ORIGINAL ARTICLE

# PREVALENCE OF WHEAT ALLERGY IN AL-KHARJ, SAUDI ARABIA

**Ali S. Al-Ghonaim, Saad R. Al-Qassam, Omar Kujan*, Bassel Tarakji*, Azzegahiby S*, Azzegahiby I*, Richard K.H. Wyse**, Mohammed W. Al-Rabia***, Faris Q.B. Alenzi†**

College of Medicine, Salman bin Abdulaziz University, Al-Kharj, *Al-Farabi Dental College, Riyadh, KSA,
**Department of Surgery, Imperial College of Medicine, London, UK W12 ONN,
***Department of Immunology, College of Medicine, King Abdulaziz University, Jeddah, KSA,
†Med Lab Sci, College of Applied Medical Sciences, Salman bin Abdulaziz University, Al-Kharj, KSA

**Background:** Wheat allergy has been suggested to represent an important allergic disease. This study collates clinical and laboratory aspects in patients with wheat allergy in Al-Kharj city, Saudi Arabia. **Methods:** Total and specific IgE were measured in 15 suspected cases of wheat allergy. Protein allergenicity was assessed with Western blotting. **Results:** Significant elevation of total and specific IgE was found in 4 cases. Basophlia was also demonstrated on blood film. Western blotting results showed 2 bands (83 and 40 kDa). **Conclusion:** Wheat allergy must also be considered when planning treatment of asthma and eczema of adult patients.

**Keywords:** Allergy, IgE, Asthma, Eczema, Wheat, Saudi Arabia

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## INTRODUCTION

Due to the large number of different wheat flours that are used in bakeries and mills, it is important to detect flour that was representative of those used in industry and other sites where flour exposure also occurs. Flours were responsible for 3% of the reported cases of occupational asthma in the KSA in 2013.[1]

No systematic study of the different cereal flours involved in hypersensitivity and associated with respiratory diseases amongst bakers and millers has been previously undertaken. Different allergens in wheat, rye, barley and Soya flour have been identified in the previous reported studies.

An investigation of the nature of different wheat flours was 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.[2]

There are three main criteria for establishing allergy diagnosis. One must identify the allergen, establish a causal relationship between exposure to allergen and the occurrence of symptom, and demonstrate an IgE -mediated immunologic reaction.

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 an 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.[3–7] 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 majorities of the *in vitro* assays.[4,5] Thus, *in vitro* allergen specific assays, which make use of well-characterised and in-house standardised 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 are generally able to be detected by most systems. Sensitivity ranges from 60–95% and specificity ranges from 30–95%. Discordance 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 tests and *in vitro* tests are approximately 70–90%. Most *in vitro* allergy tests show a test concordance of 80–90%. It is appropriate to discuss the presence of positive specific serum IgE test results in a clinical, and a 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., immuno-blotting) and inflammatory cell activation may occur (e.g., histamine release from basophils), patients will not experience symptoms when exposed to the appropriate allergen. When patients demonstrate increased levels of specific IgE without clinical symptoms, it indicates sensitisation to allergens; however, no clinical symptoms have developed and may never become apparent. 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.

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 allergens and 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. It is more appropriate to recommend another test modality, e.g., skin test and to report the *in vitro* test results as an 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. Periplaneta americana (American cockroach) tropomyosin showed 80%, 81% and 82% sequence identity to tropomyosins from D. Pteronyssinus, D. Farinae, and shrimp. Likewise, the immunochemical similarity of several of the groups of well-studied homologous grass allergens is extensive. 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 related trees with certain foods. 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 unequivocally to determine the identity of the symptom-producing allergen. Finally, it has also been observed that certain allergens contain cross-reacting carbohydrate.[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 in-vitro specific IgE will be positive.

