DOI: 10.7860/JCDR/2017/8567.9436

Original Article

Internal Medicine Section

Effect of Anti-Cyclic Citrullinated Peptide and HLA-DRB1 Subtypes on Clinical Disease Activity Index in Rheumatoid Arthritis Patients

AKBAR SOLEIMANI[1], ZAHRA MOBEDI[2], MARYAM AL-E-RASUL[3], ABOLGHASEM SHARIFI[4], ABDOLRAHIM KAZEMI VARDANJANI[5]

ABSTRACT

Introduction: Rheumatoid arthritis (RA) is a crippling disease with a global prevalence of approximately 0.5%-1% in adults. Genetic, environmental and immunologic factors contribute importantly to pathogenesis of RA. American College of Rheumatology (ACR) assists in early diagnosis of the disease.

Aim: The aim of this study was to investigate the effects of *HLA-DRB1* gene and anti-Cyclic Citrullinated Peptide (CCP) antibody on Clinical Disease Activity Index (CDAI) and to determine the frequency of HLA-DRB1 alleles in the patients with RA.

Materials and Methods: In this descriptive-analytical study, 64 patients with RA referring rheumatology clinic of Hajar Hospital, Shahr-e-Kord, Iran were enrolled based on ACR criteria (1987) by convenience sampling. All patients were examined to assess primary CDAI and referred to laboratory for serologic tests [Rheumatoid Factor (RF) and anti-CCP]. After the patients' DNA was extracted, HLA-DRB1 was determined per single specific primer-polymerase chain reaction by inno-train kits. The patients were re-examined six months later.

Results: The most prevalent type of HLA-DRB1 in the studied patients was 04. In patients with HLA-DRB1 (04), HLA-DRB1 (01), and HLA-DRB1 (15), CDAI decreased pronouncedly after six months, but in other patients it did not ($p<0.05$). Of the patients, 81.3% had high titers of anti-CCP, but no association between anti-CCP and CDAI was found.

Conclusion: RA could be a multifactorial disease. The patients with HLA-DRB1 (04), HLA-DRB1 (01) and HLA-DRB1 (15) showed a good response to routine treatments. The patients with HLA-DRB1 (04) are likely to have no decrease in secondary CDAI. High titers of anti-CCP in patients may indicate the severity of RA in the studied region and perhaps environmental, genetic and unknown or idiopathic factors are aetiologically crucial.

Keywords: Human leukocyte antigen, Rheumatic diseases, Rheumatoid Factor

INTRODUCTION

RA is a chronic autoimmune disease and the most common inflammatory joint disease has a global prevalence of approximately 0.5%-1% in adults. RA characterized by the presence of RF and anti-citrullinated protein/peptide autoantibodies [1-3]. The incidence and prevalence of RA vary depending on the geographical region [4].

RA is a chronic disease with numerous degenerative effects on joints. Auto immune disease and genetic factors which are HLA-DRB1 and its subtypes follow different patterns in different races and communities [1]. RA course varies widely, making the prognosis difficult in individual patients.

Although no factor has been determined as the main and primary beginner of the disease, there is some evidence indicating that synovial inflammation is likely to result from the complex interactions among environmental, genetic and immunologic factors, leading to deregulation in the immunity system and disruption of auto tolerance process. The finding that auto antibodies such as RF and anti-CCP could be found in patients' serum long before clinical stage confirms the above theory [4,5].

The rate of contribution of genetic factors to RA is varied in different studies due to the interaction between the genes and environment. System of Human Leukocyte Antigen (HLA) plays a role in development of rheumatic diseases in different individuals with different characteristics. The risk of acquiring RA is mainly associated with allelic variation in HLA-DRB1 gene which encodes the beta chain of MHC-II molecules [4].

Preliminary studies indicated that 70% of RA patients had HLADR4, much higher than 28% of the control group. This association was more pronounced in the patients with anti-CCP production. Pathogenic alleles of HLA-DRB1 have a shared amino acid sequence at position 70-74 in the third hypervariable region of the HLA-DR beta chain, called Shared Epitope (SE) [6].

