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Association of Hla-B27 and D3 Vitamin Level in Iraqi Ankylosing Spondylitis Patients

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Abstract

Major histocompatibility complex (MHC), that plays pivotal role in self/non-self-discrimination in order to survive, is called 'Human Leukocyte Antigens (HLA)' in humans; is encoded on the short arm of chromosome 6 by MHC locus and is divided into 4 classes: Class I HLA molecules are composed of a 3-domained α chain together with a β2-microglobulin chain while Class II HLA molecules are heterodimers involving 2 α and 2 β domains.β2-microglobulin is encoded on chromosome 15. Class III HLA molecules are encoded by the region located between Class I and II genes and are not involved in antigen presentation. Class III and IV HLA molecules are primarily important in inflammation and autoimmune diseases.

The main duty of HLA molecule is to present antigen to T cells. Class I HLA molecules present antigens to CD8+ cytotoxic T cells whilst Class II Molecules present to CD4+ hepler T cells. Besides Classical Class I and II HLA molecules, there are non-classical HLA Class I and II molecules with various functions.

Key words: Histocompatibility antigens; histocompatibility antigens class I; histocompatibility antigens class II; antigen presentation; molecular structure.

Introduction

The diseases caused by the combination of immunological, viral, and genetic factors in a living organism are called as autoimmune diseases .Ankylosing Spondylitis (AS) is an autoimmune-derived disease, and it can also be defined as a rheumatological disease that causes pain and involvement in the vertebrae and sacroiliac joints causing chronic, inflammatory arthritis (Landewe et al. 2009, Schett and Sieper 2009). The causes of this autoimmune-derived ankylosing spondylitis disease are mostly caused by genetic factors. It is reported that the strongest association revealed so far belongs to HLA-B27. In the studies conducted to date, numerous HLA-B27 subtypes showing varying distributions in different populations have been found. HLA-B27 types are MHC Class I member antigens. It is known that the difference between HLA-B27 antigens is due to the difference in the a sequence found in the peptide binding groove (Reveille,2012 ,Uchanska et al.2013, Khan 2013). While 5% of a population transports HLA-B27, AS disease is seen only in 1-5% of HLA-B27 positive people (Yang et al. 2014). Recently, the

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increase in pathogenic diseases in people and the avoidance of chemical drugs have led them to natural supplements and vitamins. One of these vitamins is Vitamin D3. Vitamin D efficiency can cause different failures in the organism.In this paper study, the presence of the HLA-B27 gene, which has an effect on the disease, and whether the vitamin D3 level contributes to the presence and development of IRAQI AS patients are investigated. Also, the role of Vitamin D in the interaction between the immunity and inflammation factors in AS is analysed.

AS patients generally apply to the doctor with lumbar pain and back stiffness, however, %75 have lumbar pain (Silman, 2002). At the onset of the disease, it progresses slowly and insidiously. Patients mostly cannot state the onset of the AS symptoms and they cannot localize the pain. The pain is blunt, and the initial site of pain can be one or both hips and the gluteal region. Pain can spread to the posterior thigh and can be confused with sciatica and hip pathology. The most prominent features of inflammatory lumbar pain, that are pain and involvement, become evident in the morning and increase with resting (gel phenomenon), and decrease with exercise. Back involvement occurring in the morning sometimes last for 3 hours. AS can be suspected if such symptoms are seen in the individuals under the age of forty (Khan 1995, Van- Der 1984, Calin 1998). In addition, pain may occur at nights and this pain does not decrease when lying down, the patient can feel better when he gets up and moves. Rarely, the patients apply with peripheral arthritis complaints. Shoulder and hip involvement are the first symptoms with a rate of 15% and this rate increases to 35% as the disease continues.

It is revealed that peripheral arthritis is generally associated with HLA-DR4 (Calin 1998). Enthesis is defined as the insertion site of a ligament, tendon, or an articular capsule into the bone. Inflammatory process in AS is primarily seen in enthesis. It continues with new bone formation and fibrosis and then, with the appearance of bone spurs in these areas.

Materials and Methods

This study was conducted on 20 patients with ankylosingspondylitis (16 male, average age 41, 35 and 4 female, average age 38,42; demographic and clinical findings of the patients are given in Table 1.1) among 40 AS patients applied at Iraq-Baghdad Hospital in 2020 and DNA samples of 50 health individuals were used. Quantities and purities of the DNA samples were measured by using a Nanodrop ND-1000 spectrophotometer and 100 ng/µl working dilutions were prepared, and these dilutions were kept at -20°C throughout the study period.

