitamin D Supplementation for Polycystic Ovarian Syndrome: A Metformin Comparative Study

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ABSTRACT

Background: Among women of the reproductive age, the most prevalent endocrine disease with (15-44) percentile is the polycystic ovarian syndrome (PCOS). Last decades, many studies indicated that vitamin D deficiency in women with PCOS may aggravate hyperandrogenism and insulin resistance.

Objectives This study was designed to investigate the role of some biochemical markers and hormonal status in evaluation the efficacy of vitamin D supplementation in treatment of polycystic ovary syndrome.

Subjects and Methods: This cross-sectional study was carried out at Department of Biochemistry, College of Medicine, University of Baghdad. It included: group I: Twenty-five women with PCOS who were treated with metformin and followed for 8 weeks, and group II: Twenty-five women with PCOS who were treated with vitamin D3 and followed for 8 weeks. Serum investigations included measurements of HOMA-IR, 25 hydroxyvitamin D3, adiponectin, androgen indices by ELISA technique

Results: The mean (±SD) values of HOMA-IR, free testosterone, dihydrotestosterone, and adiponectin levels were significantly improved after treatment in both groups of metformin and vitamin D supplement compared with before their treatment ($p < 0.05$). In addition, there was no significant differences after treatment between women treated with metformin and those treated with vitamin D supplement for all the measured parameters ($p > 0.05$). PCOS women treated with vitamin D showed significant negative correlation between SHBG and BMI ($r= -0.976$, $p <0.001$)

Conclusions: Measurements of serum free testosterone, dihydrotestosterone and adiponectin are good indicators for efficacy of vitamin D supplement in improvement of insulin resistance and androgen disturbances of PCOS.

Introduction

Among women of the reproductive age, the most prevalent endocrine disease with (15-44) percentile is the polycystic ovarian syndrome (PCOS), it affects between 20% and 50% of women all over the world (1, 2). The Rotterdam criteria, are the most widely accepted and appropriate, that are internationally used for significant diagnosis of PCOS, are required women to meet two out of the following three criteria: Ultrasonography evidence of polycystic ovaries and/or biochemical or clinical indicators of hyperandrogenism, such as oligo- or anovulation (2, 3).

Noticeably, PCOS is heterogeneous disorder, so women may associate with several of; reproductive (infertility or menstrual dysfunction, or may be pregnancy complications), or endocrine as (acne, hirsutism or hyperandrogenism), or metabolic (glucose intolerance, insulin resistance, compensatory hyperinsulinemia, dyslipidemia, increases the risk of diabetes mellitus type II) and psycho-social (anxiety, depression and poor quality of life) symptoms (1, 3).

Hyperandrogenism and Insulin resistance are considered as the most typical features of the PCOS syndrome, with their adjacent relationship and influences on reproductive function, and the metabolic profile of PCOS patients, irrespective of BMI (4, 5). Insulin receptors that have been found in ovaries promote the ability of insulin for steroidogenesis and ovarian growth, thus result in increased intra ovarian androgen synthesis along with disruption in normal folliculogenesis, resulting in the development of innumerable ovarian cysts and enlarged ovary (6).

Metformin as one of the insulin sensitizers, at a dose of (1700-2000) mg/day with or without lifestyle is widely used as a very effective and adaptive drug for PCOS treatment, due to its efficacy and safety (7, 8).

Metformin beneficial effects are increasing, especially in combined with lifestyle modifications, leading to the improvement of the pathogenetic mechanisms that underlying PCOS, by restoring the ovarian function and also improving the metabolic profile, particularly insulin sensitivity (4, 9). Also, Metformin with and without lifestyle may associated with a significant improvement on BMI and menstrual cycles (10).

Last decades, many studies have revealed women with PCOS frequently associated with vitamin D deficiency, serum 25-hydroxyvitamin D concentrations of <20 ng/ml (11, 12). Adequate vitamin D levels (≥30 ng/ml) should be required in women with PCOS, otherwise deficiency of vitamin D may aggravate hyperandrogenism and insulin resistance (11, 13).

Few investigations have reported that vitamin D deficiency may have a role in the development of metabolic syndrome because vitamin D is essential for the formation of the adrenal cortex hormones. Therefore; the management of PCOS needs treatment of vitamin D deficiency (12, 14). Vitamin D as an oral, relatively safe and almost inexpensive vitamin, may enhance in the improvement of the common ovulation dysfunction in PCOS, by promoting the follicular development and enhance menstruation, and it may be used in all infertile women of childbearing age (15, 16).

Subjects and Methods

This cross-sectional study was carried out at Department of Biochemistry, College of Medicine, University of Baghdad, and at Kamal Al-Samarraei hospital for infertility management and IVF, during the period from September 2022 to June 2023. It included 50 infertile women who priorly diagnosed with polycystic ovary syndrome with age range (18-40 year) and without any related treatment for two months at least. They were diagnosed by Gynecologist after proper physical, biochemical and gynecological examinations and confirmed by ultrasound. According to Rotterdam consensus; the presence of two over three of the following criteria (oligo/anovulation, hyperandrogenism and polycystic ovaries [≥ 12 follicles measuring with diameter of (2-9) mm and/or an ovarian volume more than 10 mL in at least one ovary], would confirm the polycystic ovarian syndrome morphology (17).

All women enrolled in this study were without any infertility related treatment for two months at least. They were classified into two sub-grouped: **Group I:** Twenty-five women who were treated with metformin 850 mg/twice a day and followed for 8 weeks and **Group II:** Twenty-five women who were treated with vitamin D3 50.000 IU/wk. and followed for 8 weeks.

This study excluded women with any type of cancer, acute and chronic illness, DM, chronic liver disease, pregnant women, smokers, endocrine disorders and chronic renal failure.

Blood samples was collected from each included women of the two studied groups after (10-12) hours of overnight fasting state, in the follicular phase between the 2nd and 7th day of the menstrual cycle, before starting of their designed treatment, left to clot for 15 minute, then centrifuged at 2500 rpm for 10 minute to obtain serum that stored in aliquots at -20 °C till the day of measurement of fasting serum glucose, insulin, 25 hydroxyvitamin D3, adiponectin, free Testosterone, dihydrotestosterone, dehydroepiandrosterone-sulfate (DHEA-S) and sex-hormone binding globulin (SHBG) by the quantitative sandwich and competitive enzyme immunoassay technique for the in vitro determination of human serum and plasma. HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) was calculated by equation: HOMA-IR = [insulin (mU/L) * glucose (mg/dl)]/ 405 (18). Weight and height of the included women was measured and the body mass index (BMI) was calculated by equation: BMI (Kg/m^2) = Weight (Kg)/height (m^2) (19). Both ovaries were scanned using transvaginal ultrasound at Kamal Al- Samarraei hospital for infertility management and IVF, in order to determine the total number of early antral follicles using a 6.5 MHz transducer.

Results

The results of the PCOS studied groups revealed that, there were no significant differences between the two groups before metformin or vitamin D treatment regarding to the comparison of demographic, biochemical and hormonal parameters as represent in table (1).

The results of follow up study of PCOS women showed that 18 of women who were treated with metformin and 13 of women who were treated with vitamin D supplement were completed the 8 wk. follow up of their treatment.

Table (2) shows the comparison of demographic, biochemical and hormonal parameters before and after metformin treatment. Women after metformin treatment compared with before showed significantly lower mean values of BMI (27.56 ± 5.30 kg/m^2 vs. 30.76 ± 6.40; $p<0.001$), HOMA-IR (2.84 ± 1.25 vs. 4.37 ± 1.19; $p < 0.001$), free testosterone (3.22 ± 0.42 pg/ml vs. 4.24 ± 0.28; $p < 0.001$) and dihydrotestosterone (450 ± 33.25 pg/ml vs. 496 ± 15.88; $p < 0.001$). On the other hand, there was significantly improved by increasing the mean values of adiponectin (4.25 ± 0.39 ng/ml vs. 2.49 ± 0.29; $p < 0.001$), 25 hydroxyvitamin D (13.63 ± 1.64 ng/ml vs. 12.35 ± 1.65; $p=0.01$), and SHBG levels (17.64± 4.66 ng/ml vs. 14.68 ± 4.67; $p < 0.001$) after treatment compared with before. However, there was no

significant changes in the levels of DHEA-S after metformin treatment ($p > 0.05$).

Table 1: Mean (±SD) values of BMI, 25 hydroxyvitamin, adiponectin and Androgen profile between the studied groups before the treatment

Parameter	Before metformin treatment (n=25)	Before vitamin D treatment (n=25)
BMI (kg/m²) NS	30.76 ± 6.40	28.62 ± 6.15
HOMA-IR NS	4.37 ± 1.19	4.17 ± 1.69
Adiponectin NS (ng/ml)	2.49 ± 0.29	2.54 ± 0.30
25 hydroxyvitamin NS D (ng/ml)	12.35 ± 1.65	12.26 ± 1.61
Free testosterone NS (pg/ml)	4.24 ± 0.28	4.28 ± 0.41
Dihydrotestosterone NS (pg/ml)	496 ± 15.88	498 ± 16.03
DHEA-S NS (ng/ml)	315 ± 7.99	315 ± 7.58
SHBG NS (ng/ml)	14.68 ± 4.67	16.43 ± 5.06

t- test revealed NS: Not significant (p>0.05); BMI: Body mass index, DHEAS: Dehydroepiandrosterone; SHBG: Sex hormone binding globulin

Table 2: Mean (±SD) values of Demographic, Biochemical and Hormonal parameters, before and after metformin treatment (Group 1) patients

Parameter	Before metformin treatment (n=25)	After metformin treatment (n=18)	p value
BMI (kg/m²)	30.76±6.40	27.56±5.30	< 0.001 Ŧ S
HOMA-IR	4.37 ± 1.19	2.84 ± 1.25	< 0.001 Ŧ S
Adiponectin (ng/ml)	2.49 ± 0.29	4.25 ± 0.39	< 0.001 Ŧ S
25 hydroxyvitamin D (ng/ml)	12.35±1.65	13.63±1.64	0.01 Ŧ S
Freetestosterone (pg/ml)	4.24 ± 0.28	3.22 ± 0.42	< 0.001 Ŧ S
Dihydrotestosterone (pg/ml)	496± 15.88	450± 33.25	< 0.001 Ŧ S
DHEA-S (ng/ml)	315 ± 7.99	316 ± 7.35	0.122 Ŧ NS
SHBG (ng/ml)	14.68±4.67	17.64±4.66	< 0.001 Ŧ S

BMI: Body mass index, DHEA-S: Dehydroepiandrosterone-sulfate; SHBG: Sex hormone binding globulin; Ŧ: paired sample t test, NS: Not significant (p>0.05); S: Significant ($p \leq 0.05$).

Table (3-3) depicts the comparison of demographic, biochemical and hormonal parameters before and after vitamin D treatment. PCOS women after vitamin D treatment in comparison with before showed significantly lower mean values of BMI (24.75 ± 3.28 kg/m² vs. 28.62 ± 6.15; p<0.007), waist circumference (89.46 ± 8.59 cm vs. 97.04 ± 12.07; p<0.012), HOMA-IR (2.88 ± 1.72 vs. 4.17 ± 1.69; p < 0.001), free testosterone (3.19 ± 0.55 pg/ml vs. 4.28 ± 0.41; p < 0.001) and dihydrotestosterone (450 ± 33.25 pg/ml vs. 496 ± 15.88; p < 0.001).

In addition, there was significantly improvement by elevating the mean values of adiponectin (4.23 ± 0.32 ng/ml vs. 2.54 ± 0.30; p < 0.001) and 25 hydroxyvitamin D levels (18.20 ± 1.93 ng/ml vs. 12.26 ± 1.61; p < 0.001) after vitamin D treatment compared with before treatment. However, there was also no significant changes in the levels of DHEA-S (p=0.472) and SHBG (p=0.196).

