HLA-DQB1 genotyping of H. pylori Associated Gastritis Patients

Dataset · August 2013

6 authors, including:

Ihsan E Al-Saimary
University of Basrah - college of medicine
149 PUBLICATIONS **75** CITATIONS
SEE PROFILE

Ma Luo
National Microbiology Laboratory, Canada
121 PUBLICATIONS **1,291** CITATIONS
SEE PROFILE

Trevor A Peterson
National Microbiology Laboratory, Canada
15 PUBLICATIONS **64** CITATIONS
SEE PROFILE

ADVANCES IN BIORESEARCH, Vol. 2 [1] June 2011: 180 - 158

http://www.soeagra.com/abr.htm
ISSN 0976-4585

RESEARCH ARTICLE
Accepted Article

HLA-DQB1 genotyping of *H. pylori* Associated Gastritis Patients

Nibras S. Al-Ammar[1], Ihsan Al-Saimary[1*] , Saad Sh. Hamadi[2], Ma Luo, Trevor Peterson and Chris Czarnecki
Department of Microbiolody ,
Department of Medicine,
College of Medicine , University of Basrah, Basrah, Iraq

Abstract
To study HLA-DQA1 genotyping in *H. pylori* associated gastritis patients.This study was carried out in College of Medicine, University of Basrah. HLA-DQB1 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. DQB1*060101 allele frequency was statistically not significant in *H. pylori* associated gastritis patients, but there was a strong association (odds ratio = 3.44), 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.

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 [1]. 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 [2,3]. Moreover, the HLA loci display an unprecedented degree of diversity and the distribution of HLA alleles and haplotypes among different populations is considerably variable [4,5]. The expression of particular HLA alleles may be associated with the susceptibility or resistance to some diseases [6]. Heterozygosity within the MHC genomic region provides the immune system with a selective advantage of pathogens [7,8]. *H. pylori* infection is, in addition to being the main etiologic agent for chronic gastritis, a major cause of peptic ulcer and gastric cancer [9]. Many studies performed in Iraq about bacteriological and immunological aspects of *H. pylori* [10-13], 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%-50%. Approximately 10%-15% of infected individuals will develop peptic disease and 3% a gastric neoplasm [14]. 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 [15].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 [16].

MATERIAL AND METHODS

A total of 100 patients (41 males and 59 females with age groups from (15-66) 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 (15-61) years, without any symptoms of gastritis were included in the present study. Blood samples were drawn from gastritis patients and subjected to HLA-DQB1 genotyping. The study was carried out during the period from (17^{th} of April 2009 to 15^{th} of July 2010). DNA Isolated from the Blood Samples by using Wizard Genomic DNA purification Kit, Promega Corporation, USA; Protocol [17].

HLA-DQB1 Genotyping:

HLA-DQB1 genotyping protocol had done according to Sequence-Based-Typing (SBT), which had been developed in National Microbiology Laboratories (NML), Winnipeg, Canada [18]. All the steps of HLA-DQB1 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. Ethedium bromide, Himedia
4. DNA ladder marker, Sigma St. Louis, Mo
5. 1X Tris- Borate buffer (TBE)

Preparation of Agarose Gel [19]

1. 1g Agarose was dissolved in 100ml Tris Borate Buffer (1%)
2. Agarose was heated in microwave until bubbles appeared
3. 2ul of ethedium bromide was added and mixed

Casting of the Horizontal Agarose Gel [19]

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 [19]

1. 2µl loading buffer was mixed with 5ul DNA on paraffin paper, then added to its place in the gel
2. 5µl 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.
4. After 30 minutes, the gel was visualized under U.V. transilluminator, Vilber Iourmant, EEC.

PCR Amplification of HLA-DQB1 Gene

The PCR Amplification of HLA-DQB1 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 DQB1 gene (NML, CA, personal communication) were illustrated in
    - Primer for DQB1F
    - Primer for DQB1R
3. Taq polymerase (Gibco/BRL, Life Technologies, Burlington, Canada)
4. Reagents and samples were placed on ice after thawing
5. Reagents and samples were spin quickly
    - Eppendorf centrifuge (spin to ~ 6000 rpm)
    - plate centrifuge (BECKMAN CS-6R) spin to ~ 1500 rpm
6. Autoclaved D.W. (23ul) was added to each well
7. Autoclaved D.W. (25ul) was added to the well of the negative control
8. 2ul DNA was added to each well accept the well of the negative control
9. 25ul of Master Mix was added to each wall
10. Plate was covered by special plastic covering
11. Plate was placed in PCR System (9700 Eppendorf thermal cycler, USA).

