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Evaluation of The Immune Response to Wheat Allergy in Wasit Province

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Abstract

Wheat allergy has become increasingly prevalent worldwide, although the mechanisms of wheat protein allergenicity remain unclear. This study aimed to evaluate the immune response to wheat allergy in Wasit Province by measuring serum levels of total IgA, IgG, tTG-IgA, and tTG-IgG. Samples were collected from clinically and laboratory-confirmed patients at Kut Hospital between January 2024 and April 2025. Antibody concentrations were assessed using the ELISA technique. The concentration of IgA ranged from 0.7-32.3 U/ml. The concentration range of IgG ranged from 4.2-48 U/ml. Range of serum concentrations of antibodies to transglutaminase tTG-IgA and tTG-IgG were 0.7-32.3 and 4.2-48 U/ml respectively. Serum concentration of studied antibodies (except tTG-IgA) does not statistically effect by aging. Also, all antibodies not significantly affected by patients, all these immune markers increased in females compared to males. Linear positive correlation appeared among studied antibodies. Moreover, the strongest positive relationship is determined between IgA/ tTG-IgA, followed by IgA/IgG, IgA/ tTG-IgG.

Keywords: Wheat Allergy, IgA, IgG, tTG-IgA, tTG-IgG, and ELISA.

1.Introduction

In many nations, including Iraq, wheat flour is one of the most essential food sources. Particularly among youngsters, certain foods have been linked to food allergies [1]. The most significant element aggravating food allergies is the delay in determining the cause, which leads to the patient's condition worsening. The production of immunological mediators from mast cells and basophils, including histamine, platelet-activating factor, and leukotrienes, is a sign of wheat allergy [2].

The response to wheat antigens is also thought to be influenced by antibodies. Celiac disease is one of the many allergic

reactions that are indirectly caused by IgG and IgA antibodies. It is believed that the breach of oral tolerance leads to a skewed immune response to type 2 helper T cells, which sensitizes the body and drives B cells to produce IgE [3].

A particular autoantibody against tissue transglutaminase 2 (anti-tTG2), endomysium, and deamidated gliadin peptide is what distinguishes wheat allergy from celiac disease. Gliadin peptides that cross the epithelial barrier activate CD4 T-lymphocytes, which in turn produce high levels of pro-inflammatory cytokines [4]. These cytokines activate T-helper 1 pattern interferon-gamma and a T-helper 2 pattern, which cause B-lymphocytes to proliferate and eventually differentiate into plasma cells that secrete anti-gliadin and anti-tissue-transglutaminase proteins.

The role of IgG food antibodies in mediating food intolerance is still unclear, though, and some laboratories have used and continue to use the measurement of serum concentrations of these antibodies as a guide for dietary interventions. According to expert societies and food allergy guidelines, there is insufficient evidence to support the use of these tests; it has been suggested that the presence of IgG food antibodies simply reflects exposure to the corresponding foods and indicates immunological tolerance [2-5].

Wheat-associated conditions include celiac disease, wheat allergy, and non-celiac gluten-sensitivity. A subset of patients with wheat-associated symptoms do not fit into these recognized conditions. In the absence of biomarkers their diagnosis remains a clinical challenge [6]. In this study we examined the utility of wheat-specific, IgA, IgG, tTG-IgA and tTG-IgG in the diagnostic and management of non-IgE mediated wheat allergy.

2. Material and Methods

2.1. Study Design and Samples Collection

The current study is a cross-section study that includes collecting questionnaires and blood samples from 173 patients with wheat allergy of different ages. Clinical examinations were conducted by specialists at Al-Kut Hospital. Ethical approval was obtained from the hospital and patients before collecting information and samples. The most important symptoms used in selecting patients were digestive disorders, such as poor growth, abdominal pain, constipation, vomiting, diarrhea, or skin rash.

Moreover, to avoid a false positive test result, patients with autoimmune diseases were excluded, especially those with concomitant autoimmune disorders such as type 1 diabetes, autoimmune liver

disease, Hashimoto's thyroiditis, psoriatic or rheumatoid arthritis, and heart failure.

2.2. Immunological Study

Study main aim is to help physicians detect celiac disease, specifically wheat allergy, the tissue transglutaminase (tTG) IgA (tTG-IgA) and IgG (tTG-IgG) tests were utilized as part of the examination. The immune system misinterprets gluten as a foreign material, leading to an autoimmune disease. Tissue transglutaminase is an intestinal enzyme that is attacked by tTG-IgA, which is produced by the immune system.

