RESEARCH STUDY

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HLA Typing in Iraqi Patients with Obesity and Primary Osteoarthritis

ARTICLE INFORMATION

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ABSTRACT

Background: Osteoarthritis (OA) is a degenerative joint disease. It is one of the major causes of disability in developed and developing countries. Human leukocyte antigen (HLA) as part of immune system has a role in the disease process.
Objectives: To investigate whether there is an association between HLA class II-DRB and OA.
Methods: A case control study with 26 patients with osteoarthritis and 22 apparently healthy obese control persons matching in ethnicity were enrolled in this study during the period between October 2012 till March 2013. Direct interview was done with each patient and HLA typing was done by molecular method using Sequence Specific Primer (PCR-SSP) method using One Lambda Kit-USA.
Results: The results showed that females were more affected than males with disease when compared with control. Odds ratio were used to test level of significance. This study showed that HLA DR4 (DRB1*04), DR2 (DRB1*15 and DRB1*16), DR9 (DRB1*09), DR10 (DRB1*10, DRB5*, DRB4* and DRB3*) (odds ratio: 14.26, 9, 9, 9, 14.26, 9.5 and 4.5) respectively are associated with OA.
Conclusions: OA is highly associated with HLA class II DR4 (DRB1*04), DR2 (DRB1*15, DRB1*16), DR9 (DRB1*09), and DR10 (DRB1*10).DR5 (DRB1*05) is not associated with OA.

Introduction:

Osteoarthritis (OA) is a degenerative joint disease. It is one of the major causes of disability in developed countries. Osteoarthritis is characterized by degeneration of articulate cartilage accompanied by sclerosis of the subcondral bones, joint space narrowing and osteophytes [(1)]. The affected joints include the hands, spine, knee and hip. Osteoarthritis can be restricted to one joint or involve more than three joints or group of joints. There are multiple risk factors for OA which include obesity, female gender, elderly and genetics [(2,3)]. Several published studies show that there is a significant correlation between obesity and OA [(4)], with the observation that weight reduction reduces the risk of developing knee OA [(5)].

Another risk factor for OA is the genetic factor with several epidemiological studies showing a strong genetic combination with primary OA, 5% with hand OA, 40% for knee and 60% for hip joint [(6)]. The genetic risk factor of this disease is complex and it does not follow the Mendelian pattern of inheritance. This complex association can be explained by many aspects. The first aspect is a linkage analysis to identify chromosomal region that contain OA genes and there are 12 chromosomes associated with this disease and they found that IL1 cluster which reside in the chromosomal region of 2q12-2q21 have a role in distal interphalangeal OA by linkage analysis [(7)]. Inflammatory episodes are generally accepted as a component in the disease course of most patients with symptomatic OA. However, inflammation is considered to be of low grade, with only low cell counts in the synovial fluid [(8)].

Another aspect is the polymorphism in genes encoding estrogen receptor alpha and Vitamin D Receptor (VDR) [(9)]. Also single nucleotide polymorphism also has a role in this process [(10)]. The involvement of inflammatory cytokine in the disease process with the low grade inflammation observed shade the light on the involvement of immune system in balancing inflammation versus degenerative reaction.

Human leukocyte antigen (HLA) as part of immune system has a role in the disease process. Many reported studies have pointed to different HLA class I and II association [(11,12)].

This study is designed to show the relation between Class II HLA-DRB 1,3,4,5 and patient with OA.

Methods:

A total of 26 Iraqi Arab Muslims patients with primary OA and obesity with an age range between 35-70 years were included in this study. They were among a patients admitting to Al-Kindy teaching hospital (orthopedic consultation unit) and obesity unit in Al-Kindy College of Medicine during the period from October 2012 till March 2013. The patients were selected by the orthopedician according to the clinical presentation and X-ray changes. Any patient with secondary OA was excluded from the study.

Twenty two persons with obesity but without clinical or radiological evidence of OA were selected as a control group.

This study was approved by the scientific and ethical committee of Al-Kindy College of Medicine. All the patients and controls were assigned a confirmed consent about the study.

An interview with patient was done. Weight and height of the patient was taken and BMI was calculated from the following formula BMI= (wt)(kg) /Ht(m)^2.