DNA Purification

The Purification of the amplified HLA-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
- Then transferred into a 96-well Millipore plate (SV 96-well plate)
- Plate placed on vacuum (Vac-Man 96 Vacuum Main Fold) and turn on (the pressure read at approx. 15-25 psi (for 5-10 minutes)
- 100ul Of TE buffer (pH 8.0) added and vacuum again (5-10 minutes)
- Plate removed, blotted lightly on Kim-wipe, vacuum again for 1 minute
- 30ul water added, then placed on shaker for 10 minutes
- Samples transferred into a new 96-well plate, sealed with foil

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

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

- Purified PCR DNA
- BigDye Terminator, v 1.1 (Applied Biosystems).
- **P**rimers (NML, CA)
- Master Mix (46ul) was added to the first column of the 96-well microplate
- 3.5ul of master mix was added to the remaining columns by using multichannel electronic micropipette (BIOHIT-e 1200)
- 2ul of purified PCR DNA was added to each column
- Plate was sealed with silicone foil
- Spin the plate quickly in plate centrifuge (Thermo Electron Corporation-IEC CL 30).
- 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

- Plates were spin quickly in plate centrifuge (Thermo Electron Corporation-IEC CL 30) following sequencing-PCR
- Plate racks were attached to each plate
- 5ml ethanol and 250µl sodium acetate (Gainland Chemical Co., UK) were added into a reservoir, 21µl of the mixture was added to all columns of 96-well plates by using multichannel electronic micropipette (BIOHIT-e 1200)
- Plate was sealed with silicon foil
- Plate was vortex (Lincolnshire, IL) and spin quickly
- Plate was placed in -20^0C for at least 1 hour
- Plate was spin at 4000 rpm for at least 1 hour (long spin)

Sequencing-using the (3100 Genetic Analyzer, USA)

HLA-DQB1 genotyping protocol had done according to Sequence-Based-Typing (SBT), which had been developed in National Microbiology Laboratories (NML), Winnipeg, Canada [18].

Statistical Analysis

For qualitative variables, frequency data were summarized as percentage. Statistical significant of differences 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, 51 (92.73%) were typed for HLA-DQB1 and 4 (7.27%) were not typed. Also results in (Table 1) showed that out of 15 patients with chronic gastritis, 14 (93.33) were typed for HLA-DQB1 and 1 (6.67%) was not typed.

Agarose Gel Electrophoresis After Amplification for DQB1 gene

For DQB1 amplification results, (Figure 1) showed the bands of amplified DQB1. As seen in this figure, DQB1 (DQB1=~300 base pairs) moves fast because shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel.

Genotype frequency of HLA-DQB1 in gastritis patients

Genotype frequencies of HLA-DQB1 alleles were studied in 65 gastritis patients and compared with 28 controls. Results shown in (Table 2), indicated that HLA-DQB1*060101 allele was present in 3 out of 65 gastritis patients and in 4 out of 28 controls, the frequencies of the allele were 4.61 and 14.29 respectively. The decreased allele frequency in gastritis patients was statistically not significant, but there was a strong association (χ^2 = 2.63, P =NS, OR= 3.44, 95% CI= 0.72-16.55) as compared with controls (Table 4.15).

Homozygosity of HLA-DQ in gastritis patients and controls

Studying HLA-DQB1 homozygosity indicated that out of 65 gastritis patients, 14 were homozygous in one or both loci and out of 28 controls, 8 were homozygous in one or both loci with frequencies of 21.54 and 28.57 respectively. No significant differences were observed in frequencies of homozygous HLA-DQB1 genotypes of gastritis patients and controls. (χ^2= 0.54, P=NS, OR= 0.69, 95% CI= 0.25-1.89) (Table 3).

