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Major Histocompatibility Complex gene polymorphism and some blood parameters in Romatiod Erythritis patients

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Abstract

The present study was carried out in Hilla city to conduct demographic, some blood parameters and major histocompatibility complex (MIC) gene polymorphism in Romatiod Erythritis (RA) patient, using PCR-SSCP technique , the results show that there are significant differences in some demographic variability's between patients and control and all blood parameters were in the normal value for patients while ESR tests were very high in all patients, MIC gene polymorphism significant association with diseases in four alleles.

Key words: PCR-SSCP, Erythrits, Hilla city

INTRODUCTION

Rheumatoid arthritis (RA) is a progressive inflammatory, chronic autoimmune disease related with articular, extra-articular and systemic effects autoimmune disease cause loss in bone and chronic inflammation of the joints, its appear as mild self-limited disease in some cases , also joint destruction, severe physical disability and multiple comorbidities most prominently in some populations [1,2]. Study shows that the dispersal of the RA in women is more than men and it associated with progress in age Over the past 40 years [3] there are many methods have been developed to determination the relation between the genes polymorphisms and RA susceptibility.

Investigators consider RA that is genetically complex, many genes polymorphisms improved that it associated with RA , one of these genes major histocompatibility complex (MHC) class I chain related gene A (MIC-A), it has been proposed as a candidate gene for some disease especially autoimmune disease [4]. The MIC-A gene is one of the MHC class I related genes which located 46 kb centromeric to the HLA-B gene on chromosome 6. [5] . This gene is mainly expressed in endothelial, epithelial cells , fibroblasts, cells, and monocytes.[6,7] To now studies don't improved the function of MIC , but its amino acid sequence suggests that it may bind peptides or other short ligands and participate in antigen presentation or T cell recognition During nucleotide sequence analysis of the MIC-A gene[6] .

MATERIALS AND METHODS

About 60 Samples (30 patients , 30 healthy) were collected from Marjan Hospital in Hilla city with data of patients and control then DNA was extracted according to Favorgene extraction kit (21). PCR condition : MIC gene was amplified used the fallowing primer MIC-A5F5′CCTTTTTTTCAGGGAAAGTGC-3 MIC-A5R, 5′CCTTACCATCTCCAGAAACTGC-3 [4] the amplification condition was 95Cº for 5 min, (95Cº for 30 sec. 57Cº for 30 sec, 72Cº for 20 sec) 30cycle and the final step was 72 for 10 min, The products were electrophoresis in garose 1% , 70 v , 20 Ma for 90 min.

SSCP technique, PCR products were denaturation using SSCP day (EDTA, formamid and bromophynol blue) 1/1 V:V in water bath for 5 min at 95Cº then its child in ice for 2 min.;

SSCP electrophoresis, the products were electrophoresis as a following About 10 µl of the samples into wells of an 8% acrylamide/bis gel (37.5:1), containing 7% glycerol, and 1x TBE buffer. And for recipe a 20 x 20 x 0.1 cm gel format. 8 ml of 40% acrylamide/bis (37.5:1) mixed with 8 ml of 5x TBE , 2,8 ml 100% glycerol, then 40 µl TEMED and 400 µl of 10% ammonium per sulfate were added with 20.8 ml of dH_2O After gel was casting sample were loaded and Run under the following conditions.

Buffer 1x TBE, Buffer temperature 10°C ,Run time 3.5 hours and 100V. Then gell was staining using ethedium bromide for 15 min (22).

RESULTS AND DISCUSSION

The results of present study included some blood parameters in patients group (HB%, PCV, WBC and ESR) and polymorphism of MIC gene using PCR-SSCP technique.

The characteristics of patient and control group were shown in table (1), according to sex; females were more than males in patients with significant increment; this results deal with international studies that improved that female was more susceptible to the disease than male, this may be because the difference of hormonal activity between male and female also the deference's of behavior and life style [8,9]. Disease recorded in urban more than rural, this may because urban life style in Iraq is differs from urban like environmental factors which effect in human health and immunity such as air pollution and stress.

The distribution of RA patients was studied according to age, BMI. In present study tow age categories were higher than others its (30-39) and (60-69), this results partial agreement with Widdifield *et al.*,[10] they found that the prevalence of diseases was increased with age in male and female. This variation may be because variation between population in the world which effect on the habitat, behavior and health awareness, also present study need more investigation about other factors that effect in disease incidence [18].

