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ORIGINAL ARTICLE

Polymorphism of HLA-B27 among Ankylosing Spondylitis Patients in Basrah, Iraq

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ABSTRACT

Objectives: Ankylosing Spondylitis is an autoinflammatory disease which influence in the sacroiliac joints and the spine lead to damage and inflammation in bones and tendons; this disease associated strongly with gene human leukocyte antigen B27 that infect male more than female. The aim of his study was detect about gene HLAB27 and subtyping for it in AS patients.

Methods: in this study, the number of each patients and control were 50 that were from south Iraq which the samples collected during October 2018 to March 2019. The steps of working was involving DNA extraction from whole blood by using ReliaPrep tm Blood g DNA MINIPREP SYSTEM (Promega, USA); HLAB27 detection by Real time RT-PCR and sequencing for HLAB27 by using primer design.

Results: the prevalence of HLAB27 in patients was 72% and in control is 6%, the more prevalence of subtyping was HLAB27:05.

Conclusion: : in this study, the prevalence of HLAB27 was 72% in Ankylosing Spondylitis patients and the subtyping were more prevalence of HLAB27 was HLAB27:05.

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INTRODUCTION

Getting Ankylosing Spondylitis is a type of arthritis that affects the joints and the spine, AS is also called spodyloarthritis (SpA) [1]. Ankylosing Spondylitis infects in men more than women at a ratio 3:1. The prevalence of the disease is 0.1 and 1.4% of general people [2]. Ankylosing Spondylitis is a chronic inflammatory disorder that firstly effects the sacroiliac joints and the axial skeleton, AS is strongly associated with HLAB27, the strong familial combination with HLA-B27 have a role for an immunogenetic abnormality in the pathogenesis of AS [3]. The primary clinical features of AS involve inflammatory back pain result from sacroilitis, inflammation in different locations in the pivotal skeleton, circumferential arthritis, enthesitis, frontal inflammation in the uvea, abnormal stiffening and immobility of a joint due to fusion of the bones [4]. Chronic inflammation due to occur the thickening and scarring of connective tissue, usually as a result of injury and this inflammation lead to

ossification at the rims of the intervertebral dics, causing the ankylosing [1]. Ankylosing Spondylitis is a systemic rheumatic disorder and can cause inflammation in the joints and other organs as eyes, heart, lungs and kidney, AS lead to non-skeletal problems such as iritis, uveitis, aortitis, pulmonary fibrosis and amyloidosis [3]. Ankylosing Spondylitis is unnamed causes but it took into consideration as an autoimmune disease that occur by environmental factor(exogenous factor) and genetic factor (endogenous factor) [5]. The environmental factors are including stressful, traumatic life events, infection, smoking, epigenome and DNA methylation. In the stress, especially biochemical stresses can excite this disorder by inducing formerly immune-sequestered autoantigens or by supplying a channel for bacterial seeding [2]. The genetic factor plays a great role, the association between AS and HLA-B27 is the one of the strongest among HLA-associated disease [6]. Ankylosing spondylitis is not occurred by special work, actions, damages but AS have a history in the family, there is a gene called HLA-B27 that is associated with AS, nearly nine from ten people with AS test positive for HLA-B2 [7]. There are several genes linked with AS but AS associated with HLAB27 is more strongly from other genes [5], other genes are including non-HLA-B27 (MHC genes) as human leukocyte antigen (B60, B61, DRB1*1, DRB1*04, DR8); low molecular weight proteasome 2 & 7 (LMP-2 and LMP-7) are proteins encoded by MHC genes which control proteasome activities [8] ; hot shock protein (HSP-70) are protein that protect the cells of thermal and oxidative stress [9] ; non-MHC genes are including IL-1 gene cluster is located on chromosome 2 which play important role in mediating inflammatory response and connected with several disorders [10] and another gene is transforming growth factor beta (TGF-Beta) which is multifactorial regulate of peptides that controls on proliferation, differentiation and other function in many types [11]. Human Leukocyte Antigen B27 is one of the antigens of class I major histocompatibility complex (MHC) that has robustly been connected with AS [12]. The ability of the disorder connection with B27 differs significantly according to racial and ethnic people and which reaches to more than 90% in patients with AS. It is an allele family that comprise about 31 subtypes with a major geographic and ethnic variations in prevalence [13]. There are different theories that are advanced with observance to molecular pathogenetic function of HLA-B27 in ankylosing spondylitis. These theories include: (1) Arthritogenic peptide presentation, (2) Aberrant surface heavy chain ,(3) HLA-B27 misfolding and (4) Enhanced microbial survival [3]. Distribution of HLA-B27 for AS patients in European regions and other countries are involving 72.1% in Romania, 68-76% in Italy, 93% in Norway and 90.2-94% in United Kingdom, 56% in UAE, 67% in KSA, 25.7% in Kuwait, 92% in Iran, 62% in Tunisia and 58.6% in Egypt [14]. The gene (HLA-B27) have large polymorphism that are more than 100 subtypes (alleles) that encoded by 132 alleles and different alleles influence biochemical and three-dimensional (3D) structure and also a charge of the peptide-binding groove of HLA-B27 alpha chain [15]. The gene (HLA-B27) is situated on short arm from chromosome 6 at 6q21.3, crossing an area of 4287 base pairs (bp); it consists of 7 exons and 6 introns overtime to a TATA that a poly A and four alternative connectivity sign [16]. Exon 1 encodes chief peptide, exon 2 and 3 encode alpha 1 and alpha 2 domains, exon 4 encodes alpha 3, exon 5 encodes the transmembrane region, and exon 6 and exon 7 encode cytoplasmic tail [17]. The distribution of AS differs in several parts of the world, almost African people do not found significantly. AS distribution is 6% in Native American people in Canada, subtypes of HLA-B27 distribute in Northern Norway are HLA-B27*02 and HLA-B27*05 [15]. The most subtypes of HLA-B27 connected with AS are HLA-B27:05 in Caucasians; HLA-B27:04 in Chinese, in Chinese people HLA-B27:04 is linked more risk than HLA-B27:05 and HLA-B27:02 in Mediterranean people. The most distribution for HLAB27 subtyping are HLAB27:05 and then HLAB27:02 [14].