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

## MATERIAL AND METHODS

A total of 15 flour-exposed individuals at Al-Kharj city were enrolled in this study. All patients were subjected to clinical examination and were assessed for eligibility. Blood was obtained by venipuncture suspected of having allergic symptoms associated with

direct or indirect exposure to animal products. Blood was allowed to coagulate for 2 hours, serum separated from the clot by centrifugation at 1,500 rpm for 10 minutes. Serum was stored in closed vials at -20 °C. The study was conducted between June and September 2013 in Salman Bin Abdul Aziz University Hospital, Al-Kharj, KSA. All participants provided informed consent before enrolment in the study. Determination of eligibility was based on medical history and physical examination. Additionally, 5 healthy controls age ranging 29–55 years (40.56±9.13), were run in parallel.

1. **Selection of flours:** Five additive-free flours were initially obtained from the Al-Kharj area; these were: Saudi wheat meal, Dubai wheat meal, spring self-rising flour, soya flour, and Saudi brown wheat. The brands of flour were representative of those used in the KSA.
2. **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 min. at 2,000 rpm at 4 °C. The supernatants were dialysed with 4 times on cold water. The resultant extract was stored at -20 °C.
3. **SDS-page:** This is an electrophoretic method of protein sulfate in polyacrylamide based on molecular weight (MW) first described by Laemmli *et al* (personal communication). Protein samples were denatured with the strong detergent SDS in the ratio 1:1–4. Electrophoresis was performed in a solid phase of polyacrylamide using constant carreat. The distance travelled towards a node is inversely proportional to the size of the protein.
4. **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).
5. **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 Fluroroenzyme Immunoassay (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 uncommon, when total IgE is less than 2 U/L.

Specific IgE is usually measured to confirm allergic aetiology symptoms. All our specific IgE testing was performed on the Phadia-immuno CAP 250 and Immuno CAP 1000 systems. 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.

## RESULTS

IgE level was measured from the sera of 15 patients used to analyse 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 of mucosal irritation and higher IgE and Basophilia (R=0.01) [data not shown].

Table-1 shows the protein details, Basophil percentage and measurement of total and specific IgE. Table-2, shows how much different allergens weight 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).

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; this suggests that many of allergens may be monomer or polymers without intra-chain disulphide bands.

**Table-1: Protein details, Basophil percentage and measurement of total and specific IgE**

| Sample No. | Age | Nationality | Medical History | Years in Bakery | IgE total | IgE Specific | Basophils |
|---|---|---|---|---|---|---|---|
| 1 | 45 | Bangladeshi | 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 |

