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Full Length Research Paper

Human leukocyte antigens (HLA) HLA-DQA1 and -DQB1 genotyping in *Helicobacter pylori*-seropositive individuals

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This work aimed at studying the HLA-DQA1 and HLA-DQB1 genotyping in gastritis patients with positive rapid urease test (RUT). This study was carried out in College of Medicine, University of Basrah. HLA-DQA1 and HLA-DQB1 genotyping was done in College of Medicine, University of Manitoba, Winnpeg, Canada during the period from 17th of April 2009 to 15th of July 2010. A total of 70 gastritis patients (29 males and 41 females) and 30 controls were included in this study. A significant increased frequency of DQA1*050101 and DQB1*020101 alleles was found in individuals (patients + controls) who showed positive rapid diagnostic test (+RDT), but the association was weak (odds ratio = 0.39 and 0.33), as compared with individuals (patients + controls) with (-RDT). A significant decreased frequency of DQB1*050201 allele was found in individuals (patients + controls) with (+RDT). The association was very strong (odds ratio = 5.31), as compared with individuals (patients + controls) with (-RDT). A significant decreased frequencies of DQA1*0201 and DQB1*020101 alleles were found in gastritis patients with (+RDT). The association in DQA1*0201 was very strong (odds ratio = 6.38) and for -DQB1*020101 allele, the association was weak (odds ratio = 0.08), as compared with controls with (RDT). A significant increased frequency of DQA1*0201 allele was found in controls with (+RDT) and the association was very strong (odds ratio = 6.18), as compared with controls with (-RDT). A significant increased frequency of DQB1*020101 allele was found in controls with (+RDT) but with weak association (odds ratio = 0.09), as compared with controls with (-RDT).

Key words: HLA-DQA1, HLA-DQB1, DQA1, HLA-DQB1, +RDT, RDT.

INTRODUCTION

Human leukocyte antigens (HLA) are an inherent system of alloantigens, which are the products of genes of the major histocompatibility complex (MHC). These genes span a region of approximately four centimorgans on the short arm of human chromosome 6 at band p21.3 and encode the HLA class I and class II antigens, which play a central role in cell-to-cell-interaction in the immune system (Conrad et al., 2006). They encode peptides involved in host immune response; also they are important in tissue transplantation and are associated with a variety of infectious, autoimmune, and inflammatory diseases (Gregersen et al., 2006; Nair et al., 2006). Moreover, the HLA loci display an unprecedented degree of diversity and the distribution of HLA alleles and haplotypes among different populations is considerably variable (Shao et al., 2004; Blomhoff et al., 2006). The expression of particular HLA alleles may be associated with the susceptibility or resistance to some diseases (Wang et al., 2006). Heterozygosity within the MHC genomic region provides the immune system with a selective advantage of pathogens (Fu et al., 2003; Kumar et al., 2007). *Helicobacter pylori* infection is, in addition to

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being the main etiologic agent for chronic gastritis, a major cause of peptic ulcer and gastric cancer (Suerbaum and Michetti, 2002). Many studies performed in Iraq about bacteriological and immunological aspects of *H. pylori* (Al- Janabi, 1992; Al-Jalili, 1996; Al-Baldawi, 2001; Al-Dhaher, 2001; Al-Saimary, 2008), but no study was performed yet on HLA genotyping, so results of the present study compared with studies done in other countries. In developing countries, prevalence of *H. pylori* infection is > 80% among middle-aged adults, whereas in developed countries prevalence ranges from 20 to 50%. Approximately 10 to 15% of infected individuals will develop peptic disease and 3% a gastric neoplasm (Torres et al., 2005). Therefore, *H. pylori* infection is a necessary but not a sufficient cause of severe forms of gastric disease. *H. pylori* induce a host immune response, but the persistence of the infection suggests that the response is not effective in eliminating the infection. Furthermore, multiple lines of evidence suggest that the immune response contributes to the pathogenesis associated with the infection. As a result, the immune response induced by *H. pylori* is a subject of continuous study that has encouraged numerous questions (Azem et al., 2006). The inability of the host response to clear infections with *H. pylori* could reflect down-regulatory mechanisms that limit the resulting immune responses to prevent harmful inflammation as a means to protect the host (Yoshikawa and Naito, 2000).

