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Electrophoretic characterization of possible allergens in population of Hyderabad, Sindh.

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Abstract: Wheat is the most consumable food in the diet worldwide especially in Pakistan and important dietary source of proteins. Some proteins of wheat may be harmful for some people because it triggers the immune response of the person. The present study was an effort to explore the prevalence of food allergy in adult and children in population of Hyderabad Sindh. The participants included in our study are in age ranged 04 - 40 yearsin which 03 children are in infant age while other included different age groups and gender. Ingestion of wheat products in the children can escort the variety of problems mainly abdominal pain and respiratory symptoms. The SDS-PAGE pattern of the selected allergic individual shows at least 02 possible allergens present in all the patients with allergic symptoms asthma, vomiting, urticaria, wheezing, gastrointestinal symptoms, abdominal pain, and respiratory symptoms. Serum of these selected allergic patients mainly contains 15 kDa, 17 kDa, 20 kDa, 47 kDa and 77 kDa major allergenic proteins. The allergic response of individuals was investigated by ELISA, The antibody (IgE) response confirmed that level of IgE of these individual are very high ranged varied from 300-1200 IU/ml.

Keywords; Enzyme Link Immune Sorbent Assay, Protein, Asthma, Respiratory Symptom

1. INTRODUCTION

Allergy to food is the most common term in scientific literature which is widely refers to adverse reaction to any food. Food allergy involves both immunological and non immunological type of response. Food allergy arise when immune system respond to the harmless food, when a person exposed to the food allergy first time no symptoms will occur, but this primary response initiate the secondary response .the second time exposure to the same food can cause the allergic response. The food allergy is common in children as compare to adult globally, it is reported that 1-2% of food allergy in adult while 8% reported in children (Schäf *et al*. 2001, Bock 1987Food allergy mainly instigate in the first 1-2 year of life with the course of sensitization with the immune system retort to specific food proteins, mainly with the of allergen specific protein IgE (immunoglobulin). (Robert *et al,* 2003).Wheat is most consumable food (cereal) in the diet worldwide and important dietary source of proteins. Some protein of wheat may be harmful for some people because it triggers the immune response of the person, while ingested wheat and inhaled in the form of flour (Shaista khan *et al,* 2015). Inhalation of wheat proteins are a major cause of occupational asthma (baker's asthma), a well-known occupational respiratory problem cause by the inhalation of wheat flour (Baur *et al.* 1998). The allergenic proteins are mainly glycoproteins with molecular weight range 3 kDa – 80 kDa), however there is no evidence relate with single structural, functional or chemical property that will describe a glycoprotein protein as allergenic. (Liebers.1996).The protein having a molecular weight 14-17 kDa belong to tryp inhibitor/ alpha amylase called water soluble proteins and these proteins involve in allergic reactions(Posch 1995).Any protein present in food may provoke an allergic reaction. Wheat allergy is kind of food allergy which involve the respiratory tract, food dependent exercise induced anaphylaxis (FDEIA), skin, occupational asthma (baker's asthma), gastrointestinal tract, contact urticaria, and rhinitis. While, ingestion of wheat protein (gluten)causes the skin disorders, T cell-mediated inflammation of small intestine and most common is celiac disease (CD) (Palosuo 2003 and Sicherer 2000). Wheat allergy cause to the patient when specific amount of wheat or wheat product ingested or inhaled cause an IgE mediated symptoms e.g. asthma, abdominal pain, bronchial problems, urticaria, and anaphylactic shock (Morita 2009).

2. MATERIALS AND METHODS

Questionnaire

Questionnaire was filled by 135 patients with positive allergic history; the questionnaire composed of questions regarding complete food allergic history, age, symptom of allergic reaction such as respiratory symptoms asthma, eczema,cough, allergic rhinitis, GIT problems,, rash, abdominal pain andexposure duration, family history of allergy, smoking habits.

Sample collection

Asthmatic/allergic patients (male/female) different age group who were suspected asthma with clinical positive IgE history included in this study. The sera were obtained from patients after consent form was signed. Sera were stored at -40°C until used. Sera were used to perform electrophoresis and the detection of IgE level through ELISA.

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ELISA procedure

ELISA was performed by the kit method (Genesis Diagnostic Ltd, Eden Research Park, Henry Crabb Road, Little port, Cambridge shire CB6 ISE, UK). Characteristically, asthma is often considered as a specific IgE mediated disease, more common in atopic individuals. Common immunological test estimation of specific IgE which is frequently measured by using the enzyme-linked immunosorbent assays (ELISA).

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Characterization of allergic protein SDS – PAGE was performed by following procedure. 30% of acrylamide solution was used for preparation of 15 %Resolving gel with buffer: pH 8.8, and 30% of acrylamide solution was used for preparation of 4.5 % Stacking gel with buffer: pH 6.8 other ingredients for gel were sodium dodecyl sulphate($C_{12}H_{25}NaO_4S$), ammonium per sulphate ($N_2H_8S_2O_8$), N,N,N',N'-tetramethylenediamine(TEMED) and water. For Staining we used Coomassie brilliant Blue R/250(CBB), for decolorizing acetic acid and methanol were used, destaining was continued by changing the destaining solution until the background of the gel turned into transparent and clear band appeared on gel. After destaining the gel was imaged on Gel documentation system.

3. RESULTS AND DISCUSSION

The food allergens mainly embrace protein with diversified biological functions such as enzyme inhibitors, enzymes and storage proteins (Breithender and Ebner 2000), The familiar allergens of wheat are mainly glycoprotein having a property (water soluble and heat stable) with molecular weight ranged 10-70 kDa).

