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Research Article

Measurement of plasma fibrinogen and D-dimer in a sample of Iraqi patients with solid malignant tumors

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Abstrct

Background: Multifactor affect the pathogenesis of thrombosis in solid malignancy; however, a significant role is attributed to the cancer cells' ability to interact with and activate the host hemostatic system.
Hemostasis is highly correlated to tumor growth, angiogenesis, and metastasis, modulation of these pathways reflects interesting and promising treatment options in the future.
Most patients with cancer frequently suffer from chronic compensated DIC and have abnormal laboratory coagulation tests without clinical manifestations of thrombosis, which is a subclinical hypercoagulable state that can be detected by varying degrees of activation of blood clotting. The results of laboratory tests in these patients reflect continuous fibrin formation and lysis during the course of malignancy.
Aim of the study: To study the effect of solid malignant tumors on blood coagulation via measurements of plasma fibrinogen and D-dimer.
Subjects and methods: Thirty patients (9 males and 21 females) attending the oncology consultatory out-patient clinic at Baghdad Teaching Hospital/ Medical City were randomly selected and included in this study.
These patients were newly diagnosed as having malignant solid tumors depending on histopathological reports from private and governmental sectors.
All the laboratory tests were done at the hematology and biochemistry departments of Teaching Laboratories/ Medical City.
Results: Plasma fibrinogen level was significantly higher in the patients' group rather than control group (3.863 ±0.706) Vs (2.497±0.457 g/L} respectively, (P-value 0.001). The mean value of factor VIII activity was {181% ±58.4)and (99.3% ±11.1)for patient and control groups respectively, the P-Value was significant (> 0.001). D-dimer was negative for all members of the control group, for patients group (66.7%) of them showed positive D-dimer and (33.3) were negative for D-dimer, P-value was significant (>0.001). Conclusions: There was an increase in plasma fibrinogen level and positive D-dimer in cancer patients compared to the control group reflecting subclinical thrombophilia and a higher risk of VTE in patients with solid tumors due to activation of prothrombotic and fibrinolytic pathways by malignant cells which is vital for the use of primary prophylaxis by anticoagulants.

Introduction

Numerous vascular lesions in the malignant tumor microvasculature promote activation of plasma coagulation cascade through the extrinsic pathway of coagulation. Multiple microvascular lesions are the foci of intravascular coagulation, flow occlusion, and also extravasations and bleeding. Resulting in aberrant tumor vascular supply. An enlarged malignant tumor mass, neoangiogenesis, and metastasis are related to high concentrations of pro coagulation factors. The tissue factor (TF) is a protease activating coagulation cascade. TF is a procoagulant expressed on numerous normal cells, i.e. endothelial, and on tumor cells. It activates clotting factor VIIa along with other extravasated plasma-clotting proteins and generates thrombin consequently. Thrombin degrades fibrinogen to fibrin monomer and creates an extracellular fibrin barrier [1,2]. The extravascular fibrin deposits render tumor blood vessels hyperpermeable to fibrinogen at the tumor/host interface [3].

In the cancer population enhanced thrombogenicity has been correlated with high levels of circulating soluble TF [4,5].

Due to increased procoagulative activity, the serum levels of plasma soluble fibrin monomer complex, fibrinogen, and fibrin degradation products (D-dimer) values are considerably higher in patients with malignancy than in nonmalignant disease and healthy controls [4,6,7].

The median D-dimer level (reflecting local thrombin and fibrin formation) showing a parallel increase with the stage of the tumor and is higher in a large-sized tumor and a tumor showing deep wall penetration [4,8]. higher D-dimer and CEA levels in colorectal cancer are correlated with Lymphatic invasion, metastasis to lymph node or liver, and peritoneal dissemination [9].

Subjects and methods

Thirty patients (9 males and 21 females) attending the oncology consultatory out-patient clinic at Baghdad Teaching Hospital/ Medical City were randomly selected and included in this study.
These patients were newly diagnosed as having malignant solid tumors depending on histopathological reports from private and governmental sectors.
All the laboratory tests were done at the hematology and biochemistry departments of Teaching Laboratories / Medical City. Ethical approval was obtained from all the participants.

Control Group

A total of 30 healthy individuals, age and sex-matched were taken from blood donors attending the National Blood Transfusion Center in Baghdad/Iraq. The age of subjects of the control group ranged between 18-58 years and both males (9) and females (21) were included.

