Wheat Allergy at Al-Kharj City

Researchers:
Ali Suliman Al-Ghonaim
Saad Rashed Al-Qassim

Supervisor:
Prf. Faris Q Al-Enzy

Salman Bin AbdulAziz University
College Of Medicine

Health Research

Abstract:

Background:

Wheat allergy has been suggested as one important allergic disease in urban and rural areas.

Methods:

In this study, total IgE were measured from 15 cases using Pharmacia CAP system IgE and Pharmacia CAP fluroroenzymeImmunoassay (FEIA).
Specific IgE wew measured using PhadiaImmuno CAP 250 and Immuno CAP 1000 system.
We also assessed the protein allergenicity by western blotting.

Results:

Significant elevation of total and specific IgE was found in 4 cases . Basophlia was also shown by blood film. Western blotting result showed a 2 bands as (20) and 60 KD.

Conclusion:

The result indicates that we have to take wheat allergy in account when discussing asthma and eczema in adult patient at Alkharj.

Introduction:

Due to the large number of different wheat flours that are used in bakeries and mills, it was importance to detect a flour representative of those used in industry, so that the result of this project would be applicable in other sites where flour exposure occurs.

Flours were responsible for 3% of the reported cases of occupational asthma in the KSA in 2013.

No systematic study of difference cereal flours involved in hypersensitivity and associated with respiratory diseases amongest bakers and millers has been previously undertaken.The different allergens in wheat, rye, barley and soya flour have been identified in the studies reported in this project.

Investigations of the nature of different wheat flours have been needed, because although several allergens studies have been carried on in different countries, including: UK, USA, Spain, Germany, and Australia. No information is available on whether wheat from different countries contains similar immunogenic proteins.

The allergy history if critical, not only in selecting the appropriate allergens for testing, but also for testing the allergy test results in order to diagnose food allergy, allergic asthma or allergic rhinitis. An IgE-related mechanism must be demonstrated, since many exogenous substances may cause otherwise clinically indistinguishable syndromes including bronchospasm and urticaria by mechanisms not related to IgE sensitization of mast cells and basophils. The absence of an allergen specific IgE-induced response argues against an allergic mechanism as a cause of the symptoms. To establish the immunologic mechanism, it is necessary to

demonstrate the presence of allergen-specific IgE antibodies at a level sufficient to induce an immunologic response following appropriate antigen challenge in vivo or by measuring the quantity of allergen specific IgE in vitro.

Test results for the presence of allergen specific IgE, whether in vitro (RAST) or in vivo (skin test) may be considered clinically relevant only if there is a history compatible with symptoms induced by exposure to the allergen. Additionally, test results indicating merely the presence of allergen-specific IgE in the serum do not necessarily indicate that the patient has clinical disease related to exposure to this allergen. All test results must be interpreted in the context of the patient's allergy history, and laboratory tests should not be used as the sole criteria for establishment of the diagnosis of allergy.

A number of studies which have compared in vitro specific IgE tests with skin tests and allergen bronchial challenges have reported good correlations (2-4). Generally, the degree of quantitation in the measurement of allergen-specific IgE values is acceptable considering that the allergen extract composition may differ between companies, this correlation being supported by a concordance of approximately 80% between the majority of the in vitro assays (5-6). Thus, in vitro allergen specific assays, which make use of well-characterized and in-house standardized allergens with a high level of quality control and quality assurance, provide the most reliable clinical test results. All allergy test results must be interpreted in the context of the test sensitivity (the ability to detect true positive test results), specificity (freedom from false positive results) and efficiency (likelihood of the test detecting only true positives or true negatives).

Allergen-specific IgE assay sensitivity may vary and depends on the system being used and the quality of the allergens. A number of studies show that sensitivity and specificity are allergen dependent, although allergen specific IgE directed against the majority of inhalant allergens generally are able to be detected by most systems. Sensitivity ranges from 60% to 95% and specificity ranges from 30%-95%. When discordance is noted this is largely due to differences in the antigens bound on the solid phase matrix of the systems, causing discrepant test results when different systems are employed for detection of allergen specific IgE.