An ELISA based on recombinant human tTG (Pharmacia Diagnostics, Freiburg, Germany) was used to measure the levels of IgA, IgG, tTG-IgA, and tTG-IgG antibodies. Samples that produced a value higher than 100 U/ml were reexamined using larger dilutions. The antibody levels in patient serum samples diluted 1:101 were assessed by comparing them with the levels on a standard curve (antibody concentration range, 0 to 100 U/ml).

2.3. Statistical Analysis

Numbers and percentages (%) of totals and ranges are used to represent descriptive statistics. In the direction of find differences between wheat allergy patients with high or low anti-tTG IgA titers, univariate analyses were conducted using the t-test, Fisher's exact test, and/or chi-squared test for continuous or categorical variables. P values with two tails < 0.05 were deemed statistically significant. MedCalc© Statistical (MedCallc Software bv, Ostend, Belgium) conducted the statistical analysis.

3. Results

The study is a quarterly study conducted on wheat allergy patients in Wasit Governorate. It included collecting samples and a questionnaire from 173 patients diagnosed by specialists. Patients with autoimmune diseases and other types of allergies were excluded. Ages of patients ranged from 1 to 78 years, with an average age of 19.22 ± 9.57 years. Half of the patients (87/173) were under the age of 15, while a very small percentage (3%) were over the age of 60, as shown in (table 1). On the other hand, most of patients were female with 58%, while the percentage of males was 42%.

Table 1: Age and sex properties of patients.

Properties	Cases
Age range	1-78 years
Age mean ± SD	19.22 ± 9.57
SE	0.73
Age groups	
1-15	87 (50%)
15- 30	57 (33%)
30- 45	13 (8%)
45- 60	11 (6%)
>60	5 (3%)
Gender	
Females (%)	100 (58%)
Males (%)	73 (42%)
Total number	173

Immunologically, the total concentration of antibodies was evaluated using ELISA for patients' serum. The concentration range of IgA ranged from 0.7-32.3 U/ml with mean ± SD concentration of 6.64 ± 5.55 U/ml. The concentration range of IgG ranged from 4.2-48 U/ml with mean ± SD concentration of 7.42 ± 3.95 U/ml, as listed in (table 2). Range of serum concentrations of antibodies to transglutaminase tTG-IgA and tTG-IgG were 0.7-32.3 and 4.2 -48 U/ml respectively with mean ± SD of serum concentration equal to 6.64 ± 5.55 U/ml and 7.42 ± 3.95 U/ ml respectively as described in (table 3).

Table 2: Total serum concentration of IgA and IgG.

Serum Conc. (U/ml)	IgA	IgG	P value
Range	0.7 – 32.3	4.2 - 48	
Mean ± SD	6.64 ± 5.55	7.42 ± 3.95	0.291
SE	0.43	0.26	

Table 3: Serum concentration of antibodies to tissue transglutaminase tTG-IgA and tTG-IgG.

Serum Conc. (U/ml)	tTG-IgA	tTG-IgG	P value
Range	1.1-60	1.3- 64.3	
Mean ± SD	10.89 ± 9.54	15 ± 11.07	0.111
SE	0.73	0.84	

Distribution serum concentration of patients according to age groups counted in (table 4). Total concentration of IgA mainly increased in age group 30-45 years (9.6 U/ml) followed by age group 45-60 years (7.47 U/ ml) and clearly decreased in older patients over 60 years (4.15 U/ ml).

Total concentration of IgG mainly increased in age groups over 60 years (9.68 U/ml) and under 15 years (8.21U/ml) and decreased in age group 30-45 years (6.18 U/ml). serum concentrations of antibodies to transglutaminase tTG-IgA and tTG-IgG mainly determined in age group 45-60 years 21.62 U/ml and 17.78 U/ml respectively.

At the same time tTG-IgA significantly decreased in age group 30-45 years (3.50 U/ml) while tTG-IgG slightly decreased in other age groups especially age group > 60 years (12.02 U/ml).