Table 3: Mean (±SD) values of Demographic, Biochemical and Hormonal parameters, before and after vitamin D treatment (Group 2) patients

Parameter	Before vitamin D treatment (n=25)	After vitamin D treatment (n=13)	p value
BMI (kg/m²)	28.62 ± 6.15	24.75 ± 3.28	0.007 Ŧ S
HOMA-IR	4.17 ± 1.69	2.88 ± 1.72	< 0.001 Ŧ S
Adiponectin (ng/ml)	2.54 ± 0.30	4.23 ± 0.32	< 0.001 Ŧ S
25 hydroxyvitamin D (ng/ml)	12.26 ± 1.61	18.20 ± 1.93	< 0.001 Ŧ S
Free testosterone (pg/ml)	4.28 ± 0.41	3.19 ± 0.55	< 0.001 Ŧ S
Dihydrotestosterone (pg/ml)	498 ± 16.03	444 ± 15.26	< 0.001 Ŧ S
DHEA-S (ng/ml)	315 ± 7.58	316 ± 4.47	0.388 Ŧ NS
SHBG (ng/ml)	16.43 ± 5.06	20.23 ± 4.56	0.196 Ŧ NS

BMI: Body mass index; DHEA-S: Dehydroepiandrosterone-sulfate; SHBG: Sex hormone binding globulin; Ŧ: paired sample t test, NS: Not significant (p>0.05); S: Significant ($p \leq 0.05$).

Table (3-4) shows that there were non-significant differences in the mean values of the measured and calculated parameters after treatment between the metformin and vitamin D groups including BMI, HOMA-IR and hormonal status (for all, $p > 0.05$).

PCOS women treated with metformin revealed significant negative correlation between HOMA-IR with SHBG (r= -0.593, *p*=0.009), SHBG with BMI (r= -0.880, *p*<0.001) as well as significant positive correlation between HOMA-IR and BMI (r= 0.631, *p*=0.003). while PCOS women treated with vitamin D showed significant negative correlation between SHBG and BMI (r= -0.976, *p* <0.001).

Table 4: Mean (±SD) values of Demographic, Biochemical and Hormonal parameters among the two studied groups after the treatment

Parameters	After metformin treatment (n=18)	After vitamin D treatment (n=13)
BMI (kg/m²) NS	27.56 ± 5.30	24.75 ± 3.28
HOMA-IR NS	2.84 ± 1.25	2.88 ± 1.72
Adiponectin (ng/ml) NS	4.25 ± 0.39	4.23 ± 0.32
Free testosterone NS (pg/ml)	3.22 ± 0.42	3.19 ± 0.55
Dihydrotestosterone NS (pg/ml)	450 ± 33.25	444 ± 15.26
DHEAS (ng/ml) NS	316 ± 7.35	316 ± 4.47
SHBG (ng/ml) NS	17.64 ± 4.66	20.23 ± 4.56

t- test revealed NS: Not significant ($p>0.05$); BMI: Body mass index, DHEAS: Dehydroepiandrosterone; SHBG: Sex hormone binding globulin

Discussion

This study revealed that, the PCOS patients had higher levels of BMI, HOMA-IR, free testosterone, dihydrotestosterone and DHEA-S, On the other hand, there were low levels of; adiponectin, 25 hydroxyvitamin and SHBG, with no significant differences between the studied groups before metformin and vitamin D treatment.

Saleh BO (2015) demonstrated that, there were high levels of BMI and free testosterone among the PCOS groups (20). The higher mean of testosterone level in the PCOS women can prevent regular periods and fertilization even with a small increase (21). Alawad (2018) revealed that, there were mostly deficient or insufficient levels of 25 hydroxyvitamin in PCOS women, which was consistent with this study (22).

A study done by Qasim MN et al., (2022) mentioned that, the mean values of BMI were significantly more significant in the PCOS group, while serum level of vitamin D3 have decreased significantly in PCOS group. They also revealed that, women who suffer from PCOS were more prone to lack vitamin D levels than those without PCOS; resulting that obesity contributes to vitamin D deficiency risk (23).

Teede et al., (2019) demonstrated that there were statistically significant improvements with metformin for fasting insulin, management of weight and metabolic outcomes (reduction of BMI and HOMA-IR) for PCOS women across all BMI categories (24), which was consistent with this study. Witchel et al., (2019) confirmed that metformin uses with and without lifestyle changes in PCOS women resulted in significant and beneficial effects on BMI (10). It has been also reported that therapeutic intervention with metformin in women with PCOS were improved several effects on reducing weight; reduction of BMI and in turn HOMA-IR (25), which was also consistent with this study.

In addition, it has been demonstrated that metformin treatment was associated with significantly increased serum adiponectin concentrations and consequently improvement of metabolic complication (26), which was concordant with this study.

After metformin treatment PCOS women of the present study also improved significantly lower levels of free testosterone and dihydrotestosterone compared with before treatment. On the other hand, there was significantly higher levels of SHBG (table 3-2). Williams et al., (2020) confirmed that metformin can reduce the hyperandrogenic signs and symptoms of PCOS patients by reducing the levels of androgen (2). Other revealed that there was statistically significant difference post-treatment with metformin for testosterone for all participants (24). As well Lashen et al. (2010) mentioned that several effects related to metformin therapeutic intervention in PCOS women as reducing circulating androgen levels with confirmed increased serum levels of SHBG (25). Upon these findings, their results were consistent with this study.

Williams et al., (2020) revealed that vitamin D supplementation for PCOS patients improved insulin resistance and other metabolic profiles (reduction of BMI and HOMA-IR) (2), and a significantly increased in serum 25 hydroxyvitamin D, which was consistent with this study.

However, other revealed that vitamin D supplementation did not change the HOMA-IR in women with PCOS (26). There was improved evidence of the therapeutic uses of Vitamin D in PCOS patients depend on the prognosis of PCOS (27). Vitamin D had an important role in the development of metabolic, endocrine and reproductive abnormalities or dysfunctions in PCOS, it might be mediated through an overall effect on insulin resistance (6, 28).

Vitamin D revealed a significant impact on insulin synthesis via enhancing the expression of insulin receptors and suppressing pro-inflammatory cytokines, thus improving glucose metabolism (6, 27). Though, after vitamin D treatment there were a significant reduction in fasting plasma glucose, insulin resistance with a significant improvement (28).

The present study revealed that PCOS women after vitamin D treatment also improved free testosterone and dihydrotestosterone compared with before treatment. A study by Alomda et al., 2019 confirmed that vitamin D supplementation can help significantly in decreasing the androgenic profile in a woman PCOS (29). The existence of Vitamin D receptors (VDRs) in the granulosa cells and the cumulus oophorus cells in humans and animals supports the assertion that, Vitamin D plays a crucial role in the appropriate regulation of the female reproductive cycle (30). PCOS women with vitamin D supplementation can restore their normal concentration of vitamin D, with disappearance of acne include (31). A study by Xue et al., (2017) mentioned that there was no significant change in DHEA-S levels after vitamin D supplementation in women with PCOS (32).

Conclusion

Serum measurements of free testosterone, dihydrotestosterone and adiponectin along with HOMA-IR and BMI are good indicators for efficacy of vitamin D supplement in treatment of PCOS women. Vitamin D supplements (50.000 IU/wk.) alone can significantly improve metabolic, hormonal, and androgens disturbances that seen in vitamin D-deficient/insufficient PCOS women.

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References

[1] Zhao JF, Li BX, Zhang Q. Vitamin D improves levels of hormonal, oxidative stress and inflammatory parameters in polycystic ovary syndrome: a meta-analysis study. Annals of Palliative Medicine. 2021 Jan;10(1):16983-183. https://doi.org/10.21037/apm-20-2201

[2] Williams A, Babu JR, Wadsworth DD, Burnett D, Geetha T. The effects of vitamin D on metabolic profiles in women with polycystic ovary syndrome: a systematic review. Hormone and Metabolic Research. 2020 Jul;52(07):485-91. https://doi.org/10.1055/a-1160-9902

[3] Deswal R, Narwal V, Dang A, Pundir CS. The prevalence of polycystic ovary syndrome: a brief systematic review. Journal of human reproductive sciences. 2020 Oct 1;13(4):261-71. https://doi.org/10.4103/jhrs.jhrs_95_18

[4] Armanini D, Boscaro M, Bordin L, Sabbadin C. Controversies in the pathogenesis, diagnosis and treatment of PCOS: focus on insulin resistance, inflammation, and hyperandrogenism. International journal of molecular sciences. 2022 Apr 8;23(8):4110. https://doi.org/10.3390/ijms23084110

[5] Pasquali R. Contemporary approaches to the management of polycystic ovary syndrome. Therapeutic advances in endocrinology and metabolism. 2018 Apr;9(4):123-34. https://doi.org/10.1177/2042018818756790

[6] Hamdi RA, Abdul-Qahar ZH, Kadhum EJ, Alsaeed FA. Assessment of Serum Vitamin D Levels in Women with Polycystic Ovary Syndrome. Journal of the Faculty of Medicine Baghdad. 2018 Sep 2;60(2):93-7. https://doi.org/10.32007/jfacmedbagdad.60212

[7] Singh S, Pal N, Shubham S, Sarma DK, Verma V, Marotta F, Kumar M. Polycystic ovary syndrome: etiology, current management, and future therapeutics. Journal of Clinical Medicine. 2023 Feb 11;12(4):1454. https://doi.org/10.3390/jcm12041454

[8] Luque-Ramirez M, Nattero-Chavez L, Ortiz Flores AE, Escobar-Morreale HF. Combined oral contraceptives and/or antiandrogens versus insulin sensitizers for polycystic ovary syndrome: a systematic review and meta-analysis. Human reproduction update. 2018 Mar 1;24(2):225-41. https://doi.org/10.1093/humupd/dmx039

[9] Guan Y, Wang D, Bu H, Zhao T, Wang H. The effect of metformin on polycystic ovary syndrome in overweight women: a systematic review and meta-analysis of randomized controlled trials. International journal of endocrinology. 2020;2020(1):5150684. https://doi.org/10.1155/2020/5150684

[10] Witchel SF, Oberfield SE, Peña AS. Polycystic ovary syndrome: pathophysiology, presentation, and treatment with emphasis on adolescent girls. Journal of the Endocrine Society. 2019 Aug;3(8):1545-73. https://doi.org/10.1210/js.2019-00078

[11] Morgante G, Darino I, Spanò A, Luisi S, Luddi A, Piomboni P, Governini L, De Leo V. PCOS physiopathology and vitamin D deficiency: biological insights and perspectives for treatment. Journal of clinical medicine. 2022 Aug 2;11(15):4509. https://doi.org/10.3390/jcm11154509

[12] Israel NB, Shamdeen MY. The relation between polycystic ovary syndrome and Vitamin D deficiency. Medical Journal of Babylon. 2019 Jul 1;16(3):234-7.

[13] Gokosmanoglu F, Onmez A, Ergenç H. The relationship between Vitamin D deficiency and polycystic ovary syndrome. African health sciences. 2020 Dec 16;20(4):1880-6. https://doi.org/10.4314/ahs.v20i4.45

[14] Menichini D, Forte G, Orrù B, Gullo G, Unfer V, Facchinetti F. The role of vitamin D in metabolic and reproductive disturbances of polycystic ovary syndrome: A narrative mini-review. International Journal for Vitamin and Nutrition Research. 2020 Dec 7. https://doi.org/10.1024/0300-9831/a000691

[15] Mohan A, Haider R, Fakhor H, Hina F, Kumar V, Jawed A, Majumder K, Ayaz A, Lal PM, Tejwaney U, Ram N. Vitamin D and polycystic ovary syndrome (PCOS): A review. Annals of Medicine and Surgery. 2023 Jul 1;85(7):3506-11. https://doi.org/10.1097/ms9.0000000000000879

[16] Zhuang L, Wei CU, Jianxiang CO, Zhang Y. Efficacy of vitamin D combined with metformin and clomiphene in the treatment of patients with polycystic ovary syndrome combined with infertility. Iranian Journal of Public Health. 2019 Oct;48(10):1802.

[17] Rotterdam ES. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81(1):19-25. https://doi.org/10.1016/j.fertnstert.2003.10.004

[18] Lewandowski KC, Skowrońska-Jóźwiak E, Łukasiak K, Gałuszko K, Dukowicz A, Cedro M, Lewiński A. How much insulin resistance in polycystic ovary syndrome? Comparison of HOMA-IR and insulin resistance (Belfiore) index models. Archives of Medical Science. 2019 May 1;15(3):613-8. https://doi.org/10.5114/aoms.2019.82672

[19] World Health Organization, editor. International Statistical Classification of Diseases and related health problems: Alphabetical index. World Health Organization; 2004.

