Extrapituitary prolactin –1149 G/T promoter polymorphism in some rheumatoid arthritis patients

*Adnan F. AL-Azzawie MSc biology**

ABSTRACT

Background: Prolactin is a hormone, as well as a cytokine which is synthesized and secreted from the anterior pituitary gland and various extra pituitary sites including immune cells under control of a superdistal promoter that contains a single nucleotide polymorphism -1149 G/T. Rheumatoid Arthritis has been associated with increased serum prolactin levels.

Objectives: To investigate the association of the extra pituitary -1149 G/T promoter polymorphism among Iraqi rheumatoid arthritis patients and prolactin levels.

Methods: We tested 73 patients with rheumatoid arthritis and 40 healthy individuals. The DNA samples were genotyped using the Polymerase Chain Reaction-Restriction fragment Length Polymorphism method and the levels of prolactin serum were measured using Enzyme linked immunosorbent assay.

Results: The parameters rheumatoid factor, C-reactive protein, erythrocyte sedimentation rate and serum prolactin concentrations of rheumatoid arthritis patients were significantly increased compared with healthy controls. The frequency of T allele and TT genotype was highest in control group compared with their frequency in rheumatoid arthritis patients, vice versa the G allele and GG genotype. There is significant differences in prolactin levels in rheumatoid arthritis patients compared with healthy controls according to PRL-1149 G/T polymorphism. In rheumatoid arthritis patients, carriers of the GG, GT genotypes had higher prolactin levels in comparison to TT genotype but did not show any significant difference.

Conclusions: There is a possible association between prolactin levels and rheumatoid arthritis. We found correlation between rheumatoid arthritis and prolactin -1149 G/T polymorphism. The prolactin -1149 G allele is a genetic marker for increased rheumatoid arthritis susceptibility in Iraqi population. In addition, influence of prolactin -1149 G/T polymorphism on prolactin levels.

Keywords: Prolactin –1149 G/T promoter polymorphism, Rheumatoid arthritis.

Al-Kindy College Medical Journal 2015: Vol.11 No. 1
Page: 40-44

**Department of Biology, College of Science, Tikrit University.*
Received 26th Oct 2014, accepted in final 17th Dec 2014
Corresponding to Adnan Fadel AL-Azzawie, email: adnan_f1980@yahoo.com, mobile 07702082063

Rheumatoid arthritis (RA) is a complex, autoimmune genetic disorder affects up to 1 % of adults worldwide, characterized by chronic inflammation and cellular proliferation in the synovium of joints leading to cartilage and bone destruction. Although the etiology and pathogenesis of RA are not clearly defined, a genetic component has been established by twin and family studies, in which it was estimated to contribute as much as 60 % to RA susceptibility [1, 2].

The higher incidence of RA in women (3:1ratio) has generated much interest about the hormonal influence on disease risk. Prolactin (PRL) levels are higher in women than in men, though there is considerable overlap in the ranges. It has been suggested that excessive PRL secretion may contribute to the pathogenesis of RA. Furthermore, hyperprolactinemia is observed in 6% of RA patients compared to 3% in the normal population. A characteristic feature of RA is remission of the disease during gestation with exacerbation in the postpartum period. During pregnancy PRL concentration is low, but start to rise during the second trimester, preparing the breasts for lactation, reaches their peak at the end of the pregnancy, and this explain the fact that RA the remits during gestation and exacerbate postpartum [3,4,5].

The relationship between PRL and the immune system has been demonstrated in the last two decades, opening new windows in the field of immunoendocrinology [6]. Researchers focused on the role of PRL in the immune response. They are based on the fact that PRL enhanced immune responses in vivo [7]. PRL acts as a cytokine and plays a role in the pathogenesis of systemic autoimmune diseases such as RA and systemic lupus erythematosus (SLE) [8]. Early studies in human showed normal serum PRL levels, but a decrease in PRL bioactivity in patients with RA was found [9]. The first evidence of high PRL levels in RA was in children with juvenile arthritis and antinuclear antibodies positivity[10]. A study reported high PRL in men with RA associated to long evolution and disease activity [6].