The overall concordance (efficiency) between skin test and in vitro tests are approximately 70%-90%. Most in vitro allergy tests show a test concordance of 80-90%. Clinical relevance off positive test results It is appropriate to discuss the presence of positive specific serum IgE test results in clinical and laboratory context separately. Elevated levels of specific IgE may indicate presence of allergy. The specific IgE measurement may be useful because it can alert the physician to the possibility of an allergic disease. A strong correlation exists among positive skin tests with common inhalation antigens, high total serum IgE concentration, and the presence of specific IgE against particular antigens. However, some patients develop specific IgE against allergens without showing clinical symptoms. The exact mechanism of this is unknown. Although, the presence of specific IgE may also be documented by other laboratory methods (e.g. immunoblotting) and inflammatory cell activation (e.g. histamine release from basophils) may occur, patients will not experience symptoms when exposed to the appropriate allergen.
When patients demonstrate increased levels of specific IgE without clinical symptoms, it indicates sensitization to allergens, however, no clinical symptoms have developed and may never become apparent. An example is beekeepers serum, which may show high levels of specific IgE against insect venoms, however, non-

allergic beekeepers never experience allergic reactions when stung by the insects. Low levels of specific IgE to allergens may be perfectly normal as often observed in patients with increased levels of total IgE, e.g. patients with atopic dermatitis. Thus, the positive laboratory result may be correct, however, symptoms are unlikely to appear.

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Higher serum IgE levels support the diagnosis of allergic disease, but a low IgE does not exclude the presence of an allergic disease. In general, patients with hypersensitivity to several allergensand multiple allergic diseases have elevated serum specific lgE to multiple allergens and those with hypersensitivity to fewer allergens and limited end-organ involvement (e.g. rhinitis) usually have fewer positives. When interpreting multiple positives it always necessary to examine the analytical specificity by checking the negative control included in the run. Rarely will patients be positive to all allergens tested. When such patient samples appear the analytical precision must be checked. In some assays affected by matrix increased serum IgE levels may affect the test results and show binding of specific IgE.

Typically, the test will show low levels of binding, e.g. class 1 test results When the test shows all positives at increased levels of IgE, e.g. class 4 results one should consider repeating the test to check for analytical imprecision One possible use of the total IgE test is in relation to certain in vitro tests for specific IgE measurements, which are influenced by high levels of total IgE leading to false positive test results. In such situations a high total IgE test may help the physician to interpret and disregard low class false positives on the specific IgE test. If it is suspected that the test matrix is affected by high levels of total IgE it is not recommended to dilute the serum sample. This way results in diluting out low levels of specific IgE however clinically relevant. It is more appropriate to recommend another test modality, e.g. skin test and to report the in vitro test results as analytical indeterminate

The effects of allergen cross-reactivity extend beyond those experienced by the patient. Cross-reactivity may also affect test results. For example, some mite allergens such as tropomyosin, are widely cross-reactive Periplanetaamericana (American cockroach) tropomyosin showed 80%, 81% and 82% sequence identity to tropomyosins from D. pteronyssinus, D. farinae and shrimp.1 Likewise, the immunochemical similarity of several of the groups of well-studied homologous grass allergens is extensive. Consequently, when a patient with a grass allergy is tested with a test that has a low specificity for grass allergens, the patient may also test positive to other grasses as well.

The same holds true for several free pollens. Several studies have documented clinically important cross-reactivity between pollens from the related trees: birch, alder and hazel with certain foods, e.g. apple, nuts. This is a key concern when testing patients in northern parts of Europe, Asia and North America where birch is a common allergen. Assays with lower specificity make it difficult to unequivocally determine the identity of the symptom-producing allergen. Finally it has also been observed that certain allergens contain cross-reacting carbohydrate (7-8). These molecules bind to IgE, however they cannot crosslink IgE molecules. Hence skin test or basophil histamine release will produce negative test results although the invitro specific IgE will be positive.

Unfortunately, one single measurement or observation cannot determine clinical allergy. The specific IgE only constitute one part of the allergic cascade including inflammatory cells, mediator releasability and end organ sensitivity. This makes the use of serum specific IgE as the sole criteria to determine atopy impossible. Any utilization of a serum specific IgE level must be in the clinical context of the likelihood of the presence of an allergic disease.