Two and a half ml of blood were withdrawn from each subject and collected into EDTA tube, kept at -20C. The DNA was extracted by using Reliaprep ® spin column kit (Promega, USA). All DNA were stored at -20C until tested. HLA Class II-DRB typing were performed by PCR-SSP according to the manufacturer instruction using Micro SSP generic Class II DNA typing tray –DRB only with lot number of SSP2LB-004 from One Lambda (USA) company. HLA typing was carried out in the HLA research unit at Al-Kindy College of Medicine.

Data were analyzed using Mini Tab version 16. Descriptive statistics were used as frequencies and percentages. Odds ratio (OR) was used as inferential statistics to evaluate the association between allele and osteoarthritis. Fisher's exact test used to determine the P-value for the significance of association. P-value of less than 0.05 is considered significance.

Results:

In this study, the age of patients range from 35-70 years with a mean age of 50.29 ± 11.57 years. Control ages range from 25-60 years with a mean of 32.28 ± 12.87. There is a significant association between age of both patients with OA and control group with P-value = 0.002 as shown in table1.

Table1: Age distribution in patient and control groups.

Groups	Age in years (Mean± SD)	P-value
Control	32.28 ±12.87	0.002
Patient	50.29 ±11.57	

For the gender, in this study 2 (7.7%) of the patients are male compared to 12 (54.5%) for the control group with P-value of 0.017, while the female forming 24 (92.3 %) for the patient with 10 (45.5%) for the control group with a significant difference with a P-value of 0.018.

Table 2: Gender distribution in patients and control group.

Gender	Patient No. (%)	Control No. (%)	P Value
Male	2 (7.7%)	12(54.5 %)	0.017
Female	24(92.3%)	10 (45.5%)	0.018

Regarding HLA-class II (DRB) alleles, this study noticed that allele of DRB1*03:01, *03:76, *0401, *07:01, *08:32, *09:01, *10:01, *11:17, *12:01, *13:01, *15:01, *16:01, DRB3*02, DRB4* and DRB5* have a significant odds ratio (2.61, 4.6, 14.26, 2.6, 4.6, 9, 9, 4.6, 4.6, 4.6, 4.6, 4.6, 4.6, 9.5, 14.26, respectively), as shown in table 3.

Table 3: HLA-DRB genotypes in patient with OA in comparison to obese healthy control group.

Allele	Patient No. (%)	Control No. (%)	OR	P value
DRB1*03:01:01:01	10 (38)	4 (18)	2.61	0.08
DRB1*03:01:06	0 (0)	2 (9)	0.15	NT
DRB1*03:17	0 (0)	2 (9)	0.15	NT
DRB1*03:76	2 (8)	0 (0)	4.6	NT
DRB1*04:01:01	6 (23)	0 (0)	14.26	NT
DRB1*07:01:01:01	6 (23)	2 (9)	2.6	NT
DRB1*08:01:01	0 (0)	2 (9)	0.15	NT
DRB1*08:32	2 (8)	0 (0)	4.6	NT
DRB1*09:01:02	4 (15)	0 (0)	9	NT
DRB1*10:01:01	4 (15)	0 (0)	9	NT
DRB1*11:01:01:01	4 (15)	2 (9)	1.64	0.28
DRB1*11:03	0 (0)	4 (18)	0.07	NT
DRB1*11:17	2 (8)	0 (0)	4.6	NT
DRB1*11:67	0 (0)	4 (18)	0.07	NT
DRB1*12:01:01	2 (8)	0 (0)	4.6	NT
DRB1*12:09	0 (0)	2 (9)	0.15	NT
DRB1*13:01:07	2 (8)	0 (0)	4.6	NT
DRB1*13:18	0 (0)	4 (18)	0.07	NT
DRB1*13:116	0 (0)	2 (9)	0.15	NT
DRB1*13:119	0 (0)	2 (9)	0.15	NT
DRB1*14:01:01	0 (0)	2 (9)	0.15	NT
DRB1*14:02	0 (0)	2 (9)	0.15	NT
DRB1*14:16	0 (0)	2 (9)	0.15	NT
DRB1*14:57	0 (0)	4 (18)	0.07	NT
DRB1*15:01:01	2 (8)	0 (0)	4.6	NT
DRB1*16:01:01	2 (8)	0 (0)	4.6	NT
DRB3*01:01:02:01	14 (54)	21 (95)	0.02	0.001
DRB3*02:18	2 (8)	0 (0)	4.6	NT
DRB4*01:01:01:01	14 (54)	2 (9)	9.5	0.009
DRB5*01:01:01	6 (23)	0 (0)	14.26	NT

Table 5 shows the Body mass index and HLA-DRB allele frequency. There is no significant association between BMI and allele frequency although there are 5 of the patient with an allele of DRB1*03 and 7 of them with DRB4* with BMI >35.