PRL, a versatile hormone with more than 300 separate functions, is produced not only by the anterior pituitary gland but also by various extra pituitary sites as neurons, prostate, decidua, mammary epithelium, skin and immune cells. PRL is structurally similar to members of cytokine/hemopoietic family and plays an important role in animal and human immune response. Because PRL is involved in the activation of many immunological responses, it enhances the progression of the immune process in autoimmune diseases. The finding of hyperprolactinemia, often described in autoimmune diseases, supports an immunomodulatory role of PRL itself [11, 12].

A single gene encoding PRL is found in the human genome, located on chromosome 6. The PRL gene is

10.215 kb in size and is composed of 5 exons and 4 introns. Transcription of the PRL gene is regulated by two independent promoter regions; the proximal 5,000-bp region directs pituitary-specific expression, and an upstream (distal) promoter region which is responsible for extra pituitary expression [5]. The extra pituitary promoter contains functional single nucleotide polymorphism (SNP) at the position 1149 G/T (rs1341239) in the GATA sequence; G allele leads to higher PRL mRNA in lymphocytes and subsequently high PRL levels [13]. GG genotype has been associated with (SLE), while TT genotype was identified as protective for non-Hodgkin and follicular lymphoma, and RA development [14].

For our knowledge, there are no studies evaluated the association of the extra pituitary PRL promoter polymorphism with RA in Iraqi population, therefore this study is designed to investigate the association of the PRL −1149 G/T promoter polymorphism and serum PRL level among Iraqi RA patients in Salahaddin governorate.

Methods. A total of 73 eligible patients had confirmed RA by a rheumatologist according to the Revised 1987 American College of Rheumatology (ACR) criteria [15] were included in the study. Patients who had comorbid diseases, overlapped with other connective tissue diseases or inflammatory arthritis, and vasculitis were excluded from the study. Patients were recruited from private clinic in different region of Salahaddin governorate. 40 apparently healthy individuals (30 females and 10 males; with matching age with patients group) considered as a control group. We used paper clinical research form through interview and questionnaires. Full case history was taken (including age, sex, disease duration, and disease activity) and complete clinical exam of participants was done.

Sample Collection; Under complete aseptic conditions, 5 ml of venous blood were collected from all the participants at the time of clinical examination & divided as follows: Tube A, 1ml of blood for the determination of erythrocyte sedimentation rate (ESR), 1ml of blood collected in Ethylene Diamin Tetra Acetic Acid tube (to prevent blood clotting) for DNA extraction, kept immediately at -20 °C. Tube B, 2 ml of blood were left to clot, serum was separated and used for immediate assay of C-reactive protein (CRP), presence of Rheumatoid Factor (RF), the remaining serum were kept at -20 °C for determination of PRL levels.

Laboratory Measurements; All the cases were screened for RF, CRP and ESR. Serum PRL levels were measured in both RA and healthy controls using Enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Abcam-UK).

DNA Extraction; Genomic DNA was isolated from whole blood samples of all the patients and control subjects using a method described by AL-Azzawie (2012)[16], R.B.Cs were lysed with R.B.Cs lysis buffer, the pellet were mixed with cell lysis buffer and sodium perchlorate then incubated in water bath for 60 min at 65ºC. After centrifugation the supernatant was transfer to new tubes. DNA was precipitated with 2 volume of ice-cold absolute ethanol. DNA concentrations (50 - 900 ng) and purity (1.6 - 1.8) were determined by measuring the absorbance of diluted DNA solution at 260 and 280 nm using Nano drop (Thermo scientific, Germany). The quality of the DNA was determined using 1 % agarose gel electrophoresis stained with ethidium bromide, samples were stored at -20 °C.

