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Assessment the Levels of Antibodies (Anti-TTG and Anti-AGA) in the Serum of Patients with Celiac Disease

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ABSTRACT

Celiac disease (CD) is an autoimmune disorder defined by distinct serological and histological alterations induced by the consumption of gluten in genetically predisposed individuals. This study aims to assess the levels of antibodies (anti - tTG and anti - AGA) as in the serum of patients with celiac disease. This case-control study involved 85 celiac disease patients (35 males and 50 females) recruited between July 2023 and December 2023. Samples were collected from the Internal Medicine Unit at Al-Sadder Medical City and the Specialized Hospital for Gastroenterology and Hepatology in Al-Najaf province. The age of the patients ranged from 5 to 60 years old. All patients underwent comprehensive general and clinical examinations to validate the diagnosis of CD using various serological kits (tTG - IgA, tTG - IgG, AGA - IgA,& AGA – IgG). A healthy control groups comprising 30 individuals comparable for (age and sex) was also included. The findings of the present study indicated that no significant differences were observed among patients with age groups, and most patients with celiac disease were between (15-24) years. The present data revealed that females in CD patients presented in a significant high percentage (58.82%) than male (41.17%).

In relation to serum levels of anti-tissue transglutaminase (anti-TTG) antibodies, including IgA and IgG, in patients with celiac disease (CD), the level between 12 and 18 AU/ml considered as an equivocal threshold serves as the distinguishing point between positive and negative results. There was no significant difference observed ($p > 0.05$) between level of anti-TTG IgA& IgG in celiac disease patients. The mean of anti-tissue transglutaminase (anti-TTG) IgA antibody level was 72.06±91.45 AU/ ml in the patient groups. 75 out of 85(88.23%) of CD patients appeared with positive values (>18 AU/ml) with no significant difference with negative value. The mean of anti-tissue transglutaminase (anti-TTG) IgG antibody level was 51.88±57.69 AU/ ml. The patients with negative results falling below the 12 AU/ml was 14.11% while those with positive values (>18 AU/ml) were 85.88% with significant difference. There was a significantdifference ($p<0.05$) between level of anti-AGA IgA& anti-AGA IgG in CD patients. the positive anti-AGA (IgA&IgG)cases of patient group were higher (68.9% and 88.23%) than negative results (31.1% and 11.76 %)**,** respectivelly with no significant difference.

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INTRODUCTION

Celiac disease (CD), characterized by chronic small intestinal enteropathy, results from T-cell-mediated inflammatory responses to dietary gluten (1). A lifelong adherence to a gluten-free diet (GFD) is the only treatment available; it eliminates the antigenic trigger and stops inflammation but does not repair the tolerance breakdown (2). Several adaptive and innate immunological disruptions that lead to a lack of gluten tolerance are the cause of celiac disease. Patients who express human leukocyte antigen (HLA-DQ2/ HLA-DQ8) have severe major histocompatibility complex (MHC) class II reliance, which is indicative of dysregulation of adaptive immunity (1,3).

The novel serologic tests for diagnosing celiac disease (CD) exhibit superior accuracy compared to most antibody-based tests used for other inflammatory or autoimmune disorders. Remarkably, the sensitivity and specificity of many of these novel CD tests are similar to the antibody tests used for infectious diseases. Nonetheless, the history of serologic testing for CD is marked by variability, with earlier generations of assays exhibited unacceptably (low specificity and sensitivity), along with significant inconsistencies in reported test characteristics (4,5).

The serologic detection of (antibodies and autoantibodies) is frequently utilized as a diagnostic tool to identify individuals who are likely to have celiac disease (CD) and to minimize the need for unnecessary intestinal biopsies in suspected cases (6). Endomysial antibody (EMA), tissue transglutaminase antibody (TTG), and gliadin antibody (AGA) are commonly employed serologic tests for both (diagnosing and monitoring) celiac disease in clinical settings (7). Endomysial antibody is recognized for its high sensitivity and specificity in diagnosing CD; however, it is less suitable for screening and follow-up due to its limitations, including cost, qualitative nature, and subjectivity (5). While AGA and TTG address some of EMA's limitations, AGA's variable sensitivity and specificity (ranging from 52% to 100% and 71% to 100% for IgA, and from 57% to 100% and 47% to 94% for IgG, respectively) limits its clinical applicability. Consequently, tissue-transglutaminase antibody-IgA is recommended as the initial screening test for CD because it is more affordable than EMA and has better sensitivity compared to AGA (8, 9).

In this article, we explore the available serologic tests, and propose and rationalize a strategy for their optimal and cost-effective utilization in diagnosing celiac disease (CD). The study aims to assess the levels of antibodies (anti-TTG and anti-AGA) in the serum of patients with celiac disease to gain insights into the best serological method for diagnosis of this disease.