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

Genotype frequencies of HLA-DQB1 alleles were studied in 51 acute gastritis patients and compared with 14 patients with chronic gastritis. Results showed in (Table 4), indicated that HLA-DQB1*050101 was present in 5 out of 51 acute gastritis patients and in 3 out of 14 chronic gastritis patients, frequencies of the allele were 9.80 and 21.43 respectively. The increased allele frequency in patients with chronic gastritis was statistically not significant, but showed very strong association (χ^2 = 1.38, P=NS, OR= 2.51, 95% CI= 0.52-12.12) as compared with acute gastritis patients.

Homozygosity of HLA-DQB1 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-DQB1, of 51 acute gastritis patients, 11 were homozygous in one or both loci and out of 14 patients with chronic gastritis, 3 were homozygous in one or both loci with frequencies of 21.57 and 21.43 respectively. No significant differences were observed in frequencies of homozygous HLA-DQB1 genotype between acute and chronic gastritis patients (χ^2 = 0.00, P=NS, OR= 0.99, 95% CI= 0.24-4.19) (Table 5).

No significant differences were observed in frequencies of homozygous HLA-(DQA1+-DQB1) genotype between acute and chronic gastritis patients (χ^2 = 0.14, P=NS, OR= 0.76, 95% CI= 0.18-3.25).

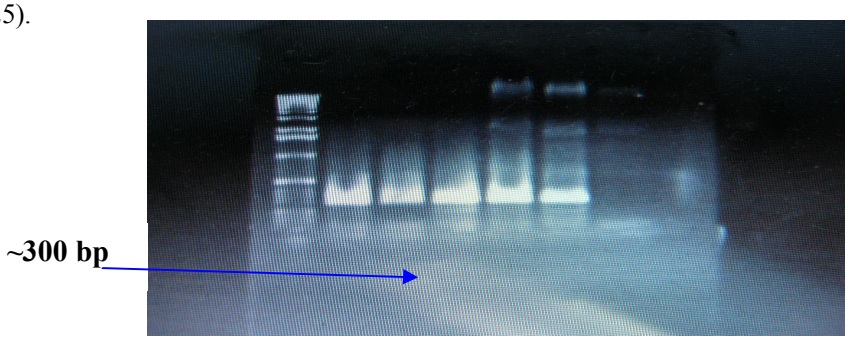

Figure 1. Bands of Amplified DNA (HLA-DQB1)(DQB1= ~300 base pairs)

Table 1: Frequencies of acute & chronic gastritis patients typed for DQB1 alleles

Type of gastritis	Typed for DQB1 alleles	Not typed	Total N= 70
	N (%) 65	N (%) 5	
Acute	51 (92.73)	4 (7.27)	55
Chronic	14 (93.33)	1 (6.67)	15

Table 2 HLA-DQB1 genotype frequency of Gastritis Patients and Controls

DQB1 allele	Gastritis Patients (n=65)		Controls (n=28)		χ^2	P	OR	95% CI
	No	%	No	%				
020101/0202/0204	33	50.76	13	46.42	0.15	NS	0.84	0.35-2.04
030101/030104/0309/0321/0322/0324/030302	25	38.46	12	42.85	0.16	NS	1.20	0.49-2.95
030201	10	15.38	2	7.14	1.18	NS	0.42	0.09-2.07
030302	3	4.61	2	7.14	0.01	NS	1.59	0.25-10.08
0402	5	7.69	2	7.14	0.01	NS	0.92	0.17-5.07
050101	8	12.31	4	14.29	0.07	NS	1.19	0.33-4.32
050201	10	15.38	4	14.29	0.02	NS	0.92	0.23-3.22
050301	1	1.53	0	0	0.44	NS	N/A	N/A
060101/060103	3	4.61	4	14.29	2.63	NS	3.44	0.72-16.55
060201	3	4.61	2	7.14	0.25	NS	1.59	0.25-10.08
060301/061401	6	9.23	2	7.14	0.11	NS	0.76	0.14-4.00
060401/0634	6	9.23	1	3.57	0.90	NS	0.36	0.04-3.18
060801	1	1.53	0	0	0.44	NS	N/A	N/A
0609	1	1.53	0	0	0.44	NS	N/A	N/A