According to BMI normal W. recorded high percentage of RA disease it was (57.77%) , and obesity was the second percentage (26.66%) , some studies referred to association of RA with obesity , Qin *et al* ., [11] found that there was association between BMI and RA especially in female. Others disagree with Qin and deal with present study. Lu *et al* ., [12]found that there was not a significant association between BMI and RA when restricting to RA cases diagnosed after 55 years of age, and 83% of RA cases in that study were diagnosed at or before 55 years of age. The present results that different from other study can be clarified by the chance of samples collected which were among younger population than older age.

Disease was recorded in unemployed patient in high percentage Table (2), This may be because lifestyle of patients and their activity also adaptation to lifestyle changes and maintenance or improvement of psychosocial health [13,18] . A small percentage of smokers in present study table (2), as known, the effect of smoking in human health were improved that its causes immune suppression and passive effect in human health.

Some blood parameters were studied in present study for patients and compare with the normal value of these parameters, the results of present study were clarified in table (3)

In table (3) all patients in the HB%, PCV% and WBC tests were in the normal value in all classifications, while in ESR test all patients in all classification were high and some of them were significant increments except in age categories (40-49), the highest ESR was in over obesity categories, These variations were effected by many factors like immune response of the individuals, environmental factors, nutrition, family history and psychological of individuals. Also genetic architecture is important factors in in pathological and progressive disease [14,16] .

Table (1) Some Characteristics of Study Groups

Categories	C	P	Statics	P-value
Age (year)	42.439±1.868	48.22±2.564	t = 1.8451	0.0685
Sex				
Male	36.58%	22.22%	X^2=2.146	0.1429
Female	63.41%	77.77%		
Residence				
Urban	31.70%	64.44%	X^2=9.202	0.0024
Rural	68.29%	35.55%		

Table (2) Demographic Distribution of Romatiod Erythritis patients

Categories	Percentage %
Age (years)	
<29	13.13
30-39	22.22
40-49	13.33
50-59	17.77
60-69	22.22
>70	11.11
BMI	
Normal W,	57.77
Obese	26.66
Over W.	11.11
Over obese	4.44
Occupation	
employed	26.66
unemployed	73.33
Smoking	
Yes	15.55
No	84.44

Table (3) Some Blood Parameters Values in study subjects according to demographic distribution

