

Full Length Research Paper

HLA-DQA1 genotyping of *Helicobacter pylori* associated gastritis patients

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To study HLA-DQA1 genotyping in *Helicobacter pylori* associated gastritis patients. This study was carried out in College of Medicine, University of Basrah. HLA-DQA1 genotyping was done in College of Medicine, University of Manitoba, Winnipeg, Canada during the period from 17th of April 2009 to 15th of July 2010. A total of 100 patients (41 males and 59 females and a total of 30 controls (18 males and 12 females) were included in this study. DQA1 alleles frequencies were studied in 70 *H. pylori* associated gastritis patients and 30 healthy controls. DQA1*0201 decreased allele frequency was statistically not significant in *H. pylori* associated gastritis patients, but there was a strong association (odds ratio= 2.61), as compared with controls. In the present study many alleles from both locus showed high frequencies with a very strong association, but statistically not significant, this association has not been reported and it is important to note that a larger sample size should be studied.

Key words: HLA-DQA1 genotyping, DQA1*0201, *Helicobacter*.

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 4 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 (Gegersen 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 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).

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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).

MATERIALS AND METHODS

A total of 100 patients (41 males and 59 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 included in the present study. Blood samples were drawn from gastritis patients and subjected to HLA-DQ genotyping. The study was carried out during the period from (17th April, 2009 to 15th July, 2010).

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

HLA-DQA1 genotyping

HLA-DQA1 genotyping protocol had 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 genotyping were done under supervision of Dr. Ma Luo in Medical Microbiology Laboratory, College of Medicine, University of Manitoba and in Dr. Ma Luo Laboratory in National Microbiology Laboratories (NML).

Reagents

- 1) Agarose, Gibco/BRL, MD
- 2) Bromophenol blue, Sigma St. Louis, Mo
- 3) Ethidium bromide, Himedia
- 4) DNA ladder marker, Sigma St. Louis, Mo
- 5) 1X Tris- Borate buffer (TBE)

Preparation of agarose gel (Brody and Kern, 2004)

- 1) 1 g agarose was dissolved in 100 ml Tris Borate Buffer (1%)
- 2) Agarose was heated in microwave until bubbles appeared
- 3) 2 µl of ethidium bromide was added and mixed

Casting of the horizontal agarose gel (Brody and Kern, 2004)

- 1) Both edges of the gel tray were sealed and the comb was positioned at one end of the tray.
- 2) Gel was poured into the tray, waited to harden.
- 3) Combs were removed gently and the gel tray was replaced in electrophoresis chamber.
- 4) TBE was added to the chamber until it reached 5mm over the surface of the gel.

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

- 1) 2 µl loading buffer was mixed with 5ul DNA on paraffin paper, then added to its place in the gel.
- 2) 5 µl of ladder DNA was added to its place in the gel (only used

with amplified DNA and not with whole DNA).

3) Electrophoresis instrument set on 100 V. After 30 min, the gel was visualized under U.V. transilluminator, Vilber lourmant, EEC.

PCR amplification of HLA-DQA1 gene

The PCR Amplification of HLA-DQA1 gene was done in Medical Microbiology Laboratories in College of Medicine, Manitoba University, Winnipeg, Canada.

Reagents

- 1) 2X mix (Tris-HCL buffer, dNTP's, Mgcl₂) (Gibco/BRL, Life Technologies, Burlington, Canada)
- 2) Primers for amplifying exon 2 of DQA1 gene (NML, CA, personal communication) were:

- a) Primer for DQA1F
- b) Primer for DQA1R

- 1) Taq polymerase (Gibco/BRL, Life Technologies, Burlington, Canada)

- i) Reagents and samples were placed on ice after thawing
- ii) Reagents and samples were spin quickly

- Eppendorf centrifuge (spin to ~ 6000 rpm)
- Plate centrifuge (BECKMAN CS-6R) spin to ~ 1500 rpm

- iii) Autoclaved D.W. (23 µl) was added to each well.
- iv) Autoclaved D.W. (25 µl) was added to the well of the negative control.
- v) 2 µl DNA was added to each well accept the well of the negative control.
- vi) 25 µl of Master Mix was added to each wall.
- vii) Plate was covered by special plastic covering.
- viii) Plate was placed in PCR System (9700 Eppendorf thermal cycler, USA) DNA.