Detection of PRL −1149 G/T polymorphism; The PRL−1149 G/T polymorphism was detected using the Polymerase Chain Reaction-Restriction fragment Length Polymorphism (PCR-RFLP) method as described by Donn et al.(2002)[17]. Amplification of the polymorphic promoter fragment was done with the following primers supplied by Promega Company (USA): 5′-AGA ATT GGA GTT CCA GTG CC-3′ (Forward) and 5′-ATC ACA CTC AAC CAG TTG GC-3′ (Reverse) according to Donn et al.[17]. Primary several experiments were carried out for optimization and to arrive to the optimum conditions, then, the PCR amplification was performed in a total volume of 25 µL including 12.5 µL of 2X Go Taq green master mix supplied by Promega company (USA), 4µL(100 ng) of genomic DNA, 1µl of each primer (10 pmol/1µL) and 6.5 µL of nucleases free water. The cycling parameters were as follows: initial denaturation at 95 °C for 5 min, 35 cycles including denaturation at 95 °C, annealing at 55 °C, extension at 72 °C for 1min of each them, and final extension at 72 °C for 5 min. The resulting PCR product (338 bp size) was visualized on a 3% agarose gel electrophoresis stained with ethidium bromide. 5 µL of the PCR product was incubated with 3 U of ApoI enzyme (New England, BioLabs, Inc.) at 50 °C for 1 hour and the restriction fragments were visualized on 3% agarose gel electrophoresis stained with ethidium bromide in the presence of 1Kb DNA ladder (Biolabs-England) as a molecular marker. The GG homozygote was identified as 2 fragments (280 and 58 bp), the GT heterozygote as 3 fragments (338, 280, 58 bp) and the TT homozygote as a single fragment of 338 bp.

Statistical analysis**;** The mean and standard deviation (SD) of serum PRL levels were calculated. The *p*-value was calculated using student's t-test (Which considered significant when $p<0.05$ and highly significant when $p<0.01$). Hardy-Weinberg equilibrium was evaluated with the chi square test. The allelic and genotype frequencies of the PRL −1149 G/T polymorphism in the RA patients and control groups were calculated. Differences in serum PRL levels among RA patients vs. control groups or among RA patients carriers of the GG, GT and TT genotypes were evaluated by the student's t test.

Results. The demographic and clinical parameters of the 73 RA patients enrolled in the study compared with 40 healthy subjects as a control group are presented in Table 1. The control group had an average age of 41.1 years and the RA group had 44.7 years, without statistical differences ($p=0.061$) between two groups. The percentage of women was higher in the RA group compared with the control group (83.54% vs. 50%). Comparing the other clinical parameters of the studied groups shown there were statistical significant differences between the two groups in ESR, RF and CRP, Table 1.

Serum PRL concentrations in RA patients (7.29 ± 8.11) ng/ml were significantly increased than in healthy controls (2.69 ± 1.51) ng/ml, (t=3.55, *p* value 0.0006), Figure 1.

The PCR analysis indicated the presence of three genotypes (GG, GT, TT) of the PRL-1149 G/T polymorphism, Figure 2.

Table 1: Demographic and clinical characteristics of the studied groups.

Parameter		RA Group	Control Group	*P* value
Average age (years) (±SD)		44.7±4.17	41.1±5.12	0.061
Gender	Males %	16.46	50	-
	Females %	83.54	50	-
ESR(mm /1 hour)		28 ±7.5	8.5±5.1	0.041*
RF UI/mL	Positive%	85.2	11.5	0.005**
	Negative%	14.8	88.5	0.009**
CRP, mg/dL		2.1±1.2	0.251±0.312	0.035*