[20] Saleh BO, Ibraheem WF, Ameen NS. The role of anti-Mullerian hormone and inhibin B in the assessment of metformin therapy in women with polycystic ovarian syndrome. Saudi medical journal. 2015 May;36(5):562. https://doi.org/10.15537/smj.2015.5.11112

[21] Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocrine reviews. 2016 Oct 1;37(5):467-520. https://doi.org/10.1210/er.2015-1104

[22] Alawad ZM. Level of follicular fluid vitamin D and embryo quality in a sample of Iraqi women undergoing IVF. Journal of the Faculty of Medicine Baghdad. 2018;60(4):215-21 https://doi.org/10.32007/jfacmedbagdad.604758

[23] Qasim MN, Kadhem HK, El-Yassin HD. CORRELATION BETWEEN VITAMIN D3 DEFICIENCY AND SERUM LEPTIN LEVELS IN A PATIENT WITH POLYCYSTIC OVARY SYNDROME. Biochemical & Cellular Archives. 2022 Oct 1;22(2). http://dx.doi.org/10.51470/bca.2022.22.2.3773

[24] Teede H, Tassone EC, Piltonen T, et al. Effect of the combined oral contraceptive pill and/or metformin in the management of polycystic ovary syndrome: A systematic review with meta-analyses. Clinical Endocrinology. 2019; Volume91, Issue4: 479-489

https://doi.org/10.1111/cen.14013
[25] Lashen H. Role of metformin in the management of polycystic ovary syndrome. Therapeutic advances in endocrinology and metabolism. 2010 Aug;1(3):117-28. https://doi.org/10.1177/2042018810380215
[26] Duan X, Zhou M, Zhou G, Zhu Q, Li W. Effect of metformin on adiponectin in PCOS: A meta-analysis and a systematic review. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2021 Dec 1;267:61-7. https://doi.org/10.1016/j.ejogrb.2021.10.022
[27] Angellotti E, D'Alessio D, Dawson-Hughes B, Nelson J, Cohen RM, Gastaldelli A, Pittas AG. Vitamin D supplementation in patients with type 2 diabetes: the vitamin D for established type 2 diabetes (DDM2) study. Journal of the Endocrine Society. 2018 Apr;2(4):310-21. https://doi.org/10.1210/js.2018-00015
[28] Menichini D, Facchinetti F. Effects of vitamin D supplementation in women with polycystic ovary syndrome: a review. Gynecological Endocrinology. 2020 Jan 2. https://doi.org/10.1080/09513590.2019.1625881
[29] Alomda FA, Hashish MA, El-Naggar WE, Mosallam AM. The effect of vitamin D supplementation on the androgenic profile in patient with polycystic ovary syndrome. The Egyptian Journal of Hospital Medicine. 2019 Apr 1;75(3):2461-6. https://dx.doi.org/10.21608/ejhm.2019.30963
[30] Mohan A, Haider R, Fakhor H, Hina F, Kumar V, Jawed A, Majumder K, Ayaz A, Lal PM, Tejwaney U, Ram N. Vitamin D and polycystic ovary syndrome (PCOS): A review. Annals of Medicine and Surgery. 2023 Jul 1;85(7):3506-11. https://doi.org/10.1097/ms9.0000000000000879
[31] Sulaiman EA, Dhiaa S, Merkhan MM. Overview of vitamin D role in polycystic ovarian syndrome. MMSL. 2022 Mar 4;91(1):37-43.
[32] Xue Y, Xu P, Xue K, Duan X, Cao J, Luan T, Li Q, Gu L. Effect of vitamin D on biochemical parameters in polycystic ovary syndrome women: a meta-analysis. Archives of Gynecology and Obstetrics. 2017 Feb;295:487-96. https://doi.org/10.1007/s00404-016-4247-y

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Research Article

Evaluation of Collagen Triple Helix Repeat Containing-1 protein in Postmenopausal Women with Osteoporosis

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ABSTRACT

Background: The collagen triple helix repeat containing 1 (CTHRC1) protein has been connected to decreased levels of vitamin D and calcium, as well as obesity. This study aimed to investigate the relationship between CTHRC1 and osteoporosis in post-menopausal women and compare with healthy subjects.
Subjects and Methods: A cross-sectional study consisted of 86 women were enrolled in this study and divided into three groups based on the results of dual-energy densitometry (DXA): 30 women with osteoporosis, 30 women with osteopenia, and 26 healthy women. Data on demographic and clinical features and laboratory values of Calcium (Ca) and Vitamin D3 (V.D3) were recorded.
Results: Women with osteoporosis had significantly increased levels of CTHRC1 (P = 0.0001) compare to HS groups. The CTHRC1 showed negative correlation with Ca and V.D3 value at (r= -0.453, P=0.001 and r= -0.415, P= 0.022) respectively in osteopenia patients. CTHRC1 alone show excellent discrimination power (AUC= 0.9) in identifying women with osteoporosis.
Conclusions: Serum CTHRC1 is a valid biomarker that can distinguish women with osteoporosis from HS group with high accuracy. Low calcium and vitamin D levels in this age group may be linked to an increase in the CTHRC1 levels and this could make it a Therapeutic target in future studies.

Introduction

Osteoporosis is a disorder in which the replacement of lost bone mass is not balanced by producing new bone. Mostly, it affects post-menopausal women (1,2).

The drop in estrogen levels during menopause is one of the main risk factors for the development of osteoporosis in women (3). Surgery related to the stomach like bariatric surgery or corticosteroid medication, can interfere with the process of rebuilding bone over time (4).

The human protein has an N-terminal hydrophobic signal peptide of 30 amino acids that commands Collagen triple helix repeat containing-1 protein CTHRC1 to secrete (5,6).

Since CTHRC1 is absent from healthy arteries, it is clear that the protein functions specifically in the healing of wounds and encourages the remodelling of arteries in the event of arterial injury (7,8). Mechanistically, elevated CTHRC1 levels are linked to a marked reduction in the mRNA and protein levels of collagen type I

and type III, which enhance migration and decrease collagen deposition (9,10).

The CTHRC1 dimer or trimer may be encouraged by the CTHR domain. By secreting extra factors, osteoblasts and osteocytes also regulate the differentiation of osteoblasts and osteoclasts (11–13). Significantly, owing to the regulation of osteoblast–osteoclast cross-talk, CTHRC1 has been identified as a critical coupling factor that connects bone resorption to bone formation. Studies on mice have provided unambiguous in vivo evidence of the critical CTHRC1's regulatory function in bone homeostasis. Because of this, it has been shown that decreased bone mass and decreased bone formation in mice with loss of CTHRC1 function are caused by impaired coupling processes. On the other hand, in transgenic animals, overexpression of CTHRC1 stimulated the formation of new bone, increasing bone mass. The exact function of secreted CTHRC1 in bone biology and its cellular source are still up for debate (7,14).

In both calcium homeostasis and bone metabolism, vitamin D is essential. Sufficient consumption of calcium and vitamin D promotes bone health and lowers the risk of developing osteoporosis and osteopenia (15). Therefore, this study aimed to estimate validity of CTHRC1, Ca, and Vitamin D_3 levels in women with osteoporosis, osteopenia and compare them with those in HS.

Subjects and Methods

A cross-sectional study was carried out in this regard from June 2023 to September 2023. This study included 86 subjects in total (divided into three groups based on the results of DXA: 30 women with osteoporosis, 30 women with osteopenia, and 26 seemingly HS at the Yarmouk Hospital. The inclusion criteria werean age range of 56–62 years old and no history of oral corticoid therapy or calcium or vitamin D supplementation within the previous six months. Every participant had to undergo a DXA scan to identify whether they had osteoporosis, osteopenia, or were healthy. The subjects' weight and height were measured.Total body weight divided by height squared was used to calculate the body mass index (BMI) (kg/m2) (16).

The DXA was used to measure the BMD at the lumbar spine (L2-L4) level. The BMD is expressedas g/cm^2. The DXA scan results were displayed as a "T-score," or the standard deviation (SD) of each participant from the young adult mean. By comparing the T-score and Z-score with reference values, the diagnosis of osteopenia and osteoporosis was made in compliance with the WHO criteria and National Osteoporosis Foundation (NOF) guidelines (17). A T-score of less than -1.0 but more than -2.5 at any site was classified as osteopenic, and a T-score of less than -2.5 was classified as osteoporotic (18). On the day of the bone densitometry, blood samples were obtained and transferring the sample into a regular tube and allowing it to clot at room temperature. Subsequently, the serum was extracted by centrifuging the sample at 3000 r.p.m. Serum was divided into aliquots and kept in the hospital at -80 °C until analysis. Serum calcium levels (Linear chemical, Spian) were assessed by colorimetric method, vitamin D levels (Cobas kit, Roche, Germany) were examined using electrochemiluminescent immunoassay, and the levels of CTHRC1 (Mybiosource, USA) kit were ascertained using the ELISA plate reader.

The study was approved by local ethnics Mustansiriyah University/National diabetes Center Ethics Committee and the Baghdad University/ College of Science for Women Ethics Committee. each participant provided informed consent.

Statistical Analysis

The statistical package for the social sciences SPSS (ver. 25), MedCalc (ver. 20.027), and GraphPad Prism (ver. 8) were used for statistical analysis. The conformity of continuous parameters to the normal distribution was evaluated using the Kolmogrov-Smirnov/ Shapiro-Wilk tests. The parameters that did not show normal distribution were expressed as median and interquartile range [IQR] values. Variables of groups were comparedusing Kruskal-Wallis tests. Spearman was used for correlation analysis. The predictive value of CTHRC1 was evaluated by measuring the area under the curve (AUC) in the receiver operating characteristic curve (ROC). The optimal cut-off value was obtained by calculating the Youden index. Significant result was those with p-value equal to or less than 0.05

Results

Characteristic features in patients and healthy subjects

Out of the 86 women who underwent screening, divided Based on the DXA scans, the patients were divided into three groups: 30 women with osteoporosis (T-score ≤ -2.5), 30 women with osteopenia (T-score: -1 to -2.5), and 26 HS (T-score > -1),. Differences between the groups in terms of age were negligible. 60 had low vitamin D levels (≤ 25 mmol/l) in comparison to the HS group. Table 1 illustrates that there were significant differences between the groups in all clinical measures indicating bone resorption, calcium, and vitamin D3.

Serum CTHRC1 in patients and HS groups

The serum CTHRC1 levels in HS and patients with osteopenia orosteoporosis are presented in table 1. The CTHRC1 levels found a statistically significant difference increase between osteoporosis, osteopenia patients and the HS group (P=0.001),with 93.97 (69.41-148.62) ng/ml, 35.34 (27.27-39.09) ng/ml, and 17.75 (16.99-18.16) ng/ml,respectively, as shown in table 1.

Table 1: Comparison of characteristic features between study groups

Parameter	Osteoporosis (N=30)	Osteopenia (N=30)	HS (N=26)	P-value
Age (year) *	58.03 ± 3.057	56.38 ± 4.639	56.92 ± 4.971	0.947
BMI (Kg/m^2) *	28.786 ± 5.427	30.905 ± 4.397	32.23 ± 5.42	0.072
Ca (mg/dl) †	7.99 (7.81-8.91)	8.11 (7.94-8.725)	9.2 (8.51-9.755)	0.0001
D3 (mg/dl) †	6.44 (4.00-9.33)	12.47 (9.83-13.00)	72.13 (57.85-79.63)	0.0001
DXA(T score %)*	-2.8967± 0.504	-1.63 ± 0.341	-0.12 ± 0.072	0.0001

All data are represented as mean±SD * and median (IQR) †.

Table 2: A comparision of serum CTHRC1 levels among osteoporosis, osteopenia and HS group.

Parameter	Osteoporosis (N=30)	Osteopenia (N=30)	HS (N=26)	P-value
CTHRC1 (mg/dl) *	93.97(69.41-148.62)	35.34(27.27-39.09)	17.75 (16.99-18.16)	0.0001

All data is represented as median (IQR) *.

