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Role of Human Leukocyte Antigens HLA-A in Gastroesophageal Reflux Disease Liability

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Abstract:

Background:
Gastro-oesophageal reflux disease had multi factorial causes both genetic and environmental. One of the genetic causes is HLA alleles. Human Leukocyte Antigens considered as a good marker for population genetic analyses and disease association. **Aim of the study:** This study aimed to investigate the association between human leucocyte antigen genes HLA-A that inherited from both parents and its role in Gastro-oesophageal reflux disease**.**

Methods:
Forty Iraqi Arab Muslims patients with a history of heartburn, epigastric pain and dyspepsia were recruited from Gastrocolonoscope Unit at Al-Kindy Teaching Hospital (Baghdad-Iraq) between January 2014 and July 2016. They were compared with 100 Iraqi Arab Muslims controls. Upper gastroesophageal endoscopic examinations was done for them and HLA-A genotyping using sequence specific oligoneucleotide primer (SOPP).

Results:
The frequencies of HLA-A*11:01 was significant higher in control group(Odd ratio=0.0812, 95% confidence interval was 0.0106-0.6228, P – value =0.0157) compared with patients with Gastro-oesophageal reflux disease which had a protective effect against the development of this disease. Other HLA-A alleles showed no significant difference between two groups

Conclusions:
This study identified HLA-A*11:01 had a protective role against Gastro-oesophageal reflux disease development.

Key words:GERD, HLA, genetic.

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a common complex gastrointestinal problem characterized by symptoms and or complications associated with reflux of gastric contents to the lower part of the esophagus (1). It had a wide spectrum of clinical presentations like heartburn and regurgitation which are the most common symptoms and it is diagnosed by endoscopy and Gastroesophageal Reflux Disease Questionnaire (2). It was treated by antiacids, proton pump inhibitor (PPI) and micro nutrients (3,4,5). The causes of this disease is multifactorial like central obesity rather than body mass index which is more associated with GERD and its complications like erosive esophagitis, Barrett esophagus and esophageal adenocarcinoma (6). Hiatal hernias is other important cause in many patients (7).

Other important factor is genetic factors that are important in causation and determination the severity of GERD symptoms (8). The role of this factor is confirmed by familial predisposition to GERD symptoms(9). One of the genetic factors is human Leukocyte antigens (HLA) which is highly polymorphic alleles that predispose to different diseases and malignancies and had an important role in immune response regulation (10). For example HLA-B*07 had a relation with Barrett's esophagus (11).This study try to shed a light on association between GERD and HLA-A (Class I MHC molecule) in a sample of Iraqi Arab patients.

PATIENTS AND METHODS

Forty Iraqi Arab Patients were included in this study who had a history of heartburn and dyspepsia at least three times a week for a period of more than 3 months diagnosed by gastroscope as GERD (12) (GIF-H260; Olympus, Tokyo, Japan and Display screen; Olympus OEV-261H liquid crystal display monitor; Olympus, Tokyo, Japan) were recruited from Endoscope center at Al-Kindy Teaching Hospital (Baghdad-Iraq) between January 2014 and July 2016. Other group was one hundred healthy individuals as control from volunteer donors for kidney transplantation in Al-Karamah Teaching Hospital. Written informed consent was obtained from all patients and control group for this study. The study protocol was reviewed and approved by the Scientific and Ethical Committee of Al-kindy medical college and Al-Kindy Teaching Hospital. The exclusion criteria were patients with Barrett's esophagus, esophageal varices, patients on antacids, H2 blockers, proton pump inhibitors (PPIs), non-steroidal anti-inflammatory drugs (NSAIDs), alcohol, *Helicobacter pylori* infection, gastrointestinal surgery, peptic ulcer, and gastric cancer.