METHODS

A total of 70 (70%) showed abnormal endoscopic findings; 29 (41%) males and 41 (58.57%) females, with age groups from (15 to 66) years, with various gastritis symptoms attending endoscopy unit at Al-Sadder Teaching Hospital in Basrah and a total of 30 controls (18 males and 12 females), with age groups from (15 to 61) years, without any symptoms of gastritis, were selected randomly. 2 ml of venous blood drawn for serological test (rapid diagnostic test) was collected in plain tube and centrifuged (Janetzki T24, Germany) for 10 min (1500 rpm/min), then serum used for rapid diagnostic kit in screening for the presence of antibodies against *H. pylori*. 3 ml of the blood was collected in tubes containing EDTA, kept under 18°C and later used for HLA-DQ genotyping. The study was carried out during the period from (17th of April 2009 to 15th of July 2010).

Rapid diagnostic test for *H. pylori* (RDT) (Maysiak–Budnik and Megraud, 1994)

A rapid one step test was done for the qualitative detection of IgG antibodies to *H. pylori* in human serum by using rapid diagnostic test kit, ACON Laboratories, Inc, USA.

DNA Isolated from the blood samples by using Wizard Genomic DNA purification kit, Promega Corporation, USA; Protocol (Beutler et al., 1990)

HLA-DQA1 and –DQB1 genotyping

HLA-DQA1 and –DQB1 genotyping protocol was done according to sequence-based-typing (SBT), which had been developed in National Microbiology Laboratories (NML), Winnipeg, Canada (Luo et al., 1999). All the steps of HLA-DQA1 and –DQB1 genotyping were done under supervision of Dr. Ma Luo in Medical Microbiology Laboratory, College of Medicine, Manitoba University in Dr. Ma Luo Laboratory in National Microbiology Laboratories (NML).

Loading and running DNA in agarose gel (Brody and Kern, 2004)

A 2 µl loading buffer was mixed with 5 µl DNA on paraffin paper, and then added to its place in the gel. 5 µl of ladder DNA was added to its place in the gel. Electrophoresis instrument set on 100 V. After 30 min, the gel was visualized under U.V. transilluminator, Vilber lourmant, EEC.

PCR (polymerase chain reaction) amplification of HLA-DQA1 and –DQB1 gene

The PCR amplification of HLA-DQA1 and –DQB1 gene was done in Medical Microbiology laboratories in College of Medicine, Manitoba University, Winnipeg, Canada.

Reagents

2X mix (Tris-HCL buffer, dNTP's, $Mgcl_2$) (Gibco/BRL, Life Technologies, Burlington, Canada). Primers for amplifying exon 2 of DQB1 genes and the primers for exon 2 of DQA1 gene (NML, CA, personal communication); Primer for DQA1F, Primer for DQA1R, Primer for DQB1F, Primer for DQB1R; Taq polymerase (Gibco/BRL, Life Technologies, Burlington, Canada). Reagents and samples were placed on ice after thawing then spin quickly. Autoclaved D.W. (23 µl) was added to each well, autoclaved D.W. (25 µl) was added to the well of the negative control, 2 µl DNA was added to each well except the well of the negative control and 25 µl of master mix was added to each well. Plate was covered by special plastic covering and placed in PCR system (9700 Eppendorf thermal cycler, USA)

DNA purification

The Purification of the amplified HLA-DQA1 and –DQB1 gene was done in National Microbiology laboratories (NML), in Dr. Ma Luo Lab, in College of Medicine, Manitoba University, Winnipeg, Canada.

Three methods had been used for purification of the amplified PCR DNA samples:

1. **DNA purification by using vacuum:** Amplified PCR DNA samples thawed, then quick spin and then transferred into a 96-well millipore plate (SV 96-well plate). The plate is placed on vacuum (Vac-Man 96 Vacuum Main Fold) and turn on (the pressure read at approximately 15 to 25 psi (for 5 to 10 min), then 100 µl Of TE buffer (pH 8.0) added and vacuum again (5 to 10 min). The plate is then removed, blotted lightly on Kim-wipe, vacuum again for 1 min and 30 µl water added, then placed on shaker for 10 min. the samples are finally transferred into a new 96-well plate, sealed with foil.