Correlation of CTHRC1 with study parameter

The CTHRC1 showed negative correlation to the Ca and D3 value at (r= -0.453, P=0.001 and r= -0.415, P= 0.022) respectively in osteopenia patients, as shown in Table 2, while the rest of the variables did not show a correlation with CTHRC1 in both groups.

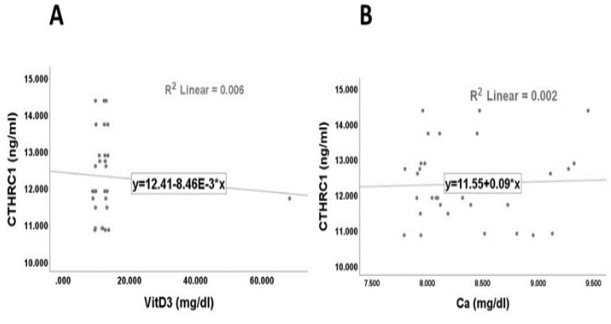

Figure 1: Spearman correlation of serum CTHRC1 in patient study groups, A. show Scatter/Dot between CTHRC1 and V.D3 in osteopenia patients at r= -0.453.P= 0.011. B. show Scatter/Dot between CTHRC1 and Ca in osteopenia patients at r= -0.415, P= 0.022.

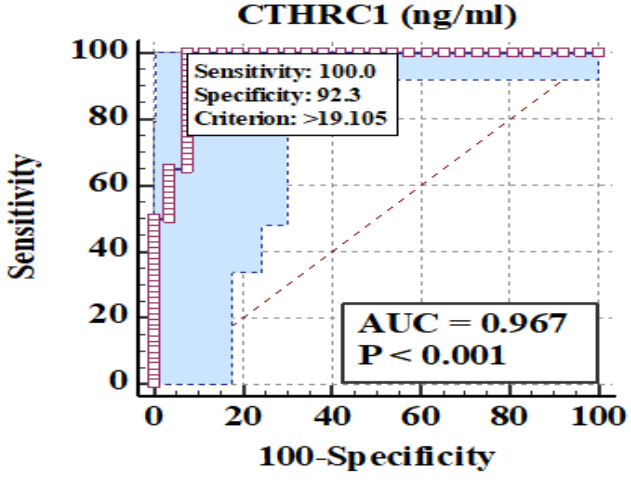

Figure 2: ROC curve for serum CTHRC1 for the prediction of Osteoporosis

ROC curve of CTHRC1 in osteoporosis women

Using the ROC analysis, statistically significant and optimal cut-off values of roughly >19.105 were determined to test the predictive power of the CTHRC1 values in the diagnosis of osteoporosis (Fig. 3, Table 3). The sensitivity to diagnose osteoporosis from the CTHRC1 value was 100% based on the cut-off values and the DXA-defined definition, while the specificity was calculated to be 92.3% at the Youden index equal to 0.92.

Table 3: Criteria to assess the diagnostic power of serum CTHRC1.

Area under the ROC curve (AUC)	0.967
Standard Error	0.0235
95% Confidence interval	0.905 to 0.994
Significance level P (Area=0.5)	<0.0001
Optimal criterion	>19.105
95% Confidence interval	>18.653 to >19.105
Sensitivity	100.00
Specificity	92.31
+PV	96.8
-PV	100.00

Discussion

Bone is a very active and complex tissue and the target of various endogenous and exogenous factors. Osteoporosis is the most common bone disorder, characterised by low BMD (19). Prevention and recognition of osteoporosis are first-step measures to lessen the impact of this condition.

To our knowledge, this is the first study to estimate the role of CTHRC1 in osteopenia and osteoporosis sample of women after menopause,as well as the interaction between this protein and serum calcium and vitamin D.

Bone mass is controlled by continuous remodelling, which is based on the balance between osteoblastic bone formation and osteoclastic bone resorption (20,21).

In our study the level of calcium was also lower in the osteoporosis group than the osteopenia group in comparison to the control group; this can mainly be attributed to a poor calcium diet, especially during the post-menopausal period, or may be due to a defect in the parathyroid gland, which would need further evaluation. In addition, both groups with osteoporosis or osteopenia had low levels of vitamin D3. Osteoporosis and osteopenia are associated with low bone density, as vitamin D3 plays a vital role in calcium absorption, and insufficient levels of it can lead to weak bones (22,23).

In subjects with osteopenia and normal BMD, the CTHRC1 levels were significantly increased in those with osteoporosis. These results indicate that CTHRC1 functions as a positive regulator of osteoblastic bone formation by promoting bone mass and may play a significant role in the anabolic strategy used to treat osteoporosis. In vivo and in vitro osteoblast proliferation has been demonstrated to be stimulated by CTHRC1, while osteoclast bone resorption remains unaffected (24).

Bone resorption is necessary for the synthesis of CTHRC1 with $PO4^{-3}$ and Ca^{+2} playing a vital roles as regulators of CTHRC1 expression in mature osteoclasts (6,12), there is a correlation between the calcium level and the collagen triple helix. Placing osteoclasts in an environment rich in extracellular calcium and phosphate can help understand the close relationship between CTHRC1 production and osteoclast attachment to calcified tissue (25,26). Here, it might catalyze the drawing of stromal/osteogenic cells toward bone resorption and subsequent triggering of bone formation (11).

Because osteoblasts with high phenotype of bone mass and stimulated formation of bone exhibit CTHRC1 expression, CTHRC1 may be crucial in the identification and management of bone disorders like osteoporosis, even though more research is required to fully understand its involvement in age-related bone loss (6,12).

Information was compared to that of the general population even though a sizable portion of the subjects were supplemented. Low levels of vitamin D have been linked to BMD in a variety of populations; however, this correlation was only discovered when comparing individuals with osteopenia and osteoporosis, presumably due to the high proportion of those who took supplements.

Based on the findings of the present study, neither age, BMI, nor Vitamin D are correlated to CTHRC1 values and cann't be considered predictive markers of osteoporosis.

In the current study, the calcium levels and T scores were significantly correlation with CTHRC1, based on BMD values, indicating that it may be considered an independent predictor of low BMD.

When comparing the osteoporosis and osteopenia groups to the control group, there were significant differences in BMI. All of the subjects' T-scores revealed a significant difference between the osteoporosis and osteopenia subjects. Further, the HS T-scores, which correlated with total BMI, were lowest in the osteoporosis group. One of the key variables influencing BMD is BMI. Women who have attained menopause and have a low BMI are more vulnerable to osteopenia and osteoporosis. It is believed that increased adipose tissue production of oestrogen and elevated mechanical loading are responsible for the protective effect of obesity on bone. So, BMI can be used as an important index of osteopenia to prevent osteoporosis (27).

Despite these encouraging findings, more long-term intervention-based studies are advised to validate and broaden the associations reported in this research to obtaina complete picture of the correlation between CTRHC1 and bone health.

The limitation of this study that it is a cross-sectional study conducted at a single centre, with a limited number of patients might limit the power of the study. In addition, in our study did not report other bone markers. In addition, the study depended on just women. While several studies have focused on various aspects of the topic, none of them deals with this particular idea discussed in present study. Notably, no previous research has been conducted on the same subject; thus, the results could not be compared.

Conclusion

In our study, serum CTHRC1 levels were significantly higher in women with osteoporosis and negatively correlated with Ca, V.D3 in women with osteopenia. These findings suggest a potential association between CTHRC1 and the bone health of post-menopausal women. This suggests the possibility that targeting the CTHRC1 may serve as a treatment strategy against osteoporosis. The ROC showed that the optimum cut-off value for CTRHC1 was less than 19.105 with 100% sensitivity and 92.3% specificity, which establishes the presence of osteoporosis with a high confidence interval and is compatible with the diagnosis. further studies will require large sample size and obtain date from multicentre to better validate this conclusion.

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Conflict of Interest

The authors declare no conflict of interest**.**

Data availability

Data are available upon reasonable request

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References

[1] Choi MH, Yang JH, Seo JS, Kim Y ji, Kang SW. Prevalence and diagnosis experience of osteoporosis in postmenopausal women over 50: Focusing on socioeconomic factors. Moran JM, editor. PLoS One. 2021 Mar 2;16(3):e0248020. https://doi.org/10.1371/journal.pone.0248020

[2] O. Farhan L, M. Farhan A, Al Obaidi S, M. Taha E. A Case-control Study to Determine Metalloproteinase-12 and Lysyl Oxidase Levels in Iraqi women with Osteoporosis. Res J Pharm Technol. 2022 Jun 28;39(1.30):2655–60. https://doi.org/0.52711/0974-360X.2022.00444

[3] Chandran M, Brind'Amour K, Fujiwara S, Ha YC, Tang H, Hwang JS, et al. Prevalence of osteoporosis and incidence of related fractures in developed economies in the Asia Pacific region: a systematic review. Osteoporos Int. 2023 Jun 3;34(6):1037–53. https://doi.org/10.1007/s00198-022-06657-8

[4] Siddiqui JA, Le Henaff C, Johnson J, He Z, Rifkin DB, Partridge NC. Osteoblastic monocyte chemoattractant protein-1 (MCP-1) mediation of parathyroid hormone's anabolic actions in bone implicates TGF-β signaling. Bone. 2021 Feb;143:115762. https://doi.org/10.1016/j.bone.2020.115762

[5] Wu Y, Li D, Li M. Osteoclasts may play key roles in initiating biomaterial-induced ectopic bone formation. Med Hypotheses. 2023 Mar;172:111033. https://doi.org/10.1016/j.mehy.2023.111033

[6] Kim JH, Kim N. Bone cell communication factors provide a new therapeutic strategy for osteoporosis. Chonnam Med J. 2020;56(2):94. https://doi.org/10.4068/cmj.2020.56.2.94

[7] Matsuoka K, Kohara Y, Naoe Y, Watanabe A, Ito M, Ikeda K, et al. WAIF1 Is a Cell-Surface CTHRC1 Binding Protein Coupling Bone Resorption and Formation. J Bone Miner Res. 2018 Aug 1;33(8):1500–12. https://doi.org/10.1002/jbmr.3436

[8] Lefta NA, Abed AY, Abed BA. Estimation of asprosin levels in female Iraqi patients with type 2 diabetes and hypothyroidism. J Med Chem Sci. 2023;6(2):433–9. https://doi.org/10.26655/JMCHEMSCI.2023.2.23

[9] Oleiwi AR, Zgair AK. Estimation levels of CTHRC1and some cytokines in Iraqi patients with Rheumatoid Arthritis. Baghdad Sci J. 2023 Jun 20;20 (3(Suppl.)):0928. https://doi.org/10.21123/bsj.2023.8036

[10] Abed BA, Farhan LO, Dawood AS. Relationship between serum nesfatin-1, adiponectin, resistin concentration, and obesity with type 2 diabetes mellitus. Baghdad Sci J. 2024;21(1):117. https://doi.org/10.21123/bsj.2023.8119

[11] Jin YR, Stohn JP, Wang Q, Nagano K, Baron R, Bouxsein ML, et al. Inhibition of osteoclast differentiation and collagen antibody-induced arthritis by CTHRC1. Bone. 2017 Apr;97:153–67. https://doi.org/10.1016/j.bone.2017.01.022

[12] Takeshita S, Fumoto T, Matsuoka K, Park K ae, Aburatani H, Kato S, et al. Osteoclast-secreted CTHRC1 in the coupling of bone resorption to formation. J Clin Invest. 2013 Sep 3;123(9):3914–24. https://doi.org/10.1172/JCI69493

[13] Hassan HJ, Mohammad TU, Hameed EK. Assessment of Serum Metalloendopeptidase level in Patients with Double Diabetes. AL-Kindy Coll Med J. 2023 Dec 30;19(3):21–5. https://doi.org/10.47723/kcmj.v19i3.999

[14] Saadi MT, Mohammed NUG, Abed BA, Farhan LO, Salman IN. Validity of galactin-3 in acromegaly: comparison with traditional markers. Irish J Med Sci (1971 -). 2024 Mar 23;1–5. https://doi.org/10.1007/s11845-024-03674-w

[15] Liu C, Kuang X, Li K, Guo X, Deng Q, Li D. Effects of combined calcium and vitamin D supplementation on osteoporosis in postmenopausal women: a systematic review and meta-analysis of randomized controlled trials. Food Funct. 2020;11(12):10817–27. https://doi.org/10.1039/D0FO00787K

[16] Lee LJ. Exploring Asian and Asian American Specific Body Mass Index Cut-Points. 2022; Thesis.

