HLA-DRB1*03 And DRB1*15 Frequency In *Helicobacter Pylori* Superficial Gastritis

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ABSTRACT

Background: **Background:** *Helicobacter pylori* is an important gastrointestinal bacteria related to the development of superficial atrophic gastritis, peptic ulcer and gastric cancer. Human leukocyte antigens (HLA) may play an important roles in host immune responses to *H pylori* antigens.

Aim of the study: to investigate the association between HLA-DRB1 genotypes and superficial gastritis with *H. pylori* infection in an Iraqi patients.

Patients and methods: Sixty patients with superficial gastritis and 100 individuals with apparently normal results after endoscopic examination were recruited from Al-Kindy Teaching Hospital - Gastrocolonoscope Unit between January 2014 and July 2016. All study patients and controls group underwent upper gastrointestinal endoscopic examinations. They were analyzed for CagA antibodies Immunoglobulin G (IgG) for *H. pylori* and HLA Class II genotyping (HLA-DRB1).

Results: Patients with superficial gastritis with *H pylori*. The infection showed significant expression (P=0.0001) DRB1*03:01 genotypes and DRB1*15:01 (P= 0.004) as compared to control group.

Conclusions: Genetic factor may play a role in gastritis. HLA-DRB1*03 and HLA-DRB1*15 may increased the susceptibility to superficial gastritis in H pylori infected patients.

Key words: Gastritis, *H pylori*, HLA

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Gastritis is the term of inflammation of stomach walls inflammation to various degrees. This is either including the superficial inner lining of the stomach (mucosa) or it can extend through the entire wall of the stomach (1). Persistent inflammation of the gastric mucosa leads to gastritis caused by many external different factors like *H.pylori* (*Helicobacter pylori*) infection or the excessive and/or long term use of medication suchas NSAIDs (non-steroidal anti-inflammatory drugs) (2). However, anyone can get gastritis; but there are certain factors can raise the risk. These include: weakened immune systems for example immune-mediated reaction and is known as autoimmune atrophic gastritis (3) and genetic factors that predispose them to developing gastritis (4). Gastritis might be associated with polymorphisms of specific allelic that involved in specific inflammatory response genes (interleukin-1 gene cluster and Toll-like receptors (TLRs) (5)., detoxification enzymes, and cancer-related processes like single A/T SNP at position -251 from the transcription start site in the promoter region of the IL-8 gene (6). Consequently polymorphisms in inflammation-related genes are factors important in the development of gastroduodenal diseases in Helicobacter pylori-infected individuals. Other important polymorphic genetic factor is Human Leukocyte Antigen (HLA) which is play particular roles in host immune responses to bacterial antigens of *H pylori* (7). Class II molecules of HLA is a hetrodimer membrane glycoproteins expressed on the surface of antigen presenting cells (8). It presents exogenous antigenic peptide to T helper cells, so the interaction between T cell receptors, antigenic peptide and MHC molecule determines T cell activation and an immune response to various antigens(9). It was reported that there is an association of Class II (HLA-DQA1 and DQB1)alleles with gastric disease (10). The present study was done in order to investigate the association of HLA-DRB1 genetic diversity in individuals infected with *H. pylori* in an Iraqi Arab Muslims population.

Methods: Patients with superficial gastritis after gastroscope examination were done to them and individuals with apparently normal results after endoscopic examination were recruited from Al-Kindy Teaching Hospital - Gastrocolonoscope Unit between January 2014 and July 2016. Written informed consent was obtained from all patients with superficial gastritis and control group for this study. The study protocol was reviewed and approved by the Scientific and Ethical Committee of Al-kindy medical college and Al-Kindy Teaching Hospital. The patient and control groups were in the same aged for both sexes.

Sixty Iraqi Arab Muslims patients with a history of upper gastrointestinal symptoms mostly recurrent epigastric and abdominal pain and had been referred for gastrointestinal endoscopy Unit at Al-Kindy Teaching Hospital, and diagnosis of superficial gastritis was done. Exclusion criteria included those patients who were treated with antisecretory, antimicrobial, anti-inflammatory medications, non-steroidal anti-

inflammatory drugs (NSAIDs), for the three months preceding the endoscopy. Patients with peptic ulcer, food allergy, giardiasis, inflammatory bowel disease and celiac disease were also excluded. The 100 Iraqi Arab Muslims controls consisted of people undergoing upper gastrointestinal endoscopy. Those subjects did not have any clinical evidence of chronic gastritis, peptic ulcer disease, gastric cancer, and personal or familiar history of autoimmune diseases. This group, their gastroscop was normal and being investigated for anaemia, chronic diarrhoea for unknown reason requiring small bowel biopsy, irritable bowel syndrome and screening for familial adenomatous polyposis.