Methodology:

1- Selection of flours:

Five additive free flours were initially obtained from Al-Kharj area, there were: Saudi wheat meal, Dubai wheat meal, Spring self-rising flour, soya flour, and Saudi brown wheat. The flours brands are representative of those used in the KSA.

2- Collection of hypersensitive serum:

Blood were obtained by venipuncture from 15 flour-exposed individuals at Al-Kharj city. Blood was allowed to coagulate for 2 hours, serum separated from the clot by centrifugation at 1500 rpm for 10 minutes. Serum was stored in closed vials at -20 °C.

3- Execration of flour:

Water and salt soluble proteins were extracted from the flours by vigorous shaking as 10% weight/volume mixture overnight at 20 °C in 0.1 M ammonium hydrogen carbonate. The resultant solution was centrifuged for 30 minutes at 2000 rpm at 4 °C.

The supernatants were dialyzed with 4 time cold water.

The resultant extract was stored at -20 °C.

4- SDS-page:

It is an electrophoretic method of protein sulfate in polyacrylamide based on MW first described by Laemmli (ref. 133).

Protein samples are denatured with the strong detergent SDS in ratio 1:1-4.

Electrophoresis is preformed in a solid sulfate of polyacrylamide using constant carreat. The distance travelled towards a node is inversely proportional to the size of the protein.

5- Western bloating of different wheat flours:

Non-reduced and reduced extracts of the five different flours were transferred to Nitrocellulose and probed with a pool of sera (antibodies).

6- Total and specific IgE:

Total IgE concentration in serum was determined by Pharmacia CAP system IgE (Uppsala, Sweden). The determination of specific IgE antibodies to (Wheat), (Gluten), (Brown wheat) was reformed using pharmacia CAP fluroroenzymeImmunoassay (FEIA). Total IgE measurements provide a useful insight into an individual's allergy derive. We measure total IgE level, on the Immuno CAP 1000 system. Clinically significant specific IgE to allergens is on common when total IgE is less than 2 U/L.

Specific IgE is usually measured to confirm allergic etiology symptoms. All our specific IgE testing is performed on PhadiaImmuno CAP 250 and Immuno CAP 1000 system. Reference range is 0.1 – 0.35 IU/L.

Reference range of total IgE is 0.35 – 200 U/L.

Reference range of specific IgE is 0.1 – 0.4 U/L.

