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EFFECT OF HUMAN LEUKOCYTE ANTIGEN HLA-DRB1 ON DEVELOPMENT OF BAGHDAD SORE IN PATIENTS

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ABSTRACT :*Leishmania tropica* is a species of flagellate parasites that infects humans and the cause of the disease cutaneous leishmaniasis, which is the most common form of leishmaniasis. It is one of the major parasites, which have high prevalence than other parasites in Iraq. The aim was to investigate the role of HLA alleles in susceptibility to cutaneous leishmaniasis infection in Baghdad in a sample of Iraqi patients. Cross-sectional study (thirty Iraqi Arab Muslims patients with *Leishmania tropica* infection and thirty Iraqi Arab Muslims healthy persons) were participated in this study. Patients were consulted Department of Dermatology in Medical city Teaching hospital and AL-Yarmook Teaching hospital for the period between March 2014 till May 2015. HLA-DRB1 was created by SSOP method. There were obvious increased in frequency of HLA-DRB1*07:0101 and *08:0101 in leishmaniasis patients, *P*-values = 0.0001, Odds Ratio = 13.14 and Confidence interval (CI) = 3.60 - 47.96 and for *08:0101 allele *P*-values = 0.0077 with OR = 9.33 and CI= 1.80 - 48.24.

Key words : HLA-DRB1, major histocompatibility complex, *Leishmania tropica*, leishmaniasis.

INTRODUCTION

Leishmania tropica is a species of flagellate parasites that infects humans and cause the disease leishmaniasis (Aoun and Bouratbine, 2014), which is a form of cutaneous disease and the most common form that affecting humans (James *et al*, 2006). It is spread by the bite of sand fly insect (Louis *et al*, 2013). The virulence factors of *Leishmania* are cysteine proteases (Mahmoudzadeh-Niknam and McKerrow, 2004). Cutaneous leishmaniasis severity depends on replication ability of the parasite and inducing of immunologic responses. Genes are the most important factors for modulation host immune response (Castellucci *et al*, 2014).

Number of researches have cleared the relationship between cutaneous leishmaniasis and polymorphisms at class I of Human Leukocyte Antigen (HLA) locus A, B, and C, class II (DR/DQ) and class III (Tumor Necrosis Factor/Lymphotoxin-α) (TNF) / (LTA) (Cabrera *et al*, 1995). The susceptibility and resistance to pathogens may cause infectious disease, detected by environmental factors, pathogen and genetic factors of the host (Prahalad *et al*, 2001).

Major Histocompatibility Complex (MHC) or (HLA), is the most important studied of genetic systems because of its effects in resistance to pathogens, autoimmunity and self -compatibility or non (Langamba Angom Longjam, 2017).

T-helper cells effect immune response of anti-*Leishmania*, it produce cytokines, provoke B cells to convert to mature antibody, increase differentiation responses of cytotoxic lymphocytes ($CD8^+$) and increase the capacity responses of immunity (Kara *et al*, 2014). Cells of $CD8^+$ contribute in production of Interferon-gamma (IFN-γ) and response differentiation of Type1 T helper (Th1) (Da Silva Santos and Brodskyn, 2014). *Leishmania* use different mechanisms to avoid immunity including changes in the expression of interleukin 10 (IL-10) (Schwarz *et al*, 2013). In the early infection, promastigotes of *Leishmania major* target macrophages, which in turn cause cellular response in addition to signals expression, that induce parasite to survive and multiply in these cells (Alessandra *et al*, 2014).

The aim of this study was to investigate if there was possible relation of HLA genes with *Leishmania tropica* in a sample of Iraqi patients.

MATERIALS AND METHODS

The study was a cross sectional comparative. Approval of medical morals board was taken from the scientific unit of AL-Kindy College of Medicine. Thirty Iraqi Arab Muslims patients were consulted at

Department of Dermatology in Medical City Teaching hospital and AL-Yarmook Teaching hospital for the period between March 2014 till May 2015, infected with *Leishmania tropica*, which were confirmed by physician inspection. There were matchingin gender and age for both groups (patients and control).

Blood samples : About 2 milliliter were collected by vein puncture using plastic disposable syringe from all patients and control group. Blood was placed in sterilized test tube with Ethylene diaminetetra acetic Acid (EDTA) and stored at -20°C till used. Extraction of DNA was carried out by using Qiagen DNA extraction kit. Polymerase chain reaction (PCR) and sequence specific oligonucleotide probes (SSOP) were used for typing of HLA-DRB1, by using amplification and hybridization kits in automated AutoLipa 48 machine (Innogenetics-Belgum). Results were analyzed by using LIRAS software (Innogenetics Belgium).