** p < 0.05 (significant), ** p < 0.01 (highly significant).*

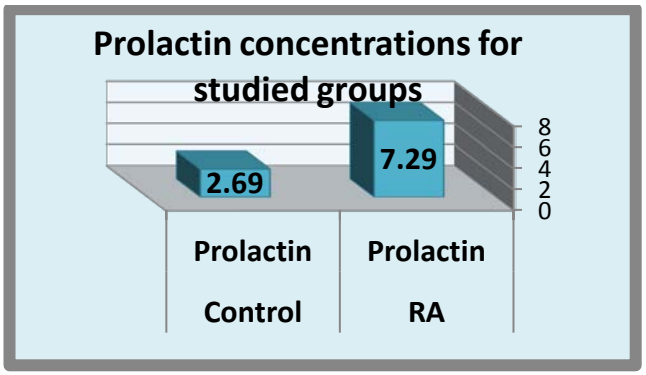

Figure 1: Comparison between RA patients and healthy controls according to PRL concentrations.

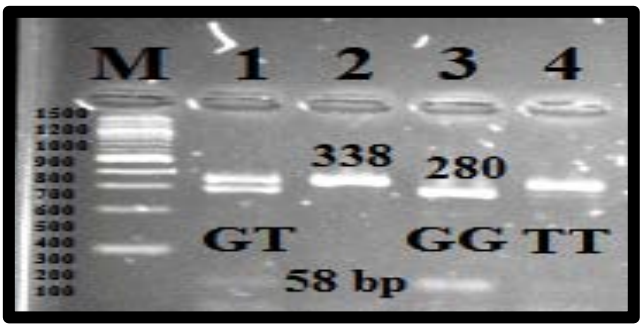

Figure 2: Agarose gel of PCR products showing genotypes of PRL −1149 G/T polymorphism: lane (M) DNA ladder, lane (1) GT heterozygote (338,280 and 58 bp bands), lane (2) and (4) TT homozygote (338 bp band) and lane (3) GG homozygote (280 and 58 bp bands).

Table 2 demonstrates the distribution of allelic frequency of the PRL-1149 G/T polymorphism in RA patients and control group. We found differences in allelic polymorphism frequency between the two groups. The frequency of G-allele was higher in RA patient compared with control group, on the contrary the T allele. The differences between the allelic frequencies indicated that the presence of G allele seems to confer a risk for expressing high level of PRL and T allele seems to be protective.

Table 2: Distribution of allelic frequency of PRL-1149 G/T promoter polymorphism for both RA and control groups.

Group	Allele	Frequency
Patient No. = 73	T	0.34
	G	0.66
Control No. = 40	T	0.4
	G	0.6
Total		1.000

The frequency of TT genotype was highest (0.2) in control group compare with frequency (0.109) in RA patients. On the other hand, the GG and GT genotypes were observed more frequent in the RA patients compare with control group (0.424, 0.465 vs. 0.4, 0.4), Table 3.

Table 3: Distribution of PRL-1149 G/T promoter genotypes and χ2 values for RA and control groups.

Group	Genotype	Observed No.	Frequency	Expected No.	Frequency	χ2
Patient No 73	TT	8	0.109	31.56	0.115	
	GT	34	0.465	32.88	0.448	
	GG	31	0.424	8.56	0.435	
Total		73	1.000	73	1.000	0.09
Control No 40	TT	8	0.2	9.22	0.16	
	GT	16	0.4	36.55	0.48	
	GG	16	0.4	36.22	0.36	
Total		40	1.000	40	1.000	1.11

The relationship between the −1149 G/T polymorphism and serum PRL levels was evaluated. PRL levels of each genotype were compared among studied groups. As shown in Table 4, we could observe statistically significant difference (p<0.05, p<0.01) in PRL levels in RA patients compared with control group. We analyzed the PRL levels according to the PRL−1149 G/T polymorphism in RA patients, the carriers of the GG and GT genotypes had higher mean PRL levels (6.15, 8.76) in comparison to the TT genotype (5.49), but did not show any significant differences.

Table 4: Comparison between PRL serum levels according to genotypes of PRL-1149 G/T promoter polymorphism for RA and control groups.