2. **DNA purification by using:** GenElute™ PCR Clean-Up Kit (Sigma- Aldrich, Inc. USA).

Table 1. HLA-DQA1 genotype frequency of individuals (patients + controls) with positive and negative RDT.

HLA-DQA1 allele	Individuals with +RDT		Individuals with -RDT		X^2	P	OR	95%CI
	n = 61	%	n = 26	%				
010101/010102/010401/010402/0105	8	13.11	6	23.08	1.34	NS	1.99	0.61 to 6.45
010201/010202/010203/010204	16	26.23	10	38.46	1.30	NS	1.76	0.66 to 4.66
0103	8	13.11	8	30.77	3.78	NS	2.94	0.96 to 8.99
0201	14	22.95	6	23.08	0.00	NS	1.01	0.34 to 2.99
030101/0302/0303	16	26.23	4	15.08	1.21	NS	0.51	0.15 to 1.71
040101/040102/0402/0404	3	4.91	3	11.54	1.24	NS	2.52	0.47 to 13.42
050101/0503/0505/0506/0507/0508/0509	42	68.85	12	46.15	3.98	<0.05	0.39	0.15 to 1.00

3. Purification in DNA core section in NML (NML, Canada): The amplified PCR DNA was purified in DNA Core laboratory in National Microbiology Laboratories (NML) in Winnipeg, Canada.

Sequencing –PCR

Sequencing–PCR was done in National Microbiology laboratories (NML), under supervision of Dr. Ma Luo.

Reagents

(1) Purified PCR DNA. (2) BigDye Terminator, v 1.1 (Applied Biosystems). (3) Primers (NML, CA).

Master mix (46) was added to the first column of the 96-well microplate.

Master mix of 3.5 µl was added to the remaining columns by using multichannel electronic micropipette (BIOHIT-e 1200), 2 µl of purified PCR DNA was added to each column and plate was sealed with silicone foil. The plate is spanned quickly in plate centrifuge (Thermo Electron Corporation-IEC CL 30) after which the plate is then placed on thermocycler (Eppendorf Mastercycler Gradient, USA.).

Ethanol precipitation

Ethanol precipitation was done under supervision of Dr. Ma Luo in National Microbiology laboratories (NML) in College of Medicine, Manitoba University, Winnipeg, Canada.

The plates were spin quickly in plate centrifuge (Thermo Electron Corporation-IEC CL 30) following sequencing-PCR and plate racks were attached to each plate. Then 5 ml ethanol and 250 µl sodium acetate (Gainland Chemical Co., UK) were added into a reservoir, 21 µl of the mixture was added to all columns of 96-well plates by using multichannel electronic micropipette (BIOHIT-e 1200).The plate was sealed with silicon foil and vortex (Lincolnshire, IL) and spin quickly The plate was then placed in 20°C for at least 1 h then spin at 4000 rpm for at least 1 h (long spin).

Sequencing-using the (3100 genetic analyzer, USA)

HLA-DQA1 and –DQB1 genotyping protocol was done according to sequence-based-typing (SBT), which had been developed in National Microbiology Laboratories (NML), Winnipeg, Canada (Luo et al., 1999).

Statistical analysis

For qualitative variables, frequency data were summarized as percentage. Statistical significant of differences between two groups was tested by Pearson Chi-square (x^2) with Yates' continuity correction. Risk was estimated using odds ratio (OR) and 95% confidence interval (95% CI). P-value was determined by Fisher's exact test, P- value of (< 0.05) was considered statistically significant. Data were analyzed using SPSS program for window (Version 10).