The expression spondyloarthritis (SpA) acts a case described by a wide chain of clinical features according to modified New York Criteria , laboratory tests as Complete blood count (CBC), Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and plasma viscosity that indicate to inflammation in patient's body [17], imaging characteristics as X-ray, MRI scan and CT scanner and genetically lead to be detected gene HLA-B27 [18].The treatment for AS but there are some medications to ease signs of disease; in some states, the treatment includes connection of treatment, exercise and self-help measures. In some states are using surgery to edit the damage joints and bones result from AS [7].

MATERIALS AND METHODS

A total of 50 patients (47male), their age range 19-56 years. They suffered from ankylosing spondylitis referred to Biologic Therapy Center –Rheumatology Center, Basrah, Teaching Hospital. Other 50 healthy people matched with the patients in age, sex, ethnicity and other demographic features. Five ml of venous blood was collected from all participants. The collected blood samples were transported to the tubes containing anticoagulants (EDTA) used for DNA extraction which were kept in -20 c (deep freezing). DNA were extracted from whole blood by using ReliaPrep tm Blood g DNA MINIPREP SYSTEM (Promega, USA). According to Gel electrophoresis, 4 µlof genomic DNA mixed with 5 µl of bromophenol blue and loaded in agarose gel (0.25 g of high melting temperature agarose (Promega) in 25 ml of 1 x TBE buffer with 0.25 microgram of ethidium bromide). Genomic DNA and PCR products were separated by horizontal electrophoresis for 45 minutes at 100 volts with 1 x TBE buffer25 ml of 1 x TBE buffer (0.01 M Tris-HCL, pH 7.2, 0.01 M EDTA). Genomic DNA and PCR products were then visualized and photographed using a gel documentary machine (UV trans-illuminator, Aquaris, UK). In the Real-Time PCR technique was used to detect gene HLAB27 by using Real- TM Real Time Amplification Kit (Sacace Biotechnologies, Italy) and the Real Time PCR is fast 7500 (Applied Biosystemvitri,

USA). In subtyping of HLA-B27 was including amplification of Exon 2 & 3 of HLAB27. Exon 2& 3 were amplified by using covenantal PCR. The primer for Exon 2&3 were designed and synthesized at National Institute For Genetic And Engineering And Biotechnology In Tehran/ Iran.