DNA purification

The purification of the amplified HLA-DQA1 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:

DNA purification by using vacuum

- I) Amplified PCR DNA samples thawed, then quick spin.
- II) Then transferred into a 96-well Millipore plate (SV 96-well plate).
- III) Plate placed on vacuum (Vac-Man 96 Vacuum Main Fold) and turn on (the pressure read at approx. 15 to 25 psi (for 5 to 10 min).
- IV) 100 ul of TE buffer (pH 8.0) added and vacuum again (5 to 10 min).
- V) Plate removed, blotted lightly on Kim-wipe, vacuum again for 1 min.
- VI) 30 ul water added, then placed on shaker for 10 min.
- VII) Samples transferred into a new 96-well plate, sealed with foil.

DNA purification by using GenElute™ PCR clean-up kit (Sigma-Aldrich, Inc. USA). GenElute™ PCR clean-up kit

Purification in DNA core section in NML (NML, Canada)

The amplified PCR DNA was purified in DNA core laboratory in

Table 1. Frequencies of acute and chronic gastritis patients typed for DQA1 alleles.

Type of gastritis	Typed for DQA1 alleles N (%)	Not typed N (%)	Total
	62	8	N = 70
Acute	49 (89.09)	6 (10.91)	55
Chronic	13 (86.67)	2 (13.33)	15

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)
- i) Master Mix (46 µl) was added to the first column of the 96-well microplate
- ii) 3.5 µl of master mix was added to the remaining columns by using multichannel electronic micropipette (BIOHIT-e 1200)
- iii) 2 µl of purified PCR DNA was added to each column
- iv) Plate was sealed with silicone foil
- v) Spin the plate quickly in plate centrifuge (Thermo Electron Corporation-IEC CL 30).
- vi) Plate was 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.

Protocol

1. Plates were spin quickly in plate centrifuge (Thermo Electron Corporation-IEC CL 30) following sequencing-PCR
2. Plate racks were attached to each plate
3. 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)
4. Plate was sealed with silicon foil
5. Plate was vortex (Lincolnshire, IL) and spin quickly
6. Plate was placed in -20°C for at least 1 h
7. Plate was spin at 4000 rpm for at least 1 h (long spin)

Sequencing-using the (3100 genetic analyzer, USA)

HLA-DQA1 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 difference between two groups was tested by Pearson Chi-square (χ^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

HLA-DQ genotyping of DNA samples of acute and chronic gastritis patients

Results shown in Table 1 indicated that out of 55 patients with acute gastritis, 49 (89.09%) were typed for HLA-DQA1 and 6 (10.91%) were not typed. Also results in Table 1 showed that out of 15 patients with chronic gastritis, 13 (86.67%) were typed for HLA-DQA1 and 2 (13.33%) were not typed.

Genotype frequency of HLA-DQA1 in gastritis patients

Genotype frequencies of HLA-DQA1 alleles were studied in 62 gastritis patients and compared with 25 controls. Results shown in Table 2, indicated that HLA-DQA1*0201 allele was present in 11 out of 62 gastritis patients and in 9 out of 25 controls, the frequencies of the allele were 17.74 and 36.00, respectively. The decreased allele frequency in gastritis patients was statistically not significant, but with strong association ($\chi^2 = 3.36$, $P = \text{NS}$, $\text{OR} = 2.61$, $95\% \text{ CI} = 0.92-7.41$) as compared with controls (Table 2). No other allele showed any significant differences between gastritis patients and controls.

Homozygosity of HLA-DQ in gastritis patients and controls

Homozygosity of HLA-DQ genotype was studied in gastritis patients and compared with controls. Results shown in Table 3 indicated that for HLA-DQA1, out of 62

Table 2. HLA-DQA1 genotype frequency of gastritis patients and controls.