In 2010, American College of Rheumatology classification criteria (1987) for RA was revised [7]. Recently revised criteria (in 2010) specifies the scores 0-10 and the score ≥ 6 confirms diagnosis of RA. The criteria are as follows: the number of involved joints, serology tests (titres of RA and anti-CCP), markers of acute phase response and duration of symptoms [7].

Van Beers JJ et al., study in the Netherlands reported that anti-citrullinated fibronectin antibodies were positively correlated with HLA type and SE alleles in RA patients [8]. Ucar F et al., found HLA*01, HLA*04, and HLA*09 as the most frequent alleles and HLA*013 as the least frequent allele associated with RA in east Black Sea Turkish population [9].

Mackie SL et al., conducted a study in England to investigate different HLA in anti-CCP-positive patients with RA and found that subgroups of HLA-DRB1*0401 and HLA-DRB1*0404 contributed to the pathogenesis of RA [10]. Naqi N et al., concluded that HLA-DRB1 (*04) was highly frequent in patients with RA, but in the control group, HLA-DRB1 (*11) was found as the most frequent allele with potentially protective role against RA [11].

S Zodaray P et al., found that anti-CCP was highly important for RA diagnosis and an important factor in RA progression and radiologic changes. Also, anti-CCP production was highly associated with genetic, underlying factors like HLADRB1 [12].

In Chaharmahal va Bakhtiari province (Southwestern Iran), with regard to consanguineous marriages and specific ethnicities, no study on rheumatologic diseases, including RA and genetic and

serological associations, has been yet conducted and no data on RA gene mapping and prevalence, especially with RF or anti-CCP positivity, are available. Therefore, this study was conducted for the first time in this province to determine the relative frequency of HLA-DRB1 alleles and to investigate the effect of these alleles and anti-CCP antibody on CDAI in RA patients to pave the way for more comprehensive research.

MATERIALS AND METHODS

In this retrospective, case control and prospective study, the study population consisted of all patients with definite diagnosis of RA according to American College of Rheumatology criteria. The study was performed in rheumatology clinic of Hajar Hospital affiliated to Shahr-e-Kord University of Medical Sciences, Iran. Sample size was 64 patients referring the clinic from May 2010 to August 2012 with RA definite diagnosis made by rheumatologist were enrolled. Data were recorded in the checklists after giving the initial explanations to the patients, a complete physical examination, and obtaining history of the disease carefully. Necessary information including demographic data (such as age and gender), disease period, taken drugs, preliminary examination results, serologic markers (RF, anti-CCP, ESR, and CRP), and CDAI was recorded in a questionnaire. Patients with concurrent septic arthritis were excluded from the study. Data were recorded in checklists to make a detailed comparison between the indicators in question after six months. Almost 5 ml blood sample was taken from the patients enrolled for serologic tests (RF, anti-CCP, ESR, and CRP).

Then, blood samples containing EDTA, as anticoagulant, were transformed to a cellular and molecular research center for molecular tests and HLA-typing, and DNA was extracted by phenol-chloroform method.

Spectrophotometry was run to check the quality and quantity of the extracted DNA. After extraction of DNA in the laboratory by phenol chloroform method, various subtypes of HLA-DRB1 were detected per single specific primer-PCR using inno-train kits (Germany).

In this study, a modified PCR, namely Sequence Specific Priming (SSP) PCR was used, in which only 3' end of the primer is responsible for building the specific allele under investigation. For complete analysis of SSP, numerous amplifications were run simultaneously. Only the samples that contain the sequences complementary to the primers in each well produced the PCR product, and indeed each sample produced product in only one or few wells (depending on the type of serotype). Therefore, each sample's serotype was specified. In addition, there was a pair of common primers (internal primer) inside all the wells, which was complementary to the sequence existing in all serotypes and hence a band was developed in all 24 wells (except negative control). PCR products were analyzed by agarose gel.

The results were interpreted based on the tables provided by the kit manufacturer. These tables provided us with the data regarding the length of each well product (the expected size of DNA bands and internal control band in each well).

