ISSN 0975-2366

DOI: https://doi.org/10.31838/ijpr/2020.SP3.115

Research Article

A study of the relationship of protein antigens extracted from house flies (Musca domestica) to allergy patients in Basra province , Iraq

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Received: 02.07.20, Revised: 29.08.20, Accepted: 10.09.20

ABSTRACT

The spread of hypersensitivity disease worldwide is similar to that of any epidemic disease. The present study dealt with one of the most important human allergens source, which are common house flies (*Musca domestica*). The current study carried out in Basrah city on 96 healthy people (control) and 96 people with allergies, as they were divided in to two groups depending on gender and age. The present study revealed that there was no significant difference (P>0.05) of ELISA test between the two age groups of patients. However, the high rate of sensitivity (12.5%) was seen in age >45. In addition, the current study revealed that there was no significant difference (p>0.05) of ELISA test between males and females of patients. However, the higher rate of sensitivity (16.6%) was seen in male. Furthermore, the present study revealed that there was a significant difference (P<0.05) in the HLA alleles distribution between patients and controls according to age. The patients higher than 45 age showed higher frequency for the HLA- DQB1*0602 (31.25%) and HLA-DQB1*0604 (15.63%) alleles. The current study revealed that there was a significant difference (P<0.05) in the HLA alleles distribution between patients and controls according to sex. The female patients showed higher frequency (29.36%) for the HLA- DQB1*0602. However, the male patients revealed high frequency (14.29%) for HLA- DQB1*0604. The seropositive showed higher frequency for HLA-DQB1*0602 (37.5%) and HLA-DQB1*0604(25%) compared to frequency of HLA- DQB1*0602 (29.55%) and HLA- DQB1*0604 (12.5%) alleles in seronegative patients. In conclusion,

Keywords: Antigen, house flies, ELISA assay, IgE , HLA ‖, PCR

INTRODUCTION

Musca domestica (*M. domestica*) is the most common pet found in poultry farms, horse stables and ranches. It is adapted well via feeding on garbage in a variety of environments [1]. House flies not only a nuisance, but they are an allergic rhinitis to human. *M. domestica* fly dust Inhalation can cause sneeyzing and irritation of the eyes [2]. There are more and more new species of insects, plants and microorganisms are considered as potential "donor" allergens. Long time exposure to allergens housefly promotes the development of allergies. An allergen is an antigen that evokes IgE antibody specific for environmental allergens [3]. In addition, Human Leukocyte Antigen (HLA) is a system paid more attention because it is very important in polymorphous immunological reactions [4]. To understand the role of genetic factors in the *M. domestica*, this work amid to study IgE responses to the *M. domestica* allergens and if HLA alleles have a general effect on the reaction to house flies allergens.

MATERIALS AND METHODS

Experimental design

A current study was carried out on 192 healthy non-allergic volunteers and patients with bronchial asthma (96 in each). Both groups were between 16 and 70 age. Forty one patients were male and 55 were female. While, 45 healthy non-allergic were male and 51 were female. The common house flies (*M. domestica*) utilized during this experimental work was collected from the environment.

Sample collection: Blood sample (5 ml) was collected from venous in plain tube. Then, blood sample (2 ml) was centrifuged to collect serum (1500 rpm for 10 min). While, remain blood (3 ml) was kept under -18 C° in tube containing EDTA for HLA-DQB1 A and HLA-DR genotyping.

Extraction of house flies allergens: Extraction of house flies allergens were done as described previously [5]. Briefly, the common house flies (*M. domestica*) were grinded using a mortar and pes-

tle, defatted with ethyl ether and dried. The weighed powder was soaked (4°C for 48 h) in phosphate-buffered saline (PBS; pH 7.4) containing phenol(0.2%). Then, the extract was centrifuged (13,000 ×g for 15 min) and the supernatant filtered using filter paper (0.22-µm pore; Millipore). The extract was then kept (under – 18C°) until use.

Estimation of protein concentration

Determination of protein concentration was done as described previously using spectrophotometry [6]. Briefly, 3 ml of extract was pipette in quartz cuvette and measured by spectrophotometry based on UV absorption at 260 nm and 280 nm absorbance. The following equation was then applied to calculate protein content: Protein concentration mg/ml= 1.55×A 280 -0.77× A260.

Detection of specific IgE antibodies

Specific IgE antibodies Detection was done by using enzyme-linked immunosorbent (ELISA) assay [7]. Briefly, disposable polyvinyl well plates were filled with serum (250 µl). Well plates were then filled with serum biotin (250 µl) and mixed wall for 30 sec. The plate was then kept at room temperature for 30 min and washed. Then, horseradish peroxidase was added to each well. The plate was then kept at room temperature for 30 min. The well rewashed again. A and B enzymes (100 µl) was then added and the plate was kept at room temperature for 15 min. Stop reaction (50ml) was then added. The results were recorded using on wave 450nm.