Genotype	Patient Group No. = 73		Control Group No. = 40		*p* value
	No.	PRL levels (Mean ± SD)	No.	PRL levels (Mean ± SD)	
TT	8	5.49 ± 5.47	8	1.70 ± 0.937	0.0025**
GT	34	8.76 ± 10.5	16	2.73 ± 1.06	0.027*
GG	31	6.15 ± 2.89	16	2.94 ± 0.302	0.025*

* P < 0.05 (significant), ** p < 0.01 (highly significant)

Discussion. It is suggested that production of PRL by lymphocytes can play a role in the pathogenesis of some autoimmune diseases; some researchers found increased level of PRL has been described in serum of patients suffering from (SLE) [18]. In humans, rheumatoid synovial T-cells produce PRL. Also PRL receptors are found on T,B, fibroblast and like synovial cells. Addition of PRL to rheumatoid synovial cells in rats causes increased production of proteolytic enzymes causing cartilage destruction and increased production cytokines which indicates that PRL injected in joints caused inflammation [19, 20, 21]. This study showed a significant difference (p<0.01) in PRL concentrations in RA patients compared with control group and this finding was in accordance with the results reported by [22] , found raised serum PRL levels up to 40.20±5.6 (p< 0.01) in women with RA than the healthy adults. Other studies have reported increased serum PRL levels in RA patients in correlation with some parameters like duration of RA [23, 24, 25].

The PRL -1149 G/T extra pituitary promoter polymorphism has been studied in several autoimmune diseases such as SLE, systemic sclerosis, multiple sclerosis, polymyositis and psoriatic arthritis in other countries [21,26,27,28]. However, this is the first study that evaluates the association between the PRL-1149 G/T promoter polymorphism with RA Iraqi patients. We found significant differences in allelic frequencies for genotype polymorphism between patients and controls, with higher frequency of the G allele in the RA group than the control group. This is similar for other study [29] who found an association between the T allele and decreased RA susceptibility. According to the results of this study, there is an association between the GG and GT genotype and increased RA susceptibility. This is agreement with Fojtíková et al (2007) [30] who found the GT genotype more frequent in the RA group compared to the healthy group, thus postulating it as a predisposing genetic factor. Since the distribution of the GG genotype in our study is more frequent in RA patients, we postulate it as risk genotype in our population rather than a protective genotype. These differences in the distribution of the PRL promoter polymorphism among populations can be due to ethnogenetic heterogeneity, which refers to the specific genetic background in a population, or ethnic group that might reflect migration history and the natural selection that shaped genetic variation in the population [31, 32].

There was statically difference in the total PRL level between RA patients and healthy control when we analyzed according to PRL -1149 G/T promoter genotype polymorphism. In RA patients, carriers of the GG, GT genotypes had higher PRL levels in comparison to TT genotype, but the difference did not reach statistical significance, most probably owing to relatively small sample size. This data are in agreement with the study of Stevens et al [28], who showed a greater induction of PRL mRNA in peripheral blood leucocytes in patient with GG genotype in response to T cell activation by PHA than those with the TT genotype. These findings suggest specific regulatory mechanisms and different mode of action of PRL-1149 G/T polymorphism from PRL produced by anterior pituitary. It is conceivable that PRL-1149 G/T polymorphism acts rather locally than systemically (in lymph nodes, spleen or organs infiltrated by immune cells) and small amounts of immune cell-derived PRL may achieve high concentrations in the adjacent micro-environment [33].

In summary, this study indicates that there is a possible association between serum PRL level, PRL -1149 G/T promoter polymorphism and the development of RA. The results confirm that the PRL-1149 G allele is a genetic marker for increased RA in Iraqi population. There was influence of PRL-1149 G/T polymorphism on PRL levels and the PRL levels were highest in GG and GT in comparison with TT genotypes without any statistical differences.