Oesophagogastric examinations: All study patients and control groups underwent upper gastrointestinal endoscopic examinations using gastroscope: GIF-H260; Olympus, Tokyo, Japan and Display screen; Olympus OEV-261H liquid crystal display monitor; Olympus, Tokyo, Japan. Biopsies were taken from gastric antrum (three or four) and gastric body (one or two) by the surgeon who did the gastroscope . Biopsy specimens were stained with haematoxylin and eosin for conventional histological examination.

Serological Tests: Separated serums from aspirated blood were analyzed for CagA antibodies Immunoglobulin G (IgG) for *H. pylori* using an immunological test (immunochromatography test) (ACON, USA). The positive ones were confirmed by ELISA test.

HLA Class II genotyping (HLA-DRB1): Two mL of venous blood were collected in EDTA containers for DNA extraction from human anticoagulated blood using blood kit (QIAmp DNA blood Mini Kit, QIAGEN INC-Germany). After DNA extraction, concentration and purification DNA product was estimated using Nanodrop -South Korea. DNA was verified by electrophoresis in a 2% agarose gel containing ethidium bromide and was visualized under UV light (Figure-1-).

In situe hybridization using Locus- and alleles-specific amplification of genomic patients and control DNA was performed for DRB1. DNA Amplification and Hybridization from both groups were performed using a sequence-specific oligonucleotide probes (SSOP) by HLA-DRB1 amplification and hybridization kits (SSO HLA type DRB1 plus and Mastermix for HLA type DRB1 Amp plus kits -Innogenetics-Belgium) by AutoLipa -48Innogenetics-Belgum. The results were interpreted using LiRas version-5.0 software- Innogenetics-Belgium.

Statistical Analysis: HLA-DRB1 frequencies were determined by direct counting. The frequency of each allele was compared between two groups using chi-square test fisher exact test using MiniTab version. 3.0 software. In each comparison, the Odds ratio (OR) along with the 95% confidence interval (95% CI) was calculated. Gene frequencies for both groups wereestimated. P-value less than 0.05 were considered statistically significant.

Results : A total of 60 patients with superficial gastritis were evaluated, together with 100 control group. The mean age of patients was 44.37 ±11.54, as compared with 46 ± 10.12 for the controls .The males were 50% and the rest was females. The male to female sex ratio in the control group was 1:1 There is a significant increase of H. pylori infection (p=0.0001) in superficial gastritis patients than control group. The Odd ratio (OD)= 18.878 with 95% CI= from 8.190-43.513. The relative risk =6.363 that indicates an association between H. pylori and superficial gastritis disease as shown in table-1- . The observed and expected phenotypes of all alleles for the patients group as demonstrated in table- 2- were in a good agreement with Hardy-Weinberg equilibrium as shown in table-3-. The frequencies of HLA*DRB1 were investigated in the control and patients groups of Iraqi Arab Muslims and analyzed for identifying the DRB1* alleles using DNA based methodology (PCR-SSOP). There was an increased frequency of HLADRB1*03:01 and DRB1*15:01 in patients group compared with control group (P=0.0001, P=0.004 respectively). Other alleles like HLA-DRB1* 04:02 and 11:01 were significantly increased in control group (P=0.036, P=0.0003 respectively) as shown in table-4-. The highest genotype frequency in patients was 03:01 which is equal to 0.106 while in the control group was 11:01 which is 0.117 as shown in table-5-.

Table-1- *Helicobacter pylori* in superficial gastritis patients compared with control group.

H. pylori Cag A+ Status	superficial gastritis + patients No. %	Control No. %	P- value	Odd ratio 95% CI	RR Relative risk
H. pylori Positive	42 70.0	11 11	0.0001	18.878 8.190-43.513	6.363
H. pylori Negative	18 30.0	89 89			
Total	60	100			

Table-2- Observed and expected numbers and percentages of HLA-DRB1 alleles in patients with superficial gastritis disease.