RESULTS

Genotype frequency of HLA-DQ in individuals (patients + controls) with positive and negative RDT

Results shown in (Table 1) indicated that HLA-DQA1*050101 was present in 42 out of 61 individuals (patients + controls) with positive RDT and in 12 out of 26 individuals (patients + controls) with negative RDT with frequencies of 68.85 and 46.15 respectively. The increased allele frequency in individuals with positive RDT was statistically significant but the association was weak (x^2 = 3.98, P < 0.05, OR = 0.39, 95%CI = 0.15 to 1.00) when compared with individuals with negative RDT. Results shown in (Table 2) indicated that HLA-DQB1*020101 was present in 37 out of 64 individuals (patients + controls) with positive RDT and in 11 out of 29 individuals (patients + controls) with negative RDT with frequencies of 57.81 and 31.03 respectively. The increased allele frequency in individuals (patients + controls) with positive RDT was statistically significant but with weak association (x^2 = 5.73, P < 0.05, OR = 0.33, 95%CI = 0.13 to 0.83) when compared with individuals (patients + controls) with negative RDT. Results in (Table 2) showed that HLA-DQB1*050201 was present in 5 out of 64 individuals (patients + controls) with positive RDT and in 9 out of 29 individuals (patients + controls) with negative RDT with frequencies of 7.81 and 31.03. The decreased allele frequency in individuals (patients + controls) with positive RDT was statistically significant with a strong association (x^2 = 8.42, P < 0.05, OR = 5.31,

Table 2. HLA-DQB1 genotype frequency of individuals with positive and negative RDT.

HLA-DQB1 allele	Individuals with +RDT		Individuals with -RDT		X^2	P	OR	95%CI
	n = 64	%	n = 29	%				
020101/0202/0204	37	57.81	9	31.03	5.73	< 0.05	0.33	0.13 to 0.83
030101/030104/0309/0321/0322/0324/030302	26	40.63	11	37.93	0.06	NS	0.89	0.36 to 2.20
030201	10	15.63	2	6.90	1.35	NS	0.40	0.08 to1.96
030302	1	1.56	0	0.00	1.12	NS	0.98	0.95 to1.02
0402	3	4.69	4	13.79	2.38	NS	3.25	0.68 to15.60
050101	6	9.38	6	20.69	2.28	NS	2.52	0.74 to 8.63
050201	5	7.81	9	31.03	8.42	< 0.05	5.31	1.59 to17.72
050301	1	1.56	0	0.00	0.46	NS	0.98	0.95 to1.02
060101/060103	3	4.69	4	13.79	2.38	NS	3.25	0.68 to15.60
060201	4	6.25	1	3.45	0.31	NS	0.54	0.06 to 5.02
060301/060401	4	6.25	4	13.79	1.44	NS	2.40	0.56 to10.36
060401/0634	6	9.38	1	3.45	1.01	NS	0.35	0.04 to3.01
060801	1	1.56	0	0.00	0.46	NS	0.98	0.95 to 1.02
0609	1	1.56	0	0.00	0.46	NS	0.98	0.95 to1.02

95%CI = 1.59 to 17.72) when compared with individuals (patients + controls) with negative RDT.

Homozygosity of HLA-DQ in individuals (patients + controls) with positive and negative RDT

HLA-DQ homozygosity was studied in individuals (patients + controls) with positive and negative RDT. Results shown in (Table 3) indicated that for HLA-DQA1, out of 61 individuals (patients + controls) with positive RDT, 15 were homozygous in one or both loci and out of 26 individuals (patients + controls) with negative RDT, 3 were homozygous in one or both loci, with frequencies of 24.59 and 11.54 respectively. No significant differences were observed in frequency of homozygous HLA-DQA1 genotype between individuals (patients + controls) with positive and negative RDT (x^2 = 1.89, P = NS, OR = 0.40, 95%CI = 0.11 to 1.52). For HLA-DQB, out of 64 individuals (patients + controls) with positive RDT, 17 were homozygous in one or both loci, and out of 29 individuals (patients + controls) with negative RDT, 5 were homozygous in one or both loci, with frequencies of 26.52 and 17.24 respectively. No significant differences were observed in frequency of homozygous HLA-DQB1 genotype between individuals (patients + controls) with positive and negative RDT x^2 = 0.96, P = NS, OR = 0.59, 95%CI = 0.19 to 1.75 (Table 3). For HLA-DQ- (A1+B1), out of 56 individuals (patients + controls) with positive RDT, 20 were homozygous in one or both loci, and out of 26 individuals (patients + controls) with negative RDT, 5 were homozygous in one or both loci with frequencies of 64.29 and 80.77, respectively. No significant differences were observed in frequency of homozygous HLA-(DQA1+DQB1) genotype between individuals (patients + controls) with positive and negative RDT x^2 = 2.28, P = NS, OR = 0.43, 95%CI = 0.14 to 0.31.