[17] Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. World Health Organ Tech Rep Ser. 1994;843:1–129. https://iris.who.int/handle/10665/39142

[18] Amarnath SS, Kumar V, Das SL. Classification of Osteoporosis. Indian J Orthop. 2023;57(Suppl 1):49–54.

[19] Costa N de S da, Lima LS, Galiciolli MEA, Ribeiro DHF, Ribeiro MM, Garica G de PJ, et al. Drug-induced osteoporosis and mechanisms of bone tissue regeneration through trace elements. J Trace Elem Med Biol. 2024 Jul;84:127446. https://doi.org/10.1016/j.jtemb.2024.127446

[20] Omi M, Mishina Y. Roles of osteoclasts in alveolar bone remodeling. genesis. 2022 Sep 27;60(8–9):e23490. https://doi.org/10.1002/dvg.23490

[21] Mohammed NUG, Gorial FI, Khaleel FM, Abed BA, Mutar SA, Farhan LO, et al. Role of Human β-Defensin-3 in Rheumatoid Arthritis: An Observational Single-Center Study. Al-Rafidain J Med Sci (ISSN 2789-3219). 2023 Nov 2;5(1S):S71-75. https://doi.org/10.54133/ajms.v5i1S.289

[22] González-Molero I, Morcillo S, Valdés S, Pérez-Valero V, Botas P, Delgado E, et al. Vitamin D deficiency in Spain: a population-based cohort study. Eur J Clin Nutr. 2011 Mar 22;65(3):321–8. https://doi.org/10.1038/ejcn.2010.265

[23] Assaf RH, Farhan LO. Studying Myostatin as a Diagnostic Indicator in Sera of Iraqi Women with Metabolic Syndrome. Baghdad Sci J. 2023;

[24] Kimura H, Kwan KM, Zhang Z, Deng JM, Darnay BG, Behringer RR, et al. Cthrc1 Is a Positive Regulator of Osteoblastic Bone Formation. Rutherford S, editor. PLoS One. 2008 Sep 9;3(9):e3174. https://doi.org/10.1371/journal.pone.0003174

[25] Šromová V, Sobola D, Kaspar P. A Brief Review of Bone Cell Function and Importance. Cells. 2023;12(21). https://doi.org/10.3390/cells12212576

[26] Abed BA, Salman IN, Hassan EA, Mohammed NUG. Role of stanniocalcin-1 and proenkephalin-A as novel biomarkers in prediction of newly diagnosed type 2 diabetic patients. Int J Diabetes Dev Ctries. 2024 May 20;1–7. https://doi.org/10.1007/s13410-024-01353-1

[27] Gkastaris K, Goulis DG, Potoupnis M, Anastasilakis AD, Kapetanos G. Obesity, osteoporosis and bone metabolism. J Musculoskelet Neuronal Interact . 2020;20(3):372.

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Al-Kindy College Medical Journal (KCMJ)

Research Article

Assessing the Effect of Apremilast on Serum Leptin levels in Obese Patients with Psoriasis

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ABSTRACT

Background: Psoriasis is a common inflammatory condition worldwide, with an average prevalence ranging from 2% to 3%. However, the incidence of psoriasis varies among different ethnic groups and regions. Elevated leptin levels have been associated with increased cellular proliferation, including T-cells. Additionally, high leptin levels may stimulate the synthesis of proinflammatory cytokines such as IL-6 and TNF-α.
Objectives: To evaluate the effect of Apremilast on Leptin in obese psoriatic patients.
Subjects and Methods: Thirty patients with psoriasis were included in This prospective cohort study to measure the levels of serum Leptin by using the ELISA technique, before and after receiving Apremilast.
Result: The present work found the concentration of Leptin before receiving Apremilast was 2.365 ng/ml, and after six months from baseline, it was reduced to 1.933 ng/ml, which was statistically significant ($P<0.05$).
Conclusion: This prospective cohort study provides evidence that Apremilast can decrease elevated Leptin levels in individuals diagnosed with psoriasis. The study observed a 0.42 ng/ml reduction in Leptin levels after 6 months of Apremilast treatment.

Introduction

Psoriasis is a prevalent inflammatory illness, with a worldwide incidence ranging from 2% to 3%. The prevalence of this condition is variable with variation based on different ethnic groups and geographical locations (1). Psoriasis is characterized by the presence of keratinocyte hyper-proliferation and abnormal cell differentiation, leading to epidermal hyperplasia(2). Psoriasis is infiltrated with inflammatory cells, leading to scaling of the skin, as the cells of the skin come to the surface quickly before their complete maturation (3).

Leptin plays a crucial role in the regulation of various physiological processes, including food intake, body mass control, proinflammatory immunological responses, angiogenesis, and lipolysis (4). Leptin, a hormone synthesized by adipose tissue, exhibits proinflammatory properties and serves as a regulator in the T-helper cell response(5). While there is growing evidence to suggest the association between psoriasis and obesity as well as cardiovascular disease, the relationship between psoriasis and leptin remains a topic of debate in the academic literature (6). Individuals diagnosed with psoriasis have a heightened prevalence and incidence

of obesity in comparison to the broader community. Moreover, individuals with severe psoriasis demonstrate an increased risk of obesity when contrasted with those with mild psoriasis (7).

Further research is needed to examine the effectiveness and potential metabolic adverse effects of recently developed biologic medicines through additional prospective studies. It is important to note that Apremilast, a phosphodiesterase 4 inhibitor, has demonstrated the ability to decrease body weight, promote lipolysis, improve insulin sensitivity, and reduce the buildup of adipose tissue in the liver, particularly in those with elevated levels of glycated hemoglobin (8). The administration of Apremilast resulted in a significant decrease in plasma concentrations of crucial cytokines implicated in the development of psoriasis. However, there were no significant changes observed in the levels of IL-23, leptin, adiponectin, apolipoproteins, Th17 cells, or regulatory T-cell populations (9).

The evidence of the association between smoking and the development of PsA among psoriasis patients is unclear and controversial. Some studies detected an inverse association whereas others found a positive association or no effect between smoking and the development of PsA(10). There aren't many studies on how Apremilast affects Leptin and its effects as a treatment for psoriasis sufferers. For that reason, the purpose of this study was to find out how the Apremilast medication affected the Leptin levels in obese psoriatic patients.

Subjects and Methods

This prospective cohort study was conducted at the Dermatology Center, Medical City in Baghdad, Iraq at trial registration no. 133 in 23/1/2022, between November 2021 and December 2022. After a full explanation for each patient about the nature of the disease, course prognosis, treatment and complications by a Dermatologist, in addition to the target of this study, formal consent was obtained from each patient before starting the study. Ethical approval was obtained from the Development Department of the Medical City Directorate in Baghdad, Iraq.

The study included a sample of thirty participants who were registered at the outpatient clinic. The patients were provided Apremilast (Aprezo)®, daily after meals, with a time interval of roughly 12 hours, for a duration of six months. Out of the total number of patients included in the trial, a subset of 6 individuals did not successfully finish their participation in the study due to various causes. All patient is assessed the status of obesity by the body mass index (BMI), [BMI= weight (kg) / height (m)²]. According to the international standard measurement of body mass (BMI). Baseline body weight, height and BMI were measured and monitored monthly.

Prior to administering Apremilast, blood samples were collected from all patients at the baseline in order to evaluate Leptin levels. The practical component of the study was conducted at Baghdad Hospital-Medical City Directorate and the International Centre for Research and Development (ICRD). The Human Leptin hormone kit used in this study was obtained from ELK Biotechnology-China. The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference between the two groups in study parameters. T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability).

Results

Sex, Smoking and Age group:

In the present work, sex group was found that nineteen (63.3%) of the participants were males and eleven (36.7%) were females. Moreover, in this work, cigarette smokers only four (13.3%) of the participants were smokers, while twenty-six (86.7%) were non-smokers. In addition, the average age of the group was 38 years. The most common age group was 30-40 years, about 60%, followed by >40 years which was about 26.6%, as illustrated in Table 1.

Table 1: General characteristics of the study sample

	No	Percentage (%)
Sex		
Male	19	63.33
Female	11	36.67
Total	30	100%
P-value	---	0.074 NS
Smoking		
Yes	4	13.33
No	26	86.67
Total	30	100%
P-value	---	0.0001 **
** (P≤ 0.01)		
Age group (year)		
<30 yr.	4	13.33
30-40 yr.	18	60.00
>40 yr.	8	26.67
Total	30	100%
P-value	---	0.0052 **

** (P≤ 0.01), NS: Non-Significant

Table 2: Comparison of Leptin levels and BMI before and after receiving Apremilast

Group	**Mean ± SE**
Leptin (ng/ml)	
Patients: Before treatment	2.365±0.134
Patients: After treatment	1.933±0.075
P-value	0.0114 (P≤0.05)
BMI (kg/m2)	
Patients: Before treatment	32.97 ±1.07
Patients: After treatment	30.48 ±1.14
P-value	0.349

Leptin levels and BMI:

In this study, the concentration of Leptin before receiving Apremilast was found to be 2.365 ng/ml. After six months from

baseline, the concentration was reduced to 1.933 ng/ml, which was a statistically significant difference (P<0.05) as illustrated in Table 2.

The international standard measurement of body mass index was used as an indicator to measure the body mass index of all cases. There was no significant difference (P>0.05) in BMI before and after receiving Apremilast, but after six months of treatment with Apremilast the BMI was reduced by 2.5 Kg/m^2 as shown in Table 2.

Discussion

Psoriasis is the most prevalent chronic inflammatory dermatological condition. This research study represents an important in examining the impact of Apremilast on Leptin levels in individuals diagnosed with psoriasis in Iraq. The findings of this study revealed a significant decrease in serum Leptin levels after a six-month treatment period with Apremilast.

The majority of participants in this study were male, which is in agreement with the findings of *Hägg et al* (11). The observed distribution of psoriasis across males and females shows inconsistency across several studies. Several studies have reported a higher prevalence of psoriasis in males compared to females. However, contrasting findings have also been documented, suggesting comparable incidence rates between the two sexes (12). One study posits that women may exhibit less severe manifestations of psoriasis compared to men. Hence, drawing a definitive conclusion regarding the distribution of psoriasis between males and females is challenging (13).

There is a strong correlation between smoking and the occurrence and severity of psoriasis. Research indicates that individuals who smoke cigarettes long-term are about twice as likely to develop psoriasis compared to those who have never smoked (14). Existing research also shows that current and former smokers have a higher risk of developing psoriasis compared to those who have never smoked (15). Numerous research findings demonstrate a correlation between cigarette smoking, alcohol consumption, and psoriasis severity. Several studies have shown an association between smoking cigarettes and drinking alcohol and increased severity in individuals with psoriasis (16). Furthermore, heavy smokers have over double the likelihood of developing psoriasis compared to non-smokers (17).

Leptin affects appetite and is one of the major adipokines associated with obesity. Leptin also affects immune cells, including dendritic cells (DC), neutrophils, natural killer (NK) cells, and T and B cells, through leptin receptors located on the surface of immune cells, and regulates the production of various cytokines. These signaling patterns induce a wide range of physiological effects by altering immune and inflammatory responses(18). The research conducted by *Yan et al* (19). observed a notable reduction in leptin levels following a 12-week course of Apremilast medication among a cohort of 20 individuals diagnosed with moderate to severe plaque psoriasis. The observed decline exhibited a strong correlation with a decrease in the severity of the condition. Previous research has indicated that the administration of Apremilast could potentially lead to a decrease in leptin levels in individuals with psoriasis, possibly attributable to its anti-inflammatory properties(8). Furthermore, a study by *Gisondi et al* (20). observed a reduction in leptin levels among 18 patients following a 16-week treatment with Apremilast. This decrease in leptin levels was found to be associated with the clinical improvement of psoriatic lesions.