Reading of sequencing by program the International ImMunoGeneTics (IMGT/HLA) Database ®(The IMGT/HLA Database [19].

RESULTS

In this study, the characters of patients and control were equal significantly in number, gender and residence. The range of age in patients and control were 19 – 57 years. The gender in the patients and control were 47 for male and 3 for female. The geographical distribution for patients and control were urban more than rural regions.

The total result of HLA-B27 detection for patients are including 36 positives from 50 (72%) and 14 negative from 50 (28%), the result of HLA-B27 detection for control are including 3 positive from 50 (6%) and 47 negative from 50 (94%) with significant differences (p-value 0.0001), as shown in Table 1, Figure 1.

Table 1: **The detection of HLA-B27 by Real time PCR in population study.**

Detection of HLA-B27	Type of sample		Total	Chi^2	P -value
	Patients	Control			
Positive	36 (72%)	3(6%)	39 (39%)	45.776	0.0001
Negative	14 (28%)	47(94%)	61 (61%)		
Total	**50(100%)**	**50(100%)**	**100(100%)**		

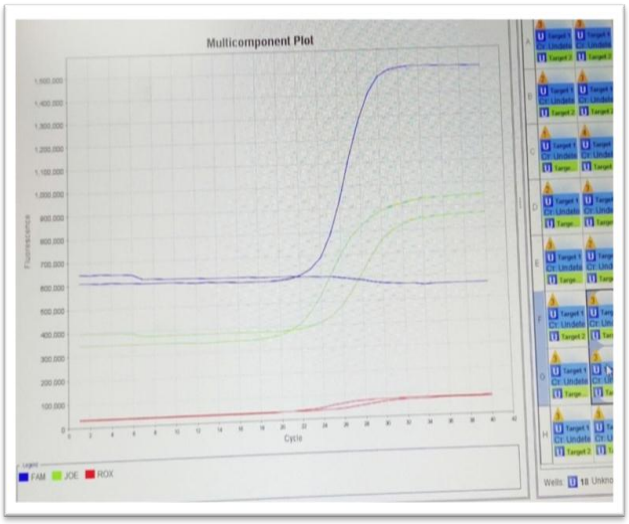

Figure 1: **The results of Real time PCR for detection of HLA-B27; the blue curved lines represent positive result and blue straight lines represent negative result and other green lines represent ready kit for working.**

The total positive results in patients are 36 (72%) including 34 (68%) male and 2 (4%) female. The detection of HLAB27 in control is 3 (6%) including 2 (4%) in male and 1 (2%) female, as shown in Table 2.

Table 2: **The total results of HLAB27 and in association with gender in patients and control.**

No. of patients	Gender	Positive for HLAB27 detection	% of total positive results	% Total results
47	Male	34	68%	72%
3	Female	2	4%	
No. of Control				
47	Male	2	4%	6%
3	Female	1	2%	

The sequencing of PCR product of exons 2 and 3 for ten samples which were selected from positive results to HLAB27 detection (Figures 2, 3 and 4) was studied by using the International ImMunoGeneTics (IMGT/HLA) Database ® [19]. The results showed three different HLA-B27 subtypes including HAL- B27:05, HAL-B27:02 and HAL- B27:04. The prevalence of subtype HLAB27 showed the highest number in HLAB27:05 and lowest number in HLAB27:04, Table 3.

Table 3: **HLA B27 Subtypes.**

Subtyping for patients (N= 10)	Frequency	Percent (%)
HLAB27:05	5	50%
HLAB27:04	2	20%
HLAB27:02	3	30%
Total	**10**	**100%**

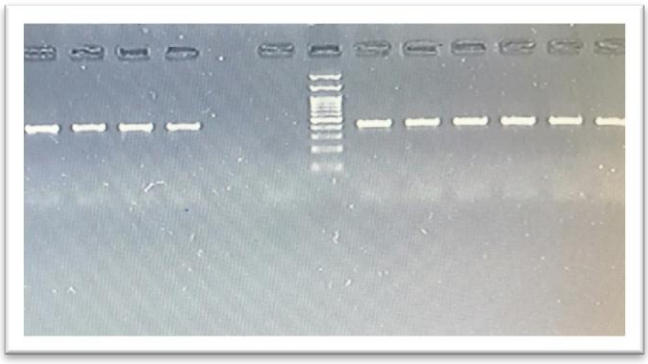

Figure 2: **Exon 2 result in thermo cycle PCR which size for positive band is 430 base pair.**