HLA-DQ allele frequencies in gastritis patients and controls with positive RDT

A comparison was done between gastritis patients with (+RDT) and controls with (+RDT). Results shown in (Table 4) indicated that HLA-DQA1*0201 allele was present in 11 out of 61 gastritis patients with (+RDT) and in 4 out of 7 controls with (+RDT) with frequencies of 17.74 and 57.14 respectively. The decreased allele frequency in gastritis patients with (+RDT) was statistically significant with strong association (P < 0.05, OR = 6.38, 95%CI = 1.52 to 7.48) when compared with controls with (+RDT). Results shown in (Table 5) indicated that HLA-DQB1*020101 allele was present in 33 out of 61 gastritis patients with (+RDT) and in 6 out of 7 controls with (+RDT) with frequencies of 50.76 and 85.71 respectively. The decreased allele frequency in gastritis patients with (+RDT) was statistically significant (P < 0.05, OR = 0.08, 95%CI = 0.01 to 0.83) when compared with controls with (+RDT).

HLA-DQ alleles frequencies in controls with positive and negative RDT

A comparison was done between controls with positive RDT and controls with negative RDT. Results shown in

Table 3. Homozygosity of HLA-DQ in individuals (patients + controls) with positive and negative RDT.

HLA-DQ Homozygousity*		Cases			
		+RDT		-RDT	
		No	%	No	%
DQA1**	Homozygous	15	24.59	3	11.54
	Heterozygous	46	75.41	23	88.46
	Total	61	100	26	100
DQB1***	Homozygous	17	26.52	5	17.24
	Heterozygous	47	73.48	24	82.76
	Total	64	100	29	100
DQA1+DQB1****	Homozygous	20	35.71	5	19.23
	Heterozygous	36	64.29	21	80.77
	Total	56	100	26	100

*Homozygous at one or both loci; **x^2 = 1.89, P =NS, OR= 0.40, 95% CI= 0.11-1.52; ***x^2 = 0.96, P = NS, OR = 0.59, 95% CI = 0.19-1.75 ****x^2 = 2.28, P = NS, OR = 0.43, 95%CI = 0.14-0.31.

Table 4. DQA1 alleles frequencies in gastritis patients & controls with +RDT.

DQA1 allele	Patients (+RDT) (n = 61)	Controls (+RDT) (n = 7)
010101/010102/010401/010402/0105	10 (16.13%)	0 (0.00%)
010201/010202/010203/010204	20 (32.23%)	2 (28.57%)
0103	10 (16.13%)	0 (0.00%)
0201*	11 (17.74%)	4 (57.14%)
030101/0302/0303	16 (25.81%)	1 (14.29%)
040101/040102/0402/0404	4 (6.45%)	0 (0.00%)
050101/0503/0505/0506/0507/0508/0509	38 (61.29%)	6 (85.17%)

*(P < 0.05, OR = 6.38, 95%CI = 1.52 to7.48).

Table 5. DQB1 alleles frequencies in gastritis patients and controls with +RDT.

DQB1 allele	Patients (+RDT) (n=61)	Control (+RDT) (n=7)
020101/0202/0204*	33 (50.76%)	6 (85.71%)
030101/030104/0309/0321/0322/0324/030302	25 (38.46%)	2 (28.57%)
030201	10 (15.38%)	0
030302	3 (4.61%)	0
0402	5 (7.69%)	0
050101	8 (12.31%)	0
050201	10 (15.38%)	1 (14.29%)
050301	1 (1.53%)	0
060101/060103	3 (4.61%)	0
060201	3 (4.61%)	1 (14.29%)
060301/061401	6 (9.23%)	0
060401/0634	6 (9.23%)	0
060801	1 (1.53%)	0
0609	1 (1.53%)	0

*(P < 0.05, OR = 0.08, 95%CI = 0.01 to 0.83).