Table 4: HLA-II serotypes in patient with OA in comparison to obese healthy control group.

HLA serology allele	Number of alleles		OR
	patients	control	
DR2	4	0	9
DR3	12	8	1.17
DR4	6	0	14.26
DR5	10	12	0.53
DR6	2	18	0.02
DR7	6	2	1.33
DR8	2	2	0.83
DR9	4	0	9
DR10	4	0	9

Table 5: Allele frequency and BMI in patients group.

Allele	BMI 30-35	BMI >35	P-value
DRB1*03	1	5	0.5
DRB1*04	0	3	NT
DRB1*07	0	2	NT
DRB1*08	0	1	NT
DRB1*09	0	2	NT
DRB1*10	1	1	0.28
DRB1*11	2	2	NT
DRB1*12	0	1	NT
DRB1*15	0	1	NT
DRB1*16	0	1	NT
DRB3*	1	6	0.53
DRB4*	1	7	0.51
DRB5*	0	1	NT

Discussion:

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 [13, 14, 15].

This study is designed to investigate the involvement of HLA antigen in the disease process. In this study, DR4 (DRB1*04) is highly associated with OA with an odds ratio of 14.26. Several published studies show that DR4 is associated with chronic inflammation rheumatoid arthritis but in a Japanese study [16], the antigen frequencies of HLA-DRB1*0101, *0401, *0405, *1001, and *1402 in the generalized OA group were not significantly different from those in the control group but the percentage of DRB1*1402 in the patient group is 1.6% in comparison with 0.1 % in the control group. This difference with other studies may be due to race and religion; method used, and sample size.

Another study [17] showed an association of DR4 with rheumatoid arthritis, they take the control as patient with Knee OA, and they found that 19% of the control populations have DR4, although DR4 is not significantly associated with OA. This can explain the probability that HLA-DR4 is associated with OA and not only with rheumatoid arthritis.

For HLA-DR2 (DRB1*15, DRB1*16), this study also demonstrate that DR2 is highly associated with OA with an odds ratio of 9, this is in agreement with several studies that demonstrate that OA is associated with DR2 as it is intriguing to speculate that DR2 as a haplotype allows the development of a degenerate joint disease with only low-grade inflammatory episodes [11, 12].

DR9 (DRB1*09) and DR10 (DRB1*10) is also associated with OA, no published article could associate DR9, DR10 with OA. , it has been shown for autoimmune diseases that HLA class II associations are different for the various ethnic groups and the same seems to apply to OA.

DR5 (DRB1*05) is negatively associated with OA and this is in agreement with many studies [12, 18]. However, it cannot be ruled out that an association with DR2 or DR4 is only indicative of an association with a polymorphic gene linked to the DRB1 locus. Indeed, HLA and the gene encoding α 2 chain of type XI collagen (COL11A2) are tightly linked on the short arm of chromosome 6 (6p21.3) and evidence of linkage of female hip OA to HLA/COL11A2 has been shown [19]. It remains to be determined whether a specific COL11A2 gene polymorphism segregates with the DR2 and/or DR4 haplotypes.

However this study can be considered as a preliminary study because the number of the patients is low with no new results for the allele frequency in Iraqi populations.

Conclusions:

1-Osteoarthritis is associated with HLA- class II DR2 (DRB1*15,DRB1*16),DR4(DRB1*04), DR9(DRB1*09), DR10(DRB1*10), DRB5*, DRB4* and DRB3*.
2-HLA class II DR5 (DRB1*05) is negatively associated with OA.

Recommendations:

There should be another study for larger number with involvement of class I HLA.

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