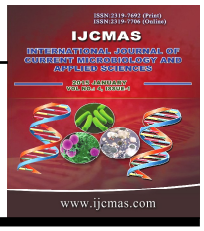
Int.J.Curr.Microbiol.App.Sci (2015) 4(3): 499-503

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 3 (2015) pp. 499-503**
http://www.ijcmas.com



Original Research Article

Human Leukocytes Antigens Determine Susceptibility to *Blastocystis hominis*

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Keywords

HLA, Blastocystis, genetic

A B S T R A C T

The human major histocompatibility complex (HMC) is known as human leukocyte antigen (HLA). The gene products of this region are encoded by a cluster of genes located on the short arm of chromosome 6. Genetic susceptibility plays a role in the development of most human diseases caused by infections like parasitic infection. The aim of the study is to determine the association between HLA-DRB1 alleles and *Blastocystis hominis* infection in a sample of Iraqi Arab Muslims. Thirty Iraqi Arab Muslims patients with *Blastocystis hominis* infection, confirmed by stool examination and culture were selected. All of them were diagnosed as irritable bowel syndrome .The other group was control group was sex and age matched with patient study group, they were consisted of thirty Iraqi Arab Muslims healthy individuals. DQB1genotyping was done using a panel of sequence-specific oligonucleotide probes (SSOP) using HLA-DRB1amplification and hybridization kits (SSO HLA type DRB1 plus and Mastermix for HLA type DRB1 Amp plus kits -Innogenetics-Belgium) using automated method by AutoLipa – 48Innogenetics-Belgum. There was an increased frequency of HLA-DRB1*03:0101and *140101 in patients group compared to control group (P=0.002, Odds ratio= 7.4286, 95% CI: 2.0782 to 26.55370.0164) and (P=0.03, Odd ratio=6.00, 95% CI: 1.1717 to 30.7255) respectively. Other alleles like HLA-DQB1* 08, 11 and 12 were not significantly difference with control group. HLA alleles like HLA-DRB1*03 and 14 increased susceptibility to *Blastocystis hominis* infection in Iraqi Arab Muslims individuals.

Introduction

The human major histocompatibility complex (HMC) is known as human leukocyte antigen (HLA). The gene products of this region are encoded by a cluster of genes located on the short arm of chromosome 6 that are involved in interactions with T-cells (Cunliffe and Trowsdale, 1987). HLA system is highly highly polymorphic and polymorphisim is the existence of two or more alleles of a gene at a single locus in a population, where the occurrence of the alleles is greater than can be explained by recurrent mutation alone. Gene polymorphism in the MHC region is the most extensive yet described

with the number of known allelic forms of genes increasing constantly with the application of molecular methods of HLA typing generates the diversity of MHC molecules within a population. Every cell in one individual, however, expresses the products of the same set of MHC genes (Benjamini *et al.*, 2000). The frequency of some HLA alleles and the association with diseases are distinctly more common in individuals with a particular HLA allele or haplotype (Chaudhuri *et al.*, 2000). HLA alleles are associated with susceptibility or protaction against parasitic infection and can influence immune clearance of the parasite (Glaser, 2000). The products of the MHC genes play a key role in the amounting and recruiting of T lymphocytes against parasitic antigens. These lymphocytes are MHC restricted class II molecules which recognize processed antigenic peptides only in the context of products of MHC class II genes (Glew *et al.*, 1992). The possible mechanism that parasitic epitopes the immune responses are influenced by the presence of a human leukocyte antigen (HLA) type that restricts the immune response to parasitic epitopes (Gilbert *et al.*, 2003). Sadissou *et al.* (2014) found that high levels of sHLA-G were associated with a significant high incidence ratio of malaria infection.

In the current study, we studied the association between HLA-DRB1 alleles and susceptibility to *Blastocystis hominis* infection in a sample of Iraqi Arab Muslims.

Patients and methods

Thirty Iraqi Arab Muslims patients with *Blastocystis hominis* infection were confirmed by stool examination and culture. All of them were diagnosed as irritable bowel syndrome. Their ages were range from 20 to 53 years. Males were 80% and the rest were females. The patients were consulted medical city and AL-Karaama Hospital for the period between September 2013 till September 2014.The personal information for each patient were obtained, which included: name, age, address, Family history and laboratory data. The control groups were sex and age matched with patient study group, they were consisted of thirty Iraqi Arab Muslims healthy individuals, Their ages were range from 18 to 55 years. Males were 82% and the rest were females. Ethical approval from Ministry of health and Al-Mustundeansrya University and confirmed consent was obtained.

Two mL of venous blood were collected by venipuncture from study population, patients and normal Iraqi Arab Muslims individual's ethnicity, age and sex were matched. The blood was put in EDTA containers for DNA extraction by blood kit (QIAmp DNA blood Mini Kit, QIAGEN INC- Germany). DNA product 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 DNA was performed for DRB1. Amplification and Hybridization was performed using a panel of sequence-specific oligonucleotide probes (SSOP) using HLA-DRB1amplification and hybridization kits (SSO HLA type DRB1 plus and Mastermix for HLA type DRB1 Amp plus kits -Innogenetics-Belgium) using automated method by AutoLipa – 48 Innogenetics-Belgum. The results were interpreted using LiRas version-5.0 software- Innogenetics-Belgium.