Table 6. DQA1 alleles frequencies in controls with positive and negative RDT.

DQA1 allele	+RDT Controls (n = 7)	-RDT Controls (n = 18)
010101/010102/010401/010402/0105	0 (0.00%)	4 (22.22%)
010201/010202/010203/010204	2 (28.57%)	4 (22.22%)
0103	0 (0.00%)	6 (33.33%)
0201*	4 (57.14%)	5 (27.78%)
030101/0302/0303	1 (14.29%)	3 (16.67%)
040101/040102/0402/0404	0 (0.00%)	2 (11.11%)
050101/0503/0505/0506/0507/0508/0509	6 (85.17%)	10 (55.56%)

*(P = 0.03, OR = 6.18, 95%CI = 1.21 to 6.18).

Table 7. DQB1 alleles frequencies in controls with positive and negative RDT.

DQB1 allele	+RDT controls (n = 7)	-RDT controls (n = 21)
020101/0202/0204*	6 (85.71%)	7 (33.33%)
030101/030104/0309/0321/0322/0324/030302	2 (28.57%)	10 (47.62%)
030201	0	2 (9.52%)
030302	0	2 (9.52%)
0402	0	2 (9.52%)
050101	0	4 (19.05%)
050201	1 (14.29%)	3 (14.29%)
050301	0	0 (0.00%)
060101/060103	0	4 (19.05%)
060201	1 (14.29%)	1 (4.76%)
060301/061401	0	2 (9.52%)
060401/0634	0	1 (4.76%)
060801	0	0
0609	0	0

*(P < 0.05, OR = 0.09, 95%CI = 0.01 to 0.63).

(Table 6) indicated that HLA-DQA1*0201 allele was present in 4 out of 7 controls with (+RDT) and in 5 out of 18 controls with (-RDT) with frequencies of 57.14 and 27.78 respectively. The increased allele frequency in controls with (+RDT) was statistically significant (P < 0.05, OR = 6.18, 95%CI = 1.21 to 6.18) when compared with controls with negative RDT. Results shown in (Table 7) indicated that HLA-DQB1*020101 allele was present in 4 out of 7 controls with (+RDT) and in 7 out of 21 controls with (-RDT) with frequencies of 85.71 and 33.33 respectively. The increased allele frequency in controls with (+RDT) was statistically significant (P < 0.05, OR = 0.09, 95%CI =0.01 to 0.63) when compared with controls with (-RDT).

DISCUSSION

Serological tests used for diagnosis of *H. pylori,* are fast and non invasive, also these tests are capable of reducing the endoscope workload. In the present study a rapid one step test was done for the qualitative detection of IgG antibodies to *H. pylori* in human serum by using (Rapid Diagnostic Test) Kit. Results of rapid diagnostic test showed that, out of 70 gastritis patients, 61 (87.14%) were positive rapid diagnostic test (RDT) and 9 (12.86%) were negative RDT; these results were compatible with a study performed by Maysiak–Budnik and Megraud (1994). The test has been tested for interference from visibly hemolysed as well as specimens containing high bilirubin levels. In addition, no interference was observed in specimens containing hemoglobin up to 1.000 µg/dL, bilirubin, and up to 2.000 µm/dL human serum albumin (Marshall et al., 1985; Faigel et al., 2000). This evidence indicated that this test is sensitive, fast and convenient and should be used in screening for anti *H. pylori* antibodies. Many factors such as socioeconomic status, ethnic group, different populations, geographical location and the type of the trouble associated with the infection also contribute to the observed variations in prevalence in the present study. Asymptomatic and untreated patients continue to test IgG seropositive as long as the *H. pylori*