However, serum concentration of studied antibodies (except tTG-IgA) does not statistically effect by age staging (P value 0.05). In (table 5), serum concentration of IgG, IgA, tTG-IgA and tTG-IgG antibodies not significantly affected by patients 'gender (P value> 0.05) although all these immune markers increased in females (8.02, 7.23, 11.72, 15.31 U/ml respectively) compared to males 6.61, 5.82, 9.61, 14.61 U/ml respectively.

Table 4: Distribution of antibodies concentration according to patients' gender.

Age groups (years)	Serum Conc. (U/ml)			
	IgA	IgG	tTG-IgA	tTG-IgG
1-15	6.59	8.21	9.62	17.33
15- 30	5.77	6.52	9.19	13.82
30- 45	9.6	6.18	3.50	14.73
45- 60	7.47	6.35	21.62	17.78
>60	4.15	9.68	16.85	12.02
P value	0.299	0.333	0.048	0.274

Table 5: Distribution of antibodies concentration according to patients' gender.

Serum Conc. (U/ml)	Females	Males	P value
IgA	7.23	5.82	0.331
IgG	8.02	6.61	0.204
tTG-IgA	11.72	9.61	0.306
tTG-IgG	15.31	14.61	0.513

Linear positive correlation appeared among studied antibodies (table 6). Moreover, the strongest positive relationship determined between IgA/ tTG-IgA (r = 0.7000), followed by IgA/IgG (r = 0.6493), IgA/ tTG-IgG (r = 0.5935) while the weakest correlation detected between IgG/ tTG-IgA (r = 0.3004) and tTG-IgA/ tTG-IgG (r = 0.4988). IgA, IgG, tTG-IgA and tTG-IgG can be used as diagnostic or prognostic parameters at cut off values > 10.02, 8.29, 13.90, 17.35 U/ml for IgA, IgG, tTG-IgA and tTG-IgG at 100% specificity and 98%, 98%, 99% , and 98% sensitivity for IgA, IgG, tTG-IgA and tTG-IgG respectively with PPV equal to 99%, 95%, 100%, and 96% as listed in (table 7).

Table 6: Pearson Correlation Coefficient (r) among studied antibodies.

r-correlation	IgA	IgG	tTG-IgA	tTG-IgG
IgG	0.6493	------	0.3004	0.5773
IgA	------	0.6493	0.7000	0.5935
tTG-IgA	0.7000	0.3004	------	0.4988
tTG-IgG	0.5935	0.5773	0.4988	------

Table 7: Validity values of studied antibodies as diagnostic markers of wheat allergy.

Antibodies	Positive if > cut off value (U/ml)	Specificity	Sensitivity	Accuracy	Positive predictive value (PPV)
IgA	10.02	100%	98%	100%	99%
IgG	8.29	100%	98%	100%	95%
tTG-IgA	13.90	100%	99%	100%	100%
tTG-IgG	17.35	100%	98%	100%	96%

4. Discussion

The study conducted in Wasit Governorate to evaluate the role of antibodies in wheat allergies. The study found that half of the patients were under 15 years of age, with females more susceptible than males. A small group of patients over

60 years of age. Through patient follow-up and laboratory tests, including immunological tests, Symptoms of wheat allergy vary according to age, gender, and diet. Some patients suffer from stomach sensitivity, but symptoms appear mild or late due to dietary habits [7].

Gender plays a clear role in the early onset of symptoms in females. This may be due to the role of sex hormones or the nature of female bodies, which are more responsive to immune reactions [8]. However, there are no sufficient studies to clarify this.

The immature or underdeveloped immune systems in children make them more susceptible to many celiac diseases, especially wheat allergies [9]. Diagnosing antibodies to the enzyme tissue transglutaminase, especially the IgA test, is the most sensitive and effective method for testing for celiac disease. Positive results in approximately 98% of people with celiac disease who consume wheat gluten. Test results may also vary based on age, gender, medical history, and the test used [10].

The current study showed that 50% of patients were children. These findings are consistent with a previous study by Cianferoni (2016), which found that food allergies to wheat are more common in children and can be associated with severe reactions such as anaphylaxis and exercise-induced wheat-related anaphylaxis [1]. Similarly, another study found that wheat allergy was the third most common food allergy developing during childhood in Japan [11]. Koike et al. note that a history of anaphylaxis to all foods, including wheat, and elevated levels of wheat-specific antibodies or omega-5 gliadin have been identified as risk factors for persistent wheat allergy in the elderly [11].