DQA1 allele	Gastritis patients (n = 62)		Controls (n = 25)		χ^2	P	Odds ratio	95% CI
	No.	%	No.	%				
010101/010102/010401/010402/0105	10	16.13	4	16.0	0.00	NS	0.99	0.28-3.51
010201/010202/010203/010204	20	32.23	6	24.00	0.58	NS	0.66	0.23-1.92
0103	10	16.13	6	24.00	0.74	NS	1.64	0.53-5.14
0201	11	17.74	9	36.00	3.36	NS	2.61	0.92-7.41
030101/0302/0303	16	25.81	4	16.00	0.97	NS	0.55	0.16-1.84
040101/040102/0402/0404	4	6.45	2	8.00	0.07	NS	1.26	0.22-7.36
050101/0503/0505/0506/0507/0508/0509	38	61.29	16	64.00	0.06	NS	1.12	0.43-2.94

Table 3. HLA-DQ genotypes homozygosity in gastritis patients and controls.

HLA-DQ homozygosity*		Cases			
		Patients		Controls	
		No.	%	No.	%
DQA1**	Homozygous	15	24.19	3	12
	Heterozygous	47	75.81	22	88
	Total	62	100	25	100

*Homozygous at one or both loci. ** $\chi^2 = 0.61$, P = NS, OR = 2.34, 95% CI = 0.61-8.93.

gastritis patients, 15 were homozygous in one or both loci and out of 25 controls, 3 were homozygous in one or both loci with frequencies of 24.19 and 12 respectively. No significant differences were observed in frequencies of homozygous HLA-DQA1 genotypes of gastritis patients and controls. ($\chi^2 = 0.61$, P = NS, OR = 2.34, 95% CI = 0.61 - 8.93).

Genotype frequency of HLA-DQ in acute and chronic gastritis patients

Genotype frequencies of HLA-DQA1 alleles were studied in 49 acute gastritis patients and compared with 13 chronic gastritis patients. Results shown in Table 4, indicated that HLA-DQA1*050101 was present in 28 out of 49 acute gastritis patients and in 10 out of 13 chronic gastritis patients, frequencies of the allele were 57.14 and 76.92, respectively. The increased allele frequency in patients with chronic gastritis was statistically not significant, but showed very strong association ($\chi^2 = 1.69$, P = NS, OR = 2.50 95% CI = 0.61-10.23) as compared with acute gastritis patients.

Homozygosity of HLA-DQ in acute and chronic gastritis patients

Homozygosity of HLA-DQ genotype was studied in acute

gastritis patients and compared with chronic gastritis patients. Results shown in Table 5 indicated that for HLA-DQA1, out of 49 acute gastritis patients, 13 were homozygous in one or both loci and out of 13 chronic gastritis patients, 2 were homozygous in one or both loci with frequencies of 26.53 and 15.38 respectively. No significant differences were observed in frequency of homozygous HLA-DQA1 genotype between acute and chronic gastritis patients ($\chi^2 = 0.69$, P = NS, OR = 0.50, 95% CI = 0.09-2.58).

DISCUSSION

The HLA genotyping was done in NML and Microbiology Laboratories, College of Medicine, University of Manitoba in Canada, by using sequencing-based typing (SBT) method which had been developed in Dr. Ma Luo laboratory. For the patients group, out of 70 whole DNA samples, 62 (88.57%) were genotyped for HLA-DQA1 alleles. For controls group, out of 30 whole DNA samples, 25 (83.33%) were genotyped for HLA-DQA1 alleles (Table 1). Genotype frequencies of HLA-DQA1 alleles were studied in 62 gastritis patients and compared with 25 controls. Results shown in Table 2, indicated that HLA-DQA1*0201 allele was present in 11 out of 62 gastritis patients and in 9 out of 25 controls, the frequencies of the allele were 17.74 and 36.00, respectively. The decreased allele frequency in gastritis

Table 4. HLA-DQA1 genotype frequency of acute and chronic gastritis patients.