HLA-DRB1 alleles	Patients with superficial gastritis disease Observed No.=60 No. %	Patients with superficial gastritis disease Expected No.=60 No. %
01:01:01	6 10	6.07 10.11
02:01:01	------	----- ------
03:01:01	24 40	24.08 40.13
03:02:01	-----	----- ------
04:02:01	15 25	15.09 25.15
05:01:01	------	------ ------
06:01:01	------	------- --------
07:01:01	18 30	18.21 30.35
08:31:01	3 5	3.09 5.15
10:01:01	------	----- ------
11:01:01	9 15	9.17 15.28
12:01:01	3 5	3.09 5.15
12;28:01	----	--- ------
13:01:01	6 10	6.07 10.11
13:22:01	9 15	9.17 15.28
14:01:01	6 10	6.07 10.11
15:01:01	15 25	----- -----
16:01:01	6 10	6.07 10.11
17:01:01	------	----- -------

Table-3- Hardy – Weinberg equilibrium in HLA-DRB1 locus of patients with superficial gastritis disease.

HLA locus	Chi ^{2}	DF	P
DRB1	0.015	11	Not significant

Table-4- Frequencies of HLA-DRB1 alleles in patients with superficial gastritis disease compared with control group.

HLA-DRB1 alleles	Patients with superficial gastritis No.=60 No. %	Control group No.=100 No. %	Odd ratio 95% confidence interval	P - value
01:01:01	6 10	6 6	1.740 0.534-5.660	0.357
02:01:01	------	10 10	na	Na
03:01:01	24 40	12 12	4.888 2.209-10.816	0.0001
03:02:01	-----	14 14	na	Na
04:02:01	15 25	26 26	0.435 0.200-0.947	0.036
05:01:01	------	4 4	na	Na
06:01:01	------	6 6	na	Na
07:01:01	18 30	18 18	1.952 0.920-4.140	0.081

08:31:01	3 5	6 6	na	Na
10:01:01	------	6 6	na	Na
11:01:01	9 15	44 44	0.224 0.099-0.505	0.0003
12:01:01	3 5	5 5	1.00.230-4.343	1.00
12;28:01	----	1 1	na	na
13:01:01	6 10	20 20	0.444 0.167-1.178	0.103
13:22:01	9 15	------	na	na
14:01:01	6 10	6 6	1.740 0.534-5.665	0.357
15:01:01	15 25	8 8	3.833 1.513-9.708	0.004
16:01:01	6 10	4 4	2.666 0.720-9.867	0.141
17:01:01	------	4 4	na	na

Table-5- Genotypes frequencies of HLA-DRB1 alleles in patients with superficial gastritis and control group.

HLA-DRB1 alleles	Patients with superficial gastritis No.=60 Gene frequency	Control group No.=100 Gene frequency
01:01:01	0.026	0.016
02:01:01	-----	0.026
03:01:01	0.106	0.031
03:02:01	------	0.036
04:02:01	0.065	0.068
05:01:01	------	0.011
06:01:01	------	0.016
07:01:01	0.079	0.047
08:31:01	0.013	0.016
10:01:01	------	0.016
11:01:01	0.039	0.117
12:01:01	0.013	0.013
12:28:01	------	0.003
13:01:01	0.026	0.052
13:22:01	0.039	------
14:01:01	0.026	0.016
15:01:01	0.065	0.021
16:01:01	0.026	0.011
17:01:01	------	0.011