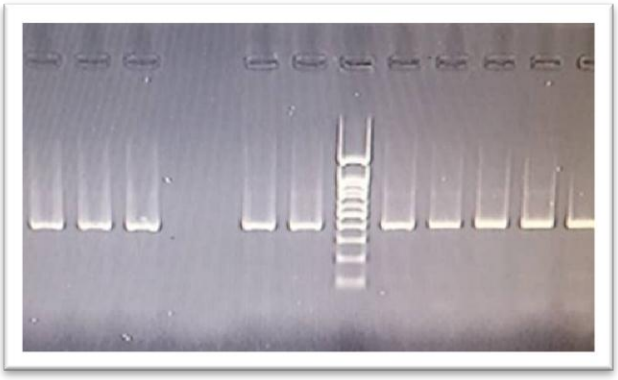

Figure 3: **Exon 3 result in thermo cycle PCR which size for positive band is 400 base pair.**

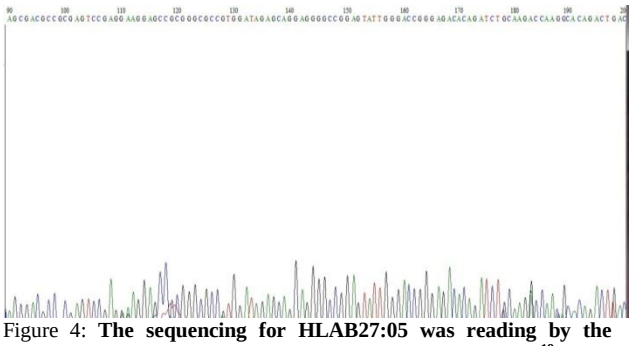

Figure 4: **The sequencing for HLAB27:05 was reading by the International ImMunoGeneTics (IMGT/HLA) Database ® [19].**

DISCUSSION

The association between AS and HLAB27 has been known at 1970s so the association between HLAB27 and AS is 90-95% and the distribution of HLAB27 and AS are depending on geographical regions and racial populations [20]. In our study, the detection of HLAB27 in AS patients was 72% positive and the detection of HLAB27 in control was 6% positive. In other studies, the prevalence of HLAB27 with AS patients in countries near from Iraq were 72% in Jordan, 25.7% in Kuwait, 82% in Iran, 60% in Syria and 70% in Turkey [14, 13], and in distant countries from Iraq were 76% in Indian, 82.2% in Germany, 68 – 76% in Italy, 13% in Sardinia, 88% in Bulgaria, 90.2 – 94% in Untied-kingdom, 72.1% in Romania, 93% in Finland and 93% in Norway [6]. The range for detection of HLAB27 in healthy people are 2.5% 4% in different countries [14].

The first 10 of subtyping for HLAB27 are studied from HLAB27:01 to HLAB27:10 to association with this disease, HLAB27 has wide range of genetic polymorphism result from variations occur in amino acid for sequence of DNA that are > 105 subtypes encoded by 132 alleles; the most prevalence subtypes of HLA-B27 in AS were HLAB27:05 and then HLAB27:02 [13]. In our study, the distribution for subtyping of HLAB27 in patients was HLAB27:05 then HLAB27:02 and then HLAB27:04. In other studies, the prevalence of HLAB27 subtyping were HLA-B27:05 in Caucasians, HLA-B27:02 in Mediterranean people, HLA-B27:04 in Chinese [21] ; HLA-B27:06 and HLA-B27:09 are common in southeast Asia and Sardinia, HLA-B27:05 and HLA-B27:02 in Gambia, HLA-B27:07 in middle east and west Asian populations, HLA-B27:03 in west Africans, HLA-B27:08 in north Europeans, HLA-B27:02 and HLA-B27:05 in northern Norway [13,15].

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