HLA-DQA1 allele	Acute gastritis patients		Chronic gastritis patients		χ^2	P	OR	95% CI
	N = 49	%	N = 13	%				
010101/010102/010401/010402/0105	7	14.29	3	23.08	0.59	NS	1.80	0.39 to 8.22
010201/010202/010203/010204	18	36.73	2	15.38	2.14	NS	0.31	0.06 to 1.57
0103	8	16.33	2	15.38	0.07	NS	0.93	0.17 to 5.03
0201	9	18.37	2	15.38	0.06	NS	0.81	0.15 to 4.29
030101/0302/0303	12	24.49	4	30.77	0.21	NS	1.37	0.36 to 5.29
040101/040102/0402/0404	3	6.12	1	7.69	0.04	NS	1.28	0.12 to 13.41
050101/0503/0505/0506/0507/0508/0509	28	57.14	10	76.92	1.69	NS	2.50	0.61 to 10.23

Table 5. HLA-DQA1 homozygosity in patients with acute and chronic gastritis.

HLA-DQ Homozygosity*		Acute		Chronic	
		No.	%	No.	%
DQA1**	Homozygous	13	26.53	2	15.38
	Heterozygous	36	73.47	11	84.62
	Total	49	100	13	100

* Homozygous at one or both loci. ** $\chi^2 = 0.69$, P=NS, OR= 0.50, 95% CI= 0.09 to 2.58.

patients was statistically not significant with strong association ($\chi^2 = 3.36$, P = NS 0.05, OR = 2.61, 95% CI = 0.92 - 7.41) as compared with controls (Table 2). No other allele showed any significant differences between gastritis patients and controls.

These results were compatible with results of a study performed by Azuma et al. (1998) who indicated that in Japan the HLA-DQA1*0102 allele has a lower frequency in *H. pylori* infection. Also Azuma et al. (1998) reported that presence of HLA-DQA1*0301 allele increase susceptibility for the infection, which disagree with the result of the present study in which HLA-DQA1*0301 allele showed no significant increased allele frequency in gastritis patients. Yu et al. (2006) reported that, among the 8 DQA1 alleles in healthy Chinese population, the most common allele was DQA1*0301/02/03 with a frequency of 28.8%. Homozygosity of HLA-DQ genotype was studied in gastritis patients and compared with controls. Results shown in Table 3 indicated that no significant differences were observed in frequencies of homozygous HLA-DQA1 genotypes of gastritis patients and controls. ($\chi^2 = 0.61$, P = NS, OR = 2.34, 95% CI = 0.61 - 8.93).

Genotype frequencies of HLA-DQA1 alleles were studied in 49 acute gastritis patients and compared with 13 chronic gastritis patients. Results shown in Table 4, indicated that HLA-DQA1*050101 was present in 28 out of 49 acute gastritis patients and in 10 out of 13 chronic gastritis patients, frequencies of the allele were 57.14 and

76.92 respectively. The increased allele frequency in patients with chronic gastritis was statistically not significant, but showed very strong association ($\chi^2 = 1.69$, P = NS, OR = 2.50 95% CI = 0.61-10.23) as compared with acute gastritis patients. Caselli and Balboni (1996) reported that DQA1*0501 contributes to susceptibility to *H. pylori* infection. No significant differences were observed in frequency of homozygous HLA-DQA1 genotype between acute and chronic gastritis patients ($\chi^2 = 0.69$, P = NS, OR = 0.50, 95% CI = 0.09-2.58).

Sequencing-based typing (SBT) is the gold standard for high-resolution tissue typing, which is required for optimal HLA matching between donor and recipient in stem cell transplantation settings. High-resolution genotyping of the HLA genes by SBT is the most comprehensive method available. The HLA-DQA1 gene presents a unique challenge for genotyping using SBT because 10 of the 22 known alleles contain a three-nucleotide deletion of codon 56. Almost all currently used SBT strategies for HLA-DQB1 typing employ amplification and/or sequencing primers located within exon 2 and 3. Complete exon 3 sequence information facilitates the resolution of allele ambiguities, for instance HLA-DQB1*030101 and HLA-DQB1*0319. Successful sequence-based DQA1 and DQB1 typing depends on several factors. These include technical issues as well as analysis issues. Among the technical issues, the quality of DNA isolation is particularly important. The genomic

DNA isolated kits and appeared to be good quality as judged by PCR amplification results. It is important to store the DNA at -20°C for the long term in order to avoid degradation.

The quantity of DNA used in the PCR amplification is important, as too much DNA can result in over amplification of one allele in the heterozygous situation, using the exact amount of good quality DNA to ensure balanced amplification of both alleles (Luo et al., 1999).

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