Statistical analysis : Data were translated by using MiniTab version 3.0 software (Minitab Inc., State College, PA, USA). Distribution of frequencies were recorded by using a table to show number and percentage of variables. In each comparison, OR with 95% CI was calculated, *P*-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Thirty persons infected with *Leishmania tropica* were enrolled in this study. The ages of them were between 24 to 59 years and 65% of them were males. Control group, matched in agea nd gender with patients study group. Results showed there was elevation in frequency of allele *07:0101 and allele *08:0101 in patients group as compared to the healthy group, other alleles that were detected show no significant association (Table 1).

Leishmaniasis is a tropical disease, its prevalence reach about 90% in poor countries. Host genes and immune response plays an important role in leishmaniasis disease (Singh *et al*, 2014), also molecules of HLA have the key role in immunity and susceptibility to disease and many researches confirmed the essential role of MHC region in diseases variety of genes of HLA included within the MHC (Shieh *et al*, 2018).

The reproduction of this parasite in non-ulcerative nodules with cutaneous leishmaniasis has been related to persistent of infection (Hernández-Ruiz *et al*, 2010). Lesions of patients with mucosal leishmaniasis due to the hyperactivity of $CD8^+$ T cells in the involved tissue (Gaze *et al*, 2006). Comparing with the results of this study, a study was carried out in Brazilian states of Maranhão and even in the north of Paraná, they found an association between the frequency of the DRB1*07 and DRB1*01, which may involve in susceptibility to cutaneous leishmaniasis (Gonçalves *et al*, 2010), while another study from Sri Lanka showed alleles DRB1*04, DRB1*07 and DRB1*15 were over represented in

Table 1 : Human leukocytes antigens (HLA-DRB1) allele's frequencies, Odds ratio, confidence interval, *P*-value in patients with *Leishmaniat ropica* and healthy control.

HLA-DRB1* alleles	Leishmaniasis patients group (N=30)	Healthy control group (N=30)	Odd ratio (95% confidence interval)	*P*- value
02:0301	NO (%)0	NO (%)2(6.7)	NA	NA
03:0101	2(8)	4(13.4)	0.7(0.09 -3.37)	0.531
03:0102	0	2(6.7)	NA	NA
03:1701	0	4(13.3)	NA	NA
03:1101	0	1(3.3)	NA	NA
04:02:01	10(40)	0	NA	NA
07:0101	20(80)	7(23.3)	13.1(3.60-47.96)	0.0001
08:0101	10(40)	2(6.7)	9.3 (1.80 – 48.24)	0.0077
08:0201	0	2(6.7)	NA	NA
11:0101	0	7(23.3)	NA	NA
11:0301	4(16)	4(13.3)	1.8 (0.003-255.626)	1.00
11:6701	0	4(13.3)	NA	NA
12:0901	0	2(6.7)	NA	NA
13:0501	2(8)	2(6.7)	1.2 (0.158-9.3126)	0.849
13:18:01	0	7(23.3)	NA	NA
14:0101	2(8)	2(6.7)	1.2 (0.155-9.326)	0.849
14:0201	0	8(26.7)	NA	NA

NA = Not Applicable.

patients (Nilakshi Samaranayake *et al*, 2016). In Mexican Mestizo, they found DRB1*15 and DRB1*16 to be protective against cutaneous lesions (Olivo-Diaz *et al*, 2004). One of the first studies assessing association between HLA and cutaneous leishmaniasis involving serological methods was done in France, revealed a low frequency of HLA-Cw7 associated with the pathogenesis of cutaneous leishmaniasis (Donaghy *et al*, 2007).

The present study found that males predominance in the infected group, also research on the epidemiological profiles of ACL shows that infected patients are predominantly male (Silveira *et al*, 1999). Even if males and females are exposed to the same environmental risk factors necessary for ACL, males are infected with the parasite much more often. The infrequency of ACL in females may be due to their infrequent exposure to risk factors, such as being near rivers and in the woods, as compared with men, and their use of repellents to protect themselves during the day and to furthermore, sample collection period for the patients group may have influenced the number of women in this group, as women were more likely than men to be home in the morning and afternoon.

This conflict with the results of this study may be related to patients' selection criteria, age of the patients and ethnicity.

CONCLUSION

Infectious parasites playthe key role in the polymorphism of MHC and HLA alleles may have an important effect on development of leishmaniasis, resistance to treatment and chronicity of the disease.

In conclusion, Alleles*07:0101 and HLA-DRB1 * 08:0101 have an obvious effect on development of leishmaniasis.

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