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Association of IL-18 cytokine and HLA-DRB1 alleles with arthritis patients

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Abstract : The current study was conducted to investigate the role of some aspects of immunogenetics that related with arthritis . Fifty blood samples were collected from people who reviewed Marjan hospitalHilla-Iraq, with 25 samples from apparently health people for period of October 2015 to March 2016. Immunological study involved estimate the level of IL-18 in certain arthritis patients using ELISA technique , the estimation of IL-18 revealed significantly increased in all patients by contrast with control group with mean value133.93 pg/ml in rheumatoid arthritis(RA) patients and 103 .3pg/ml for osteoarthritis (OA) patients with P <0.01 . The possibility of detection of histocompatibility antigens HLA(DRB1) alleles related genetically with disease by using (PCR-SSP) was performed and showed that no significant differences between RA & OA patients with DRB1 (0301 & 0701).
Keywords : Arthritis ,IL18 , HlA, Immunogenetic.

Introduction

Rheumatoid arthritis (RA) and osteoarthritis (OA) are two regular ceaseless joint issues whose etiology stays obscure. The RA synovium is described by angiogenesis, or fresh recruit's vessel development, and leukocyte penetration that prompt tissue attack and joint demolition[1].

Interleukin-18 (IL-18) is a member of the IL-1 superfamily that enhances both innate and acquired immune responses. IL-18 is produced as pro-IL-18, precursor form, in monocyte/ macrophages, dendritic cells, Kupffer cells, keratinocytes, articular chondrocytes, synovial fibroblasts and osteoblasts[2].

In RA synovium, the expression of IL-18 is associated with that of IL-1β and TNFα and correlates with the acute-phase response, indicating that IL-18 is an important proinflammatory cytokine that drives the local production of IL-1β and TNFα in RA[3]. Although IL-18 secretion has been demonstrated in normal cartilage, it is significantly enhanced in osteoarthritic cartilage[4].

Major histocompatibility complex (MHC) genes account for 50% of the genetic susceptibility in most auto immune diseases, in RA the HLA region contributes most to the genetic risk, specifically there have been reports for association of class II antigens DRB1play a key role in predisposition to most severe form of the disease[5].Osteoarthritis is a familial disorder caused by a mixture of genetic and environmental factors, environmental factors include way of life factors such as being obese, inactive job, recurring use of joints and trauma to affected joints[6]. The genes that predispose to osteoarthritis remain to be clarified, many studies have pointed to different HLA class I and II associations, perhaps indicating the heterogeneity of the condition[7].

Material &Methods

This study include one hindered patients suffering from arthritis who attend to Marjan hospital ,Hilla-Iraq for the period from October 2015 to March 2016 , in addition twenty five apparently healthy human were taken from Babylon province as a control. Blood collected from all patients and control healthy persons. Each blood sample were divided into two parts, one added to anticoagulant tube for genetic investigation and the remaining blood added to tube without anticoagulant allowed clotting at room temperature for 20 minute then serum was separated by centrifugation at 3000 rpm for 5min.for immunological assay.

Immunological Tests

Enzyme Linked Immune-sorbent Assay (EISA) technique according to manufacturer instruction of Elabscience (China) was applied to measurement concentration of IL-18 in sera of arthritis patients .

Isolation of genomic DNA:

The isolation of DNA from whole blood was applied using Wizard genomic DNA purification kits which based on five steps process using salting out methods. The genomic DNA was concentrated and desalted by Isopropanol precipitation, the genomic DNA was rehydrated using DNA Rehydration Solution. Then the typical DNA yielded from (300µl) of whole blood was about (5-15 µg) which depends on the quantity of WBCs.