Table 3 HLA-DQB1 genotype homozygosity in gastritis patients

HLA-DQB1 Homozygousity*		Cases			
		Patients		Controls	
		No	%	No	%
DQB1**	Homozygous	14	21.542	8	28.57
	heterozygous	51	78.46	20	71.43
	Total	65	100	28	100

*** Homozygous at one or both loci**
**** χ^2 = 0.54, P =NS, OR= 0.69, 95% CI= 0.25-1.89**

Table 4 HLA-DQB1 Genotype Frequency of Acute and Chronic Gastritis Patients

HLA-DQB1 allele	Acute gastritis		Chronic gastritis		χ^2	P	OR	95% CI
	N= 51	%	N= 14	%				
020101/0202/0204	25	49.02	8	57.14	0.29	NS	1.39	0.42-4.57
030101/030104/0309/0321/0322/0324/030302	18	35.29	7	50	1.00	NS	1.83	0.56-6.06
030201	9	17.65	1	7.14	0.93	NS	0.36	0.04-3.11
030302	1	1.96	1	7.14	1.25	NS	0.71	0.15-3.28
0402	4	7.84	1	7.14	0.01	NS	0.90	0.09-8.79
050101	5	9.80	3	21.43	1.38	NS	2.51	0.52-12.12
050201	9	17.65	1	7.14	0.93	NS	0.36	0.04-3.11
050301	1	1.96	0	0	0.28	NS	0.98	0.94-1.02
060101/060103	3	5.88	0	0	0.86	NS	0.94	0.88-1.01
060201	3	5.88	0	0	0.86	NS	0.94	0.88-1.01
060301/060401	4	7.84	2	14.29	0.54	NS	1.96	0.32-11.99
060401/0634	5	9.80	1	7.14	0.09	NS	0.71	0.08-6.61
060801	1	1.96	0	0	0.28	NS	0.98	0.94-1.02
0609	1	1.96	0	0	0.28	NS	0.98	0.94-1.02

Table 5 HLA-DQB1 Homozygosity in Patients with Acute and Chronic Gastritis

HLA-DQB1 Homozygosity*		Acute		Chronic	
		No	%	No	%
** DQB1	Homozygous	11	21.57	3	21.43
	Heterozygous	40	78.43	11	78.57
	Total	51	100	14	100

*** Homozygous at one or both loci**
**** χ^2 = 0.00, P=NS, OR= 0.99, 95% CI= 0.24-4.19**

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 HLA-DQB1 genotyping, Results shown in (Table 1) indicated that out of 55 patients with acute gastritis, 51 (92.73%) were typed for HLA-DQB1 and 4 (7.27%) were not typed. Also results in (Table 1) showed that out of 15 patients with chronic gastritis, 14 (93.33) were typed for HLA-DQB1 and 1 (6.67%) was not typed.

(Figure 1) showed the bands of amplified DQB1. As seen in these figures, DQB1 (DQB1=~300 base pairs) moves faster than DQA1 (DQA1=~726 base pairs) because shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel. Genotype frequencies of HLA-DQB1 alleles were studied in 65 gastritis patients and compared with 28 controls. Results shown in (Table 2), indicated that HLA-DQB1*060101 allele was present in 3 out of 65 gastritis patients and in 4 out of 28 controls, the frequencies of the allele were 4.61 and 14.29 respectively. The decreased allele frequency in gastritis patients was statistically not significant, but there was a strong association (χ^2 = 2.63, P =NS, OR= 3.44, 95% CI= 0.72-16.55) as compared with controls (Table 2). The most common DQB1 allele is DQB1*0301/09 in Jiangsu Han population, while in the present study HLA-DQB1*020101 was the most common allele, which is also the highest allele in many other groups in the world, for example, Naxi nationality [7], Northern Han [20], and Canadian [18] .

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- DQB1 genotypes of gastritis patients and controls. (χ^2= 0.54, P=NS, OR= 0.69, 95% CI= 0.25-1.89).