HLA Class I genotyping (HLA-A) : It was done in Al-Karamah Teaching Hospital Labroatary. Two mL of venous blood were collected in EDTA tubes for DNA extraction from human blood using blood kit (QIAmp DNA blood Mini Kit, QIAGEN INC- Germany). DNA concentration and purification product was estimated using Nanodrop –South Korea. DNA was verified by electrophoresis in a 2% agarose gel containing ethidium bromide and was visualized under UV light. Locus- and allele-specific amplification of genomic patients and control DNA was performed for HLA-A. DNA Amplification and Hybridization was performed using a sequence-

specific oligonucleotide probes (SSOP) by HLA-A amplification and hybridization kits (SSO HLA type A plus and Mastermix for HLA type A Amp plus kits - Innogenetics-Belgium) by AutoLipa - Innogenetics-Belgum. The results were interpreted using LiRas version-5.0 software- Innogenetics-Belgium.

Statistical analysis: HLA-DRB1 frequencies were determined by direct counting. The frequency of each allele was compared between patients and control group using chi-square test Fisher exact test using MiniTab version. 3.0 software. In each comparison, the Odds ratio (OR) along with the 95% confidence interval (95% CI) was used.

RESULTS:

A total of 40 patients with GERD (Grade II and III) (Fig-1-) were evaluated, together with 100 controls. The mean age of patients was 34.17±3.4, as compared with 33.1±1.2 for the controls. The male to female sex ratio was 1.0 in the patients and control group (table-1-). The frequencies of HLA-A*11:01 was significant higher in control group(Odd ratio=0.0812, 95% confidence interval was 0.0106-0.6228, P – value =0.015) compared with patients with Gastro-oesophageal reflux disease . Thus , this allele had a protective effect against the development of this disease. Other HLA-A alleles like (A1, A2, A3, A23, A24, A25, A26, A 28, A29, A30, A31, A32, A33, A34, A66, A68, A69 and A74.)showed no significant difference between two groups (Table-2-).

The most common gene frequency in GERD patients (HLA-A*02:01) which is 0.06 as shown in table-3- while in the control group was HLA-A*11:01 which is 0.06 (Table-4-). The results were in agreement with Hardy – Weinberg equilibrium as demonstrated in table-5-.

Table-1-Demogtaphic data of GERD patients and control group.

Parameters	Patients with GERD No.=40	Control No.=100	P-value
Age (years)(X±SEM)	34.17±3.4	33.1±1.2	0.709
Sex(Male/Female)	20/20 (50%)	50/50 (50%)	1.00
Smoking(X±SEM)	36.21±1.5	15.4±3.7	0.000

TABLE -2- Frequencies of HLA-A alleles in patients with GERD disease compared with control group.

HLA-A	**Patients with GERD No.=40**		**Control No.=100**		**Odd ratio 95% confidence interval**	**P-value**
	No.	**%**	**No.**	**%**		
01:01	8	10	10	5	2.25 0.81-6.19	0.11
01:02	3	3.75	5	2.5	1.54 0.35-6.77	0.56
01:28	2	2.5	4	2	1.26 0.22-7.18	0.79
01:30	2	2.5	5	2.5	1 0.18-5.37	1.00
02:01	10	15	15	7.5	1.88 0.76-4.65	0.16
02:11	6	7.5	6	3	2.76 0.83-9.15	0.09
02:10	3	3.75	3	1.5	2.62 0.50-13.57	0.25
02:44	2	2.5	0	0	NA	NA
02:78	1	1.25	0	0	NA	NA
02:128	1	1.25	0	0	NA	NA
03:01	3	3.75	5	2.5	1.54 0.35-6.77	0.56
03:02	0	0	14	7	NA	NA
03:31	2	2.5	3	1.5	1.7 0.27-10.58	0.56
11:01	1	1.25	24	12	0.0812 0.0106-0.6228	0.015
23:01	4	5	4	2	2.66 0.63-11.23	0.18
24:01	0	0	4	2	NA	NA
24:02	8	10	10	5	2.25 0.81-6.19	0.11
25:01	0	0	6	3	NA	NA