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Table 1.1 Demographic and clinical characteristics of Ankylosing Spondylitis Patients

Female / Male	**40 / 60**
Age	**41.35 ± 2.8**
Disease period (years)	**13.0.5 ± 9.3**
Age of onset	**25,3 ± 7,8**
Peripheral joint involvement	**44.0%**
Hip joint involvement	**28.0%**
Total hip prosthesis	**3.0%**
Uveitis	**14.0%**
Vitamin D3 (g/dl)	**140,50 ± 28,79**
Sedimentation rate (mm/hr)	**18,4 ± 17,3**
ESR (mg/dl)	**40,80 ± 2,2**
Urinary protein excretion for 24 hours (mg)	**113,9 ± 85,5**

Genomic DNA isolation

Genomic DNA Extraction DNA concentrations and purity (mg/µl) were measured by Nanodrop (ThermoFisher / ABD). PCR reaction mixture was prepared as 25 µl in total in order to include 10 µl main mixture (2 X), 3.5 µl DNA (200 ng / µl), 1.5 µl forward and backward primers (10 pm / µl) and 5 µl distilled water. at the thermal cycler: PCR was performed under 40 cycle conditions as pre-denaturation at 95°C for 10 minutes, denaturation for one cycle at 95°C for 40 seconds, annealing at 60°C for 1 minute, and extension at 60°C for 1 minute. Amplified DNA products were stained with 0.3µg / ml Ethidium Bromide (Biotium, ABD) and separated in 1.5% gel via electrophoresis at 70 volt for 120 minutes by using a 2000-bp DNA ladder/maker on 1.5% agarose gel, and DNA bands were examined under ultraviolet light. The bands on the gel were screened via the gel documentation system (Touitou, 2010).

All the PCR products were purified according to the directions of the manufacturer by using the purification kit and were taken to DNA sequencing reaction. The DNA sequences obtained were analysed by using (Macrogen / Korea sequencing services). DNA sequences, SNP (Single Nucleotide Polymorphism), and genomic regions were examined and analysed by the NCBI BLAST program in order to determine other changes.

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In general, according to the studies conducted in the world, it has been revealed that the maximum prevalence of AS is seen in 6 people of Haida Indians, 50% in healthy individuals,and 100% in individuals with AS disease (Gofton et al. 1984).
The prevalence of AS disease in the individuals carrying the HLA-B27 gene was 2%, the prevalence of AS disease was 20% in the relatives of AS patients carrying the HLA-B27 gene, and 0% in the relatives, who did not carry the HLA-B27 gene. The frequency of AS can rise up to 60% in twins and in the individuals with positive HLA-B27.
According to the study of Brown et al. (2000) conducted on twins, the concordance in monozygotic twins was found as 63% and as 24% in dizygotic twins. The differences in the concordance rate in monozygotic and dizygotic twins have revealed that the genetic factors contribute significantly to AS disease and HLA-B27 cannot play a role alone in genetic susceptibility.

Statistical analysis

A logistic regression analysis was performed by using the SPSS statistical package to calculate the statistical significance of the Kruskal-Wallis test, and non-parametric one-way ANOVA adjusted for age and sex of the putative genotypes at risk versus the control group. The statistical significance of the differences between the combined genotype groups was calculated using the x2 test.

Results

Under the light of the results, it was determined whether the vitamin D3 level is one of the factors causing this disease or contributing to the disease progression with the presence of the HLAB27 gene. Images in the agarose gel are given in Figure 1.1 and Figure 1.2.