In Pakistan food allergy is not common because there is no awareness about food allergy many people may suffer with food allergy but there is not the proper diagnosis. The respiratory symptoms manifested by these target organs are relatively limited and may be the result of both allergic and non allergic symptoms. Patients with adverse reactions to food can present at any Age. All doctors and many other health professionals should be made aware of the diagnostic criteria and dietary management of specific conditions, and about the existence of unusual organic forms of food intolerance.

In allergic individuals, exposure to an allergen against which the individual have been sensitized may lead to allergic symptoms and the symptoms may occur from the respiratory tract (asthma, rhinitis, conjunctivitis, laryngeal edema), from the oral cavity and gastrointestinal tract (OAS, esophagitis, nausea, vomiting, abdominal cramping, diarrhea), from the skin (eczema, flush, urticaria) or from the cardiovascular system (hypotension, bradycardia, syncope, loss of consciousness, death) (Johansson *et al*., 2004, Abbas 2011) .

Individual sensitive with wheat allergyshown in electrogram **(Fig. 1), (Table 01),**In present study out of 135 food allergic patients 15 individuals are sensitive to wheat in which 05(33%) of male and 10(66%) are females.

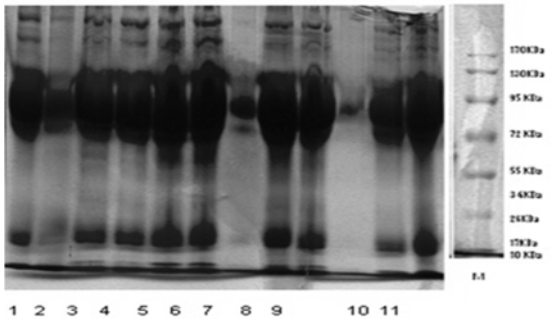

Fig: 1.a: Electrogram of Wheat Allergic patients

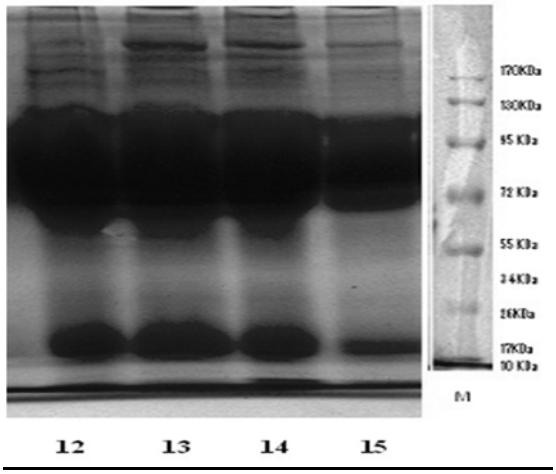

Fig. 1.b: Electrogram of Wheat Allergic patients

Table: 1: IgE concentration of wheat allergic patients with possible Allergenic Proteins and Symptoms:

Patient No	Age(year)/gender	Possible Allergenic proteins in kDa	Concentration of IgE IU/ml	Symptoms
1	20(F)	20,47,77	845	G,U,R
2	18(F)	15,17,31,47	935	U,A,W
3	30(M)	15,17,20,36	644	W,A,U
4	17(M)	15,20,36,77	856.4	A,W,R
5	15(F)	15,47,77	472.2	R,A,W
6	38(F)	15,31	350	W,A,G
7	29(F)	36,77	522	W,A,G
8	20(M)	15,36,47	659	W,A.G
9	40(M)	15,47	801.1	A,V,AP
10	04(M)	15,20	302	U,V
11	05(F)	15,20,47	300	U,V,R
12	05(F)	15,31	704	A,W,G
13	35(F)	15,47,77	564	R,W
14	22(F)	15,20,47,77	1200	R,A,G
15	25(F)	15,77	585	W,A

W = Wheezing, R = Respiratory symptom, U = Urticaria, A = Asthma, G = Gastrointestinal symptoms, AP=Abdominal pain, V=Vomiting

All the allergic patients contains possible allergens having molecular weight 10kDa,15 kDa, 17 kDa, 20 kDa, 31 kDa, 36 kDa, 47 kDa and 47 kDa, 77 kDawhich are major allergens of wheat reported by different scientists. SDS-PAGE pattern of allergic individuals show that at least 02 possible allergens present in all the patients with allergic symptoms Wheezing, Urticaria, Asthma, Gastrointestinal symptoms, abdominal pain, Vomiting and Respiratory symptoms. The patients having allergic history showed the high IgE response 300-1200 IU/ml.

Varjonen *et al* (1995) reported that the IgE binding to 26kDa, 38 kDa and 69 kDa protein in albumin/globulin fraction of wheat in over 50% of 34 serums from wheat allergic children with atopic dermatitis. Another scientist Yokta (1992) identified by immunoblotting six IgE binding wheat proteins with molecular masses between 33 and 98kDa using sera of adults with severe atopic dermatitis and positive RAST to wheat. Paloso *et al*., (2001) and Lethto *et al*., (2003) reported that the ώ-gliadin protein with molecular weight 77kDa are responsible allergen in food allergy and atopic dermatitis. James *et al*., (1997) reported that the low molecular weight proteins are responsible to cause the hypersensitivity after ingestion of wheat specially 15 kDa. The present results are related to the above findings which described the presence of allergenic proteins.

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