For anti-CCP, the patients were divided into three groups, positive low titre, positive high titre, and negative. Then, the patients' HLA was determined. HLA-DR alleles were classified based on anti-CCP classification, and the CDAI was accordingly determined and recorded.

Patients received a usual medical intervention and were re-examined six months later with no dropout in 64 patients. A new questionnaire was filled out for them and the CDAI was determined. At the end of the intervention the variations in the CDAI, as an indicator of response to the treatment, in different groups were compared. The CDAI was determined based on the number of sensitive joints, the number of swollen joints, patient's assessment, and clinical examination of patients [5]. By measuring CADI and determining anti-CCP antibody in patients, the association between them was examined.

STATISTICAL ANALYSIS

The data were analyzed by SPSS software using descriptive statistics, correlation tests, and Chi-square.

RESULTS

1. Effect of HLA-DRB1 Gene Subtypes on CDAI

The most frequent HLA in the studied patients was HLA-DRB1 (04) followed by HLA-DRB1 (11) and HLA-DRB1 (01) [Table/Fig-1].

In 64 patients under study the highest initial CDAI was obtained 24 with range 7.81±5.13. CDAI in the patients with the most prevalent HLA-DRB1 subtypes decreased in number after 6 months, with a significant difference ($p<0.05$). Initial CDAI and the CDAI six months later in HLA-DRB1 (01) was $p=0.043$ and paired t-test showed this decrease was significant.

Initial CDAI in HLA-DRB1 (11) increased six months later, but this increase was not significant. The CDAI six months later decreased compared to initial index in all patients, but this decrease was not significant [Table/Fig-2].

2. Effect of Anti-CCP Antibody on CDAI

For anti-CCP, 15.6% of the patients were negative (titre of < 30), 3.1% had low titre (less than or equal tripled the maximum normal titre), and 81.3% had high titre (more than tripled the maximum normal titre) [4].

Out of 54 patients with common HLA subtypes, the most frequent (83.3%) patients had high anti-CCP titre followed by 14.8% of the patients with negative titre and 1.9% with low titre.

CDAI decreased pronouncedly in patients with positive, high titre anti-CCP, but no significant difference between anti-CCP titre and CDAI was seen in all patients [Table/Fig-3].

No statistically significant difference between anti-CCP and the initial CDAI and the CDAI six months later ($p=0.0164$, $p=0.0372$, respectively).

3. Demographic Characteristics

Out of the studied patients, 10.9% were male and 89.1% were female. In addition, 70.3% of the patients had no family history of RA. A total of 26% of the patients were RF-negative, and the rest was of (+1) to (+4). The mean age of samples was 49.5±13.09 year and the duration of suffering from disease was 9.7±7.9 year.

HLA-DRB1 subtypes	Frequency	%
04	20	3.31
11	15	4.23
01	10	6.15
15	9	1.14
10	4	3.6
14	3	7.4
07	2	1.3
16	1	6.1
Total	64	100

[Table/Fig-1]: A comparison of frequency among the most prevalent HLA-DRB1-subtypes in the studied patients.

HLA type	CDAI	Mean±SD	p-value
HLA-DRB1(04)	Initial	9.32±5.78	0.002
	Six months next	5.22±3.23	
HLA-DRB1(11)	Initial	5.93±3.78	0.197
	Six months next	8.83±6.76	
HLA-DRB1(15)	Initial	8.4±3.93	0.045
	Six months next	4.6±3.18	
Prevalent HLAs	Initial	7.75±4.95	0.046
	Six months next	5.94±4.73	
Total	Initial	5.13±7.81	0.66
	Six months next	4.86±6.25	

[Table/Fig-2]: Comparison of severity of the initial CDAI* and the CDAI six months later in HLA-DRB1 subtypes in RA patients.
* Clinical disease activity index

Anti-CCP antibody	F	CDAI	Mean±SD	p-value
Negative	10	Initial	5.8±4.76	0.817
		Six months next	5.45±2.92	
Low	2	Initial	3.5±0.0707	0.817
		Six months next	3.5±1.41	
High	52	Initial	8.3±5.17	0.066
		Six months next	6.51±5.21	
Total	64	Initial	7.8±5.13	0.66
		Six months next	6.25±4.8	

[Table/Fig-3]: Comparison of severity of the initial CDAI* and the CDAI six months later in HLA-DRB1 subtypes in RA patients for different titers of anti-CCP
* Clinical disease activity index

No statistically significant difference between the type of HLA and gender, and between RA severity and gender was observed (p>0.05). No significant difference was observed between gender and age of the patients (M=52.4±16 and F= 49.1±12 year), between gender and disease duration (M= 8.2±9, F= 9.3±7 year), and between gender and CDAI (M=8.1±5, F=7.7±5), (p>0.05).