Data Collection

All patients were questioned about their age, current place of residence, drug intake, chemotherapy, personal and family history of any thrombotic or suspected thrombotic episodes, history of immobility, dehydration, leg edema, and history of hypertension or diabetes mellitus. Data were collected from histopathological reports about the specific histopathological type. Staging of the malignant tumor was taken from clinical and histopathological reports.
Fibrinogens assay (Clauss Technique):

Principle: This test was performed using a commercially available kit; (FORTRESS DIAGNOSTICS/U.K.)

Reagent:

1. FORTRESS Diagnostics Fibrinogen reagent: a lyophilized preparation of bovine thrombin, approximately 50 NIH U/ml. [10]

2. FORTRESS Diagnostics Calibrator [10]. STA®-Owren-Koller buffer (REF 00360). [10]

Plasma D-DIMER assay

Latex agglutination slide test for the semi-quantitative determination of D-dimer (Diagnostiga Stago, France).[11]

Principle: The latex particles provided in the D-Di test® are coated with mouse antihuman D-Dimer monoclonal antibodies.

Test samples containing D-Dimers when mixed with the latex particle suspension make the particles agglutinate [11].
At a prearranged concentration of D-Dimers that the D-Di test® is designed for, the agglutination of the latex particles produces macroscopic clumps that can be examined by the naked eye [11].

Results

This test is designed to have a positive cut-off at 0.5 µg/ml expressed in fibrinogen equivalent unit (FEU). [16] Positive and negative agglutination pattern may be interpreted as shown in table (1):

Table 1. Positive and negative agglutination pattern

Presence of agglutination	D-Dimer level (µg/ml-FEU)
-	< 0.5
+	≥ 0.5

Plasma Fibrinogen: The mean value of plasma fibrinogen level was 3.863 and 2.497 g/L for patient and control groups respectively as shown in table (2) and there is a statistically significant difference in mean plasma fibrinogen level between the patients and control.

Table 2. Plasma Fibrinogen results for patient and control groups.

Groups	Subjects	Mean	S.D.	SE. Mean
Patients	30	3.863**	0.706	0.13
Control	30	2.497	0.457	0.084

**P-value at or less than 0.05 is statistically significant*

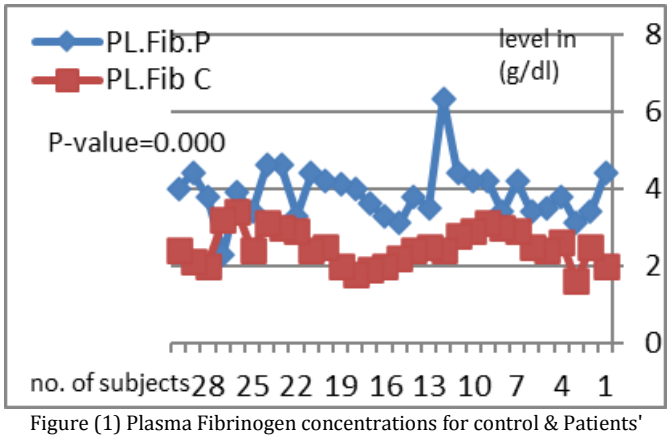

Figure (1) Plasma Fibrinogen concentrations for control & Patients' groups.

(PL.Fib.P: plasma fibrinogen for patients' group, PL.Fib.C : plasma fibrinogen for control group).

D-Dimer Results: D-dimer was negative for all members of the control group, for the patient group 20 of them showed positive D-dimer (66.7%) and 10 patients (33.3%) were negative for D-dimer see figure (2). there is a statistically significant difference in mean D-dimer level between the patients and control group (P-value was 0.000), as shown in figure (2).

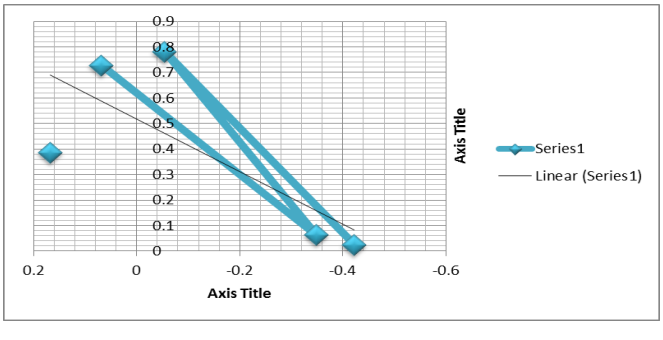

Figure (2) correlations of coagulation data with the stage of malignant tumors.