Recent studies suggested that circulating adipokine concentrations are altered in psoriatic patients and are suggested to represent the pathophysiologic link between psoriatic lesions and metabolic alterations. Both leptin and resistin are known to promote the production of pro-inflammatory mediators involved in the pathogenesis of psoriasis, such as TNFα(21). Noteworthy, the phosphodiesterase 4 inhibitor Apremilast has been shown to reduce body weight, enhance lipolysis, increase insulin sensitivity, and reduce the accumulation of adipose tissue in the liver, especially in patients with high glycated hemoglobin(22).

Conclusion

The efficacy of Apremilast as a monotherapy has been demonstrated in reducing Leptin levels, hence conferring advantageous outcomes for individuals suffering with psoriasis. This study provides evidence that supporting the efficacy of Apremilast in reducing Leptin levels among persons who have been diagnosed with psoriasis. The present study showed a decrease in Leptin concentrations during a six-month period of Apremilast treatment, in comparison to the initial values recorded before to the initiation of therapy. The findings suggest that the therapeutic effectiveness of Apremilast in the treatment of psoriasis can be attributable to its capacity to modulate various inflammatory pathways.

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Conflict of Interest

Authors declare no conflict of interest.

Data availability

Data are available upon reasonable request

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References

[1] Caputo V, Strafella C, Termine A, Dattola A, Mazzilli S, Lanna C, et al. Overview of the molecular determinants contributing to the expression of Psoriasis and Psoriatic Arthritis phenotypes. Journal of Cellular and Molecular Medicine. 2020;24(23):13554-63. https://doi.org/10.1111/jcmm.15742

[2] Sabri SA, Ibraheem SR. Comparing the Disease Severity in Iraqi Psoriasis Patients According to Some Immunological and Biological Factors. Iraqi Journal of Science. 2023:2774-85. https://doi.org/10.24996/ijs.2023.64.6.11

[3] AL-Sariay AH, Al-Ahmer SD, Muslim AM, Abood ZH, Haleem H. GENETIC STUDY OF PSORIASIS DISEASE: A REVIEW. Plant Archives. 2021;21(1):2046-8.

https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.335

[4] Obradovic M, Sudar-Milovanovic E, Soskic S, Essack M, Arya S, Stewart AJ, et al. Leptin and obesity: role and clinical implication. Frontiers in endocrinology. 2021;12:585887. https://doi.org/10.3389/fendo.2021.585887

[5] Pluta W, Radecka A, Dudzińska W, Lubkowska A. Adipose tissue and its proinflammatory properties. Journal of Education, Health and Sport. 2020;10(8):138-49. https://doi.org/10.12775/JEHS.2020.10.08.016

[6] Coimbra S, Catarino C, Santos-Silva A. The triad psoriasis–obesity–adipokine profile. Journal of the European Academy of Dermatology and Venereology. 2016;30(11):1876-85. https://doi.org/10.1111/jdv.13701

[7] Armstrong A, Harskamp C, Armstrong E. The association between psoriasis and obesity: a systematic review and meta-analysis ofobservational studies. Nutrition & diabetes. 2012;2(12):e54-e. https://doi.org/10.1038/nutd.2012.26

[8] Paroutoglou K, Papadavid E, Christodoulatos GS, Dalamaga M. Deciphering the association between psoriasis and obesity: current evidence and treatment considerations. Current obesity reports. 2020;9:165-78. https://doi.org/10.1007/s13679-020-00380-3

[9] Strober B, Alikhan A, Lockshin B, Shi R, Cirulli J, Schafer P. Apremilast mechanism of efficacy in systemic-naive patients with moderate plaque psoriasis:pharmacodynamic results from the UNVEIL study. Journal of Dermatological Science. 2019;96(3):126-33. https://doi.org/10.1016/j.jdermsci.2019.09.003

[10] Gazel U, Ayan G, Solmaz D, Akar S, Aydin SZ. The impact of smoking on prevalence of psoriasis and psoriatic arthritis. Rheumatology. 2020;59(10):2695-710. https://doi.org/10.1093/rheumatology/keaa179

[11] Hägg D, Sundström A, Eriksson M, Schmitt-Egenolf M. Severity of psoriasis differs between men and women: a study of the clinical outcome measure psoriasis area and severity index (PASI) in 5438 Swedish register patients. American journal of clinical dermatology. 2017;18:583-90. https://doi.org/10.1007/s40257-017-0274-0

[12] Guillet C, Seeli C, Nina M, Maul LV, Maul J-T. The impact of gender and sex in psoriasis: What to be aware of when treating women with psoriasis. International Journal of Women's Dermatology. 2022;8(2):e010. https://doi.org/10.1097/JW9.0000000000000010

[13] Parisi R, Symmons DP, Griffiths CE, Ashcroft DM. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. Journal of Investigative Dermatology. 2013;133(2):377-85. DOI: 10.1038/jid.2012.339

[14] Naldi L. Psoriasis and smoking: links and risks. Psoriasis: Targets and Therapy. 2016:65-71. https://doi.org/10.2147/PTT.S85189

[15] Dai Y-X, Wang S-C, Chou Y-J, Chang Y-T, Chen T-J, Li C-P, et al. Smoking, but not alcohol, is associated with risk of psoriasis in a Taiwanese population-based cohort study. Journal of the American Academy of Dermatology. 2019;80(3):727-34. https://doi.org/10.1016/j.jaad.2018.11.015

[16] Salihbegovic EM, Kurtalic N, Omerkic E. Smoking cigarettes and consuming alcohol in patients with psoriasis. Materia socio-medica. 2021;33(1):30. https://doi.org/10.5455/msm.2021.33.30-33

[17] Blackstone B, Patel R, Bewley A. Assessing and improving psychological well-being in psoriasis: considerations for the clinician. Psoriasis: Targets and Therapy. 2022:25-33. https://doi.org/10.2147/PTT.S328447

[18] Hwang J, Yoo JA, Yoon H, Han T, Yoon J, An S, et al. The role of leptin in the association between obesity and psoriasis. Biomolecules & Therapeutics. 2021;29(1):11. https://doi.org/10.4062/biomolther.2020.054

[19] Yan D AL, Jeong A, Sandoval M, Maydan A, Liao W. Treatment with apremilast is associated with decreased levels of leptin in patients with moderate-to-severe plaque psoriasis: a pilot study. J Drugs Dermatol. 2015 Aug;14(8):806-11..

[20] Gisondi P, Del Giglio M, Girolomoni G. Considerations for systemic treatment of psoriasis in obese patients. American journal of clinical dermatology. 2016;17:609-15. https://doi.org/10.1007/s40257-016-0211-7

[21] Kyriakou A, Patsatsi A, Sotiriadis D, Goulis D. Effects of treatment for psoriasis on circulating levels of leptin, adiponectin and resistin: a systematic review and meta-analysis. British Journal of Dermatology. 2018;179(2):273-81. https://doi.org/10.1111/bjd.16437

[22] Dalamaga M, Karampela I, Mantzoros CS. Commentary: Phosphodiesterase 4 inhibitors as potential adjunct treatment targeting the cytokine storm in COVID-19. Metabolism. 2020;109:154282. https://doi.org/10.1016/j.metabol.2020.154282

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Al-Kindy College Medical Journal (KCMJ)

Case Report

McKusick-Kaufman Syndrome in an Iraqi Neonate

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ABSTRACT

McKusick-Kaufman (MKS) syndrome is a rare, autosomal recessive disorder determined by a genetic mutation in the MKKS gene on chromosome 20. MKS commonly manifests with three primary components: Polydactyly, congenital heart defects, and genital and urogenital abnormalities. Most reported cases are of Amish descent, with only one Arabic documented case. We present a case of a genetic mutation of MKKS gene in an Iraqi female neonate who presented with repeated non-projectile non-bilious vomiting and abdominal distension. The examination showed a post-axial polydactyly in the hands and feet, an ejection systolic murmur, and an abdominal mass. Perineal examination revealed no vaginal orifice. Diagnosing MKS may necessitate a comprehensive approach due to its rarity and diversity in manifestation. Ruling out Bardet-Biedl syndrome, which shares MKS features, was difficult due to genetic test unavailability; for that patient was kept on follow-up. A high suspicion index, early diagnosis, and customized treatment strategies are essential to address each case's distinct symptoms and requirements, frequently using a multidisciplinary medical team to prevent complications.

Introduction

McKusick-Kaufman syndrome (MKS) is a rare, autosomal recessive disorder, determined by genetic mutation in the MKKS gene on chromosome 20, characterized by a triad of hydrometrocolpos (HMC) in females, post-axial polydactyly, and congenital heart disease (1–3), while in males the main documented genital anomalies are hypospadias, cryptorchidism, and chordee (1). MKS was first described by McKusick in 1964, who reported an inherited form of hydrometrocolpos and polydactyly in a family of Amish descent.

Later, in 1971, Kaufman discovered the presence of congenital cardiac disease, further elucidating the syndrome (2). Since that time, more than 100 cases have been described, most reported cases were in the Amish population. A single previous report of MKS was reported in an Arabic female patient 20 years ago (2).

The clinical diagnosis of MKS relies on a classical diagnostic triad, after excluding other overlapping syndromes, especially Bardet-Biedl syndrome (BBS)(4). MKS diagnosis is suspected in the neonatal period by the clinical evidence of hydrometrocolpos and post-axial polydactyly. However, confirming the diagnosis is often delayed until the age-dependent symptoms of BBS are excluded, which occur around the age of 5 years (5). This makes the documented prevalence of the disorder questionable, as some cases are labeled as MKS at a very young age before the age-dependent features of BBS can be excluded (6). Molecular diagnosis of MKS can be established by finding biallelic pathogenic variants in MKS identified by molecular genetic testing (7) as the locus for MKS syndrome has been mapped to 20p12, close to the jagged1 gene (3).

Although there is a degree of clinical overlap between MKS and BBT syndromes, it is important to note that there are also distinct characteristics that may be used to separate them. BBS is a multifaceted condition that encompasses a broader spectrum of characteristics beyond the commonly shared triad components observed in MKS (4). The other BBS traits encompass retinal degeneration, which has the potential to progress to visual impairment, obesity, compromised cognitive function, and several other endocrine irregularities (4).

There are no published clinical guidelines for the management of patients with MKS, which made the treatment of this disorder tailored to the signs and symptoms expressed by the patients. The therapeutic management begins with surgical repair of the obstructive lesion causing HMC and drainage of the accumulated fluid (8). Treatment for polydactyly and congenital heart defects and other anomalies follow the standards (1).

We present a female newborn with hydrometrocolpos (resulting from vaginal atresia), post-axial polydactyly, and congenital heart disease (atrial septal defect) in an Iraqi neonate diagnosed with McKusick-Kaufman syndrome

Case report

A female neonate born to non-consanguineous marriage at 39 weeks of gestation presented to the emergency room (ER) on the 3rd day of life with repeated non-projectile non-bilious vomiting and abdominal distension since birth. There was no bowel motion since birth and low urine output.

The newborn was vaginally delivered by a midwife at home. The mother received inadequate pre

natal care and did not have a prenatal ultrasound. The family history was negative for congenital disorders. The mother was G4 P3 A1; the abortion resulted from severe abdominal trauma.

On clinical examination, a newborn with weight of (3.5 kg) and length of (51cm) both are normal for her age, normal facial profile, and post-axial polydactyly in hands and feet. The chest exam was not significant apart from ejection systolic murmur with the fixed splitting of second heart sound in the left upper sternal border with no radiation. Abdominal examination reveals distension with a lower mid-line abdominal mass (Figure 1A). Perineal examination revealed no vaginal opening but normal anal and urethral orifices (Figure 1B).

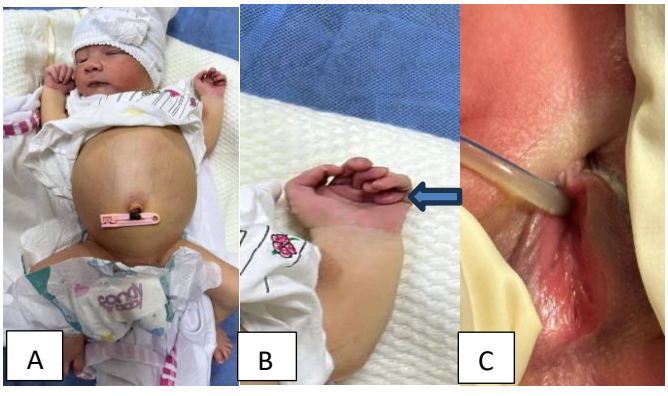

Figure 1: The patient's picture showing A: abdominal distension from the pelvic mass B: postaxial polydactyly in hand (blue arrow). C: absent vaginal orifice.

