WHEAT ALLERGY: IMMUNOLOGICAL STUDY AMONG WORKERS IN MILL INDUSTRY IN BASRAH CITY

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ABSTRACT

This is a comparative study, carried out to involve 160 individuals; 80 individuals with work-related allergic symptoms who were grain industry workers with direct contact to the grain dust, and 80 individuals (control group) were taken from Basrah General Hospital, not suffering from allergic disorders. The first group was divided into two subgroups, including 47 workers in high exposure places to grain dust and 33 workers in low exposure places. ELISA test was performed using locally prepared albumin/globulin extract to detect wheat specific IgE. The results showed that 70% of individuals with work-related allergic symptoms had allergy in comparison to nil among control group. The study also showed that the highest rate of positive ELISA result was observed in 80.8 % of individuals with work-related allergic symptoms working in highly exposed places to grain dust, compared to only 54.5 % among those working in low exposed places. The study revealed a highly significant association between specific allergy and years of service. The presence of respiratory symptoms was higher among workers in highly exposed places, followed by skin and conjunctival symptoms.

INTRODUCTION

Wheat flour is an important cause of baker's asthma, a well-known occupational respiratory allergy to inhaled flour. Baker's asthma is an IgE-mediated occupational disease of millers and bakers caused by inhalation of cereal flour. It is usually accompanied by rhinitis and often by conjunctivitis and contact dermatitis in the hands.[1] However, most patients with baker's asthma are able to ingest wheat products without symptoms. Wheat proteins are divided into two major fractions on the basis of water/salt solubility. The water/salt soluble fraction contains albumin and globulin, whereas the water/salt insoluble fraction contains gluten and gliadin.[2,3] The components of the water/salt soluble fraction have been reported to be the causative allergens in cases of baker's asthma[4,5], which is based on a typical IgE-mediated allergy to inhaled wheat flour.[6] Several wheat allergens have been identified and characterized in baker's asthma,[7] accounting for about 70-80% of the specific IgE binding activity.[8,9] The characterized allergens belong to the water/salt-soluble fraction, include several 14-17 kDa proteins from the alpha-amylase/trypsin inhibitor family.[9,10] In addition a 36 kDa wheat glycoprotein with peroxidase activity, [11,12] a 27 kDa protein similar to acyl-coenzyme A oxidase,[10] and a 35 kDa protein similar to fructose-bisphosphate-aldolase have been identified.[13] The diagnosis of wheat allergy is based on the patient's clinical history, detection of wheat-specific IgE by enzyme-linked immunsorbent Assay (ELISA) or radioallergosorbent test (RAST), and results of elimination diets and oral challenges, or bronchial challenges in cases of baker's asthma.[14,15] Baker's asthma is one of the most common forms of occupational asthma.[16] The vast majority of patients, about 60-70%, have IgE to wheat flour.[7] The incidence of bakers' asthma varies from 4% up to 10% in different reports.[17] There are several studies which have investigated the allergenicity of the water/salt insoluble gliadin and gluten proteins (prolamins) and provide information on their role in cereal hypersensitivity and demonstrated IgE binding to the gliadin and gluten fractions of wheat.[6,8] In Basrah, there was no previous study carried out to investigate wheat allergy, so this study was carried out to identify, purify and characterize clinically significant wheat allergens among mill industry workers, to explain the relationship between wheat allergen exposure and wheat sensitization among work-related allergic symptoms, and to determine the allergenic activity of allergen extract and allergen-specific immunoglobulin.