PCR -based HLA-DQB1and HLA-DRB1 genotyping

Genomic DNA isolated from whole blood of 192 healthy non-allergic volunteers and patients with bronchial asthma was amplified by the PCR procedure [8]. Three PCR primer set for amplification of sequences for detection of HLA alleles was applied (Table1). The basic three steps of PCR (denaturation, annealing, and extension) repeated 35 cycles (Initial denaturation (94°C for 30 sec), annealing (60°C for 45 sec), extension (72C° for 45 sec), and final extension (72C° for 5 min)). The amplified DNA was then stained with a fluorescent stain (Ethidium Bromide) and images by gel-documentation systems using agarose gel electrophoresis (UVIDOC UK). After that, the size of the band was determined by comparison with a standard DNA ladder (100 bp).

Table 1: HLA class II Oligonucleotide primers sequence used for PCR

HLA-	Alleles	primer sequence (5´ → 3´) Forward	Reverse	SIZ bp
DRB1	*12	AGTACTCTACGGGTGAGTGTT	CACTGTGAAGCTCTCCACAG	248
DQB1	*0602	CGTGCGTCTTGTGACCAGAT	GCTGTTCCAGTACTCGGCAT	121
	*0604	CGTGTACCAGTTTAAGGGCA	GCAGGATCCCGCGGTACC	254

Statistical analysis: The Pearson's chi-square test was done by using statistical program, SPSS[9].

RESULTS

Specific IgE based on ELISA result

The present study revealed that 64 out of 96 (66.66%) of patients were sensitive to house fly allergen.

Distribution of house fly allergen according to ELISA test based on age of patients

The present study revealed that there was no significant difference (P>0.05) of ELISA test between the two age groups of patients (Table 2). However, the high rate of sensitivity (12.5%) was seen in age >45.

Table 2: Distribution of house fly allergen according to ELISA test based on age of patients

Age groups (years)	Tested No	Specific IgE	
		Seropositive No (%)	ELISA OD D Mean ±S.E.M
45≤	64(66.66%)	4(6.25 %)	0.1468±0.00365
>45	32(3.125%)	4 (12.5%)	0.1527±0.00480
Total	96(100%)	8(18.75%)	
Chi-Square df(1)=1.091 , P value= 0.251			

*Results are expressed as Mean ±S.E.M; *OD= Optic Density

Distribution of house fly allergen according to ELISA test based on o sex of patients

The current study revealed that there was no significant difference (p>0.05) of ELISA test between males and females of patients (Table 3). However, the higher rate of sensitivity (16.6%) was seen in male.

Table 3: Distribution of house fly allergen according to ELISA test based on sex of patients

Sex	Tested No	Specific IgE	
		Seropositive No%	ELISA OD SD Mean ±S.E.M
Male	42(43.75%)	7 (16.6%)	0.1574±0.00532
Female	54(56.25%)	1(1.85%)	0.1463±0.00343
Total	96(100%)	8(18.45%)	
Chi-Square df(1)=6.788 , P value= 0.12			

*Results are expressed as Mean ±S.E.M; *OD= Optic Density

PCR -based on genotyping results

Gene sequences for the identification of HLA alleles were effectively done by using PCR. The band with 121pd (HLA- DQB1*0602) and 254pb (HLA- DQB1*0604) revealed a single band under UV illuminator (Figure 1).

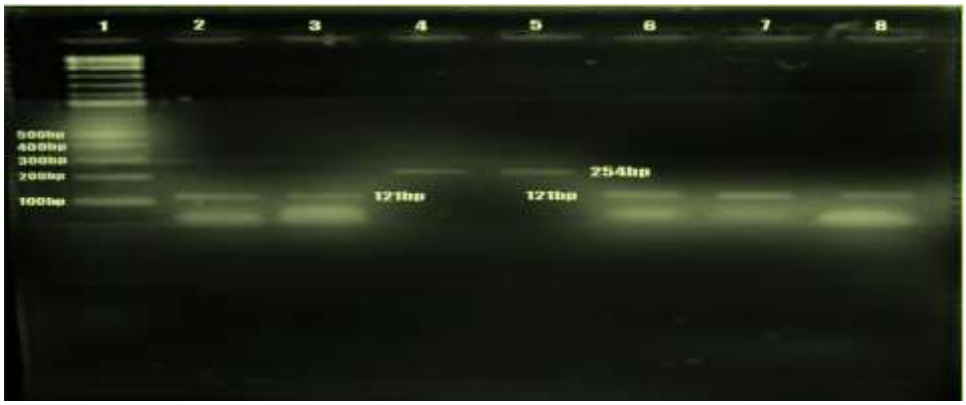

Fig.1: The amplification of HLA alleles by using PCR in agarose gel electrophoresis of DNA. Lane1: ladder, Lanes:2,3,6,7,8: HLA- DQB1*0602 (121bp).Lanes: 4,5: HLA-DQB1*0604 (254bp)

Associations of HLA alleles with allergy

The present study revealed that there was a significant difference (P<0.05) in the HLA alleles distribution between patients and controls according to age (Table 4). The patients higher than 45 age showed higher frequency for the HLA- DQB1*0602 (31.25%) and HLA-DQB1*0604 (15.63%) alleles.

