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EVALUATION OF HLA-DQA1 AND HLA-DQB1 ASSOCIATED WITH *HELICOBACTER PYLORI* INFECTION IN PATIENTS WITH ACTIVE GASTRITIS

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Abstract

Helicobacter pylori is one of the most common pathogens in the world. It has been suggested that most bacterial infectious disease related to MHC class 1 or 2 according to bacterial infection type (intracellular and extracellular). Many previous studies reported that HLADQA1 and HLADQB1 which is part of MHC 2 related to *H. pylori* infection. This study was performed in Babylon university, collage of science , department of biology, It is include 48 patients and 20 controls . Samples was include blood and biopsy from each patient and healthy that was collected in ALimam ALsadiq Hospital in Babylon city from January to April 2019. The aim of study was to evaluate quantitative HLADQA1 and HLADQB1 in *H. pylori* infected patients by ELIZA method and compare with healthy persons and also relationship of these parameters with age, gender and infection types. The results was show significant increase of local and systemic of both HLADQA1 and HLADQB1 in patients compared with control, HLADQB1 was increase more than HLADQA1 in blood and tissue. No significant differences between age groups of both parameter , while the systemic HLADQA1 was significantly increase in male compared with female only. Duodenal ulcer was show relative increase of local and systemic of both HLADQA1 and HLADQB1 without any significant differences between types of infections.

Key words: Environment; patients; HLA-DQA1

Introduction

Helicobacter pylori (*H. pylori*) is spiral or comma-shaped Gram-negative bacilli that exhibits host and tissue tropism and can be colonized in the gastric antrum and cardiac also in all gastric regions including corpus and duodenum, where gastric cell metaplasia is observed, in humans and in some primates. Human leukocyte antigens (HLA) are an inherent system of alloantigens, which are the products of genes of the major histocompatibility complex (MHC). A genomic region well- known for its high density of genes, complex linkage of equilibrium. These genes span a region of approximately 4 Mega bases 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). Its encode peptides involved in host immune response and also they are important in tissue transplantation and are associated with a variety of infectious, autoimmune and inflammatory diseases (Gregersen *et al*., 2006) As well as, 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).

Expression of particular HLA alleles may be associated with the susceptibility or resistance to some diseases , HLAs which are expressed on the human cell surface play important role in regulate the immune response, Heterozygosity within the MHC genomic region provides the immune system with a selective advantage of pathogens (Wang *et al*., 2006).

The studies continuing for years on the relation between HLA and dis-eases are rapidly increasing today. For this reason, the importance of conducting HLA studies in different populations for the same disease is obvious. There are numerous publications demonstrat-ing the relation between HLA antigens and diseases. Such type of studies are conducted to determine the risks and preventive factors for the diseases, to plan treatment, to understand whether they are genetically transmitted, and

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consequently to reach a molecular pathogenesis (Hollenbach *et al*., 2012).

HLA-DQ is α and β heterodimer of the MHC class II type. The α-and β-chains are encoded by HLA-DQA1 and HLA-DQB1, respectively. These two loci are adjacent to each other on chromosome 6P21.3. Both the α- and β-chains vary greatly. The DQ loci are in close genetic linkage to HLA-DR but less closely linked to HLA-DP, HLA-A, HLA-B and HLA-C serological (Klitz *et al* ., 2003).

Materials and Methods

Protocol of Biopsy DNA Extraction

DNA extraction was performed according to the manufacture instruction (FAVORGEN) as in the following:

1- Approximately 25 mg of tissue samples was grinded with a specialized plastic slick and then the homogenized tissue was transferred to a new eppendorf tube.

2- Homogenized tissue was mixed with 200 µl of FATIG1.

3- A 40 µl of proteinase k (10mg/ml) was added and mix thoroughly by vortex and incubated at 60°C for two hours or until the tissue is lysed completely.

4- The lysate was briefly span at 15000 rpm to remove bubbled from inside the lid.

5- A 200 µl of FATG2 buffer was added to the samples mixture, mix well and incubated at 70°C for 10 min , then the mix was span at 15000 rpm to remove bubbled from inside the lid.

6- A 200 µl of absolute ethanol was added to the samples and mix thoroughly by vortex.

7- FATG mini column was placed in a collection tube, then the mixtuue was transferred to and centrifuged at 15000 rpm for 1 minute.

8- FATG min column was washed with 500 µl W1 buffer by centrifuge at 15000 rpm for 1 minute then discard the flow- thorough , the column was washed again 750 µl wash buffer and centrifuged.

9- FATG mini column was placed in a new epenorf tube and eluted with 100 µl of elution buffer that added immediately in the center of membrane of FATG mini column and leave stand for 3 minutes.