In another study, compared with adults, children had a higher rate of comorbid allergic diseases $p = 0.004$, 0.001, respectively and a higher rate of other food allergies $p < 0.001$, < 0.001, respectively [12]. In the study by Bai et al., the variable clinical picture of wheat allergy is due to genetic and immunological underpinnings, with age of onset, extent of mucosal involvement, dietary habits, and gender influencing the clinical presentation of the disease [13].

Remarkably, majority of studies, show that females are more likely than males to have wheat allergy, with a male-to-female ratio of 2.8:1. The fact that men with wheat allergies are diagnosed later in life may be the cause of this. Indeed, because of their adoption of Western dietary practices e.g., limited or short breastfeeding, early weaning, and high gluten intake, children of immigrants from Eastern Europe, North, West, and East Africa, the Middle East, and South Asia have been reported to have cases of celiac disease.

This implies that although a high number of people may be genetically predisposed to wheat allergy, clinical symptoms do not manifest until adequate amounts of gluten are consumed [14]. Majority of earlier research verified wheat allergy by measuring serum immunoglobulin. The first screening test that should be ordered is an antibody to tissue transglutaminase (anti-tTG). Deamidated gliadin peptides-antibody of the IgG class can be utilized as a preliminary screening test for individuals with IgA deficiency. It is recommended to order an IgA anti-endomysia antibody as a confirmatory test because it has a 98% specificity for active celiac disease [15].

The immunological response to wheat, in the study was used serum concentrations of IgA, IgG, tTG-IgA, and tTG-IgG. One of the infrequent investigations on celiac disease is the investigation of IgG's function in wheat allergies. These immunological markers, particularly tTG-IgA, were shown to be elevated.

Conversely, all these indicators increased in females when antibody concentrations varied by age group. In any event, when dividing the concentrations of these antibodies by gender or age groups, we were unable to identify any discernible statistical differences. In terms of pathology, Maglio et al. (2020) expanded on the basic idea that human immune system produces anti-tTG antibodies in reaction to gluten exposure, which ultimately leads to intestinal mucosal damage [16].

The study showed that intestinal damage arises from an increase in anti-tTG antibodies produced in the small intestine during active wheat allergy, which is consistent with the findings of earlier meta-analyses. These results are consistent with the resulted study [17], which showed a strong relationship between serum anti-tTG levels and the histological severity of wheat allergy.

The intricate connection between anti-tTG antibody levels and the intensity of wheat allergy was emphasized by Aziz et al. (2015). According to Aziz study, over one-third of celiac disease individuals also had normal anti-tTG levels, but many non-celiac cases did not report elevated levels. This suggests that antibody levels can occasionally be normal even when histological damage is present.

Furthermore, this study highlights the significance of certain anti-tTG thresholds by showing that, in contrast to levels >2 x ULN, which were prevalent in both celiac and non-celiac individuals, levels >20 x ULN were only observed in wheat allergies. In contrast to results of the meta-analysis, both papers frequently emphasize the diagnostic potential of anti-

tTG antibody levels in detecting intestinal injury. Excluding the finding of higher antibody levels, Aziz et al. (2015) provided further details on limitations, including the sensitivity and specificity of anti-tTG antibodies, emphasizing the need for further research [18].

Other studies demonstrated the correlation between anti-tTG IgA titter and histological damage, arguing that the autoimmune response is strictly involved in intestinal inflammation and could be directly associated with the severity of the duodenal damage. Galli et al.'s study revealed that adult patients with a high rate of anti-tTG IgA at the time of wheat allergy diagnosis presented a more severe disease with a higher rate of total villous atrophy (Marsh 3C), hypoferritinaemia, and osteopenia/osteoporosis compared to patients with a low rate [19-20].

5. Conclusion

The current study showed that wheat allergy can occur at different ages, but the onset and severity of symptoms vary depending on the individual's nutritional and health status. Also, sex hormones play a role in exacerbating the immune response, as females were more susceptible to wheat allergy than males, and serum concentrations of the studied antibodies were higher in them. Furthermore, there is a significant increase in antibodies IgA, IgG, tTG-IgA and tTG-IgG. The evaluation of the role of tTG may contribute to future strategies for treating celiac disease, either by producing non-toxic wheat or by developing an oral vaccine that can prevent the disease.

6. References

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