METHODS

Samples Collection

A total of 85 individuals of both sexes during the period from July of 2023 to December of 2023. All sample was collected from Internal Medicine Unit/ Al-sadder Medical City and Specialized Hospital for Gastroenterology and Hepatology in AL-Najaf province. The age group of patients ranges from (5 to 60) years old. All patients were subjected to complete general and clinical examination to detected the celiac disease by using different serological kits (tissue-transglutaminase-IgA, tissue-transglutaminase-IgG, anti-gliadin antibody -IgA, and anti-gliadin antibody-IgG). A healthy control group comprising 60 individuals matched for age and sex to the patients was also included in the study.

Collection of samples Venous blood samples (5 ml each) were collected from both the patient group (n=85) and the control group (n=60) under sterile conditions. Following centrifugation at 3000 rpm for 10 minutes, the serum was separated, and the samples were stored at -80°C until they were examined by ELISA.

Serological test

Serum samples were maintained at or below -20°C until the time of the assays. All antibodies were quantified using the ELISA method. Samples were tested in accordance with the specifications provided by each manufacturer. Each assay run was validated against specified quality control criteria. AGA-IgA and AGA-IgG levels were determined using ELISA kits intended for in vitro diagnostic use (Scanlisa Anti-Gliadin-IgA Antibody and Anti-Gliadin-IgG Antibody; Scimedx Corporation, Denville, NJ). Similarly, TTG-IgA and TTG-IgG levels were measured using an ELISA kit designed for in vitro diagnostic use (BINDAZYME human IgA and IgG Anti-Tissue Transglutaminase EIA Kit, The Binding Site, Ltd, Birmingham, UK).

Statistical analysis

Data were described using mean and standard deviation. All graphs were made by GraphPad prism.9 to analyze the data statistically. The results are shown as mean ± SD.

RESULT AND DISCUSSION

The age of patients and control groups

In this study, the high percentage (29.41%) of CD patients appeared in the age group (15-24) years, while The high percentage of healthy control (46.66%) appeared in (15-24) age group followed by others as in table (1).

Table (1): Distribution of CD patients and control according to age groups.

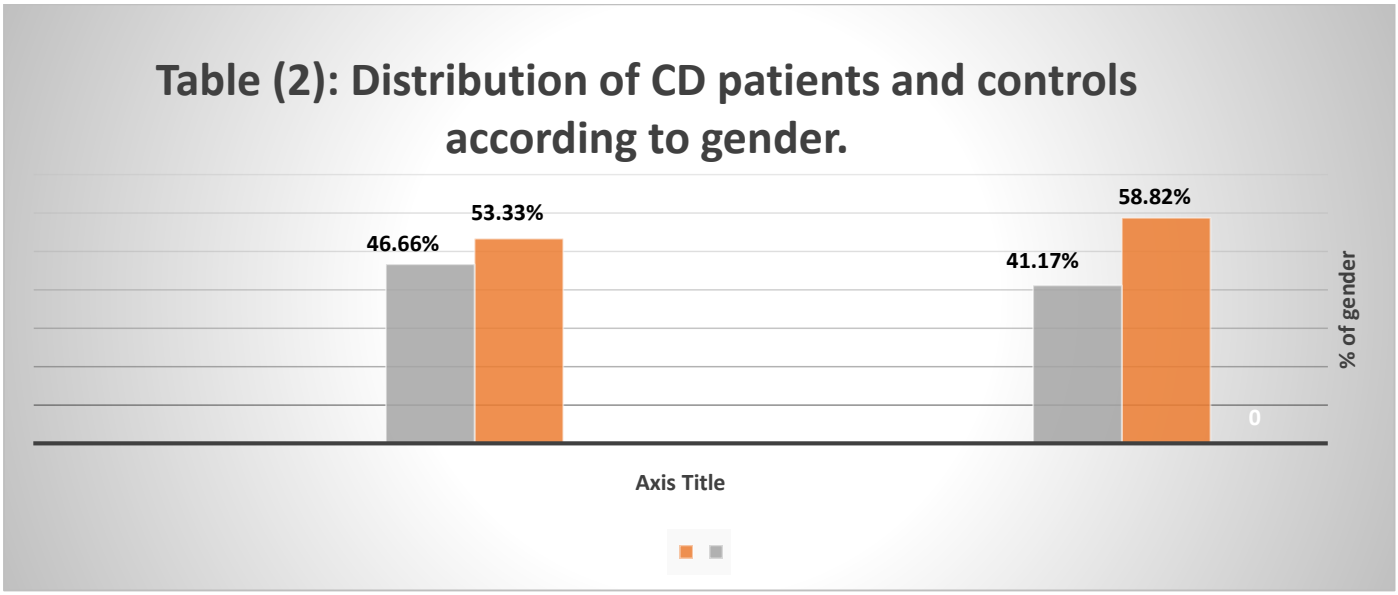
Age group	Patient		Control	
	No.	%	No.	%
5-14	13	15.29%	4	6.66%
15-24	25	29.41%	28	46.66%
25-34	22	25.88%	14	23.33%
35-44	11	12.94%	8	13.33%
45-54	13	15.29%	4	6.66%
>55	1	1.17%	2	3.33%
Total	85		60	