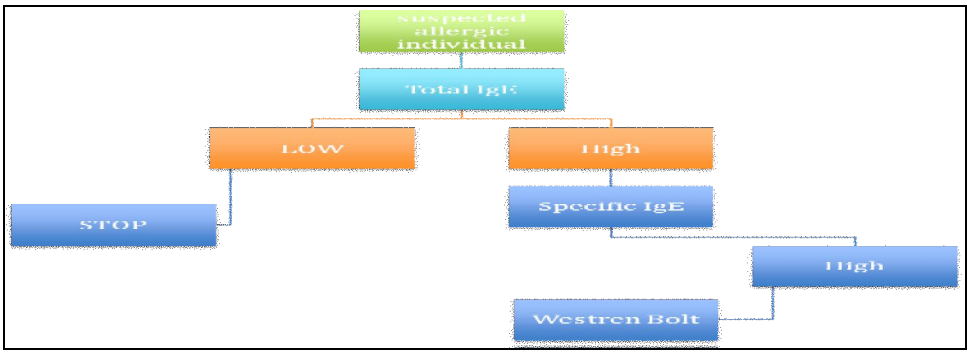


**Figure-1: The protocol used in this study**

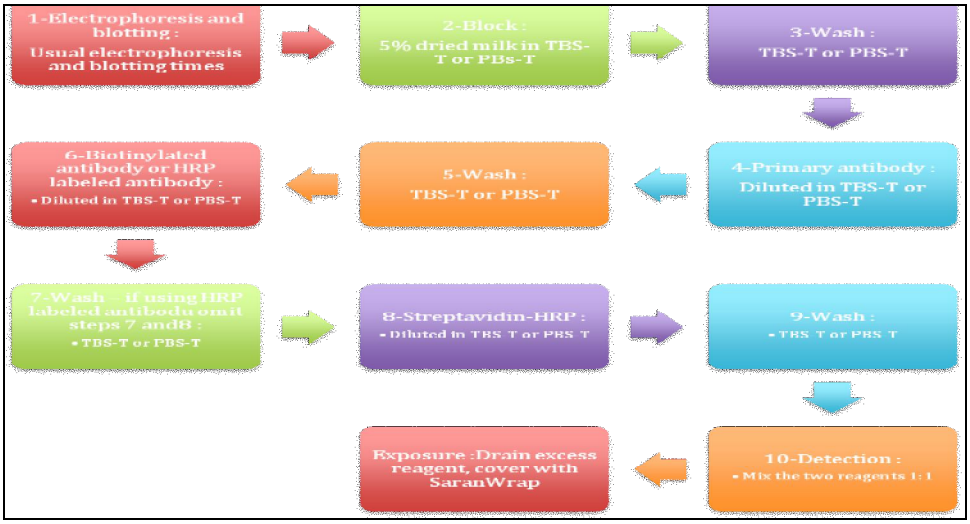


**Figure-2: Western blotting protocol used**

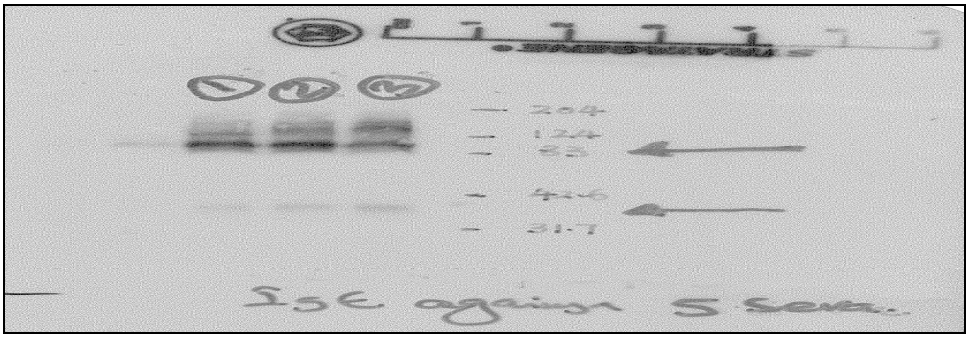

**Figure-3: Western blotting results after electrophoresis of 3 extracts**

**Table-2: Molecular weight of allergens in wheat, gluten and brown wheat**

| Parameter | Wheat (white) | Gluten | Brown wheat |
|---|---|---|---|
| Molecular Weight (KDa) | 124 | 124 | 140 |
| | 83 | 83 | 83 |
| | 40 | 40 | 40 |

## DISCUSSION

In this study, work has been conducted to identify the allergens associated with occupational flour hypersensitivity. Flour was 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 allergens in wheat, brown wheat, and wheat gluten have been identified in this study. At least two papers showed an agreement to our reported results here.[1,8] IgE data have a high degree of allergenic similarity. It would be of great interest to use RAST inhibition technique to show a high degree of immunological identity of various flours. The results of IgE and western blotting in addition to electrophoresis data obtained indicate that this can be used as a standard in immunoassay to measure airborne flour proteins. Specific IgE was 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.

The immunochemical comparison was undertaken to determine whether 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 83 and 40 kDa. Western blotting showed that different flours had a similar allergen profile. It also showed that reduction of flour samples using 5 mM DTT had little apparent effect on allergen pattern of flours.

There were 13 allergens with MW 98–35 kDa. The widespread occurrence of the majority of these allergens in wheat, wheat gluten, and 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 of specific allergens.

## CONCLUSION

Wheat allergy must also be considered when planning treatment of asthma and eczema of adult patients.

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## Address for Correspondence:

**Faris Q Alenzi,** Professor of Immunology, College of Applied Medical Sciences, Salman bin Abdulaziz University, Saudi Arabia.
**Email:** fqalenzi@ksu.edu.sa