Thermal Cycling Conditions

Duplicate reaction for HLA-DRB1 alleles set was done . The reaction contains forward primer 1.5µl , reverse primer 1.5µl , 4µl of DNA sample , 5µl of master mix , and 8µl of Nuclease Free Water .All the components was collected in a special tube called microcentrifuge tube PCR (20µl),then these entered into Thermocycler apparatus with the cycling conditions in table (1)

Allele	Sequences5'- 3'	Size of amplicon	Thermal cycler conditions	References
0301	5-TACTTCCATAACCAGGAGGAGA-3 5-TGCAGTAGTTGTCCACCCG-3	151 bp	95℃ for 5min 94℃ for 20 sec 60℃ for 40 sec 72℃ for 30 sec 72℃ for 1min	Nakajima *et al.*, 2010[7]
0701	5-CCTGTGGCAGGGTAAGTATA-3 5-CCCGTAGTTGTGTCTGCACAC-3	232 bp	95℃ for 5min 94℃ for 20 sec 56° C for 30 sec 72℃ for 30 sec 72° C for 1min	Nakajima *et al.*, 2010[7]

Agarose Gel Electrophoresis

Successful PCR amplification was confirmed by agarose gel electrophoresis. Agarose gel was prepared by dissolving 1.5 gm of agarose powder in 100ml of TBE buffer previously prepared (90 ml DW were added to 10 ml TBE buffer l0X, the final concentration was 1 X and pH: 8) in boiling water bath, allowed to cool to 50^{o}C and ethidium bromide at the concentration of 0.5µg/ml was added.

The comb was fixed at one end of the tray for making wells used for loading DNA sample. The agarose was poured gently into the tray, and allowed to solidify at room temperature for 30 min. Then the comb was removed gently from the tray. The tray was fixed in an electrophoresis chamber filled with TBE buffer that covered the surface of the gel, 5µl of PCR product was transferred into the wells in agarose gel, and in one well we put the 6µl DNA ladder.

The electric current was allowed at 100 volt for 30min, then 70 volt for 30 min. Gel documentation system was used for the observation of DNA bands by using the gel documentation apparatus (E. graphgel documentation).

Results

The present study involved different types of arthritis patients selected according to many symptoms including joint pain, swelling, stiffness and fatigue. And it insights in this subject in two types; Rheumatoid arthritis and Osteoarthritis; throughout two major aspects : immunological and genetic studies. This study is carried on 50 patients attend to Marjan hospital , these patients included slightly and severity cases of disease according to diagnosis of doctor, in addition to 25 sample of apparently healthy as control .

Distribution of patients with types of disease.

The percentage of infection was estimated according to types of arthritis which include rheumatoid arthritis and osteoarthritis. The result revealed that rheumatoid arthritis consist of 30 patients(60%), while osteoarthritis 20 patients(40%) . table (2).

Table (2) Distribution of patients according to type of disease

Arthritis types	Number of patients	Percentage %
Rheumatoid arthritis (RA)	30	60 %
Osteoarthritis (OA)	20	40 %
Total	50	100 %

Immunological assays

Sera of arthritis patients were divided according to the type of disease into two groups, apparently healthy (control) were also selected for each type for cytokines levels determination.

IL-18 concentration in Rheumatoid arthritis and Osteoarthritis patients.

The results of the current study showed an increased level of IL-18 concentration in all patients group compared with control .The statistical analysis revealed the presence of a significant difference in the IL-18 concentration between the patients and control and found the patients with RA had a higher value (133.93 ± 35.85 pg/ml) compared with control group , while osteoarthritis reached (103.3 ± 13.3pg/ml) with ($P< 0.05$), table (3).

Table (3) Concentration of IL-18 with types of arthritis .

Subject	IL-18 level pg/ml M ±SD
Rheumatoid arthritis (RA)	133.93 ± 35.85
Osteoarthritis (OA)	103.3 ± 13.3
Control	51.04 ± 19.87

The results show significant increasing in the level of IL-18 concentration in total arthritis patients which reached (125.24 ± 35.99 pg/ml) compared with control (51.04 ± 19.87 pg/ml) ,figure (1).