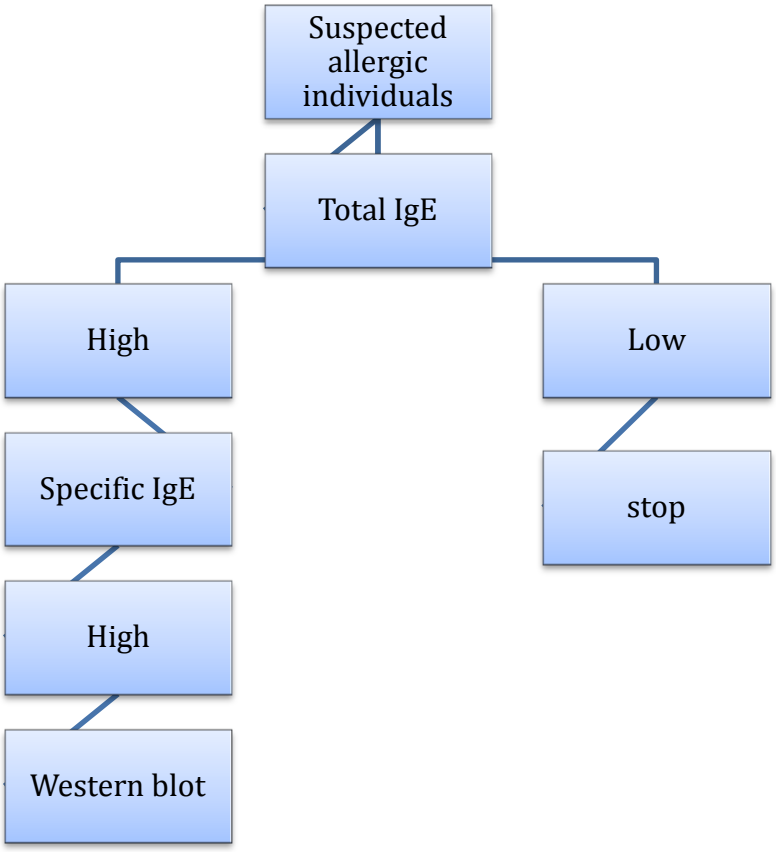

Figure no. (1) shows the protocol we used in our study.

Western blotting protocol summary:

Stage	Reagent	Volume used	Time
1- Electrophoresis and blotting	-	-	Usual Electrophoresis and blottingtimes
2- Block	5% dried milk in TBS-T or PBS-T	10 ml	1 hour
3-Wash	TBS-T or PBS-T	10 ml	1 x 15 mins 2 x 5 mins
4-Primary antibody	Diluted in TBS-T or PBS-T	10 ml	1 hour
5-Wash	TBS-T or PBS-T	10 ml	1 x 15 mins 2 x 5 mins
6- Biotiylated antibody or HRP labelled antibody	Diluted in TBS-T or PBS-T	10 ml	20 mins – 1 hour
7-Wash – If using HRP labelled antibody omit steps 7 and 8	TBS-T or PBS-T	10 ml	1 x 15 mins 2 x 5 mins
8-Streptavidin – HRP	Diluted in TBS-T or PBS-T	10 ml	20 mins – 1 hour
9-Wash	TBS-T or PBS-T	10 ml	1 x 15 mins 4 x 5 mins
10-Detection	Mix the two reagents 1:1	0,125ml/cm2	1 min
11-Exposure	Drain excess reagent cover with SaranWrap	-	Immediately expose to film for 30 seconds – 10 mins

Figure 2: shows western blotting protocol we used throughout this study.

Results:

IgE analysis of deferent wheat flours:

IgEmeasurement of 15 sera were used to analyze the different wheat flours that are used in bakeries. Barley, Corn, wheat Gluten and wheat were collected as immunologically representative of the wheat typhus used within KSA, IgE and western blotting have been conducted. A good correlation was found between the severity mucosal irritation and higher IgE and Basophilia (R = 0.01).

Table 1, shows the proteins details, Basophil percentage and measurement of total and specific IgE.

Table 2, shows how much different allergens weigh in each extract, including wheat, gluten and brown wheat. There was a significant difference between allergic group (n = 5) and control group (n = 5); (P = 0,00078) . (Mann-Whitney Test).

Table 1:

Sample No.	Age	Nationality	Medical History (Asthma)	Years in Bakery	IgE total	IgE Specific	Blood Basophils
1	45	Bangladeshi	Yes (Rhinitis)	21	755	297	5
2	37	Bangladeshi	yes	17	1031	571	9
3	48	Bangladeshi	yes	18	920	308	6
4	24	Indian	no	1	72	0,48	0
5	24	Indian	no	1	84	3,5	0
6	42	Bangladeshi	no	19	101	2	0
7	45	Bangladeshi	no	22	79	3,1	1
8	40	Bangladeshi	no	20	80	11	0
9	30	Bangladeshi	no	13	64	21	0
10	47	Bangladeshi	no	20	60	19	0
11	53	Bangladeshi	no	30	98	40	0
12	53	Bangladeshi	no	20	51	0,9	0
13	35	Bangladeshi	eczema	15	1270	350	13
14	47	Bangladeshi	yes	20	1800	411	1
15	45	Bangladeshi	no	21	108	2	1

Table 2:

Molecular Weigh (MW KDa)	Flour		
	Wheat (white)	Gluten	Brown wheat
	124	124	140
	83	83	83
	40	40	40

Western blotting of different wheat: Reduced extracts of wheat Gluten, Wheat, Brown wheat were transferred to nitrocellulose and probed with a pool of sera. A large number of proteins were present in both reducing and non-reducing flow samples, suggesting that many of allergens may be monomer or polymers without intra-chain disulphide bands.

