SHORT PAPER

The Effect of HLA-DRB1 on Cholecystitis

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ABSTRACT

Background: Cholecystitis is one of the major digestive diseases. Its prevalence is particularly high in some populations. Significant risk factors associated with cholecystitis include age, sex, obesity, diet, parity and type 2 diabetes. **Objective:** To determine the association between HLA-DRB1 and cholecystitis. **Methods:** This case-control study included forty Iraqi Arab patients who had cholecystitis with multiple calculi treated by cholecystectomy admitted in the surgical ward at Al-Kindy Teaching Hospital Baghdad between September -2013 to June -2014. The control group consisted of forty healthy volunteers among the staff of Al-Kindy College of Medicine. Control and cholecystitis patients groups were typed for identifying the DRB1* alleles using DNA-based methodology (PCR-SSOP). **Results:** There was an increased frequency of HLA-DRB1*0301 in patients with cholecystitis compared with healthy controls (p=0.0442, odd ratio=4.1111, 95% CI: 1.0372-16.2949). **Conclusion:** HLA-DRB1*0301, as a genetic factor, seems to have an association with cholecystitis.

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INTRODUCTION

Cholecystitis originated from Greek word -*cholecyst*, "gallbladder", combined with the suffix -*itis*, mean "inflammation" means inflammation of the gallbladder, which occurs due to obstruction of the cystic duct with gallstones (cholelithiasis). The blockage of the cystic duct with gallstones results in accumulation of bile and increased pressure within the gallbladder. Multiple factors like concentrated bile, pressure, and secondary infection by gut organisms, predominantly *E. coli* and *Bacteroides* species irritate and damage the gallbladder wall, causing inflammation and swelling of the gallbladder. This leads to reduce normal blood flow to areas of the gallbladder, ending in cell death due to insufficient oxygen supply to tissues (1). There are multiple causes that contribute to this disease like old age more than 60 years, female, pregnancy, hormones replacement therapy for women during the menopause, obesity, rapid weight loss or gain weight, diabetes and Native American or Mexican American ethnicity (2). Genetic factor may play a role in causation of disease because around 50%-70% of cholecystitis patients have a positive family history of the disease (3). Epidemiologic studies have concerned several environmental factors and common genetic elements in gallstone formation. Genetic factors that influence gallstone formation have been elaborated from linkage studies of twins, families, and ethnicities with gall stone formation (4). Human leukocytes antigens (HLA) is one of the genetic factor that predispose to cholecystitis, studies into the genetic characteristics of patients with chronic cholecystitis made the significance of hereditary load in the development of cholecystitis and to identify genetic markers (B (III) blood group), type Hp 1-1, HLA-A3, HLA-A30 and HLA-B5, as well genetic protectors (O(I) blood group), HLA-B8, HLA-B14 of the disease (5). The importance of HLA-DRB1 that belongs to the HLA class II beta chain. The class II molecule is a heterodimer consisting of two chains an alpha (DRA) and a beta chain (DRB), both anchored in the membrane of the cell wall. HLA DRB1 plays a central role in the immune system by presenting peptides derived from extracellular proteins and the Class II molecules are expressed in cell wall of antigen presenting cells B lymphocytes, dendritic cell and macrophages. The beta chain is approximately 26-28 kDa and is encoded by 6 exons. Exon one encodes the leader peptide; exons 2 and 3 encode the two extracellular domains; exon 4 encodes the transmembrane domain; and exon 5 encodes the cytoplasmic tail. Within the DR molecule the beta chain contains all the polymorphisms of HLA that specifying the peptide binding specificities. Allelic variants of DRB1 are linked with many diseases.

In our study we try to confirm the genetic burden of HLA*DRB1 in the development of cholecystitis with gall stone formation in Iraqi Arab Muslims patients which manifests itself in greater significance of hereditary burden and greater power of association with genetic markers of the disease.