Statistical analysis was done using MiniTab version 3.0 software. The distribution of HLA alleles in patients and control groups were compared. In each comparison, the Odds ratio (OR) along with the 95%

confidence interval (95% CI) was used. P-value less than 0.05 were considered statistically significant.

Results and Discussion

A total of 30 patients with *Blastocystis hominis* infection were enrolled in this study. Their ages were range from 20 to 53 years. Males were 80% and the rest were females. The control groups were sex and age matched with patient study group they were consisted of thirty Iraqi Arab Muslims healthy individuals. Their ages were range from 18 to 55 years. Males were 82% and the rest were females. Control and patients groups were typed for identifying the DRB1* alleles using DNA based methodology (PCR-SSOP). There was an increased frequency of HLA-DRB1*03:0101 and *140101 in patients group compared to control group (P=0.002, Odds ratio= 7.4286, 95% CI: 2.0782 to 26.55370.0164) and (P=0.03, Odd ratio=6.00, 95% CI: 1.1717 to 30.7255) respectively. Other alleles like HLA-DQB1* 08, 11 and 12 were not significantly difference with control group as shown in table 1.

HLA plays an important role in parasitic infection because it will present peptide to T cell enhance the activation of immune response thus it plays a role in control resistance or susceptibility to a disease (Alves *et al.,* 2006). MHC class I gene regulate the function of NK cell through interplay of opposing signals from surface receptors that trigger or block the function of NK cell through killer immunoglobulin-like receptors (KIR) during infections and autoimmune disorders (Parham, 2005). The frequency of the allelic variants of the HLA genes varies from area to area and is much influenced by environmental factors which is a pathogen infection which is potentiate the immune response against the pathogen. This explains why HLA alleles are associated with susceptibility or protection for a disease in one geographical area and do not confer the same vulnerability or protection in another area for example malaria infection (Riley *et al.,* 1992; Marrosu *et al.*, 1997). This study found that HLA-DRB1*030101, 030102, 031101 and 140101 is significantly increased susceptibility to *Blastocystis hominis* infection. Tejera *et al.* (2012) found that reactive arthritis due to *Blastocystis hominis* in immunocompetent patients with HLA-B27 positive patient. The mechanisms by which different parasites can cause joint disease are multiple like local invasions from neighboring bones or muscles, via the blood or lymphatic with the presence of adult individuals, larvae or eggs in the joint cavity.

They could also trigger a reactive inflammatory reaction to the presence of the parasite in the surrounding tissue, without an actual joint invasion or due to "parasitic rheumatism" in the case of inflammatory conditions without the presence of the parasite *Blastocystis hominis* in the joint or in its vicinity, probably triggered by an immune mechanism (Richi Alberti, 2010). The possible mechanism that parasitic epitopes stimulate the immune responses is influenced by the presence of a human leukocyte antigen (HLA) type that restricts the immune response to parasitic epitopes (Gilbert *et al.,* 2003).

The studies about HLA association with *Blastocystis hominis* infection is very rare because it was considered as non pathogenic protozoa and now a days it was found to be the causative agent of irritable bowel syndrome. HLA had been found to be associated with other parasitic infection like there is a significant association between malaria risk varies in parallel with MHC allele frequency across countries. Thus, the

Human leukocyte antigen molecule appears to be a good candidate for a modulation of the immune response to parasitic infection. HLA alleles like HLA-DRB1*03 and 14 increased susceptibility to *Blastocystis hominis* infection in Iraqi Arab Muslims individuals.

Table.1 Human leukocytes antigens (HLA-DRB1) allele's frequencies in patients with Blastocystis hominis and healthy control groups

HLA-DRB1* alleles	patients with Blastocystis hominis group No.=30		Healthy control group No.=30		Odd ratio (95% confidence interval)	P- value
	No.	%	No.	%		
02:0301	0	0	2	6.66	na	na
03:0101	16	53.33	4	13.33	7.4286 2.0782 to 26.5537	0.0020
03:0102	15	50:00	2	6.66	14.0000 2.8176 to 69.5639	0.0013
03:1701	0	0	4	13.33	na	na
03:1101	8	26.66	1	3.33	10.5455 1.2266 to 90.6658	0.0319
07:0101	0	0	7	23.33	na	na
08:0101	0	0	2	6.66	na	na
08:0201	6	20.00	2	6.66	3.5000 0.6454 to 18.9807	0.1464
11:0101	5	16.66	7	23.33	0.6571 0.1828 to 2.3630	0.5202
11:0301	0	0	4	13.33	na	na
11:6701	0	0	4	13.33	na	na
12:0901	1	3.33	2	6.66	0.4828 0.0414 to 5.6282	0.5611
13:0501	0	0	2	6.66	na	na
13;1801	0	0	7	23.33	na	na
14:0101	9	30.00	2	6.66	6.0000 1.1717 to 30.7255	0.0315
14:0201	0	0	8	26.66	na	na

na=not applicable

Acknowledgments

We would like to thank Baghdad University and Al-Kindy College of Medicine who facilitated this study.

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