Figure 3:

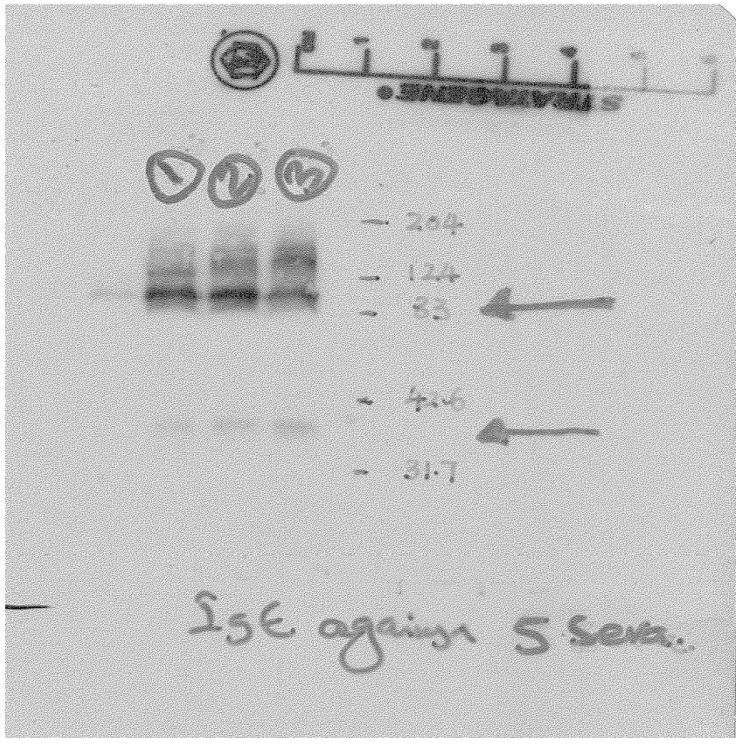

Figure 3, shows the western blotting after electrophoresis of 3 extracts.

There are 2 bands exist at 83 KDa and 40 KDa. Most importantly, this indicate that all the three extracts has 2 common allergens in between.

Discussion:

Work has been conducted in this project to identify the allergens associated with occupational flour hypersensitivity. Flours were responsible for 3 % of reported cases of occupational asthma in the KSA (1). No systemic study of different cereal flours involved in hypersensitivity and associated with RT disease among bakers has been previously undertaken. The different allergen, in Wheat, Brown wheat, and Wheat Gluten have been identified in this study.

There are at least two papers showed an agreement to our reported results here (1, 8) Analysis of IgE data indecently but have a high degree of allergenic similarity. Therefore, it would be of great interest to do RAST inhibition technique to show the various flours have a high degree of immunological identify. The result of IgE and western blotting in addition to electrophoresis data obtained. Indicates that thus can be used as a standardin immunoassay to measure airborne flour proteins. Specific IgE measured by Phamacia-CAP system, because the systems specificity for measured flour specific IgE has to be confirmed by RAST inhibition analysis using flour– human serum albumin conjugate as an inhibitor.

Western blotting was used for immunochemical comparison of 4 different wheat flours. This comparison was undertaken to determine whither wheat flours are immunologically similar and contain similar concentration of allergens. Western blotting of the different wheat flours using a pool of hypersensitivity sera, showed that different flours contained similar number of allergens with MW between 98 and 35 kDa. Western blotting showed that the difference flours had a similar allergen profile. It also showed that reduction o flours samples using 5 mM DTT had little apparent effect on allergen pattern of flours.

Examination of the data obtained from western blotting of wheat using 5 sera, showed that 13 allergens with molecular weight (MW) range from 98 – 35 kDa. The widespread occurrence of the majority of these allergens in wheat, wheat

Gluten, Brown Wheat may be responsible for high level of cross reactivity observed between these 3 flours. The 83 KDa proteins were identified as a major allergen of all 3 flours. This study may explain the cross reactivity between wheat flours.

Knowledge of allergens would allow development of monoclonal antibody based assay to many specific flour allergens.

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