Table 4: Distribution of HLA- DQB1 and HLA-DRB1 alleles according to age

Age groups (years)		Tested No (%)	HLA-DQB1		HLA-DRB1
			0602 *+ve No(%)	0604 *+ve No(%)	*12 +ve No(%)
<45	P No=96	64(66.66%)	19(29.69%)	8(12.5%)	0
	C No=96	72(75%)	0	5(6.94%)	0
>45	P No=96	32(33.33%)	10(31.25%)	5(15.63%)	0
	C No=96	24(25%)	0	3(12.5%)	0

*P=Patients, C=controls; *Chi-Square df(3)= 13.206 ,P value= 0.004

The current study revealed that there was a significant difference (P<0.05) in the HLA alleles distribution between patients and controls according to sex (Table 5). The female patients showed higher frequency (29.36%) for the HLA-DQB1*0602. However, the male patients revealed high frequency (14.29%) for HLA-DQB1*0604.

Table 5: Distribution of HLA- DQB1 and HLA- RB1 alleles according to sex

Sex		Tested No(%)	HLA-DQB1		HLA-DRB1
			0602 *+veNo(%)	0604 *+veNo(%)	*12 +ve No.(%)
Male	P No=96	42(43.75%)	13(30.95)	6(14.29%)	0
	C No=96	57(59.38%)	0	3(5.26%)	0
Female	P No=96	54(56.25%)	16(29.36%)	7(12.96%)	0
	C No=96	39(40.63%)	0	5(12.82%)	0

*P=Patients, C=controls; Chi-Square df(3)= 13.158, P value= 0.04

Distribution HLA alleles in the seropositive and seronegative allergic patients are shown in Table 6. The seropositive showed higher frequency for HLA-DQB1*0602 (37.5%) and HLA-DQB1*0604(25%) compared to HLA-DQB1*0602 (29.55%) and HLA-DQB1*0604(12.5%) alleles in seronegative patients (Table 6).

Table 6: Association of HLA- DQB1 and HLA- RB1 alleles with house fly- based ELISA seropositivity

CR-based ELISA	Tested No(%)	HLA-			Total
		DQB1*0602	DQB1*0604	DRB1*12	
Seropositive	8(8.33%)	3(37.5%)	2(25%)	0	5
Seronegative	88(91.67%)	26(29.55%)	11(12.5%)	0	37
Total	96	29(67.05%)	13(37.5%)	0	42

DISCUSSION

Changes in lifestyle increase the exposure to household allergens via spending more time in closed environments [10]. Homes contain many antigens that cause allergy. Insect antigens are one of antigen that cause allergy to human [11]. Asthma is an allergic illness represents a significant health problem [12]. The common house fly (*Musca domestica*) have been related with an allergic illness (asthma) around the world [13]. Allergens are played an important role in some atopic respiratory disorders. In addition, house fly is a mechanical carrier for many pathogens to human (bacteria, fungi, parasites, viruses, and parasites) causing serious diseases to human being [14].

In the current study, the extraction of protein via crushed whole common house fly body used to determine the hypersensitivity of allergic patients to this insect by appling IgE based indirect ELISA. This finding is in agreement with previous study, in which purified protein from common house fly body extracts is an excellent IgE antibody binding activity [15].

The present study revealed that there was higher frequency of patients with respiratory and/or skin allergic diseases for common flies protein extract. This finding is in agreement with previous study, which used skin test for the assessment of exposure [16].It has been found that patients with allergic rhinitis revealed positive reactions to the skin test [16].

The present study carried out on 96 patients of both sexes and aged (15 to 75 years) to quantitative assesses for the presence of common fly health impact. The current data revealed that the exposure and sensitivity to common fly allergens is a risk factor for the asthma in patients [17]. It has been found that there is a relationship between common fly exposure and severity of illness in the infested residences [18]. The exposure to common house fly might elevate level of infestation and explain some aspects of allergic diseases.

The current study revealed sensitivity 64 of 96 (66.66%) patients to the IgE antibody against common house fly. This finding is in agreement with previous study, in which sensitivity 36 of 60 (30%) to IgE antibody against a group of insects, including the house fly [19]. It has been found that 197 allergens including 17 insects, 4% of allergens for patients are for common house fly [20]. It is suspected that the house fly is the causative agent for rhino-conjunctivitis and mild asthma due to the presence of this insect in large numbers in the farm. The respiratory sensitisation to insects might be highly specific to Muscidae [21].

The present study revealed that there was higher frequency in seropositive patients for HLA-DQB1*0602 (37.5%) and HLA-DQB1*0604(25%) compared to seronegative patients in 96 allergic patients for HLA- DQB1*0602 (29.55%) and HLA- DQB1*0604(12.5%) alleles in. This finding might be elevated a person's susceptibility to some genes and protected other genes against these diseases [22]. It has been found that allergic reactions occur as a result of the interaction between environmental and genetic factors [23]. The presence of alleles in the HLA genetic region elevates the likelihood of developing allergic disease. HLA alleles play an important role in expansion asthma in patients with HLA-DRB1*070101. However, HLADRB1* 030101 might be protected against development of asthma [24].

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