Discussion

Malignancies are often correlated with alteration of hemostasis and thrombotic risk is increased in cancer patients. Thrombosis may occur at any time during the evolution of the oncological disease. However, an occult malignancy can be discovered by deep vein thrombosis (DVT).
The mechanism whereby cancer may modify hemostasis is still not completely clear but many pathways are likely to be involved. Yet, the majority of patients with neoplasia have active hemostasis which is asymptomatic and detected only by laboratory tests [12].

Stage: There were insignificant correlations between D-dimer and plasma fibrinogen with the stage of malignant tumors. Ahmet Ursavaş et al reported that there was no significant relationship between D-dimer plasma levels and tumor stage [13]. Sitalakshmi S. et al found that fibrinogen levels didn't show any statistically significant differences between early and advanced disease groups which agree with the current study [14].

Coagulation Data:
D-dimer: There is a statistically significant association between diagnosis of cancer and D-dimer positivity (P-Value=0.000), which is consistent with the results of Micco et al,[12] Kirwan CC et al,[15] Madkour B.S. et al,[16] Fregoni V et al,[17] Dworakowska D. et al,[18] Batshauer A. et al,[19] Gabazza et al,[20] and Mikaszewski B.qq et al. [21] High D-dimer levels in patients with cancer who do not have VTE suggest that elevated D-dimer are not only because of the presence of thrombus or fibrinolysis activation. Knowlson et al. suggest that high D-dimer levels in malignancy are likely to be due to the biology of the underlying tumor [22].
Plasma fibrinogen: Plasma fibrinogen levels in patients with a solid tumor in the current study were higher than those of the control group, which reflects activation of thrombosis by a malignant tumor. [1] Similar results were obtained by Dworakowska D. et al,[18] Mikaszewski B. et al, [21] Battistelli S. et al, [23], and Da-Yong Lu et al. [24] Fibrinogen is one of the main acute phase proteins produced by the liver and is greatly augmented in response to infection and other inflammatory disorders. Subsequently, malignancy is often associated with an inflammatory response, it may be possible that the high fibrinogen level in patients with solid malignancy is a second incident resulting from the increased systemic inflammatory response triggered by tumor progression. [25].

Conclusion

There was an increase in plasma fibrinogen level and positive D-dimer in cancer patients compared to the control group reflecting subclinical thrombophilia and a higher risk of VTE in patients with solid tumors due to activation of prothrombotic and fibrinolytic pathways by malignant cells which is vital to consider primary prophylaxis by anticoagulants.

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Conflicting Interest
No conflict of interest.