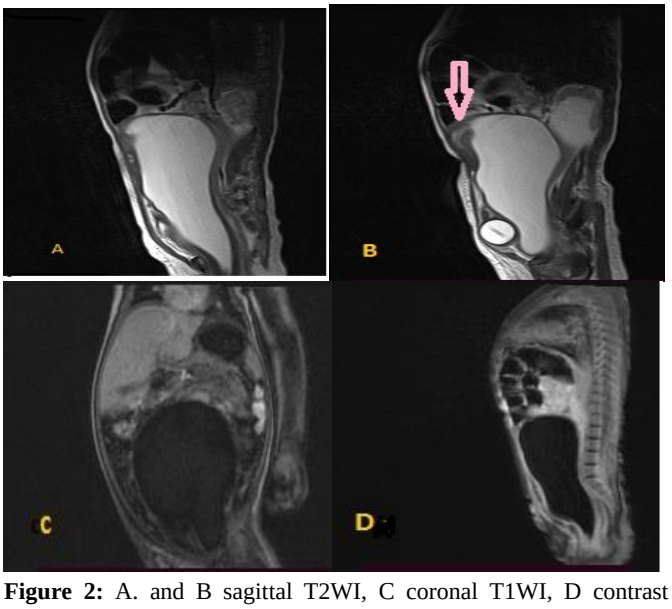

Figure 2: A. and B sagittal T2WI, C coronal T1WI, D contrast enhances sagittal T1 WI images showing that the vagina is massively distended measured (33x80x110 mm) and filled with fluid SI displaying hypointense in T1WI and hyperintense in T2 WI. The uterus (pink arrow) is superiorly and anteriorly displaced by the distended fluid-filled vagina. The urinary bladder is compressed and displaced anteriorly.

Abdominal ultrasound revealed a well-defined, thick wall cystic suprapubic lesion (82 x 41mm) filled with thick turbid fluid. Both kidneys were normally positioned but enlarged with bilateral severe pelvicalyceal system (PCS) dilatation (33 mm) on the right and (30 mm) on the left. Echocardiography showed moderate size atrial septum defect (ASD) secondum (7.8 mm) with a left to right blood flow. Abdominal MRI revealed a massively distended vagina (33 x 80

x 110 mm) filled with fluid, with an anteriorly displaced normal-size uterus, compressing the bladder (Figure 2), and bilateral moderate-severe dilatation in PCS with dilated tortious ureters (Figure 3). Surgery was done on day 4 of life. A cruciate incision was made to create a vaginal orifice, a huge amount of milky fluid was drained, and vaginoplasty was performed.

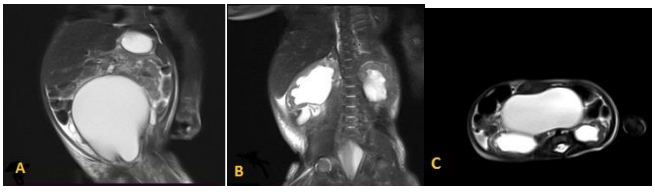

Figure 3: A, B coronal, C axial T2WI images showing that the vagina is a massively distended fluid-filled vagina. B and C show bilaterally severe hydronephrosis and dilated tortuous ureters.

Discussion

McKusick-Kaufman syndrome is rarely reported in Arabic countries, with no previous case documented in Iraq. Only one case was reported earlier in the Arabic population, among Palestinian Bedouin babies, who had the following: Epidemiologically, it was the first case ever reported in an Arabic Bedouin baby at the age of 3 months. The consanguinity marriage was not mentioned in the history. The family history showed an older brother (8 years old) with history of post-axial polydactyly on his 5th Lt hand finger, which was excised shortly following birth; this in contrast to our patients who did not have any family history of polydactyly (2).

We reported an Iraqi female neonate who presented with signs of abdominal mass and gastrointestinal symptoms, vomiting, and absent bowel motions since birth. Physical and imaging examination revealed three clinical features of MKS (hydrocolpos, post-axial polydactyly, and congenital heart disease). Subsequently, the abdominal distension and the mass were drained via cruciate incision and vaginoplasty.

The hydrocolpos has resulted from vaginal atresia that leads to the accumulation of normal secretions under the effect of maternal estrogen. Vaginal atresia is seen in other syndromes like Mayer-Rokitansky-Küster-Hawser, Robinow, and Bardett-Biedl syndrome(9).

Hydrometrocolpos is the most common clinical manifestation of MKS documented in 70% of the affected females. Other studies reported post-axial polydactyly in 60% of cases, and 15% presented with congenital heart disease (5,10,11). Other presentations ranged from hydrops fetalis (12) to malformations involving other systems like renal, respiratory, and gastrointestinal (2,8). So, whenever MKS is suspected, these malformations should be investigated for by the laboratory, radiological, and other diagnostics methods (13).

Prenatal diagnosis is usually suggested by the clinical triad during the second or third-trimester ultrasound (14,15). However, its reliability is questionable as the clinical features of the syndrome may not present prenatally (1) . The early diagnosis of hydrometrocolpos in the affected newborn is vital since the delayed diagnosis may lead to obstructive uropathy and renal failure (16). Moreover, there is the risk of ruptured hydrocolpos, peritonitis, and sepsis leading to death (17,18). Since this sequence of complications can simply be interrupted via the early diagnosis, having a high index of suspicion and increasing pediatricians' awareness about the importance of female newborn genital examination cannot be overstressed (19).

In MKS syndrome, the polydactyly is always reported as post axial type but can affect hands, feet, or both; it may present as a rudimentary skin tag or a complete extra digit (13). Our patient had post-axial polydactyly in both upper and lower extremities.

The presence of congenital cardiac malformation is not consistently observed since a significant number of verified cases have been reported with structurally and functionally normal hearts (20). The etiology of heart problems in MKS remains incompletely elucidated. Scott et al. proposed that a specific allele of the BBS6 gene, known to be mutated in MKS, disrupts a discrete cellular mechanism that plays a role in developing these cardiac abnormalities (21). Heart abnormalities have proven to be a valuable indicator for MKS diagnosis. However, individuals with Bardet-Biedl syndrome have reported heart anomalies with varying rates, as documented in previous studies (22-23).

The dilemma in making a diagnosis of neonate or infant presented with polydactyly -hydrocolpos-congenital heart disease resides in the first place; these features are shared between MKS and BBS and many patients diagnosed as MKS in early life and later turned to be BBS (10,20).

Secondly, the lack of molecular genetic testing for MKS in Iraq adds to the problem. The patient should follow the previous recommendation of the necessity of reevaluation for developing age-dependent features of BBS later in life (10).

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Conflict of Interest

Authors declare no conflict of interest.

Data availability

Data are available upon reasonable request.

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References

[1] Slavotinek, A., Searby, C., Al-Gazali, L., Hennekam, R., Schrander-Stumpel, C., Orcana-Losa, M., Pardo-Reoyo, S., Cantani, A., Kumar, D., Capellini, Q. and Neri, G., 2002. Mutation analysis of the MKKS gene in McKusick-Kaufman syndrome and selected Bardet-Biedl syndrome patients. Human genetics, 110, pp.561-567 https://doi.org/10.1007/s00439-002-0733-3

[2] Kawar B, Sakran W, Chervinsky L, Siplovich L. Unusual presentation of McKusick-Kaufman syndrome in a female Bedouin Arab baby. European journal of pediatric surgery. 2005;15(06):446-8. https://doi.org/10.1055/s-2005-865846

[3] Stone DL, Agarwala R, Schäffer AA, Weber JL, Vaske D, Oda T, et al. Genetic and physical mapping of the McKusick-Kaufman syndrome. Human molecular genetics. 1998;7(3):475-81. https://doi.org/10.1093/hmg/7.3.475

[4] Schaefer E, Durand M, Stoetzel C, Doray B, Viville B, Hellé S, et al. Molecular diagnosis reveals genetic heterogeneity for the overlapping MKKS and BBS phenotypes. European journal of medical genetics. 2011;54(2):157-60. https://doi.org/10.1016/j.ejmg.2010.10.004

[5] Slavotinek AM, Biesecker LG. Phenotypic overlap of McKusick-Kaufman syndrome with Bardet-Biedl syndrome: A literature review. American journal of medical genetics. 2000;95(3):208-15. https://doi.org/10.1159/000486781

[6] Mallmann MR, Reutter H, Mack-Detlefsen B, Gottschalk I, Geipel A, Berg C, et al. Prenatal diagnosis of hydro (metro) colpos: a series of 20 cases. Fetal Diagnosis and Therapy. 2019;45(1):62-8. https://doi.org/10.1159/000486781

[7] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine. 2015;17(5):405-23. https://doi.org/10.1038/gim.2015.30

[8] Hatti RB, Badakali AV, Vanaki R, Samalad MS. Mckusick-kaufman syndrome presenting as acute intestinal obstruction. Journal of Neonatal Surgery. 2013;2(1).

[9] Uğuralp S, Demircan M, Çetin S, Sığırcı A. Bardet Biedl syndrome associated with vaginal atresia a case report. 2003.

[10] David A, Bitoun P, Lacombe D, Lambert J-C, Nivelon A, Vigneron J, et al. Hydrometrocolpos and polydactyly: a common neonatal presentation of Bardet-Biedl and McKusick-Kaufman syndromes. Journal of medical genetics. 1999;36(8):599-603.

[11] Adnan EK, Aymen H EE, Santosh K S. McKusick-kaufman syndrome: a rare case report with review of literature. 2009.

[12] Rosen RS, Bocian ME. Hydrops fetalis in the McKusick-Kaufman syndrome: a case report. American Journal of Obstetrics & Gynecology. 1991;165(1):102-3. https://doi.org/10.1016/0002-9378(91)90234-i

[13] Pul N, Pul M, Gedik Y. McKusick-Kaufman syndrome associated with esophageal atresia and distal tracheoesophageal fistula: A case report and review of the literature. American journal of medical genetics. 1994;49(3):341-3. https://doi.org/10.1002/ajmg.1320490320

[14] Tsai H-F, Wu M-H, Cheng Y-C, Chang C-H, Chang F-M. Prenatal ultrasonography and postnatal follow-up of a case of McKusick-Kaufman syndrome. Taiwanese Journal of Obstetrics and Gynecology. 2014;53(2):241-4. https://doi.org/10.1016/j.tjog.2012.07.042

[15] Traisrisilp K, Nunthapiwat S, Luewan S, Tongsong T. Fetal hydrometrocolpos with pre-axial mirror polydactyly as a new variant of McKusick-Kaufman syndrome. Journal of Clinical Ultrasound. 2021;49(1):62-5. https://doi.org/10.1002/jcu.22882

[16] Dahal GR, Phuyal S, Agrawal P. Symptomatic imperforate hymen in early infancy: a case report. JNMA: Journal of the Nepal Medical Association. 2020;58(226):433. https://doi.org/10.31729/jnma.4922

[17] Murthy V, Costalez J, Weiner J, Voos K. Two neonates with congenital hydrocolpos. Case reports in pediatrics. 2013;2013. https://doi.org/10.1155/2013/692504

[18] Adam A, Hellig J, Mahomed N, Lambie L. Recurrent urinary tract infections in a female child with polydactyly and a pelvic mass: consider the McKusick-Kaufman syndrome. Urology. 2017;103:224-6. https://doi.org/10.1016/j.urology.2017.01.024

[19] Glavan N, Haller H, Brnčić–Fischer A, Glavan-Gačanin L, Miletić D, Jonjić N. Imperforate hymen presenting as vaginal cyst in a 16-month-old child–considerations for an early diagnosis. Scottish Medical Journal. 2016;61(1):48-50. https://doi.org/10.1177/0036933015615263

[20] Halim A, Afzal T, Fatima S, Riaz S. A newborn with rare McKusick syndrome. J Coll Physicians Surg Pak. 2018;28(6):S140-S2. https://doi.org/10.29271/jcpsp.2018.06.s140

[21] Scott CA, Marsden AN, Rebagliati MR, Zhang Q, Chamling X, Searby CC, et al. Nuclear/cytoplasmic transport defects in BBS6 underlie congenital heart disease through perturbation of a chromatin remodeling protein. PLoS genetics. 2017;13(7):e1006936. https://doi.org/10.1371/journal.pgen.1006936

[22] Chitayat D, Hahm SY, Marion RW, Sachs GS, Goldman D, Hutcheon RG, et al. Further delineation of the McKusick-Kaufman hydrometrocolpos-polydactyly syndrome. American Journal of Diseases of Children. 1987;141(10):1133-6. https://doi.org/10.1001/archpedi.1987.04460100111042

[23] Elbedour K, Zucker N, Zalzstein E, Barki Y, Carmi R. Cardiac abnormalities in the Bardet-Biedl syndrome: Echocardiographic studies of 22 patients. American journal of medical genetics. 1994;52(2):164-9. https://doi.org/10.1002/ajmg.1320520208

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Al-Kindy College Medical Journal (KCMJ)

Commentary

Metagenomics: An Unbiased Tool for Understanding and Preventing Pandemics

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ABSTRACT

Metagenomics is a branch of genomics that involves sequencing genetic material directly from environmental samples. These data could facilitate evidence-based decisions by providing a more complete view of the microbial ecosystem in a pandemic setting. It would provide essential knowledge for future pandemic preparedness programs.