MATERIALS AND METHODS

A case-control comparative study included forty Iraqi Arab Muslims patients who had cholecystitis with multiple calculi treated by cholecystectomy diagnosed by their physician admitted in the surgical ward at Al-kindy Teaching Hospital between September -2013 to June -2014. Their age of patients group was ranged from 20-45 years and. Males were 50% and the rests were females. The inclusion criteria to select

the patients were complaining from acute upper and right abdominal pain with tenderness, involuntary guarding under the right hypochondrium and/or in the flank; fever higher than 38°C, leukocytosis greater than 10×10^9/L or both, and ultrasonographic symptoms showed thickened and edematous gallbladder wall, presence of gallstones, ultrasonographic Murphy's sign positive. Exclusion criteria were acute cholangitis, other acute inflammation like acute pancreatitis, hematological disorder, anticoagulant treatment, thromboembolic disorders, renal, hepatic, rheumatic or vascular disease, pregnancy, malignancy and immunosuppressive therapy.

The second control group consisted from forty healthy volunteers among the staff of Al-Kindy College of Medicine that did not have any abdominal disorders whether recent or previously and had negative family history for this diseases or other diseases. The control group was ethnically similar to patients group; their ages were ranged from 20-48 years and fifty percent of them were males and rest was females. They are age and sex matched.

The Scientific and Ethical Committee of Al-Kindy Medical College and Medical City Hospital had approved the study. Informed consent was obtained from all patients and control group.

HLA genotyping: Peripheral venous blood samples from patients and control groups were collected in ethylenediaminetetraacetic acid-containing tubes and then stored at -20°C until testing for class II- HLA-DRB1. Genomic DNA was extracted using Promega DNA extraction Kit- USA. DNA product was verified by electrophoresis in a 2% agarose gel containing ethidium bromide and was visualized under UV light. Locus- and allele-specific amplification of genomic DNA were performed for DRB1. Amplification and Hybridization was performed using a panel of sequence-specific oligonucleotide probes (SSOP) using HLA-DRB1 amplification and hybridization kits (SSO HLA type DRB1 plus and Mastermix for HLA type DRB1 Amp plus kits - Innogenetics-Belgium) using automated method by AutoLipa–48 Innogenetics-Belgum. The results were interpreted using LiRas version-5.0 software- Innogenetics-Belgium.

Statistical Analysis. was done using using MiniTab version. 3.0 software. The distribution of HLA alleles in patients and control groups were compared using chi-square for continuous variable. Fisher's exact test was used when necessary. In each comparison, the odds ratio (OR) along with the 95% confidence interval (95% CI) was used. P-value less than 0.05 were considered statistically significant. The Bonferoni inequality method was used to correct for each comparison. The threshold for significance of a deviation for the HLA-DRB1 alleles was given by a corrected p value of <0.004545.

RESULTS and DISCUSSION

Cholecystitis is a multifactorial origins due to interaction between environment and genetic factors and associated with high morbidity that affects the economy and public health (6). Chromosomal aberrations confined on chromosome 1's long arm and translocation from the long arm of chromosome 4 to the long arm of chromosome 6 may cause gall bladder cancer (7).

Control and cholecystitis patients groups were typed for identifying the DRB1* alleles using DNA-based methodology (PCR-SSOP). Allele's frequencies of HLA-DRB1 for cholecystitis patients and control group are shown in Table1.

Table 1. Human leukocytes antigens (HLA-DRB1) alleles frequencies in patients with cholecystitis and healthy control groups.

HLA-DRB1* alleles	cholecystitis patients group, No.=40 (%)		Healthy control group, No.=40 (%)		Odds ratio (95% confidence interval)	P Value After correction
02:03	0	0	3	7.5	na	na
03:01	10	25	3	7.5	4.1111 1.0372 to 16.2949	P< 0.0442
03:06	4	10	5	12.5	na	na
04:03	2	5	0	0	na	na
04:18	2	5	1	2.5	2.0714 0.1777 to 24.1495	0.5611
04:04	2	5	2	5	1.00 0.1315 to 7.6049	1.00
04:22	2	5	0	0	na	na
07:01	8	20	8	20	1.00 0.3184 to 3.1405	1.00
08:02	4	10	3	7.5	na	na
0825	2	5	0	0	na	na
10:01	2	5	0	0	na	na
11:01	6	15	7	17.5	0.8319 0.2528 to 2.7374	P<0.7620
11:02	4	10	3	7.5	1.3704 0.2863 to 6.5587	P<0.6933
11:03	0	0	5	12.5	na	na
11:12	6	15	0	0	na	na
11:16	2	5	5	12.5	0.3684 0.0671 to 2.0226	P<0.2504
12:09	0	0	4	10	na	na
13:01	12	30	0	0	na	na
13:05	0	0	3	7.5	na	na
13:12	2	5	5	12.5	0.3684 0.0671 to 2.0226	P<0.2504
13:22	4	10	3	7.5	1.3704 0.2863 to 6.5587	P<0.6933
13:119	0	0	3	7.5	na	na
14:01	2	5	3	7.5	0.6491 0.1025 to 4.1102	P<0.6463
14:02	0	0	3	7.5	na	na
14;16	0	0	3	7.5	na	na
14:57	0	0	5	12.5	na	na
15:01	2	5	3	7.5	0.6491 0.1025 to 4.1102	P<0.6463
15:10	2	5	0	0	na	na