MATIRIALS AND METHODS

This is a comparative study involved 80 individuals working in mill industry with allergic symptoms *(related to upper and lower respiratory tract disorders)*. The study group was selected randomly from eight mill industries in Basrah City. This group was divided into two subgroups according to the

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level of exposure to wheat dust, 47 individuals working in highly exposed area *(grinder, discharge point, packing section)*, and 33 individuals working in low exposure area *(other places of mill industry)*. The control group comprised of 80 individuals; not suffering from allergic symptoms, were chosen from Basrah General Hospital *(not exposed to grain dust)*. Special questionnaire form was designed for the purpose of the study and filled by one of the authors. Blood samples were taken from the 160 individuals for detection of total and specific IgE to wheat. The study was carried out during the period from July 2004 to November 2004. (ELISA) was performed for detecting both total IgE and specific IgE to the albumin/globulin fraction. Statistical analysis was performed by using statistical package of social sciences (SPSS) version 11, Chi-squared and Fisher's Exact tests were used as tests of significance.

Antigens preparation

Sequential extraction of wheat proteins

Wheat grains were milled at 10000 rpm (3 pulses, 5) using an electric mill. Wheat proteins were extracted from 100 mg of flour according to the sequential procedure of Osborne, adapted by Nicolas et al,[18] which is performed by extracting albumins/globulins in 3mL 0.05 m phosphate/0.1m NaCl buffer pH 7.8 for an hour at 4°C with constant stirring. After centrifugation (20 000 g for 20min at 4°C), the supernatant was collected and frozen.

Determination of protein content

The protein content of the allergen extract was estimated according to Whitaker and Granum[19] method; which is performed by transferring 3-ml of the extract using a pipette to quartz cuvettes, the absorbance value was measured spectrophotometrically at 235 and 280 nm. The protein content in mg/ml was calculated by the following equation.

Protein content mg/ml= (A235-A280/2.51).

Purification and fractionation of protein extract on G-75 sephadex

Gel-filtration-liquid Chromatography used to fractionate and purify the protein extract into molecules of different molecular weights by using G- 75 Sephadex according to the method of Leslic and Frank [20], as follows:

Eight grams of dry Sephadex (G-75) were swollen in about 120 ml of 0.5 M tris buffer pH 6.8, 1M HCl. The gel poured into the column in vertical level using a glass rod to avoid any air bubbles and left to settle with opened outlet, the flow control valve was opened and the water was allowed to enter the column. Blue dextran stain at 1:100 w/v in water was added to the surface of the gel and effluent was collected into a graduate cylinder. The water was added to the surface of the gel, and the effluent was collected until the blue dye just appears, the collected liquid represents the void volume of the column. The gel was equilibrated with 0.1M tris pH 6.8 equivalents to three times the void volume of the column.The column was calibrated with standard molecular weight proteins; ribonuclease A (13.7 KDa), papine (23KDa); Ovalbumin (45KDa), Bovine serum albumin (67KDa), each one was applied individually, the volume of calibration solution should be 1-2% of total gel bed volume (Vt); the elution volume (Ve) for the calibration proteins was determined by a spectrophotometer. A calibration curve was fitted by measuring the elution volume (Ve) of several standard proteins, their corresponding Kav values, were calculated using the following equation:

$$Kav = \frac{Ve - Vo}{Vt - Vo}$$

Where:

Kav = distribution coefficient

Ve = elution volume for the protein

Vo = column void volume = elution volume of blue dextran 2000

Vt = total bed volume

The Kav values were plotted versus the logarithm of their molecular weight. The extract was applied to the column at flow rate 21 ml/hour. Fractions (3ml each) were collected in a sterile glass tubes and monitored for protein content spectrophotometrically at 280nm. The major peak for each extract was determined by plotting the eluted volume of protein extract, versus the absorbance value at wave length 280nm. Each major peak was used as an antigen in ELISA technique. Total IgE (total allergy) and specific IgE antibodies against the suspected allergens are measured by a modification of (ELISA).[21]

RESULTS

The purification of wheat protein extract was achieved by gel filtration. The protein fractionation and molecular weight determination were performed by using Sephadex G-75 (Fig-1). The molecular weight of purified antigens was estimated by gel filtration (Fig-2).