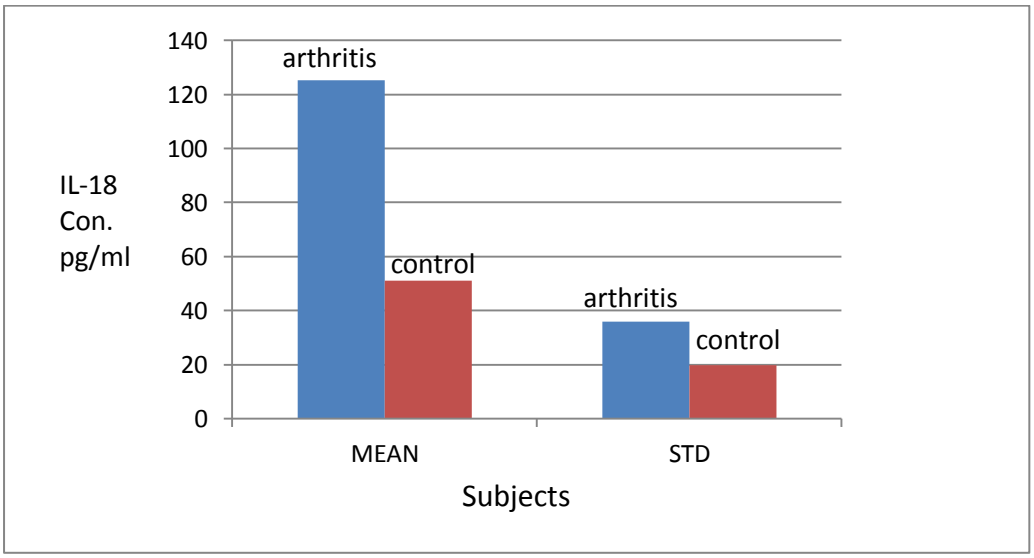

Figure (1) IL-18 concentration for arthritis patients .

Current study showed an increased level of IL-18 concentration in females with two types of arthritis compared with control, table(4).

Table (4) Distribution of IL-18 concentration in both sex according to types of arthritis .

Subjects	**IL-18 pg/ml**	**IL-18 pg/ml**
	Female Mean ± SD	**Male** **Mean± SD**
Rheumatoid arthritis	155.4 ± 39.3	**108.8 ± 6.66**
Osteoarthritis	111.17 ± 9.25	**91.5 ± 7.83**
Control	51.04 ± 19.87	**51.04 ± 19.87**

Genetic assay

The genetic investigations involved a total of 50 samples were used in this study, obviously the current results were consecrated on Human Leukocyte Antigen (HLA) class II in DRB1 alleles.

HLA DRB1 (0.0301) alleles.

Genotypic study for HLA DRB1 (0.03)allele showed variation in number and percentage of allelic prevalence, eleven case show positive result for Rheumatoid arthritis with rate of 36.7%, while two case revealed positive result in Osteoarthritis with rate 10%, but there is no significant differences, as shown in table(5).

Table (5) Distribution of HLA DRB1*0301 allele between arthritis patients and control.

Types of disease	State	Case		Control		OR(95%CI)	P value
RA n=30	Positive	11	36.7%	6	24%	1.8333 (0.563-5.970)	**0.3864**
	Negative	19	63.3%	19	76%	0.5455 (0.167-1.776)	
OA n=20	Positive	2	10%	6	24%	0.3519 (0.062-1.975)	**0.2692**
	Negative	18	90%	19	76%	2.8421 (0.506-15.956)	
RA&OA n=50	Positive	13	26%	6	24%	1.1126 (0.365-3.390)	**1.00**
	Negative	37	74%	19	76%	0.8988 (0.294-2.739)	

Agarose gel electrophoresis of PCR products for HLA DRB1 (0.03) allele reveal the positive results which reached 13 with rate 26 % for arthritis patients while 6 with rate 24 % for control , figure (2).

Figure (2) Agarose gel electrophoresis of PCR products for detection HLA DRB1*0301 allelic product in human, lane 1-12 refers to sample number .L ladder (100 bp).

HLA DRB1 (0.0701) allele with arthritis patients and control

Genotypic study for HLA DRB1 (0.07)allele showed contrast in number and percentage of allelic prevalence, (17) case show positive result for Rheumatoid arthritis with rate of 56.7% , while (5) case showed positive result in Osteoarthritis with rate 25%, but there is no significant differences ,table(6).