HLA-A	Patients with GERD No.=40		Control No.=100		Odd ratio 95% confidence interval	P-value
	No.	%	No.	%		
26:01	8	10	9	4.5	2.52 0.89-7.10	0.07
26:25	2	2.5	3	1.5	1.7 0.27-10.58	0.56
28:01	0	0	8	4	NA	NA
29:01	0	0	6	3	NA	NA
30:01	0	0	8	4	NA	NA
30:02	3	3.75	5	2.5	1.54 0.35-6.77	0.56
30:16	1	1.25	3	1.5	0.82 0.08-8.21	0.87
31:01	3	3.75	5	2.5	1.54 0.35-6.77	0.56
31:02	0	0	7	3.5	NA	NA
31:54	1	1.25	2	1	1.25 0.11-14.25	0.85
32:01	2	2.5	4	2	1.26 0.22-7.18	0.79
33:01	3	3.75	2	1	3.97 0.63-24.73	0.13
34:01	0	0	4	2	NA	NA
66:01	0	0	4	2	NA	NA
68:01	1	1.25	4	2	0.61 0.06-5.68	0.66
69:01	0	0	4	2	NA	NA
74:01	0	0	4	2	NA	NA

NA=not applicable.

TABLE -3- Observed and expected frequencies of HLA-A alleles in patients with GERD with gene frequency.

HLA-A	Observed frequencies of Patients with GERD No.=40		Expected frequencies of Patients with GERD No.=40		Gene frequency
	No.	%	No.	%	
01:01	8	10	7.8	9.75	0.05
01:02	3	3.75	3.16	3.96	0.02
01:28	2	2.5	1.59	1.99	0.01
01:30	2	2.5	1.59	1.99	0.01
02:01	10	15	9.31	11.64	0.06
02:11	6	7.5	6.27	7.84	0.04
02:10	3	3.75	3.16	3.96	0.02
02:44	2	2.5	1.59	1.99	0.01
02:78	1	1.25	1.59	1.99	0.01
02:128	1	1.25	1.59	1.99	0.01
03:01	3	3.75	3.16	3.96	0.02
03:02	0	0	0	0	0
03:31	2	2.5	1.59	1.99	0.01
11:01	1	1.25	1.59	1.99	0.01
23:01	4	5	4.72	5.91	0.03
24:01	0	0	0	0	0
24:02	8	10	7.8	9.75	0.05
25:01	0	0	0	0	0
26:01	8	10	7.8	9.75	0.05
26:25	2	2.5	1.59	1.99	0.01
28:01	0	0	0	0	0

29:01	0	0	0	0	0
30:01	0	0	0	0	0
30:02	3	3.75	3.16	3.96	0.02
30:16	1	1.25	1.59	1.99	0.01
31:01	3	3.75	3.16	3.96	0.02
31:02	0	0	0	0	0
31:54	1	1.25	1.59	1.99	0.01
32:01	2	2.5	1.59	1.99	0.01
33:01	3	3.75	3.16	3.96	0.02
34:01	0	0	0	0	0
66:01	0	0	0	0	0
68:01	1	1.25	1.59	1.99	0.01
69:01	0	0	0	0	0
74:01	0	0	0	0	0

TABLE -4- Observed and expected frequencies of HLA-A alleles in healthy control group with gene frequency.

HLA-A	Observed frequencies Of Control No.=100		Expected frequencies of control No.=100		Gene frequency
	No.	%	No.	%	
01:01	10	5	7.92	3.96	0.02
01:02	5	2.5	3.98	1.99	0.01
01:28	4	2	3.98	1.99	0.01
01:30	5	2.5	3.98	1.99	0.01
02:01	15	7.5	15.68	7.84	0.04
02:11	6	3	3.98	1.99	0.01
02:10	3	1.5	3.98	1.99	0.01
02:44	0	0	0	0	0
02:78	0	0	0	0	0
02:128	0	0	0	0	0
03:01	5	2.5	3.98	1.99	0.01
03:02	14	7	11.82	5.91	0.03
03:31	3	1.5	3.98	1.99	0.01
11:01	24	12	23.28	11.64	0.06
23:01	4	2	3.98	1.99	0.01
24:01	4	2	3.98	1.99	0.01
24:02	10	5	7.92	3.96	0.02
25:01	6	3	3.98	1.99	0.01
26:01	9	4.5	7.92	3.96	0.02
26:25	3	1.5	3.98	1.99	0.01
28:01	8	4	7.92	3.96	0.02
29:01	6	3	3.98	1.99	0.01
30:01	8	4	7.92	3.96	0.02
30:02	5	2.5	3.98	1.99	0.01
30:16	3	1.5	3.98	1.99	0.01
31:01	5	2.5	3.98	1.99	0.01
31:02	7	3.5	7.92	3.96	0.02
31:54	2	1	1.99	0.99	0.005
32:01	4	2	3.98	1.99	0.01
33:01	2	1	1.99	0.99	0.005
34:01	4	2	3.98	1.99	0.01
66:01	4	2	3.98	1.99	0.01
68:01	4	2	3.98	1.99	0.01
69:01	4	2	3.98	1.99	0.01
74:01	4	2	3.98	1.99	0.01