10- After that it was centrifuged at 15000 rpm for 2 minutes to elute DNA that was stored at -20°C until be used.

Table 1: Sequence of primers.

Reference	Product size (bp)	Sequence		Target Gene
Luo *et al*.,1999	726	ATCTTCACTCAGCTGACCA	F	DQA1
		GCTGACCCAGTGTCACGGGAG	R	
Luo *et al*.,1999	300	TCCCCGCAGGATTTCGTG	F	DQB1
		GGCGACGACGCTCACCTC	R	

Table 2: Content of PCR Reaction Mixture.

Volume	Content of PCR Reaction Mixture
12.5 µl	Master mix
2 µl	Forward primer
2 µl	Reverse primer
5 µl	Template DNA
3.5 µl	Nuclease free water
25 µl	Total volume

Polymerase Chain Reaction

All primers was used in this study was illustrated in table 1.

Primer pairs preparation

All primer pairs used in this study were dissolved using nuclease free water, firstly the primer stock prepared as 100 pmol and then the working primer would prepared from primer stock tube. According to the instruction provided by manufacture (Bioneer/Korea) nuclease free water was added to get 100 Pmol/µl as a stock solution. And then making dilution to get 10 Pmol/µl as working solution.

The PCR Mixture

Amplification of DNA was carried out in a final volume of 25 µl reaction mixture as illustrated in Table 2.

Thermal Cycles Condition

Conventional PCR was used to amplify the target DNA using specific primers. It include three consecutive steps that repeated for specific number of cycles to get amplified PCR product , all thermal cycling condition was listed in table 3.

PCR Product Analysis

Agarose gel electrophoresis to analysis the PCR products was performed according to the (Gambrook *et al*., 1989) as in the following steps:

1- A 1.5% agarose gel was prepared by using

Table 3: Thermal Cycling Conditions.

No of cycles	Final extension	Extension	Annealing	Denaturation	Initial denaturation	Genes
44	72C° / 10min	72C° / 1min	53C° / 1min	96C° / 1min	96C° / 5 min	DQA1
44	72C° / 10min	72C° / 1min	57C° / 1min	96C° / 1 min	96C° / 5 min	DQB1

1x TBE buffer and dissolving in Macrowave, after that left to cool to 50°C.

2- A 5 µl of red safe stain was added in to agarose gel solution and mix well.

3- Agarose gel solution was poured in to the tray after fixing the comb in the proper position, then left to solidify at room temperature.

4- The comb was remove gently from the tray and then PCR product was loaded in the comb well and also 10 µl of DND marker (100 bp ladder) was loaded in first lane.

5- The gel tray was fixed in the electrophoresis chamber and filled by 0.5X TBE buffer and electric was performed at 100 volts and 70 AM for one hour.

6- After finished of electrophoresis, PCR product was visualization by using UV transilluminator.

Quantitative ELIZA of Human Leukocyte Antigen (HLA-DQ Alpha 1 and HLA-DQ Beta 1) Assay Procedure

1- The following procedure was performed at room temperature according to the manufactured instructions (Bioassay Technology Laboratory).

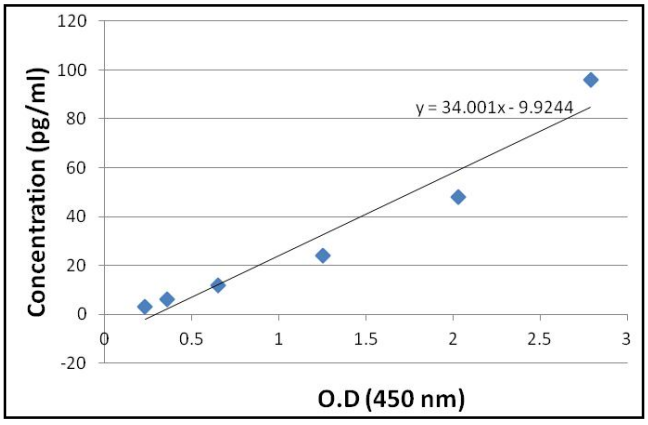

Fig. 1: Standard Curve of HLA-DQA1.