Categories	HB g/dl	PCV%	WBC_S	ESR	P-Value for ESR
Mean ±Se	13.803±0.240	36.89±1.589	9.56±1.030	36.13±4.138	
Normal value	males:14-17.5 female12–16	male 45-52 female 36-48	5000-10.000	male 10-19 female 15-23	
Sex					
Male P	14.3800±0.64	38.57±4.246	8.98±0.868	37.77±12.00	n.s
Male C	14.64±0.2249	37.65±5.34	8.800±1.090	12.45±2.90	
Female P	13.002±0.861	36.42±1.676	9.728±1.306	26.556±4.554	n.s
Female C	14.920±0.263	37.23±0.234	8.56±1.22	12.83±2.67	
Age categories					
<29 P	14.400±0.68	43.166±1.9046	10.033±0.837	33.50±12.057	n.s
C	13.45±0.45	45.87±2.78	6.78±0.34	18.47±2.98	
30-39	13.37±0.368	37.280±3.2370	11.300±3.4540	34.11±9.3548	n.s
C	12.34±0.56	40.78±0.34	10.67±2.67	15.67±3.43	
40-49	13.283±0.91	40.166±2.358	9.233±0.8815	16.66±4.5141	n.s
C	14.56±1.09	39.06±4.78	8.56±0.78	12.56±3.12	
50-59	13.150±0.68	35.112±4.893	6.687±0.4348	29.14±6.9980	0.0218
C	12.78±2.59	40.33±1.89	7.34±1.23	10.12±0.34	
60-69	13.280±0.46	32.270±4.546	10.560±3.030	52.60±10.569	0.0194
C	15.5±1.56	39.34±5.23	7.45±1.112	20.34±6.34	
>70	12.220±0.44	36.800±1.462	8.520±1.3316	43.20±15.219	n.s
C	10.26±1.45	39.87±9.67	7.49±0.67	17.98±1.23	
Residence					
Rural	13.31±0.524	36.29±2.189	10.45±2.1892	36.50±8.177	0.0038
C	12.340±2.47	38.31±4.167	9.561±1.990	15.342±2.39	
Urban	13.30±0.243	37.23±1.740	9.069±1.072	35.92±5.009	0.0065
C	11.891±2.56	35.912±11.234	7.23±2.78	14.346±0.123	
BMI					
Normal	13.42±0.342	34.97±2.622	10.67±1.735	37.34±5.506	0.0010
C	12.78±1.089	33.450±6.120	8.92±1.43	15.230±3.121	
Obesity	13.050±0.37	39.16±1.1202	8.29±0.600	32.60±9.658	n.s
C	10.234±2.99	37.340±4.78	9.00±1.890	17.34±4.12	
Over W.	13.06±0.813	39.40±2.181	7.60±0.8018	29.60±7.325	n.s
C	12.678±5.12	31.345±0,129	10.129±3.211	15.99±0.112	
Over O.	14.00±1.300	42.00±4.00	7.600±1.800	54.50±41.50	n.s
C	15.230±3.13	39.123±12.92	9.408±3.654	21.230±3.04	
Occupation					
Employee	14.69±.47997	40.30±3.561	8.791±0.733	30.20±10.616	n.s
C	13.890±4.12	35.988±2.179	10.345±0.267	18.235±0.678	
unemployed	12.80±0.2231	35.65±1.724	9.84±1.384	37.93±4.674	0.0001
C	11.456±3.12	36.097±13.23	8.234±0.912	14.67±2.14	
Smoking					
Yes	14.65±0.779	43.85±2.354	10.08±1.064	45.71±14.44	n.s
C	15.490±1.289	44.23±4.180	9.56±0.128	14.28±0.27	
No	13.06±0.228	35.61±1.762	9.4658±1.208	34.27±4.377	n.s
C	11.34±0.340	22.46±3.458	10.345±3.12	12.09±0.890	

C, control group. n.s, non-significant at ($p<0.05$).

MIC gene polymorphism was studied used PCR-SSCP as show in table (4) and figure (1,2). According to bioinformatics amplification 183 bp was produced from PCR, this product was obtained by PCR product electrophoresis as in figure (2) then the product was pressed for SSCP, there are 11 bands appeared in SSCP pattern in patient and control and the differences between patients and control was clarified in table (5), the significant between patient and control were show in C,D,H,I and k.

These results deal with other study like Mizuki *et al*.,[4] , they found a triplet repeat microsatellite polymorphism of (GCT/AGC) in exon 5, in trans membrane (TM) region of the gene product. This polymorphism is composed of six alleles named representing four, five, six, and nine triplet repeats of (GCT/AGC) respectively. one of these alleles represents an insertion (G) between the second and third triplet repeats resulting in a premature stop codon. As a result of genetic complexity of RA disease there are more than one genes interacted, allele 6 "new" stands for a C/T substitution at position 991 in some sixth alleles, which gives rise to a Cys/Arg amino acid change. The polymorphism in exon 5 of the MIC-A gene was studied in several populations. In the Japanese population the A6 was found in a significantly higher frequency in patients than in controls. In this study all HLA-B51 positive patients with BD carried the A6 allele, which was also present in some HLA-B51 negative patients [4,15,] . The present results need more investigation about other loci in MIC gene, also other factors can be effect on this polymorphism like level of gene expression, transcription factors, and microenvironments of the cells [16, 19,20].

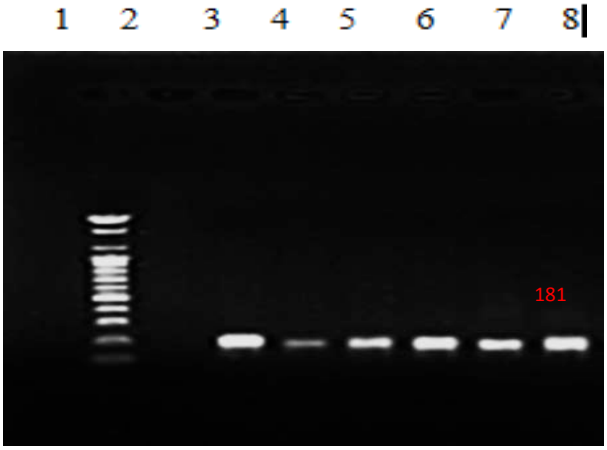

Figure (1) Electrophoresis pattern of PCR product of MIC gene , lane 1 DNA marker, 2-4 for patient , 5-8 for control.