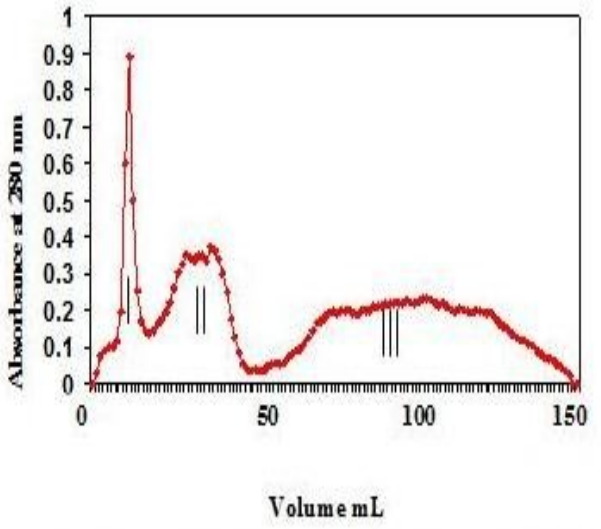

Fig. (1): Elution profile of wheat protein extracts

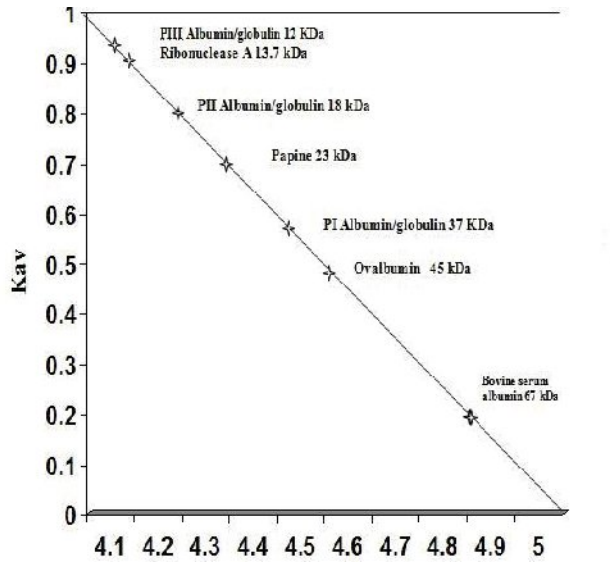

Fig 2. The calibration curve of protein extract using some standard proteins for estimation of protein molecular weight

Table-1, shows the distribution of the studied population according to age. It shows a comparable distribution regarding age, where the majority of individuals with work-related allergic symptoms and controls were at the age group 20-39 years. With no statistical significant difference (P>0.05).

Table 1. ***Distribution of the studied population according to age.***

Age groups (years)	Individuals with work-related allergic symptoms	Control	Total
	No.(%)	No. (%)	No. (%)
<19	3(3.8)	5 (6.3)	8 (5)
20-29	23(28.8)	34 (42.5)	57 (35.6)
30-39	26(32.5)	21 (26.3)	47 (29.4)
40-49	12(15)	5 (6.3)	17 (10.6)
≥ 50	16(20)	15 (18.8)	31 (19.4)
Total	80(100)	80 (100)	160 (100)

X^2=6.069 df=4 P> 0.05

Table-2, shows the distribution of the studied population according to the presence or absence of the total allergy. It shows that 70% of individuals with work-related allergic symptoms had allergy in comparison to 15% of the control group and the difference was statistically highly significant (P<0.001).

Table 2. ***Distribution of the studied population according to the presence or absence of the total allergy***

Total allergy	Individual with work-related allergic symptoms	Control	Total
	No. (%)	*No. (%)*	*No. (%)*
Positive	56(70)	12(15)	68(42.5)
Negative	24(30)	68(85)	92(57.5)
Total	80(100)	80(100)	160(100)

X^2=47.667 df=1 P< 0.001

Table-3, shows the distribution of the studied population according to the presence or absence of the specific allergy to wheat. It shows that 70% of individuals with work-related allergic symptoms had wheat specific allergy in comparison to nil allergy among control group, and the difference was statistically highly significant (P<0.001).