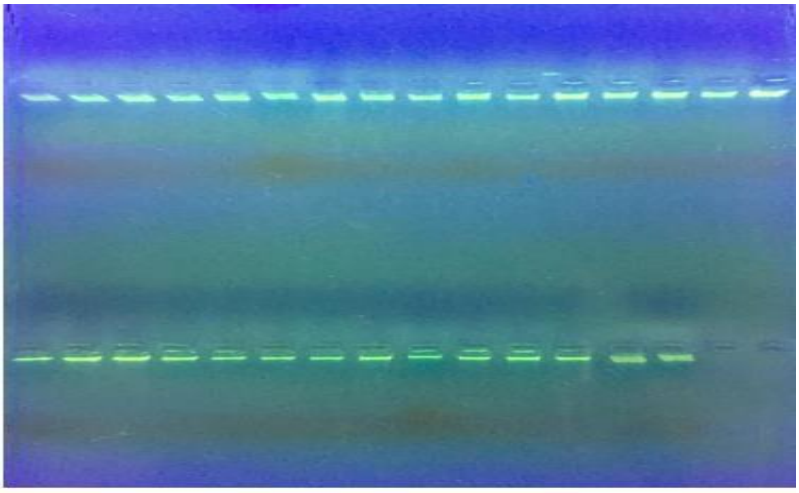

Figure 1.1 Agarose gel images of healthy individuals

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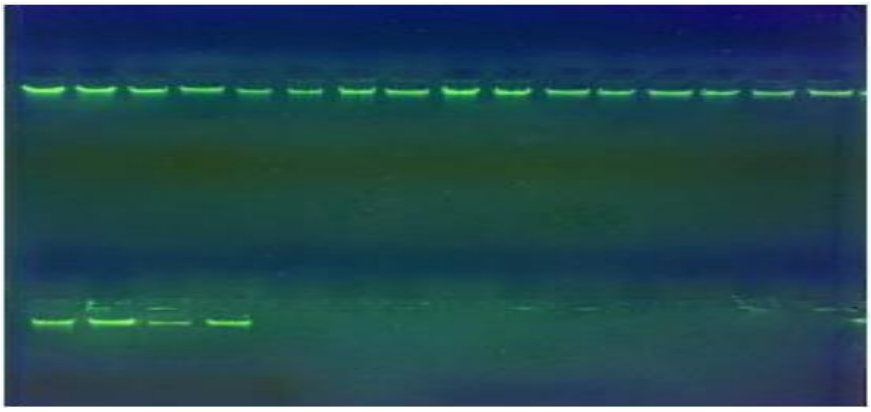

Figure 1.2 Agarose gel images of patients

Statistical Analysis

All statistical analyses were performed according to the SPSS 24.0 version. Mean standard deviation and p value were calculated for all the numerical parameters. ANOVA test was used to compare three or more parameters. Student test was also used to numerically compare the T.categorical parameter or two numerical data.

Age differences between the patients and the control group

Table 1.1 Age differences between the patients and the control group

Group	No. of people	Average Age	Std. Deviation	Mean Standard Error	P value
The findings and systemic deficiencies detected	30	26.6000	10.01241	1.82801	< 0,001
Patients	20	41.3500	12.62110	2.82216	

Correlation between the mean ESR between the ESR patient and control group is shown in Table 1.2.

Table 1.2 Mean ESR between the ESR patient and control group

Group	No. of people	ESR average	Std. Deviation	Mean Std. Error	P. value
Control	30	8.1667	3.83346	3.69989	

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Patients	20	40.8000	16.34368	3.65456	p< 0,001

Vitamin D3 differences between the patients and the control group

Table 1.3 shows the Vitamin D3 differences between the patients and the control group.

Table 1.3 Vitamin D3 differences between the patients and the control group

GROUP	No. of people	Mean Vd3	Std. Deviation	Std. Mean Error	P value
Control group	30	128.1594	103.47399	18.89168	0,710
Patients	20	140.5003	128.77118	28.79411	

The graphic showing the relationship between vitamin D3 and Genotype in the patients and control groups is given in Figure 1.3.

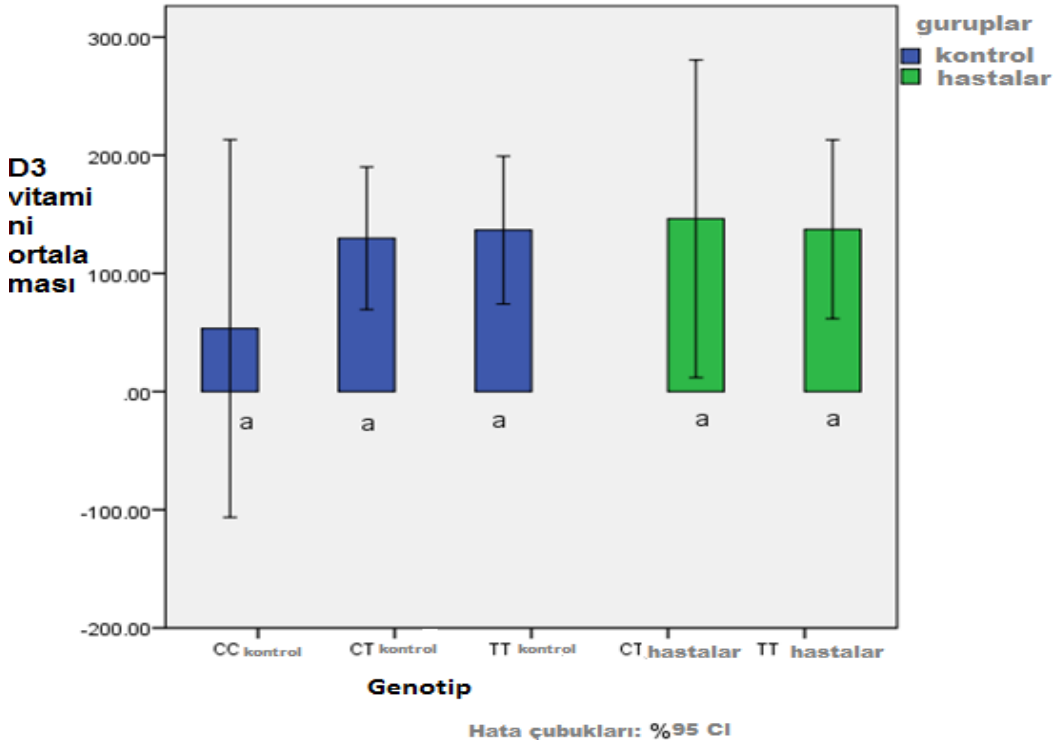

Figure 1.3 Graphic showing the relationship between vitamin D3 and Genotype in the patients and control groups