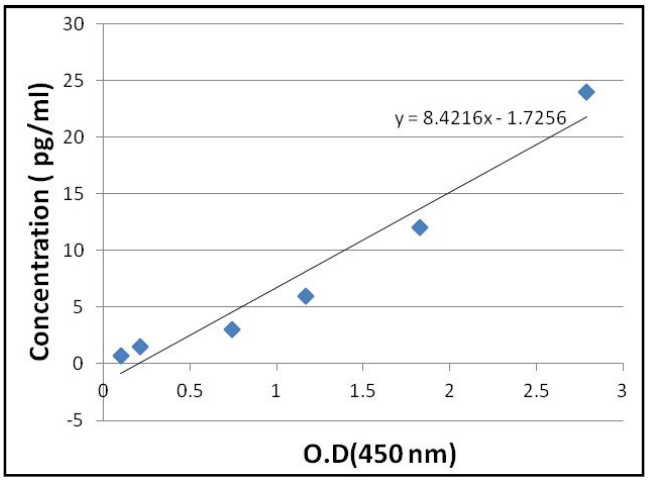

Fig. 2: Standard Curve of HLA-DQB1.

2- Standard solution preparation: Serial dilution of standard solution were prepared by adding 120 µl of standard diluent to 120 µl original standard and then allow the standard to sit for 15 min with gentle agitation prior to making next dilution.

2- Standard solution load: The volume 50µl of standard was added to standard well without adding antibody because the standard was previously containing antibody.

3- Samples load: The volume of 40 µl of each samples were added to each well and then 10 µl of biotinylated antibody was added to each well. In case of biopsy samples, a known weight (0.05 gm) of tissue was taken then minced into small pieces and rinsed in ice- cold PBS (0.01 M, pH = 7.4) to remove excess blood thoroughly, then suspended in PBS (0.5 ml) the ratio was (1:9 W/V) then gently homogenized with a glass homogenizer on ice then centrifuged for 5 min at 5000 rpm to get the supernatant.

4- HRP Conjugate: The volume of 50 µl of HRP conjugate working solution was added to each well . The plate was covered with plate sealer and incubated for 60 minutes at 37°C.

5- Wash: Each well was aspirated and repeating the process three times, then wash by filling each well

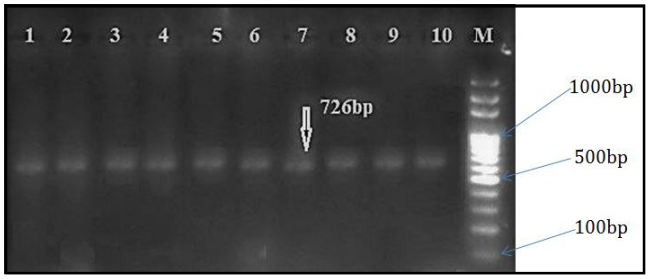

Fig. 3: Gel electrophoresis of PCR product of HLA-DQA1 gene 300 bp. 1.5% Agarose gel at 60 volt for 1 hour. Lane 1-10 : positive for HLA-DQA1 gene, M: 100-bp DNA ladder.

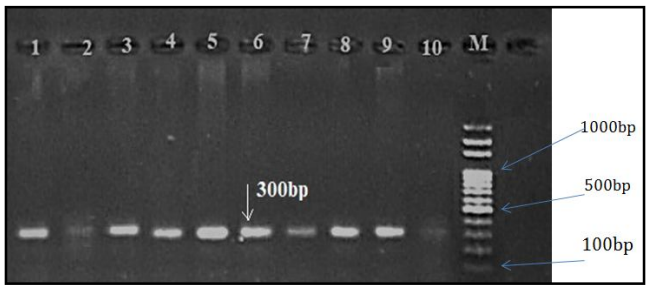

Fig. 4: Gel electrophoresis of PCR product of HLA-DQB1 gene 300 bp. 1.5% Agarose gel at 60 volt for 1 hour. Lane 1-10 : positive for HLA-DQB1 gene, M: 100-bp DNA ladder.

by wash buffer. Complete removal of liquid at each step is essential. After the last wash, removed remained wash buffer by aspirating or decanting. Invert the plate and pat it against thick clear absorbent paper.

6- Substrate: The volume of 50 µl of substrate solution A and 50 µl of substrate solution B was added to each well. The plate was covered with new plate sealer and incubated for 10 minutes at 37°C.

7- Stop solution: The volume of 50µlof stop solution was added to each well. Then the color turns to yellow immediately. The order of added stop solution should be the same as the substrate solution.

8- Measurement of Optical Density: The optical density (O.D value) of each well was read at once. Using the micro-plate reader set at 450 nm. User should be opened the micro-plate reader in advance, preheat the instrument and set the testing parameters, as show in Figures (1 and 2 respectively).

Results and Discussion

Agarose Gel Electrophoresis Results After Amplification for DQA1 and DQB1 gene

As show in Fig. 3 the amplified DNA products for DQA1 and Fig. 4 showed the bands of amplified products of DQB1. A total of 35 out of 48 biopsy were given positive result for DQB1gene, by using DQB1specific primers with products size 300bp, while only 31 out of 48 biopsy were given positive result for DQA1gene, by using DQB1specific primers with products size 300bp. As seen in these Figures, DQB1 moves faster than DQA1 this may be because shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel.