In patient with common HLA subtypes, based on one-way Kruskal–Wallis test, the age (p=0.349), anti-CCP antibody (p=0.085), and CDAI (p=0.123) did not show statistically significant difference and only disease duration in HLA 15 was significantly higher than other HLAs (p<0.05). Moreover, the chi-square test showed no statistically significant difference between gender and type of HLA (p=0.145) and between family history and the type of HLA (p=0.213). No significant association was found between HLA type and severity of RF, and between anti-CCP and HLA type in RA patients, as well (p=0.927 and 0.498, respectively).

DISCUSSION

Because of the insidious and progressive nature of RA, in the absence of timely diagnosis and appropriate treatment, it can lead to complications and disability in patients, and leaving the cycle of economic activity by the patients and their crippling lead to high costs for individual and community. This study was carried out to pave the way for further investigations regarding this disease and other rheumatologic diseases and to do appropriate planning with regard to the prevalence of seropositivity in the early detection of the disease so that RA-related complications and disability rate could be reduced as much as possible.

In this study, the frequency of HLA-DRB1 types was reported: type 04 (0.31.3%), type 11(23.4%), type 01(15.6%), and type 15 (15.6%). Therefore, the most frequent allele was type HLA-DRB1 04, called SE, in the studied patients, which is consistent with many similar studies, such as Ucar F et al., study in Turkey, Naqi N et al., study in Pakistan, Mitsunagu et al., study in Japan, Ben Hamed M et al., study in Tunisia, and Farouk HM et al., study in Egypt [9,11,13-15]. This allele can increase the predisposition to RA and may be associated with RA prognosis [16].

In different studies, the prevalence of some subtypes was obtained higher in different races and regions worldwide. For example, in a Turkish population of the patients with RA, the prevalence of HLA subtypes 09, 04, and 01, and in a Tunisian population, the prevalence of HLA-DRB1 04 was higher. Even, in different provinces of Iran varied prevalence has been obtained for different subtypes. For example, a study in Zahedan-Iran, Sistan va Baluchestan showed that the prevalence of HLA-DRB1 0110 was higher [17]. In another study on RA patients in Khorasan-Iran, the prevalence of HLA subtype DQ5 was higher [18].

Since a direct correlation between HLA system particularly HLA-DR4 subgroups, more specifically HLA-DR1, and RA, RF, and/or positive anti-CCP has been reported in several studies, determination of HLA and above factors may predict the outcome of the disease and assist in developing the type of therapy. This argument could be explained by the potential resistance, from the RA onset, to primary medications used for its treatment with increased titre of anti-CCP and RF, and presence of certain HLAs.

It seems that multifactorial behaviors contribute to RA development. We found that the patients with HLA-DRB1 15, 01, and 04 showed suitable responses to the routine therapies. It is not consistent with what has been mentioned in the scientific resources and the patients with HLA-DRB1 04 are likely to have no decrease in secondary CDAI. High titres of anti-CCP antibody in the patients may be representative of the severity of RA disease in the province under study, and other genetic and environmental factors could be aetiologically important. More comprehensive studies could further elucidate this issue.

In examining the relationship of CDAI with HLA type and anti-CCP, we found that this index significantly decreased compared to the second examination only in the groups of the patients with HLA-DRB1 01, 04, and 15, and no significant difference was seen in other groups of the patients. This finding could be due to different responses of patients with different HLAs to pharmacologic therapies including Disease-Modifying Antirheumatic Drugs (DMARDs).