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Statistical Comparison of polymorphism

Table 1.4 Statistical Comparison of Polymorphism

Genotype	Group	ODD	Fisher value	C.I.	
	Control	Patients			
CC	2 (%6.6)	0 (%0)	0.28	0.51	0.01 - 5.69
CT	13 (%43.3)	7 (%35.0)	0.7	0,769	0.22 - 2.21
TT	15 (%50.0)	13 (%65.0)	1.8	0,387	0.59 - 5.80
C	17 (%28.3)	7 (%17.5)	0.54	0,241	0.20 - 1.43
T	43 (%71.6)	33 (%82.5)	1.86	0,241	0.70 - 4.96

Discussion

One of the most common spondyloarthropathies, that are known as connective tissue diseases involving the spine, is ankylosing spondylitis and it is an autoimmune disorder that occurs in the sacroiliac joints as the first symptom and subsequently causes involvement in the whole part of the spine. Arthritis in the peripheral joints and hip is common, and this disease can affect the tendon junctions, proximal aorta, lungs, kidneys, and uvea region (Langman, 1999). Inheritance, environmental factors, and immunological factors are effective together in the occurrence of AS. Frequently, genetic factors emerge as the most important factor in the proneness to the disease. Until now, the strongest bond in pathogenesis determined in patients with AS is the HLA-B27 molecule, which is a member of the MHC Class I family (Laval et al. 2001). In this study, the allele frequency of the disease with ankylosing spondylitis was examined in Iraqi population and it was revealed that this SNP is a risk factor in the development of the disease as a result of the statistical analysis ($p< 0.001$). The statistical analysis revealed that the vitamin D3 level was one of the factors contributing to this disease and the presence and development of the HLAB27 gene, and the role of vitamin D in the interaction between immune and inflammation effectors in AS was found to be significant (Table 1.4, Figure 1.3) ($p=0.710$). Prevalence of *HLA-B27* varies according to the human populations living at different regions in the world (Mathieu et al. 2009 ; Khan , 2008).

The incidence rate of *HLA-B27* in people, who are not sick in Europe, has been revealed as 8%, and the incidence rate in Han Chinese is 3-5% (Khan 1995).The studies stated that the prevalence of HLA-B27 in patients with non-Turkish population in Turkey was reported to be 5-8% (Acar et al. 2012).In this study, the incidence of *HLA-B27* in the healthy control group in the Iraqi population was found to be 6.6%.It is also stated in various studies conducted in Turkey that the frequency of HLA-B27 was between 70% and 90% in AS patients (Birinci et al.

2006, Yang et al. 2014). In this study, the frequency of *HLA-B27* (65.5%) in AS patients in the Iraqi population was found to be similar when compared to the previously reported results, and thus, the accuracy of the study was verified. The results of this paper study have revealed the association between vitamin D and AS (p=0.710).

Conclusion

Researches in molecular area have also revealed many *HLA-B27* subtypes that produce many different rates in different populations equivalent today. This diversity between the HLA-B27 types has been reported to result from the change in the amino acid sequence located in the peptide binding groove (Uchanska-Ziegler et al. 2013, Khan 2013).

While 5% of the population has HLA-B27 gene, AS disease is only determined in 1-5% of the individuals with HLA-B27 (Yang et al. 2014). The presence of AS in 5% of HLA-B27 positive people shows that not only HLA-B27, but also other genes have a contribution to the occurrence of the formation of AS disease (Brown et al. 2000). Studies have revealed that indirect amounts of vitamin D can have a number of consequences for immune inflammations in AS (Zochling, 2005).Vitamin D response element has been found in the promoter region of genes encoding antimicrobial peptides (AMPs) (Wang et al. 2004).Studies on vitamin D and its ratio in patients with AS may have some contradictions (Durmus et al. 2009, Zou, 2003) showed that there is a relationship between the polymorphism of the vitamin D binding protein (DBP) gene, that encodes the DPB protein that carries vitamin D and its metabolites, and the development of peripheral arthritis and uveitis in Korean patients with AS.

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