Genotype frequencies of HLA-DQB1 alleles were studied in 51 acute gastritis patients and compared with 14 patients with chronic gastritis. Results showed in (Table 4), indicated that HLA-DQB1*050101 was present in 5 out of 51 acute gastritis patients and in 3 out of 14 chronic gastritis patients, frequencies of the allele were 9.80 and 21.43 respectively. The increased allele frequency in patients with chronic gastritis was statistically not significant, but showed very strong association (χ^2 = 1.38, P=NS, OR= 2.51, 95% CI= 0.52-12.12) as compared with acute gastritis patients.

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. Almost all currently used SBT strategies for HLA-DQB1 typing employ amplification and/or sequencing primers located within exon 2 and exon 3. Complete exon 3 sequence information facilitates the resolution of allele ambiguities, for instance HLA-DQB1*030101 and HLA-DQB1*0319. Successful sequence-based 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^{o} 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 [18].

REFERENCES

[1] Conrad D F, Andrews T D, Carter N P, Hurles M E, and Pritchard J K, (2006). A high-resolution survey of deletion polymorphism in the human genome. Nat Genet; 38: 75-81.

[2] Gregersen J W, Kranc K R, Ke X, Svendsen P, Madsen I S, Thmsen A R, *et al,* (2006). Functional epistasis on a common MHC haplotype associated with multiple sclerosis. Nature; 443: 574-577.

[3] Nair R P, Stuart P E, Nistor I, Hiremagalore R, Chia N V, Jenisch S, *et al,* (2006). Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene. Am J Hum Genet; 78: 827-851.

[4] Shao W, Tang J, Dorak MT, Song W, *et al* (2004) Molecular typing of human leukocyte antigen and related polymorphisms following whole genome amplification. Tissue Antigens; 64: 286-292.

[5] Blomhoff A, Olsson M, Johansson S, Akselsen H E, Pociot F, *et al,* (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.

[6] Wang CX, Wang JF, Liu M, Zou X, Yu XP, Yang XJ, et al. (2006) Expression of HLA class I and II on peripheral blood lymphocytes in HBV infection. Chin Med J; 119: 753-756.

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

[8] Kumar PP, Bischof O, Purhey PK, Natani D, Urlaub H, Dejean A, *et al,* (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.

[9] Suerbaum S, Michetti P, (2002). *Helicobacter pylori* infection. N Engl J Med; 347(15): 1175-1186.

[10] Al- Janabi, A Ah, (1992). *Helicobacter*-associated gastritis, diagnosis and clinicopathological correlation. "A prospective study" MSc-Thesis. Submitted to College of Medicine. Al-Mustansirya University.

[11] Al-Baldawi M R, (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.

[12] Al-Dhaher Z AJ, (2001). Study of some bacteriological and immunological aspects of *Helicobacter pylori*. MSc-Thesis Submitted to College of Science. Al-Mustansirya University.

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

[14] Torres J, Lopez L, Lazcano E, Camorlinga M, Flores L, Munoz O, (2005).Trends in *Helicobacter pylori* infection and gastric cancer in Mexico. Cancer Epidemiol Biomarkers Prev; 14: 1874-1877

[15] Azem J, Svennerholm AM, and Lundin BS, (2006). B cells pulsed with *Helicobacter pylori* antigen efficiently activate memory $CD8^{+}$ T cells from *H. pylori* infected individuals. Clin Immunol; 158: 962-967.

[16] Yoshikawa T and Naito Y, (2000). The role of neutrophils and inflammation in gastric mucosal injury. Free Radic Res; 33: 785-794

[17] Beutler E, Gelbart T, and Kuhl W, (1990). Interference of heparin with the polymerase chain reaction. BioTechniques; 9: 166.

[18] Luo M, Blanchard J, Pan Y, and 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.

[19] Brody, J.R., Kern, S.E. (2004). History and principles of conductive media for standard DNA electrophoresis. Anal Biochem; 333(1):1-13.

[20] Zhou L, Lin B, Xie Y, Liu Z, Yan W, Xu A. (2005) Polymorphism of human leukocyte antigen-DRB1, -DQB1, and -DPB1 genes of Shandong Han population in China. Tissue Antigens; 66: 37-43.