In the patients with HLA-DRB1 11 consisting of 15 individuals, CDA1 increased after six months unexpectedly, but this increase was not significant. Due to small number of patients, this finding cannot be definitely commented on, and definite explanation requires more comprehensive research. This HLA type may be associated with more severity of the disease.

In the present study, no significant relationship between HLA and anti-CCP, RF, and initial CDAI was found. This finding confirms Lu Z et al., study in China finding that HLA-DR4 was not associated with DAS28, anti-CCP and RF, but PAD4 was associated with disease activity (DAS28) and anti-CCP. On the other hand, STAT4 is associated with DAS28 and RF. A potential laboratory error in measuring anti-CCP titre could be another reason for this finding [19].

No relationship between CDAI and anti-CCP confirms the results of Laki J et al., in Sweden and Montes A et al., in Spain. In Montes A et al., study, lack of association was due to high specificity and negative anti-citrullinated alpha-enolase in the patients with anti-CCP [20,21].

In the present study, the initial decrease in CDAI and the decrease in CDAI six months later were not significant in all patients. It seems that a larger sample size will help to achieve more acceptable results. Notably, this decrease was significant for most patients.

The effective genetic factors (HLA-DRB1 and sub-types) follow different patterns in different communities and races. The obtained specific patterns can be further completed in future studies [1].

The CDAI decreased after six months only in the patients with HLA-DRB1 04, 01, and 15 subtypes, and no statistically significant difference was observed in other groups of the patients.

According to the results of statistical analysis of the data, and statistically significant differences in the association between HLA type and CDAI, it can be concluded that patients with HLA-DRB1 04, 01, and 15 responded to the RA routine therapies suitably.

The study of anti-CCP antibody frequency in patients with common HLAs revealed that 54 patients had the most frequent alleles. Most (83.3%) patients had anti body titres higher than 3, which is consistent with some studies [16]. Perhaps, these results indicate the severe and progressive nature of the disease in the province under study. More accurate conclusions require more comprehensive studies in this region and other regions of the country.

Consistent with other studies, the present study found that anti-CCP antibody could have an effective role in diagnosis and determination of disease prognosis. Almost 84% of the patients were positive for anti-CCP antibody. In the patients who were negative for anti-CCP antibody, the role of RF or other immunity factors could be stronger [1].

In this study, only the association between HLA-DRB1 15 and duration of disease was statistically significant in the HLAs obtained from patients. Further similar studies need to be done in this field, because no similar finding was seen in the literature to the best of our knowledge [20].

In this study, the frequency of RF with regard to its effective role in developing and diagnosing the disease (with sensitivity and specificity of about 90%) was considered and found similar to other studies' findings because 74% of the studied patients were positive for RF (1+ to 4+), which confirms the crucial role of RF in early diagnosis of RA [1].

According to the role of specific HLAs and genetic patterns of the patients in RA disease, family history of the disease was expected to be high. Almost 30% of the studied patients had family history of RA, but 70.3% of the patients did not. Therefore, the role of environmental and genetic factors could be pronounced and hence complementary studies in this area are needed to compare the effect of environmental (such as smoking, mineral oil, and silicon), infectious (such as Epstein-Barr virus, parvovirus B19, *Streptococcus*, and *Mycoplasma*), and other genetic factors to derive more acceptable results [4].

The mean age of the patients in the present study was 49.4 years, which was similar to the mean age of the patients with RA in different communities [4].

The patients with HLA-DRB1 04 in the present study exhibited a better response to the therapies compared to other patients. But in the Mori S et al., study in Japan, HLA-DRB1 04 was associated with more severity of the disease and drug resistance to DMARDs [22]. This inconsistency in the findings could be explained by the difference in genetic factors other than HLA.

LIMITATION

Limitations of this study include the cost of expensive tests to determine HLA and two stages of the study required to follow up the patients. Another problem was related to communicating with the patients to have them refer for subsequent examination and follow up and the patients migrating to other regions. Finally, 64 patients were present both at onset and till the completion of the study.