Quantitative ELIZA Test of HLA- DQA1 and HLA-DQB1 in Patients and Control

The quantitative result of ELIZA of HLA- DQA1 and HLA-DQB1 were show a significant increase in HLA- DQA1 and HLA-DQB1 of patients compared with control in both samples (blood and tissue), HLADQA1 = 1.85±0.70 versus control 0.93 ± 0.35 in blood samples, (P. value = 0.00) and 4.59 ± 1.33 versus control 2.52 ± 0.97 in tissue samples, P. value = 0.00), while HLA-DQB1 was 2.52 ± 1.11 versus contro l 1.99 ± 0.73 in blood samples (P. value = 0.00) and 7.86 ± 1.86 versus control 3.69 ± 1.66 in tissue samples (P. value = 0.00). Also there was increase in local of both HLA compared with systemic concentration, as show in Table 4.

Table 4: Concentration of HLA- DQA1 and HLA-DQB1 ELIZA (ng/ml) in blood and tissue of patients compared with control.

P. value	Controls	Patients	Parameters
	M±SD		
0.00	0.93±0.35	1.85±0.70	HLA-DQA1 Blood
0.00	2.52±0.97	4.59±1.33	HLA-DQA1 Tissue
0.00	1.99±0.73	2.52±1.11	HLA-DQB1 Blood
0.00	3.69±1.66	7.86±1.86	HLA-DQB1Tissue

Quantitative ELIZA test of HLA- DQA1 and HLA-DQB1 in patients compared with control according to the age groups:

As show in Table 5 the results was recorded a significant increase in HLA- DQA1 and HLA-DQB1 of patients compared with controls in all age groups and in both samples (tissue and blood) for all parameters. Also there was increase in concentration of local of both HLA compared with systemic once. However no significant differences between age groups for each parameter , moreover the local concentration of HLA- DQA1 and HLA-DQB1 was relatively higher than systemic and there was significant increase in HLA-DQB1 compared with HLA- DQA1.

Table 5: Concentration of HLA- DQA1 and HLA-DQB1 ELIZA (ng/ml) in blood and tissue of patients compared with control according to the age groups.

P. value between groups	Patients M±SD			Parameters	
	> 60 years	40-59 years	< 39 years		
0.33	2.09±0.98	1.74±0.69	1.74±0.57	Patients	HLA-DQA1 Blood
	1.11±0.57	1.05±0.26	0.76±0.26	Control	
	0.01	0.02	0.00	P. value	
0.59	4.10±1.55	4.61±1.13	4.70±1.42	Patients	HLA-DQA1 Tissue
	2.41±0.87	2.56±1.12	2.58±1.05	Control	
	0.03	0.00	0.00	P. value	
0.44	3.50±0.70	4.09±1.02	3.53±1.31	Patients	HLA-DQB1 Blood
	2.11±0.66	2.24±1.01	1.68±0.45	Control	
	0.02	0.00	0.00	P. value	
0.38	6.84±0.52	8.59±2.05	7.76±2.02	Patients	HLA-DQB1Tissue
	2.97±1.32	3.24±1.68	4.29±1.79	Control	
	0.00	0.00	0.00	P. value	

Quantitative ELIZA Test of HLA- DQA1 and HLA-DQB1 in patients and control according to the gender

As show in Table 6 the results was reported a significant increase of HLA- DQA1 and HLA-DQB1 of patients compared with

controls in both sex and samples (blood and tissue), also its was significantly increased in tissue samples compared with blood samples for each of HLA- DQA1 and HLA-DQB1, moreover the HLA-DQB1 have higher relationship with *H. pylori* infection than HLA- DQA1 especially in biopsy samples.

Quantitative ELIZA Test of HLA- DQA1 and HLA-DQB1 of Patients According to the Infection Types

As show in Table 7 the results was do not reported any significant differences in HLA- DQA1 and HLA-DQB1 of patients between groups of infection types. HLA-DQB1 was significantly raised in duodenal ulcer followed by gastric ulcer. It is important to note that all parameters of HLA was reach to maximum level in duodenal ulcer infection except of systemic HLADQB1.