organisms are presents, even after histological resolution. These results showed that humoral immunity was present throughout the spectrum, but does not seem to provide protection in patients, Although controls showed +RDT, they had no symptoms of gastritis, this might be explained, as in such individuals, the genetic factors may control host immune responses to the infectious agents and provide protection (Mattsson et al., 1998). Genotype frequency of HLA-DQ was studied in individuals (patients + Controls) with positive and negative RDT. Results shown in (Table 1) indicated significant increased frequency of HLA-DQA1*050101 allele in individuals with positive RDT but the association was weak (x^2 = 3.98, P<0.05, OR = 0.39, 95%CI = 0.15 to 1.00) when compared with individuals with negative RDT. Results shown in Table 4 indicated significant increased frequency of HLA-DQB1*020101 allele in individuals (patients + controls) with positive RDT, but with weak association (x^2 = 5.73, P<0.05, OR = 0.33, 95%CI = 0.13 to 0.83) when compared with individuals (patients + controls) with negative RDT. Results in Table 4 showed significant decreased frequency of HLA-DQB1*050201 allele in individuals (patients + controls) with positive RDT with a strong association (x^2 = 8.42, P<0.05, OR = 5.31, 95%CI = 1.59 to 17.72) when compared with individuals (patients + controls) with negative RDT. HLA-DQ homozygosity was studied in individuals (patients + controls) with positive and negative RDT. Results shown in Table 4 indicated that there was no significant differences were observed in frequency of homozygous HLA-DQ genotype between individuals (patients + controls) with positive and negative RDT. A comparison was done between gastritis patients with (+ ve RDT) and controls with (+RDT). Results shown in Table 4 indicated a significant decreased frequency of HLA-DQA1*0201 allele in gastritis patients with (+RDT) with strong association (P < 0.05, OR = 6.38, 95%CI = 1.52 to 7.48) when compared with controls with (+ RDT). Results shown in Table 4 indicated significant decreased frequency of HLA-DQB1*020101 allele in gastritis patients with (+RDT) (P < 0.05, OR = 0.08, 95%CI = 0.01 to 0.83) when compared with controls with (+RDT). Wu et al. 2002 reported lower seropositivity of *H. pylori* and a higher ratio of diffuse/intestinal type carcinoma in Taiwanese patients carrying the HLADQB1*0301 allele. A comparison was done between controls with positive RDT and controls with negative RDT. Results shown in Table 4 indicated significant increased frequency of HLA-DQA1*0201 allele in controls with (+RDT) (P < 0.05, OR = 6.18, 95%CI = 1.21 to 6.18) when compared with controls with negative RDT. Results shown in Table 4 indicated a significant increased frequency of HLA-DQB1*020101 allele in controls with (+RDT) (P < 0.05, OR = 0.09, 95%CI = 0.01 to 0.63) when compared with controls with (-RDT). These results were compatible with a study performed by Magnuson et al. (2001) who reported that HLADQA1*0102 was inversely associated with *H. pylori*-seropositivity. Current evidence indicates that the majority of individuals harboring *H. pylori* infection remain asymptomatic during their lifetime, with no clinical consequence from their infection. In a community-based seroepidemiologic study in Mexico (Torres et al., 1998) who reported that seropositivity for *H. pylori* infection was 66%, and > 80% of adults were infected at 25 years of age; seroprevalence remained nearly unchanged after the third decade of life, with an increment in seropositivity of < 0.5% per year in persons between 30 and 69 years. Taken together, these data suggest that risk for gastric diseases depends on factors other than *H. pylori* infection and age.

REFERENCES

Al-Baldawi MR (2001). Isolation and identification of *Helicobacter pylori* from patients with duodenal ulcer, study of pathogenicity and antibiotic resistance. MSc-Thesis. Submitted to College of Science. University of Baghdad.

Al-Dhaher ZAJ (2001). Study of some bacteriological and immunological aspects of *Helicobacter pylori*. MSc-Thesis Submitted to College of Science. Al-Mustansirya University.

Al-Jalili FAY (1996). *Helicobacter pylori* peptic ulceration in Iraqi patients, bacteriological and serological study. MSc-thesis submitted to the college of Science. Al-Mustansirya University.

Al-Janabi AAh (1992). *Helicobacter*-associated gastritis, diagnosis and clinicopathological correlation. “A prospective study” MSc-Thesis. Submitted to College of Medicine. Al-Mustansirya University.