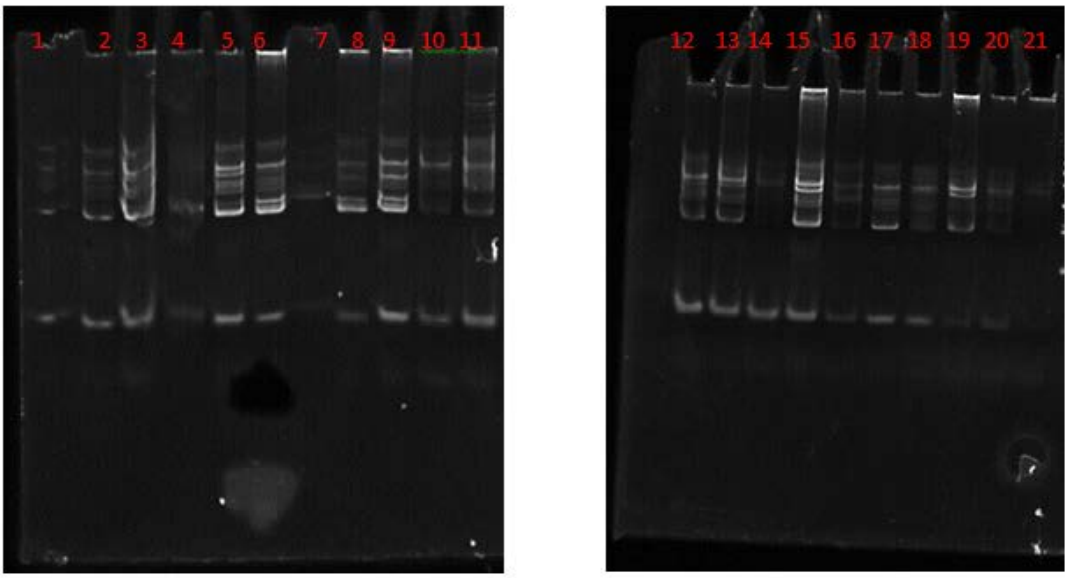

Figure (2) Electrophoresis of PCR-SSCP patterns for patients and control lane 1-11 for control, Lane 12-21 for patients.

Table (3) Sequence Descriptive of MIC Gene Amplification According to NCBI.

Descriptive	**Sequence**
Homo sapiens chromosome 6, GRCh38 Primary Assembly NCBI Reference Sequence: NC_000006.12	20.19kb region from base 31397453 to 31417644.
PCR product of 183 bp product from linear template Untitled, base 14861 to base 15043	CCTTTTTTTCAGGGAAAGTGCTGGTGCTTCAGAGTCATTG GCAGACATTCCATGTTTCTGCTGTTGCTGCTGGCTGCTGC TATTTTTGTTATTATTATTTTCTATGTCCGTTGTTGTAAGA AGAAAACATCAGCTGCAGAGGGTCCAGGTGAGAAAAGC GGGCAGTTTCTGGAGATGGTAAGG

Table (4) MIC Gene Polymorphism Descriptive in study subjects according to its appearance in figure (2)

Descriptive bands	C (%)	P (%)	Odd ratio
A	(43)	(5)	0.0688 (0.0081 to 0.5830)N
B	0	(15)	12.2000 (0.5948 to 250.2370)
C	(13)	(85)	36.8333 (7.3112 to 185.5632)*
D	(53.3)	(100)	36.0303 (1.9968 to 650.1295)*
E	(36.6)	(30)	0.7403 (0.2206 to 2.4845)
F	(20)	(15)	0.7059 0.1546 to 3.2237
G	(86.6)	(80)	0.4444 0.0880 to 2.2451
H	0	(35)	33.8889 1.8031 to 636.9389*
I	(10)	(100)	205.0000 9.8936 to 4247.7061*
K	(26.6)	(65)	5.1071 1.5012 to 17.3749*

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