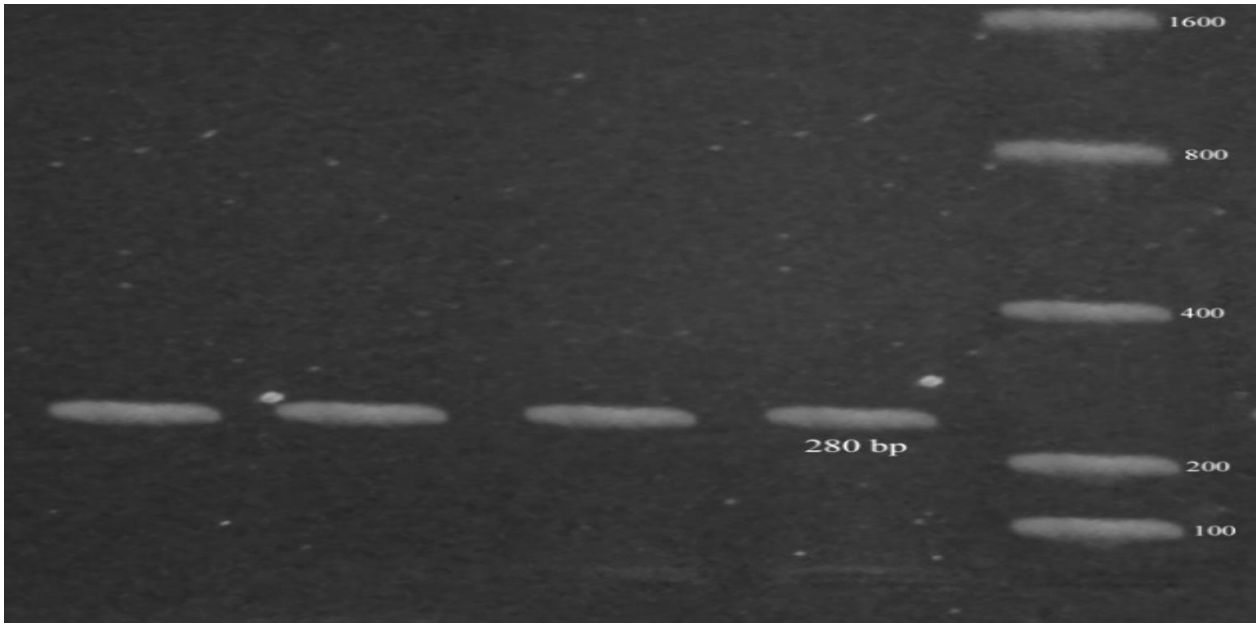

Figure-1- Results of DNA electrophoresis after amplification

Discussion: The current study investigated association between patients of superficial gastritis (with an *H pylori* infection) and HLA-DRB1 alleles in Iraqi Arab Muslims. Individuals carrying DRB1*03:01 genotypes (0.106) were noted to be at a significantly greater risk for developing superficial gastritis with *H. pylori* infection, compared to control group. Other allele was DRB1*15:01 that increased in patients group. Thus these two alleles may be considered as predisposing factors to develop disease while the HLA-DRB1* 04:02 and 11:01were increased in control group. Thus these two alleles may be considered as protective factors against the development of disease. Other result in Korea found that the frequencies of DRB1*1101 and DQA1*0505 were significantly higher in the chronic atrophic gastritis patients while in the patients with *Helicobacter pylori* (+), the frequencies of DRB1*1501 and DQB1*0602 were significantly lower in the chronic atrophic gastritis compared to other group with chronic superficial gastritis (11). In Italian patients, over 50% of patients with atrophic gastritis had HLA-DRB1*03 or *04 alleles that associated with autoimmune diseases, suggesting that this disease has a genetic predisposition to autoimmunity while DRB1*01 occurred more frequently in controls than patients with atrophic gastritis (11.5% vs. 3.4%, p=0.01) conferring an OR of 0.27(12). Other study found that HLA-DRB1*17 allele might be associated with increased susceptibility to peptic ulcer formation among *H. pylori*-positive children while HLA-DQB1*07 allele may be associated with protection against H. pylori infection (13). In Mexican study demonstrated that there is a significant increased frequency of HLA-DQB1*0401 allele in H pylori-positive Mexican Mestizo patients with chronic gastritis when compared with control group (19 vs 0%, P = 1 x 10(-7), odds ratio (OR) = 4.96; 95% confidence interval = 3.87-6.35). They also found a significant increased frequency of HLA-DQB1*0501 in Mexican Mestizo patients with gastric carcinoma in comparison with control group (P = 1 x 10(-6), OR = 13.07; 95% CI= 2.82-85.14) (14). The authors have reported that human leukocyte antigen (HLA)-DQB1*0401 plays an important role in the development of atrophic gastritis in H. pylori infected patients and it is a useful marker for determining susceptibility to intestinal type gastric cancer(15). In Lombok Indonesian patients, HLADQB1*0401 gene play important roles in *H. pylori* infection, but there was no statistically significant association between HLA-DQA1 or DQB1 haplotypes and *H.pylori* infection (16). These differences with our study may be related to race of the studying patients, religion, sample size, criteria of patient's selection and method used in detection associated alleles. Thus, early detection of atrophic gastritis and gastric cancer can be done using multiple markers like HLA-DR, HLA-DQ, age, sex and *H pylori* infection (17). Other studies done in Iraq showed that there is an association between HLA and different diseases in Iraq (18) like DR3 and DR4 is common in Insulin dependent diabetes mellitus (19,20), Inflammatory bowel disease was associated with DR8 (21). In conclusion, our results, together with other studies , support that genetic constitution through HLA-DR locus determines the mechanism and pathogenesis of disease as well as clinical and pathologic outcomes, triggered by the interaction between environmental factors and genetic factors. In other words, immunogenetic background among different population with different ethnicities is manifested as resistance or susceptibility to the development of superficial gastritis.

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