Table (6) Distribution of HLA DRB1*0701 allele of arthritis patients

Types of disease	State	case		control		OR(95%CI)	P value
RA n=30	Positive	17	56.7%	12	48%	1.416 (0.487-4.115)	**0.5934**
	Negative	13	43.3%	13	52%	7059 (0.243-2.050)	
OA n=20	Positive	5	25%	12	48%	0.3611 (0.100-1.299)	**0.1351**
	Negative	15	75%	13	52%	2.7692 (0.769-9.966)	
Total n=50	Positive	22	44%	12	48%	0.8512 (0.324-2.229)	**0.8083**
	Negative	28	56%	13	52%	1.1748 (0.448-3.077)	

Agarose gel electrophoresis of PCR products for HLA DRB1(0.07) allele show the positive results which reached 22 with rate 44 % for arthritis patients compared with control which reached 12with rate 48%, figure (3).

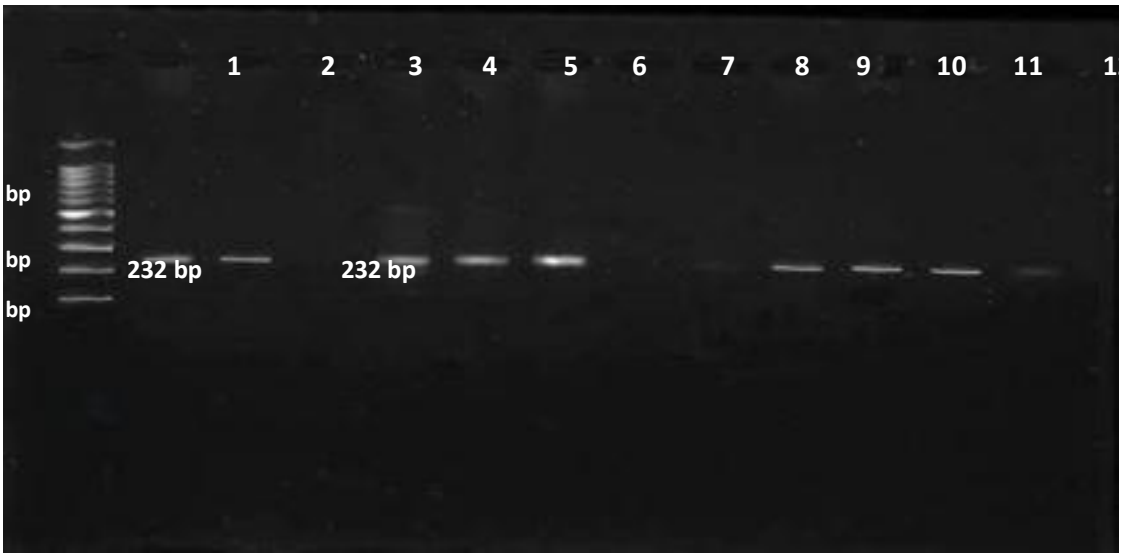

Figure (3) Agarose gel electrophoresis of PCR products for detection HLA DRB1*0701 allelic product in human, lane 1-12 refers to sample number .L ladder (100 bp).

Discussion

Distribution of types of arthritis

The highest disease frequency was noticed in age groups (71 – 80) then (61 – 70) years. The study included two types of arthritis which are Rheumatoid arthritis (RA) and Osteoarthritis (OA). rheumatoid arthritis was showed a high frequency reached 60 % of total arthritis patients .The incidence and prevalence of Rheumatoid Arthritis vary sustainably between geographical areas and this cannot be explained by genetic factors alone, rather than this variability can explained by environmental exposure[8]. Asia had the lower rate of disease (0.2% - 0.3 %),some Native American populations had a remarkably high prevalence more than 5% [9].

The lifetime risk of RA is commonly misunderstood and miscommunicated as a risk of ~1 in 100 based on the prevalence of RA, which is 0.5–1%. The overall prevalence is a poor estimate of individual risk, particularly for diseases like RA that occur more frequently at older ages and are associated with increased

mortality, the overall prevalence is an average across all ages, whereas the age-specific prevalence of RA increases with age (to > 2% among women age > 65 years)[10].