With further study to evaluate the type and dose of drugs leading to remission or low disease activity in patients with different HLA subtypes and different anti CCP titres, valuable findings on the RA severity and its association with the patients' genetic features could be obtained. Study of the association between immunologic factors, such as PTPN22, STAT4, and PAD4, and RA clinical course is recommended in future investigations.

CONCLUSION

RA could be a multifactorial disease. The patients with HLA-DRB1 (04), HLA-DRB1 (01) and HLA-DRB1 (15) showed a good response to routine treatments. The patients with HLA-DRB1 (04) are likely to have no decrease in secondary CDAI. High titers of anti-CCP in patients may indicate the severity of RA in the studied region and perhaps environmental, genetic and unknown or idiopathic factors are aetiologically crucial.

ACKNOWLEDGEMENTS

This research was obtained from an MD thesis (no. 949), approved and performed at Shahr-e-Kord University of Medical Sciences. Authors thank all colleagues who contributed to conducting this study and Deputy of Research and Technology of Shahr-e-Kord University of Medical Sciences for funding this research.

REFERENCES

[1] Al-Shukaili A, Al-Ghafri S, Al-Marhoobi S, Alkaabi J. Evaluation of anti-mutated citrullinated vimentin antibodies, anti-cyclic citrullinated peptide antibodies and rheumatoid factor in Omani patients with rheumatoid arthritis. Int J Rheumatol. 2012;2012:285854.
[2] Sakkas LI, Bogdanos DP, Katsiari C, Platsoucas CD. Anti-citrullinated peptides as autoantigens in rheumatoid arthritis-relevance to treatment. Autoimmun Rev. 2014;13(11):1114-20.
[3] Guo CJ, Lv JH, Niu DS, Ma T, Sun SX, Li LX, et al. Detection of anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis patients undergoing total knee arthroplasty. Int J Clin Exp Med. 2015;8(3):4410-14.
[4] Harrison H, Brawnwald G, Fucci T, Kasper R. Principles of intercultural medicine in an internal medicine program. 18th ed. USA: Mcgraw Hili; 2012.
[5] Molenaar ET, Voskuyl AE, van der Horst-Bruinsma IE, Schreuder GM, Zanelli E, Dijkmans BA. Influence of HLA polymorphism on persistent remission in rheumatoid arthritis. Ann Rheum Dis. 2002;61(4):351-53.
[6] Fugger L, Svejgaard A. Association of MHC and rheumatoid arthritis. HLA-DR4 and rheumatoid arthritis: Studies in mice and men. Arthritis Res. 2000;2(3):208-11.
[7] Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, et al. 2010 rheumatoid arthritis classification criteria: An American College of Rheumatology/ European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2010;69(9):1580-88.
[8] van Beers JJ, Willemze A, Stammen-Vogelzangs J, Drijfhout JW, Toes RE, Pruijn GJ. Anti-citrullinated fibronectin antibodies in rheumatoid arthritis are associated with human leukocyte antigen-DRB1 shared epitope alleles. Arthritis Res Ther. 2012;14(1):R35.
[9] Ucar F, Karkucak M, Alemdaroglu E, Capkin E, Yucel B, Sonmez M, et al. HLA-DRB1 allele distribution and its relation to rheumatoid arthritis in eastern Black Sea Turkish population. Rheumatol Int. 2012;32(4):1003-07.
[10] Mackie SL, Taylor JC, Martin SG, YEAR Consortium, UKRAG Consortium, Wordsworth P, et al. A spectrum of susceptibility to rheumatoid arthritis within HLA-DRB1: Stratification by autoantibody status in a large UK population. Genes Immun. 2012;13(2):120-28.
[11] Naqi N, Ahmed TA, Malik JM, Ahmed M, Bashir MM. HLA DRbeta1 alleles in Pakistani patients with rheumatoid arthritis. J College Physicians Surg-Pak. 2011;21(12):727-30.