na=not applicable
The threshold for significance of a deviation was given by a corrected p value of <0.004545.

There was an increased frequency of HLA-DRB1*03:01 in patients with cholecystitis compared with healthy controls (p=0.0442, odd ratio=4.1111, 95% CI: 1.0372-16.2949); also There is increase in the HLA-DRB1* 13:01 in patients with cholecystitis

while the control group did not have this allele, thus this allele is predisposing allele to diseases development. As shown in Table 2, the highest frequensies belong to HLA-DRB1*03:01 and HLA-DRB1*13:01 that are 0.14 and 0.16, respectively.

Table 2. Human leukocytes antigens (HLA-DRB1) gene frequencies in patients with cholecystitis and healthy control groups.

HLA-DRB1* alleles	cholecystitis patients group No.=40 Gene frequency	Healthy control group No.=40 Gene frequency
02:03	0.00	0.04
03:01	0.14	0.04
03:06	0.05	0.06
04:03	0.02	0.00
04:18	0.02	0.01
0404	0.02	0.02
04:22	0.02	0.00
07:01	0.10	0.10
08:02	0.05	0.04
0825	0.02	0.00
10:01	0.02	0.00
11:01	0.07	0.09
11:02	0.05	0.04
11:03	0.00	0.06
11:12	0.07	0.00
11:16	0.02	0.06
12:09	0.00	0.05
13:01	0.16	0.00
13:05	0.00	0.04
13:12	0.02	0.06
13:22	0.05	0.04
13:119	0.00	0.04
14:01	0.02	0.04
14:02	0.00	0.04
14;16	0.00	0.04
14:57	0.00	0.06
15:01	0.02	0.04
15:10	0.02	0.00

In our study, we demonstrated the role of chromosome 6 in gallbladder disease by HLA typing. We found that HLA-DRB1*13:01 is significantly higher than control group. Other study found that HLA A3, HLA A30, HLA B5, HLA B8 and HLA B14 are associated with this disease (5). Other studies had demonstrated the crucial role of this antigen presenting cells in assessing the activity of the immune system. The HLA-DR antigen expression on macrophages and monocytes plays an important role in antigen presentation to T-helper lymphocytes (8). In fact, these cells require both HLA-DR and exogenic antigens on the macrophage surface to initiate proliferation. Thus, HLA-DR is

a major histocompatibility complex class II cell surface receptor that is up-regulated in response to signaling during an infection. Therefore, decrease of human leukocyte antigen-DR leading to increased gallbladder inflammation and sepsis (9). The Cholecystitis pattern of genotypic variability in an admixed population is a function of the gene frequencies of the original contributing parental populations, the number of loci involved in a trait of interest, the mating pattern relative to those loci and the amount of admixture between populations. Native peoples of the New World, including Amerindians and admixed Latin Americans such as Mexican-Americans, are highly susceptible to Cholecystitis. This pattern differs from that generally associated with Westernization, which suggests a gene-environment interaction (10). Among women with Cholecystitis, the risk is highest among American Indians, followed by Hispanics, non-Hispanic whites, and non-Hispanic blacks. Men differ from women by having lower risk in all ethnic groups and by having a similar prevalence between Hispanics and non-Hispanic whites. Genetic markers have not been identified that would explain differences in risk among ethnic groups (11). Patients with HLA typing haplotype HLA-B*07 and DRB1*15 have a higher level of IgG4 in patients with primary sclerosing cholangitis (12). In our study, we found an alleles with high number like HLA-DRB1* 13:119 and 14:57. This may be new alleles or ambiguous allele that assign with high number. According to IMGT/HLA, these two alleles occur in Native Indian.
In conclusions, genetic factor especially HLA-DRB1*03:01 had an association with Cholecystitis.

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