Al-Saimary AE (2008). The prevalence of *H. pylori* in intra abdominal hydatid disease. Diploma-Dissertation. Submitted to College of Medicine. Kufa University.

Azem J, Svennerholm AM, Lundin BS (2006). B cells pulsed with *Helicobacter pylori* antigen efficiently activate memory CD8T cells from *H. pylori* infected individuals. Clin. Immunol., 158: 962-967.

Beutler E, Gelbart T, Kuhl W (1990). Interference of heparin with the polymerase chain reaction. Biol. Techniq., 9: 166.

Blomhoff A, Olsson M, Johansson S, Akselsen HE, Pociot F (2006). Linkage diequilibrium and haplotype blocks in the MHC vary in an HLA haplotype specific manner assessed mainly by DRB1*04 haplotypes. Genes Immun., 7: 130-140.

Brody JR, Kern SE (2004). History and principles of conductive media for standard DNA electrophoresis. Anal. Biochem., 333(1): 1-13.

Conrad DF, Andrews TD, Carter NP, Hurles ME, Pritchard JK (2006). A high-resolution survey of deletion polymorphism in the human genome. Nat. Genet., 38: 75-81.

Faigel DO, Magaret N, Corless C, Lieberman DA, Fennerty MB (2000). Evaluation of rapid antibody tests for the diagnosis of *Helicobacter pylori* infection. Am. J. Gastroenterol., 95: 72-77.

Fu Y, Liu Z, Lin J, Jia Z, Chen W, Pan D. (2003). HLA-DRB1, DQB1 and DPB1 polymorphism in the Naxi ethenic group of south-western China. Tissue Antigens, 61: 179-183.

Gregersen JW, Kranc KR, Ke X, Svendsen P, Madsen IS, Thmsen AR (2006). Functional epistasis on a common MHC haplotype associated with multiple sclerosis. Nature, 443: 574-577.

Kumar PP, Bischof O, Purhey PK, Natani D, Urlaub H, Dejean A (2007). Functional interaction between PML and SATB1 regulates chromatin-loop architectur and transcription of the MHC class I locus. Nat. Cell Biol., 9: 45-56.

Luo M, Blanchard J, Pan Y, Brunham K (1999). High resolution sequence typing of HLA-DQA1 and HLA-DQB1 exon 2 DNA with taxonomy-based sequence ananlysis (TBSA) alleles assignment. Tissue Antigens, 54: 69-82.

Marshall BJ, McGechie DB, Rogers Par, Glancy RG (1985). *Pyloric Campylobacter* infection and gastroduodenal disease. Med. J. Aust., 149:44.

Mattsson A, Quiding-Jarbrink M, Lonroth H, Hamlet A, Ahlstedt I, Svennerholm A (1998). Antibody –secreting cells in the stomachs of symptomatic and asymptomatic *Helicobacter pylori* – infected

subjects. Infect. Immun., 66: 2705-2712.
Maysiak-Budnik T, Megraud F (1994). *Helicobacter pylori* in Eastern Europe countries: What is the current status. Gut; 35: 1683-1686.
Nair RP, Stuart PE, Nistor I, Hiremagalore R, Chia NV, Jenisch S (2006). Sequence and haplotype analysis supports HLA-C as the psoriasis susceptiblilty 1 gene. Am. J. Hum. Genet., 78: 827-851.
Shao W, Tang J, Dorak MT, Song W (2004). Molecular typing of human leukocyte antigen and related polymorphisms following whole genome amplification. Tissue Antigens; 64: 286-292.
Suerbaum S, Michetti P (2002). *Helicobacter pylori* infection. N. Engl. J. Med., 347(15): 1175-1186.
Torres J, Leal-Herrera Y, Perez-Perez G, Gomez A, Camorlinga- Ponce M, Cedillo-Rivera R, Tapia-Conyer R, Munoz OA (1998). Community-based seroepidemiologic study of *Helicobacter pylori* infection in Mexico. J. Infect. Dis., 178: 1089-1094.
Yoshikawa T, Naito Y (2000). The role of neutrophils and inflammation in gastric mucosal injury. Free Radic. Res., 33: 785-794.