Metagenomics is a branch of genomics that involves sequencing genetic material directly from environmental samples. By focusing on single organisms and studying the combined genomes of all microorganisms within a sample (1). It offers a holistic view of the microbial community in an environment. Metagenomics has revolutionized our understanding of microbial communities by enabling the assessment of genetic diversity and functional capacity of entire microbiomes, obviating the need to pure culture single bacteria strains in the laboratory (2). One well-documented example is the role of metagenomics in responding to a pandemic, tracking microbial communities through culture-independent techniques that are holistic and can study all microbes at once. We explored the role of metagenomics in pandemic understanding and prevention in this short article.

We can apply metagenomics as a tool of epidemiological surveillance to characterize strains genetic diversity and relatedness in different environments, enhancing the understanding inherited or adapted equilibria of traits governing dynamics transmission within-population (within-host) and between-population (between-host) level processes. It also elucidates the source of an outbreak (wildlife, location, or host species) (3). Scientists can utilize metagenomics — the direct sequencing of genetic material from clinical samples such as blood, saliva or respiratory secretions — to identify and characterise new infections. It also helps determining if the outbreak was due to new or modified viruses (1). The quasispecies genetic diversity of a virus population can also be characterized by metagenomics, which is important to track the evolution of the virus and potentially anticipate changes in transmissibility or virulence (1).

Additionally, it helps in detecting potential zoonotic disease reservoirs and spillover events before they occur, as metagenomics can be performed on environmental specimens. (3). Metagenomics can also be used for the detection of antimicrobial resistance genes from microbial communities, providing insights into the prevalence and spread of resistance in both pathogenic as well as non-pathogenic bacteria (5). Conserved regions of pathogen genomes are targeted for metagenomic vaccine development and since antigens that elicit immune responses can sometimes indicate promising vaccine targets, that helps determine what they might use to build a vaccine. (6).

In conclusion, these metagenomic data facilitates evidence-based decisions by providing a more complete view of the microbial ecosystem in a pandemic setting. It is a powerful weapon in the pandemic response arsenal with information on pathogen identification, epidemiology, antibiotic resistance, diagnostics, vaccine development, and environmental monitoring as well as public health policy in general. Further, metagenomic studies would provide critical knowledge for future pandemic preparedness programs.

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References

[1] Nooij S, Schmitz D, Vennema H, Kroneman A, Koopmans MPG. Overview of Virus Metagenomic Classification Methods and Their Biological Applications. Front Microbiol. 2018; 9:749. https://doi.org/10.3389/fmicb.2018.00749

[2] Sawyer A, Free T, Martin J. Metagenomics: preventing future pandemics. Biotechniques. 2021 Jan;70(1):1-4. https://doi.org/10.2144/btn-2020-0166

[3] von Fricken ME, Melendrez MC, Linton YM, Takhampunya R. Editorial: Metagenomics for epidemiological surveillance in One Health. Front Microbiol. 2023; 14:1191946. https://doi.org/10.3389/fmicb.2023.1191946

[4] Pillay S, Calderón-Franco D, Urhan A, Abeel T. Metagenomic-based surveillance systems for antibiotic resistance in non-clinical settings. Front Microbiol. 2022; 13:1066995 https://doi.org/10.3389/fmicb.2022.1066995

[5] Govender KN. Precision Pandemic Preparedness: Improving Diagnostics with Metagenomics. J Clin Microbiol. 2021;59(6): e02146-20 https://doi.org/10.1128/jcm.02146-20

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Letter to Editor

Addressing Psychoactive Drug Use in Iraq: Embracing Challenges and Seizing Opportunities

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ABSTRACT

Drug use disorder is a global health issue and one of the leading causes of premature deaths and disability. There is an increase recognition of the need for a public health rather than criminal justice approach to mitigate the current health burden and prevent future health loss

Drug use disorder is a global health issue and one of the leading causes of premature deaths and disability. There is an increase recognition of the need for a public health rather than criminal justice approach to mitigate the current health burden and prevent future health loss. The issue stems from social and environmental challenges that are beyond individual control. Treatment for illicit drug use is not simple; it is a chronic health condition that may require long-term treatment. Patients often have lifetime vulnerabilities. Additionally, limited access to treatment is a common challenge that also hinders the effectiveness of some of the interventions (1). Substance abuse negatively impacts society because of the loss of productivity, premature deaths, and high healthcare costs. There is also an increased cost associated with criminal justice and social welfare. Iraq faces many pressing public health issues, among them the emerging public health crisis of substance abuse. Some reports indicate a correlation between substance use and communities experiencing higher unemployment rates and poverty, especially among adolescents and young adults.

Iraq has been consistently exposed to wide-scale traumatic experiences in the past half century, including successive wars since 1980, economic sanctions, organized violence, and terrorism. These experiences have created an unstable and unsafe environment that negatively impacts the psychological health of all Iraqi society. Adverse childhood experiences (ACEs) have been associated with an increased likelihood of early initiation into drug use by about 2-4 folds compared to youth without ACEs. Additionally, as the number of ACEs increases, the possibility of illicit drug use, addiction, and parenteral drug use increases (2).

Hypnotics, sedatives, and benzhexol are among the commonly abused substances in Iraq. Captagon, which is a psychostimulant substance, is a combination of amphetamine and theophylline, has been used widely in Iraq despite its production cessation. Psychoactive drugs in Iraq used to be among the legally available medicines, which were diverted from authorized health facilities to the black market or obtained without prescription from private pharmacies (3). However, some of them are being manufactured internally.

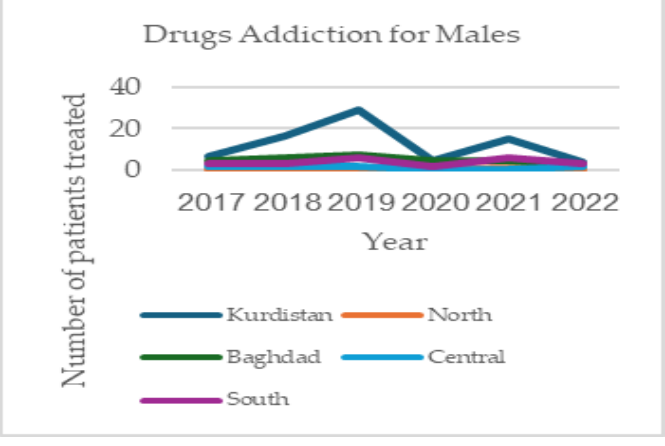

Figure 1. Rates of Treated Males for Drug Addiction per 100,000 of Population in Each Region of Iraq 2017-2022. Adopted from the annual statistical reports.

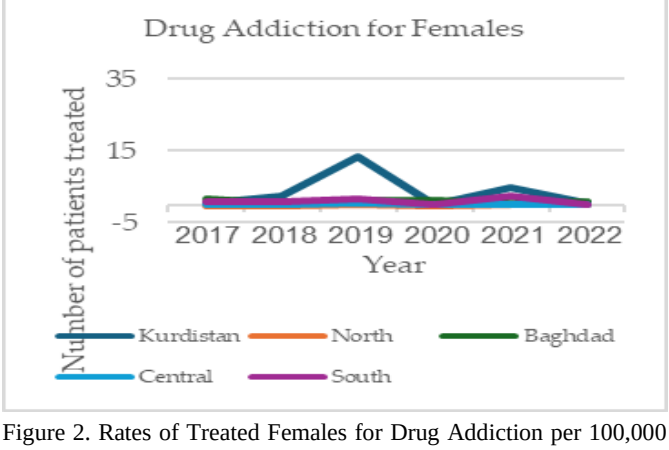

Figure 2. Rates of Treated Females for Drug Addiction per 100,000 of Population in Each Region of Iraq 2017-2022. Adopted from the annual statistical reports.

Data from the annual statistical reports from the Ministry of Health 2017-2022 showed the rates for drug addiction were significantly higher for males than females in all regions (4). Additionally, the Kurdistan region had particularly high rates of drug abuse. Moreover, the year of 2019 was significant was a particularly burdensome year.

Iraq has recognized psychoactive drug use as a public health issue. However, its response is primarily punitive, as there are penalties for those who handle drugs (transport, trade, and use). Of those who appeared in front of a judge in the Iraqi courts because of drug use for the years 2016, 2017, 2018, and 2020, 65% of them were related to personal drug use, while the rest of the cases were related to drug smuggling and trading (5). In the Kurdistan region, it was reported that from January to May 2024, at least 400 individuals were sentenced to drug-related charges, which is eight times more than in the previous years. Additionally, Iraq has a limited number of recovery and rehabilitation facilities, mainly located in Baghdad, resulting in limited treatment options. The United States has led a "war on drugs" for decades that has not been successful; it was based on bolstering the power of law enforcement and maximizing their budgets while cutting public health funding and diverting valuable resources away from the root causes of drug use.

Iraq needs collaborative and coordinated efforts from different official agencies to address the high unemployment and poverty. Additionally, the media should also actively participate in an antidrug campaign. Moreover, resources must be available for conducting research, implementing educational workshops, and establishing new rehabilitation centers. Funding must be allocated to each governorate to design its own prevention and treatment programs. A strong political will should lead a multidisciplinary approach where social work, economics, and addiction medicine intersect with public health to address psychoactive drug use.

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References

[1] Degenhardt L, Glantz M, Evans-Lacko S, Sadikova E, Sampson N, Thornicroft G, et al. Estimating treatment coverage for people with substance use disorders: an analysis of data from the World Mental Health Surveys. World psychiatry. 2017;16(3):299-307. https://doi.org/10.1002/wps.20457

[2] Dube SR, Felitti VJ, Dong M, Chapman DP, Giles WH, Anda RF. Childhood abuse, neglect, and household dysfunction and the risk of illicit drug use: the adverse childhood experiences study. Pediatrics. 2003;111(3):564-72. https://doi.org/10.1542/peds.111.3.564

[3] Al-Hemiary NJ, Al-Diwan JK, Hasson AL, Rawson RA. Drug and alcohol use in Iraq: findings of the inaugural Iraqi Community Epidemiological Workgroup. Substance use & misuse. 2014;49(13):1759-63. https://doi.org/10.3109/10826084.2014.913633

[4] MOHE. Annual statistical report 2022. Report. Baghdad: MOHE, Department of Statistics; 2022 07/06/2023.

[5] Muzil J, Abdulwahid D, Abed A, Kammad Z. Substance abuse in Iraq, quantifying the picture. J Popul Ther Clin Pharmacol. 2023;30(12):302-13. http://dx.doi.org/10.47750/jptcp.2023.30.12.036

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