Table-5-Hardy –Weinberg equilibrium in HLA-A loci of GERD patients and control group.

Studied groups	Locus	X^2	Degree of freedom	p- value
GERD patients	HLA-A	2.17	79	NS
Control	HLA-A	7.09	199	NS

NS= Not significant.

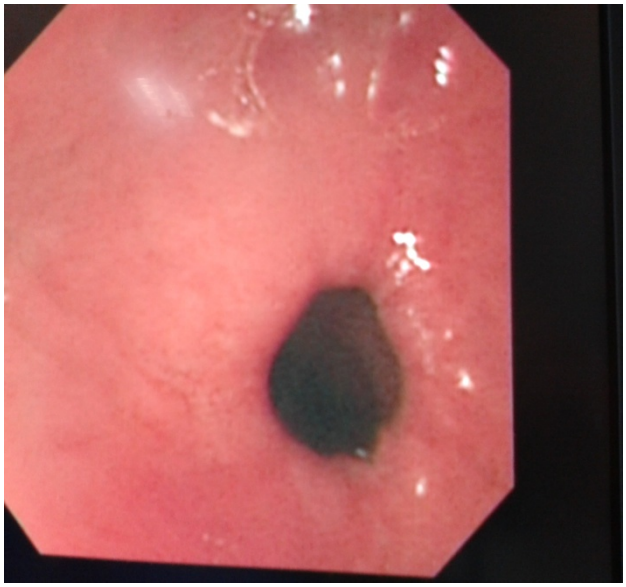

Figure -1- Patient with GERD by Endoscopy.

DISCUSSION:

GERD is a disorder in the motility of the gastrointestinal tract that leads to different symptoms and complications like erosive esophagitis, esophageal strictures, and Barrett esophagus (13).One of the causes of GERD disease in addition to abdominal obesity, age, sex is genetic factor like association of HLA alleles with this disease(14). In this study we cannot demonstrate any association between the HLA-A class I and this disease but it showed that HLA-A*11:01 was a protective against development of this disease. Other study showed that HLA-DRB1* 15:01 was significantly associated with GERD patients and HLA-DRB1*11:01 had a protective role against this disease (15). There is an association of pathophysiology of Barrett's esophagus development with allele C in FOXF1 rs9936833 and allele A in MHC rs9257809 (16). It had been found that Caucasian genetic of Indians origin are more prone to GERD than an Oriental one and the HLA-B07 gene commonly found in South Asian and Caucasian populations, but not Orientals, this may confer an increased risk for Barrett esophagus (BO) . This genetic factor with the high prevalence of H. pylori in South Asians may enhance this genetic predisposition to BO (17). In addition to that Genome-wide association studies (GWAS) have shown outlooks into the genetic background of GERD (18). Other factor that contributes to this disease is the presence of gram negative bacteria in the esophagus that detected by molecular methods in spite of the human esophagus was considered sterile (19). HLA had an association with different ethnic groups (20) and diseases (21,22,23). In spite of few researches about HLA and GERD, one can conclude from this study that HLA-A*11:01 had a protective role against Gastro-oesophageal reflux disease development.

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