Table 3. ***Distribution of the studied population according to the specific allergy to wheat.***

Specific allergy to wheat	Individual with work-related allergic symptoms	Control	Total
	No. (%)	*No. (%)*	*No. (%)*
Positive	56 (70)	0 (0)	56 (35)
Negative	24 (30)	80 (100)	104 (65)
Total	80 (100)	80 (100)	160 (100)

Fisher's Exact test **P<0.001**

Out of 80 studied individuals with work-related allergic symptoms, 56(70%) had a specific allergy to wheat. The highest rate of positive ELISA results was observed in 78.7% of individuals working in highly exposure places, compared to only 57.6% among those working in low exposure places and the difference was statistically significant (P<0.05), (Table-4).

Table 4. ***Distribution of specific allergy to wheat in individuals with work-related allergic symptoms according to the level of exposure.***

Level of exposure	Specific allergy to wheat		Total
	Positive	Negative	
	No. (%)	No. (%)	No. (%)
High exposure	37 (78.7)	10 (21.3)	47 (100)
Low exposure	19 (57.6)	14 (42.4)	33 (100)
Total	56 (70)	24 (30)	80 (100)

X^2= 6.089 **df= 1** **P<0.05**

It is evident that out of 47 individuals worked in highly exposure places, 87.2% of them were presented with respiratory symptoms and 12.8% had cutaneous and conjuctival symptoms, compared to only 33 individuals worked in low exposure places, 75.8% of them showed respiratory symptoms while 24.2% showed cutaneous and conjuctival symptoms. The difference was statistically not significant (P>0.05), (Table-5).

Table 5. ***Distribution of individuals with work-related allergic symptoms according to work place and clinical presentations***

Type of symptoms	Work place		Total
	High exposure	Low exposure	
	No. (%)	No. (%)	No. (%)
Respiratory	41 (87.2)	25 (75.8)	66 (82.5)
Dermal and conjunctival	6 (12.8)	8 (24.2)	14 (17.5)
Total	47(100)	33(100)	80(100)

X^2= 1.69 **df= 1** **P>0.05**

Table-6, shows that the highest rate of total positive ELISA result was 88.2 % of individuals with work-related allergic symptoms worked more than 5 years compared to only 37.9%, of individuals who worked for ≤ 5 years. The difference was statistically highly significant (P<0.001).

Table 6. ***Distribution of individuals with work-related allergic symptoms according to years of service and total allergy.***

Total allergy	≤5 years of service	>5 years of service	Total
	No. (%)	No. (%)	No. (%)
Positive	11(37.9)	45(88.2)	56(70)
Negative	18(62.1)	6(11.8)	24(30)
Total	29(100)	51(100)	80(100)

The final dilutions of antigens (purified extract), sera and conjugate were 1/200, 1/20 and 1/100 respectively, while the background value and negative cut off optical density (OD) values of IgE based ELISA were <0.065 and 0.034 respectively.