It has been suggested that the incidence of digestive diseases associated with *Helicobacter pylori* is influenced by strain diversity of *H. pylori*, factors involving the host or environment and the duration of infection. The result of quantitative ELIZA test to HLA- DQA1 and HLA-DQB1 was show significant association between gene expression of DQA1 and DQB1with *H. pylori* infection in both sex and all age groups, the expression of these genes was relatively higher in duodenal ulcer than other types of *H. pylori* infection. Moreover , gene expression of HLA-DQB1 was relatively higher than HLA-DQA1 in all types of infection especially in tissue samples. Its suggestive that increase level of HLA- DQ especially DQB1 may be useful marker to *H. pylori* infection.

Human major histocompatibility complex (MHC) class II molecules, such as HLA-DP, HLA-DQ and HLA DR are α–β heterodimeric membrane glycoproteins that are expressed on the surface of antigen-presenting cells such as macrophages, dendritic cells and B lymphocytes (Kaufman *et al.*, 1984). Helper T cells can only recognize peptides, derived from extracellular antigens, that are associated with HLA class II molecules. The interaction of T cell receptors, peptides and HLA class II molecules determines T cell activation and an immune response to antigens play a key role in immune reactions to pathogens, Watanabe *et al*., (2006), was reported that human leukocyte antigen (HLA)-DQB1*0401 plays an important role in the development of atrophic gastritis in *H. pylori* infected patients. The HLA-D region accounts for over 50% of the heritability in hosts and appears responsible for variation in the immune response of different individuals to various exogenous antigens, suggesting that there are varieties in the host's response to the same organism, and individuals with various HLA types differ in their immune response. Allele-specific antigenic peptides to T-cells may contribute to the differences between HLADQ genotypes and susceptibility or resistance to *H. pylori* infection (Herrera-Goepfere *et al*., 2006).

Because there is no previously study on quantitative ELIZA of HLA associated with *H. pylori* infection we compare our study with genotyping study of HLA associated with this bacteria. This results was agreement with Al-Ammar, (2010) that was reported that, out of 70 whole DNA samples, 62 (88.57%) were genotyped for HLA-DQA1 alleles and 65 (92.86%) were genotyped for HLA-DQB1 alleles. For controls group, out of 30 whole DNA samples, 25 (83.33%) were genotyped for HLA-DQA1 alleles and 28 (93.33%) were genotyped for HLA-DQB1 alleles. Also this result was homologous to Al-Ammar, (2010) according to infection types, he was find that 89% and 92.7% of acute *H. pylori* gastritis were typed for HLA-DQA1 and HLA-DQB1 respectively, while 86.6% and 93.3% of chronic *H. pylori* gastritis were typed for HLA-DQA1 and HLA-DQB1

Table 6: Concentration of HLA- DQA1 and HLA-DQB1 ELIZA (ng/ml) in blood and tissue of patients compared with control according to the gender.

P. value between groups	Patients M ± SD		Parameters	
	Female	Male		
0.01	1.52±0.51	2.09±0.77	Patients	HLA-DQA1 Blood
	0.93±0.37	0.95±0.37	Control	
	0.01	0.00	P. value	
0.18	4.07±1.30	4.95±1.31	Patients	HLA-DQA1 Tissue
	2.55±1.01	2.52±1.02	Control	
	0.00	0.00	P. value	
0.92	3.71±1.14	3.68±1.13	Patients	HLA-DQB1 Blood
	1.76±0.50	2.22±0.93	Control	
	0.00	0.00	P. value	
0.73	7.71±1.83	7.89±1.96	Patients	HLA-DQB1Tissue
	3.48±1.54	3.79±1.91	Control	
	0.00	0.00	P. value	

Table 7: Concentration of HLA- DQA1 and HLA-DQB1 ELIZA (ng/ml) in blood and tissue of patients compared with control according to the infection types.

P. value	Gastric ulcer	Duodenal ulcer	Chronic gastritis	Acute gastritis	Parameters
	M±SD				
0.61	1.82±0.46	2.04±1.00	1.58±0.61	1.92±0.76	HLA-DQA1 Blood
0.53	4.07±0.75	5.20±0.59	4.42±1.04	4.73±1.57	HLA-DQA1 Tissue
0.83	3.83±0.65	3.19±0.39	3.63±1.13	3.78±1.27	HLA-DQB1 Blood
0.77	8.19±1.68	8.77±1.75	7.78±1.71	7.71±2.01	HLADQB1 Tissue

respectively.

Other previous studies such as Azuma *et al*., (1994) reported that DQA1*0102 might contribute to resistance against *H. pylori* associated gastric atrophy and its association with intestinal type gastric adenocarcinoma. Magnusson *et al*., (2001) reported that DRB1*1601 was associated with gastric adenocarcinoma. Lee *et al*., (1996) reported that DQB1*0301 was positively associated with gastric adenocarcinoma.

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