References

1. Arman, A., Coker. A., Sarioz., O., Inanc. N., Direskeneli. H. Lack of association between IL-6 gene polymorphisms and rheumatoid arthritis in Turkish population. ***Rheumatol Int*** (2012) 32:2199-2201.
2. Lee, Y. H. Bae , S.C., Choi, S.J., Ji, J.D., Song, G.G. The association between interleukin-6 polymorphisms and rheumatoid arthritis: a meta-analysis. ***Inflamm. Res***. (2012) 61:665-671.
3. Castillo, Z.R Suárez, A.L.P., Sanchez, C.A.P.S., Villalobos, H.R. The extra pituitary prolactin promoter polymorphism is associated with rheumatoid arthritis and anti-CCP antibodies in Mexican population. ***Gene***: (2013) 525 130-135.
4. Eijsbouts,A.M.M.,Hoogen,F.H.J.,Laan,R.F.J.M.,Sweep,C.G,J., Hermus,A.R.M.M. Decreased prolactin response to hypoglycaemiain patients with rheumatoid arthritis: correlation with disease activity. ***Ann Rheum Dis***. (2005) 64:433-437.
5. Nore, B.F. ., Mahmoud. T.J., and Rashid, B.M. Sequence Verifications and Promoter Analysis of The Prolactin Gene.***Journal of Zankoy Sulaimani*** - Part A (JZS-A), (2013) 15 (1).
6. Jara, L.J., Medina, G., Saavedra, M.A., Lastra, O.V., Navarro, C. Prolactin and Autoimmunity. Clinic Rev ***Allerg Immunol*** (2011) 40:50-59.
7. Ghule, S; et al. Serum prolactin levels in women with rheumatoid arthritis. ***Biomedical Research*** (2009) 20 (1): 115-118.
8. Stolfa, J., Fojtkova,M., Cejkova, P., Cerna, M., Sedova, L., Dostal, C. Polymorphism of the prolactin extra pituitary promoter in psoriatic arthritis. ***Rheumatol Int*** (2007) 27:1095-1096.
9. Berczi, I. Cosby, H. Hunter, T. Baragar, F. McNeilly, A.S, Friesen, H.G. Decreased bioactivity of circulating prolactin in patients with rheumatoid arthritis. ***Br J Rheumatol*** (1987) 26:433-436.
10. McMurray, R.W, Allen, S.H, Pepmueller, P.H, Keisler, D. Cassidy, J.T. Elevated serum prolactin levels in children with juvenile rheumatoid arthritis and antinuclear antibody seropositivity. **J Rheumatol** (1995) 22:1577-1580.
11. Bellis, A.D., Bizzarro, A., Pivonello, R. Prolactin and Autoimmunity. Pituitary (2005) 8: 25-30
12. Vera-Lastra, O., Jara, L.J., Espinoza, L.R. ***Autoimmunity Reviews*** 1.(2002)360-364.
13. Stevens, A., Ray, D.W., Worthington, J. Polymorphisms of the human prolactin gene - implications for production of lymphocyte prolactin and systemic lupus erythematosus. ***Lupus*** (2001) 10: 676-683.