DISCUSSION

Wheat, like all other food, contains a number of proteins; some of them have been identified as allergens. Some proteins are considered as major allergens (more than 50% of wheat-allergic persons react to these allergens) while others considered as minor allergens (less than 50% of wheat-allergic persons react to these allergens).[22] Gel filtration analysis of wheat protein extract in 0.05 M phosphate/0.1 M NaCl buffer pH 7.8, demonstrated three major peaks. The molecular weight of eluted proteins were 37 KDa, 18 KDa and 12 KDa. This result was in line with many studies, which reported that the molecular weight of albumins and globulins were mainly under 40 kDa, and the albumin/globulin fraction of the a-amylase inhibitor subunits with molecular masses from 12 to 18 kDa were considered to be important allergens for subjects with baker's asthma.[2,8] The IgE recognition pattern of albumin/globulin extract was determined by the ELISA technique. Albumin/ globulin proteins regarded as the major allergic components of baker's asthma; these proteins can be recognized by human allergic sera. This result was in line with Baur et al,[1] who reported that 60% of bakers with work-related respiratory symptoms have IgE antibodies to albumin/globulin fraction of wheat flour, and in line with Mittag et al.[23] who reported that, subjects with baker's asthma, as well as other food-allergic subjects, have the most intense IgE-reactivity to the albumin/globulin fraction. It was found that proteins are the most clinically important allergens. These findings are in line with many studies which reported that the water/salt-soluble albumins and globulins are the most relevant allergenic proteins for patients with baker's asthma, accounting for about 70 to 80% of the specific IgE binding activity,[8] and with Sutton et al.[24] who found that most patients with baker's asthma have an allergy to inhaled flour where the albumins are the important allergens. The study showed no significant difference between age of individuals with work-related allergic symptoms and control groups. Houba et al.[25] reported that several potential effects e.g. *(gender and age)* were tested but none of which was significantly associated with wheat sensitization. Also, Cullinan et al.[26] stated that there were clear relationships between the risks of developing work-related symptoms and exposure to total dust of flour, and his findings were unaffected by age or sex in a case control study. The study showed that the majority of individuals with work related allergic symptoms were having total allergy. This result is in line with Park et al. [27], who reported that total IgE was increased in the exposed workers, and this could be explained by the possibility that grain dust could directly activate mast cells, which results in the release of IL-4 and the induction of IgE synthesis. Also the study showed that all individuals with total allergy had specific sensitization to wheat. This result is in line with Heederik and Houba [28] who reported that there is an increasing sensitization for specific wheat and grain dust. Also, this result is similar to that of Mittag[22] who found that subjects with baker's asthma, as well as other food-allergic subjects, had the most intense specific IgE-reactivity to the albumin/globulin fraction. This study showed that there is a significant difference between wheat allergen exposure and wheat-specific IgE sensitization, where most of persons directed to high flour dust exposure are more likely to be sensitized to wheat-specific IgE. These results are in line with Jeffrey et al.[29], who stated that specific IgE to wheat flour was significantly associated with exposure, and in line with Houba et al.[30], who reported that, patients with a high and medium wheat allergen exposure are more likely to be sensitized to wheat allergens compared with workers with low allergen exposure. This study showed a highly significant difference between individuals with work related allergic symptoms and years of work, where the most of wheat allergic individuals have worked for more than 5 years. This result is in line with Chia et al.[31] who found that workers developed asthmatic attacks 5 years after joining the mill backing. This may explain the high prevalence of respiratory symptoms presented among workers with long-term exposure to wheat flour. The present study also showed that the majority of individuals with work related allergy had respiratory symptoms and these results are similar to that of Droste et al.[32] who found that bakery workers had significantly more often respiratory symptoms. This finding is also in

line with Gimenez et al.[33] who found a higher prevalence of respiratory symptoms and a lower pulmonary function values among mill workers. This study also, showed that, the rate of respiratory allergy was higher among individuals with work-related allergy working in highly exposed place. This result is in line with several studies like, Chia et al.[31] who found that, suffered person from respiratory symptoms and asthma when transferred to another section *(with less exposure to grain dust)* showed marked improvement in the frequency of attacks. Also this result is compatible that of Harmann[34] who stated that the intensity of flour dust exposure is decisive for the incidence and severity of respiratory allergies and asthma in bakers. The present study also in line with Matsumura et al.[22] who found that, the most common symptoms related to work place being rhinitis, followed by itching, skin eruptions and ocular symptoms.

In conclusions, We can conclude that there was a positive relationship between level of exposure and sensitization where most of individuals directed to high dust flour exposure, are more likely to be sensitized. The risk for allergy was increased by current exposure to wheat dust, and a significant difference between individuals with work related allergic symptoms and the years of service. The study recommended the need to develop detailed knowledge on the structure and the immunologic and biochemical properties of major wheat allergens which can be a prerequisite for the development of novel strategies for diagnosis and treatment.

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