[12] S Zodoray P, Szabo Z, Kapitany A, Gyetvai A, Lakos G, Szanto S, et al. Anti-citrullinated protein/peptide autoantibodies in association with genetic and environmental factors as indicators of disease outcome in rheumatoid arthritis. Autoimmunity reviews. 2010;9(3):140-43.
[13] Mitsunaga S, Suzuki Y, Kuwana M, Sato S, Kaneko Y, Homma Y, et al. Associations between six classical HLA loci and rheumatoid arthritis: A comprehensive analysis. Tissue Antigens. 2012;80(1):16-25.
[14] Ben Hamad M, Mahfoudh N, Marzouk S, Kammoun A, Gaddour L, Hakim F, et al. Association study of human leukocyte antigen-DRB1 alleles with rheumatoid arthritis in South Tunisian patients. Clin Rheumatol. 2012;31(6):937-42.
[15] Farouk HM, Mansour HE, Rahman SA, Mostafa AA, Shamy HA, Zarouk WA. Effect of the human leukocyte antigen HLA-DRB1 and anti-cyclic citrullinated peptide on the outcome of rheumatoid arthritis patients. Brazilian J Med Biol Res. 2009;42(9):831-38.
[16] Kapitany A, Szabo Z, Lakos G, Aleksza M, Vegvari A, Soos L, et al. Associations between serum anti-CCP antibody, rheumatoid factor levels and HLA-DR4 expression in Hungarian patients with rheumatoid arthritis. Israel Med Asso J. 2008;10(1):32-36.
[17] Sandoughi M, Fazaeli A, Bardestani G, Hashemi M. Frequency of HLA-DRB1 alleles in rheumatoid arthritis patients in Zahedan, Southeast Iran. Ann Saudi Med. 2011;31(2):171-73.
[18] Shoja Taheri F, Sadeghizadeh M, Tavakkol Afshari J. The role of HLA-DQB1 alleles in susceptibility to rheumatoid arthritis in northeastern Iran. Modares J Med Sci. Spring. 2007;9(1):25-36.
[19] Lu Z, Li J, Feng ZT, Ren J. [Association of HLA-DR4, PAD4, and STAT4 expression in the peripheral blood with disease activity in patients with rheumatoid arthritis]. Nan fang yi ke da xue xue bao (J Southern Med Univ). 2010;30(6):1349-53.
[20] Laki J, Lundstrom E, Snir O, Ronnelid J, Ganji I, Catrina AI, et al. Very high levels of anti-citrullinated protein antibodies are associated with HLA-DRB1*15 non-shared epitope allele in patients with rheumatoid arthritis. Arthritis Rheum. 2012;64(7):2078-84.
[21] Montes A, Dieguez-Gonzalez R, Perez-Pampin E, Calaza M, Mera-Varela A, Gomez-Reino JJ, et al. Particular association of clinical and genetic features with autoimmunity to citrullinated alpha-enolase in rheumatoid arthritis. Arthritis Rheum. 2011;63(3):654-61.
[22] Mori S, Hirose J, Yonemura K. Contribution of HLA-DRB1*04 alleles and anti-cyclic citrullinated antibodies to development of resistance to disease-modifying antirheumatic drugs in early rheumatoid arthritis. Clin Rheumatol. 2010;29(12):1357-66.

PARTICULARS OF CONTRIBUTORS:
1. Faculty, Department of Internal Medicine, Shahr-e-Kord University of Medical Sciences, Shahr-e-Kord, Iran.
2. Faculty, Department of Rheumatology, Shahr-e-Kord University of Medical Sciences, Shahr-e-Kord, Iran.
3. Faculty, Department of Gynaecology and Obstetrics, Shahr-e-Kord University of Medical Sciences, Shahr-e-Kord, Iran.
4. Deputy of Research and Technology, Shahr-e-Kord University of Medical Sciences, Shahr-e-Kord, Iran.
5. Deputy of Research and Technology, Shahr-e-Kord University of Medical Sciences, Shahr-e-Kord, Iran.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Zahra Mobedi,
Faculty, Department of Rheumatology, Shahr-e-Kord University of Medical Sciences, Shahr-e-Kord, Iran.
E-mail: z_mobedi@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jan 20, 2014**
Date of Peer Review: **Jul 17, 2014**
Date of Acceptance: **Aug 26, 2015**
Date of Publishing: **Mar 01, 2017**