Obvious differences between genders exist in the prevalence, age at onset, and autoantibody production of RA ,the majority of patients with RA are middle-aged women, generally greater than 70% in any RA cohort ,although RA can occur at any age in either gender [11].

Osteoarthritis is the leading cause of lower extremity disability amongst older adults with an estimated lifetime risk for knee OA being approximately 40% in men and 47% in women. The risks are higher still among individuals who are classified as obese[12] . OA is considered a multifactorial disease, the available epidemiological studies indicate that OA affects 10-15% of the world population, with an incidence of 60% in men and 70% in women over 65 years of age, there are likely to increase the incidence of this disease in the coming decades, becoming an issue of increasingly important public health.

HLA DRB1 alleles .

The results in this study showed no significant correlation between (HLA-DRB1*0301 , *0701) in RA patients and control. This construes that these alleles have protective effects with RA susceptibility. The protective effect of certain HLA-DRB1 alleles against RA has been reported in several reviews[13].

In certain studies, *03 allele group frequency is almost lower in the RA patients than in the controls or nearly the same with no significant difference from healthy controls[14].

The marginal association of DRB1*0301 with RA protection may arise from loose linkage disequilibrium with other genetic loci near to HLA. In addition, in another study on RA patients in Korea, DRB1*0701 has been described as the protective subtype[15]. Depending upon geographical location, HLA-DR associations with RA vary from population to population, different literatures investigated the biogeographic distribution of RA-DRB1 alleles in various ethnicities and races around the world[16,17].The number of RA-associated DRB1 genes present in the genotype configuration of RA patients influences the level of DRB1 gene expression in peripheral B cells, independently of disease activity, severity and drug regimen. This particular regulation of DRB1 gene expression in RA either genetically determined or resulting from unidentified modulating factors may be part of the molecular mechanisms involved in the association of RA with the HLA class II component[18].

In this study, HLA DRB1 (*0301, *0701)alleles were no significant differences between OA patients and control. One may suggest a direct role of HLA-DR antigens in OA. HLA class II molecules are involved in the communication between T cells and antigen presenting cells. Although OA is not generally considered to be an autoimmune disorder with an inflammatory nature, several studies suggest a role for T cells and HLA class II molecules in OA[19].

Interleukin 18 (IL-18) ELISA

Results of the current showed that serum IL-18 levels were significantly higher in patients with RA than in normal healthy controls with ($P < 0.001$). This was in agreement with Ying *et al*. (2010) who similarly found high serum IL-18 levels in 30 Chinese patients with RA when compared with 30 normal subjects[25].

The current result also confirmed the results of Tanaka *et al*. (2001) who compared the serum IL-18 level in 48 Japanese RA patients with that in 52 healthy control subjects, and found a statistically significant difference[21]. Also, Sato *et al.* (2004) reported that serum level of IL-18 is significantly higher in RA patients than controls ($P<0.001$)[22]. According to the results in this study , IL-18 level record high value compared with control , this result confirmed by Wang, *et al.* 2014[23] when suggested that serum, plasma, synovial fluid and articular cartilage IL-18 levels were significantly increased in OA patients. Chronic disease express elevation in inflammatory cytokine levels, IL-17concentration raised in kidney failure diseases[24].

The increased levels of serum IL-18 in patients with RA have also been observed in other studies[25]. The increased levels of IL-18 are primarily associated with chronic inflammation in the autoimmune diseases[26].

IL-18, described for the first time by Gracie *et al.*,[27,] , was significantly elevated in the rheumatoid synovium, which may confirm its contribution to the development of arthritis in RA and the destruction of cartilage[3]. Elevated IL-18 levels are detected in 80% of patients with RA, which may result from the association between IL-18 gene polymorphisms and the occurrence of individual susceptibility to RA[28].But the exact contribution of genetic predisposition to the RA development has not been well recognized and needs further research. Additionally, the markedly higher IL-18 concentration in RA than in OA[29], and psoriatic arthritis[30].Indicates the increased local production of this cytokine[29]. High expression of IL-18 by synoviocytes in RA may regulate the production of pathogenic cytokines responsible for the local inflammatory process[31].

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