References

1.Gupta PK, Charan VD, Kumar H.Cancer related thrombophilia .clinical importance and management strategies .JAPI. 2005 OCT; 53:877-882.
2. Heath EI, Burtness BA, Kleinberg L, Salem RR, Yang SC, Heitmiller RF, et al. Phase II, parallel-design study of preoperative combined modality therapy and the matrix metalloprotease (mmp) inhibitor prinomastat in patients with esophageal adenocarcinoma. Invest New Drugs .2006; 24:135-40.
3. Dvorak HF, Rous-Whipple award lecture. How tumors make bad blood vessels and stroma. Am J Pathol. 2003; 162:1747-57.
4. Verheul H M, Hoekman K, Lupu F, Broxterman H J, van der Valk P, Kakkar A K, et al. Platelet and coagulation activation with vascular endothelial growth factor generation in soft tissue sarcomas. Clin Cancer Res. 2000; 6:71-166.
5. Kuenen B C, Levi M, Meijers J C, Kakkar A K, van Hinsbergh V W, Kostense P J, et al. Analysis of coagulation cascade and endothelial cell activation during inhibition of vascular endothelial growth factor/vascular endothelial growth factor receptor pathway in cancer patients. Arterioscler Thromb Vasc Biol. 2002; 22:1500-5.
6. Nakashima J, Tachibana M, Ueno M, Baba S, Tazaki H. Tumor necrosis factor and coagulopathy in patients with prostate cancer. Cancer Res. 1995; 55:4881-5.
7. Kim HK, Song KS, Lee KR, Kang YH, Lee YJ, Lee ES. Comparison of plasma D-dimer and thrombus precursor protein in patients with operable breast cancer as a potential predictor of lymph node metastasis. Blood Coagul. Fibrinolysis .2004; 15:9-13.
8. Nijziel MR, van Oerle R, Christella M, Thomassen LG, van Pampus EC., Hamulyak K, et al. Acquired resistance to activated protein C in breast cancer patients. Br J Haematol. 2003; 120:117-22.
9. Oya M, Akiyama Y, Yanagida T, Akao S, Ishikawa H. Plasma D-dimer level in patients with colorectal cancer: its role as a tumor marker. Surg Today. 1998; 28:373-8.
10.FIBRINOGEN (QUANTITATIVE) COAG 108A Fortress Diagnostics Limited, unit 2CAntrim Technology Park, Antrim BT411QS, United Kingdom.
11.D-DI TEST® Latex agglutination slide test for the qualitative determination (REF 00554) DIAGNOSTICA STAGO S.A.S., 9 rue des Fre'res Chaussan92600 Asnie'res sur Seine (France).stago@stago.fr
12. Micco P, Lucia D, Vita F , Niglio A, Micco G, Martinelli E, et al. Aquired Cancer Related Thrombophilia Testified by Increased Levels of Prothrombin Fragment 1+2 and D-dimer in Pateints Affected by solid tumors. Experimental Oncology. 2002;24,108-111.
13. Ursavaş A, Karadağ M, Uzaslan E, Yesilkaya S, Coşkun F, Eğe E. Prognostic Significance of Plasma D-Dimer Levels in Patients with Lung Cancer. Eur J Gen Med. 2010; 7(2):155-160.
14. Sitalahshmi S, Rameshkumar K, Damodar P. Significance of haemostatic markers in overian carcinoma. Indian Journal of medical and pediatric oncology. 2008; 29(2):610.
15. Kirwan C C, McDowell G, McCollum C N, Byrne G J. Chemotherapy-Induced Changes In Platelet Function In Early And Advanced Breast Cancer. haematologica reports 2005. 2005; 1(9),84.
16. Madkour B S., Bekheet I W, Khaled I A, El. Baz A G, Essawy F M. Platelet Activation a Link Between Angiogenesis and Haemostasis in Bladder Cancer. Patients Research Journal of Medicine and Medical Sciences .2009;4(2): 212-219.
17. Fregoni V, Regolo L, Zambelli A, Da Prada G, Vitali M, Ponchio L, et al.Correlation Between plasma D-dimer Levels And Axillary Lymph Node Status In Operable Breast Cancer Patients. haematologica reports 2005. 2005; 1(9),77.
18. Dworakowska D, Kulwas A, Lapinski M, Tomaszewski D, Dworakowski R, Skokowski J, et al. TAFI, fibrinogen and D-dimers in radically operated NSCLC. haematologica reports 2005. 2005; 1(9),81.
19. Batshauer A, Figueiredol C, Buenol E, Ribeiro M, Dusse L, Fernandes A, et. D-dimer as a possible prognostic marker of operable hormone receptor-negative breast cancer. Oxf J Med Ann Oncol. 2009); (issue 6), (21):1267-1272.
20. Gabazza E C, Taguchi O, Yamakami T, Machishi M, lbafa H, Tsufsui K, et al. Coagulation-fibrinolysis system and markers of collagen metabolism in lung cancer. Cancer J. 1992; 70(11):2632-2637.
21. Mikaszewski B, Dworakowska D, Lapinski M, Skibowska A,Stodulski D, Mital A, et al.Determination of known risk factors of thromboembolism in laryngealand non-small cell lung cancer patients. haematologica reports 2005. 2005; 1(9).82.
22. Knowlson L, Bacchu S, Paneesha S, McManus A, Randall K, Rose P. Elevated D-dimers are also a marker of underlying malignancy and increased mortality in the absence of venous thromboembolism. Journal of Clinical Pathology.2010; 63 (9): 818–822.
23. Battistelli S, Vittoria A, Stefanoni M, Genovese A, Cevenini G. *Antiphospholipid Antibodies And Acute Phase Responce In Cancer Patients.* haematologica reports 2005. 2005; 1(9):78.
24. Lu DY, Chen XL, Huang M, Xu B, Ding J. Relationship between blood fibrinogen concentration and pathological features of cancer patients: A

139-case Clinical Study, Online Journal of Biological Sciences.2007; 7 (1): 8-11.
25. Yamashit H, Kitayama J, Nagawa H. Hyperfibrinogenemia is a useful predictor for ymphatic metastasis in human gastric cancer. Jpn J Clin Oncol. 2005;35(10):595–600.