14. Marketa, F., Pavlina, C., Radim, B., Marie,C. Polymorphism of the extra pituitary prolactin promoter and systemic sclerosis. ***RheumatolInt*** (2009) 30:1691-1693.
15. Aletaha D, 3rd C.O. Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. ***Arthritis Rheum***. 2010;62 (9):2569-81.
16. AL-Azzawie, A.F. A rapid and non-enzymatic method for genomic DNA extraction from whole blood and some other mammalian tissues. ***Journal Tikrit Univ. For Agri. Sci.*** (2012) Vol. (12) No. (4)
17. Donn, R.P., Farhan, A., Stevans, A., Ramanan, A. Neuroendocrine gene polymorphisms and susceptibility to juvenile idiopathic arthritis. Rheumatology (2002) 41, 930-936.
18. Moszkorzova, L. Marek, J., Cerna, M. Hyperprolactinemia in patients with systemic lupus erythematosus. ***Clin Exp Rheumatol*** (2002) 20:807-812.
19. Erb, N., Pace, J.V., Delamere, J.P., Kitas, D. Control of unremitting rheumatoid arthritis by the prolactin antagonist cabergoline. ***British Journ of Rheumatology*** (2001) 40: 237-239.
20. Nagafuchi, H., Suzuki, N., Kaneko, A., Asai, T., Sakane, T. Prolactin locally produced by synovium infiltrating T lymphocytes induces excessive synovial cell functions in patients with rheumatoid arthritis. ***J. Rheumatol*** (1999) 26 (9), 1890-1900.
21. Neidhart, M., Gay, R.E., Gay, S. Prolactin and prolactin-like polypeptides in rheumatoid arthritis. *Biomed Pharmacother* (1999) 53: 218-222.
22. G.hule, S., Dhotre, A., Gupta, M., Dharme,P., Vaidya, S.M. Serum prolactin levels in women with rheumatoid arthritis. ***Biomedical Research*** (2009) 20 (1): 115-118.
23. Fojtíková, M., Jiri, V. Jana, T.S. Elevated prolactin levels in patients with rheumatoid arthritis: association with disease activity and structural damage. ***Clin. Exp. Rheumatol*** (2010) 28 (6),849-854.
24. Ram, S., Blumberg, D., Newton, P., Anderson, N.R., Gama, R. Raised serum prolactin in rheumatoid arthritis: genuine or laboratory artefact? *Rheumatology* (2004) 4 (43),1272-1274.
25. Seriolo, B., Ferretti, V., Sulli, A., Fasciolo, D., Cutolo, M. Serum prolactin concentrations in male patients with rheumatoid arthritis. ***Ann. N. Y. Acad. Sci.*** (2002) 966, 258-262.
26. Fojtíková, M., Radim, B., Jiri, V.V. Polymorphism of the extra pituitary prolactin promoter and systemic sclerosis. ***Rheumatol. Int*** (2010) 30 (12), 1691-1693.
27. Mellai, M., Miller, A., Shtiller, R., Touby, E. Prolactin and prolactin receptor gene polymorphisms in multiple sclerosis and systemic lupus erythematosus. ***Hum. Immunol*** (2003) 64, 274-284.
28. Stevens, A., Newton, P., Anderson, N.R., Gama, R. Characterization of a prolactin gene polymorphism and its associations with systemic lupus erythematosus. ***Arthritis Rheum***. (2001) 44 (10), 2358-2366.
29. Lee, Y.C., Sulli, A., Fasciolo, D. The PRL -1149 G/T polymorphism and rheumatoid arthritis susceptibility. ***Arthritis Rheum.(***2009) 60 (5), 1250-1254.
30. Fojtíková, M., Cerná, M., Cejková, P., Ruzicková, S., Dostál, C., 2007. Extra pituitary prolactin promoter polymorphism in Czech patients with systemic lupus erythematosus and rheumatoid arthritis. ***Ann. Rheum. Dis***. (2007) 1, 1-2.
31. Guzman-Guzman, I.P., Parra-Rojas, I., Oregón-Romero, E., Ledezma-Lozano, I.Y., Palafox-Sánchez, C.A. The PADI4 haplotypes are associated with anti-CCP levels in rheumatoid arthritis from Western Mexico. **ClinChem Lab Med** (2011) 49, S205 Special suppl.
32. Kochi, Y., Suzuki, A., Yamada, R., Yamamoto, K. Ethnogenetic heterogeneity of rheumatoid arthritis-implications for pathogenesis. ***Nat. Rev. Rheumatol***.(2010) 6, 290-295.
33. Montgomery, D.W., Steven, R., Leuis, A. Prolactin production by immune cells. ***Lupus*** (2001)10: 665-675.