Mean ± SD	16.66±9.18	16.82±14.902
***p* value**	0.0627	

CD, also referred to as gluten-sensitive enteropathy, is an autoimmune condition that affects individuals with genetic predisposition. The immunopathogenesis of this disease occurs in the small intestine, involving both the epithelium and lamina propria. (10). Celiac disease has no specific age-related group and can manifest at any age. If someone tests negative for celiac disease at age 50, they may develop symptoms later in life, such as at age 65, as the onset of gluten intolerance can occur at any point in time (11). In this study, the highest number of individuals in both groups, (patients and controls), were between 15-24 years old (adults), comprising the following percentages 29.41% and 46.66% respectively, this result is incompatible with the results of some local studies (12, 13) and global studies (14, 15). They referred to CD increased with age and it may relate to many factors including genetics factors, hormonal factors, exposure to radiation and life style.

The gender of patients and control groups

According to gender, the female in CD patients presented in high percentage (58.82%) than male (41.17%), while in control group the female had (53.33%) and male had (46.66%) with a significant difference as in Figure (1).



The prevalence of celiac disease (CD) tends to be higher in females globally compared to males. While the exact cause remains unclear, hormonal changes in females affected by celiac disease might play a role. Additionally, men with celiac disease are significantly more susceptible to developing autoimmune disorders compared to men without the condition (12).

Statically analysis revealed a significant difference between the patients and control groups concerning gender (*p*) and females had a high percent than male in both groups as shown in the table (4.2). Other studies have indicated that females are at a higher risk for celiac disease, with an estimated female-to-male ratio (2:1) (16, 12, 13).

Generally, females are higher than males for diagnosis with celiac disease and the reason is unknown (Ludvigsson *et al*.,2016). Present study identified 85 true positive CD cases , (50) of the cases diagnosed were female with percentage 80% and (35) 20% male with a highly. Other studies have established that females have a higher risk of developing celiac disease, with an estimated ratio of females to males (2:1) (17, 16, 12, 13).

Serum level of anti-TTG (IgA& IgG) in CD patients

The anti-tissue and anti-gliadin levels have been measured by Chorus trio machine for all patients to detect celiac diseases in any type (new disease, relapsed cases, gluten free diet, treated cases and healthy cases). The sensitivity and specificity of these two biomarkers were about 70-85%, and that be helpful to diagnose celiac disease. These samples were approval by these two biomarkers. Our results were compared with others researchers. Interestingly, most of our results were similar with different researchers' findings in worldwide for approving that the anti-tissue and anti-gliadin are specific bio-markers to celiac disease such as (18,19).

In this study, the mean of anti tissue-transglutaminase IgA antibody level was 72.06±91.45 AU/ml in the patient groups . Patients who tested negative, below 12 AU/ml, comprised 11.76% (10 out of 85), while those with positive results (>18 AU/ml) constituted 88.23% (75 out of 85), with no significant difference observed (2).

The range of anti-TTG-IgG levels between (12 and 18 AU/ml) was designated as the cutoff point to differentiate between positive and negative results. The mean of anti- TtG IgG antibody level was 51.88±57.69 AU/ml. The patients

with negative results below the 12 AU/ml was14.11% while those with positive values (>18 AU/ml) were 85.88% with significant difference table (4.3).

There was no significant difference ($p > 0.05$) between level of anti-TTG IgA& anti-TTG IgG in CD patients as shown in figure (3).

Table (2): The distribution of CD patients according to the level of anti-TTG IgA& IgG.

Anti-TTG IgA	Patient		Mean ± SD
	No.	%	72.06±91.45
< 12 AU/ml	10	11.76%	
>18 AU/ml	75	88.23%	
Total	85	100	
Anti-TTG IgG	**Patient**		**Mean ± SD**
	No.	**%**	51.88±57.69
< 12 AU/ml	12	14.11%	
>18 AU/ml	73	85.88%	
Total	85	100	

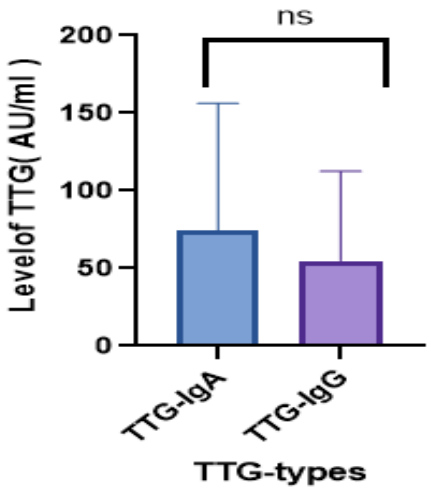


Figure (2): Level of anti-TTG-IgA&IgG in CD patients.

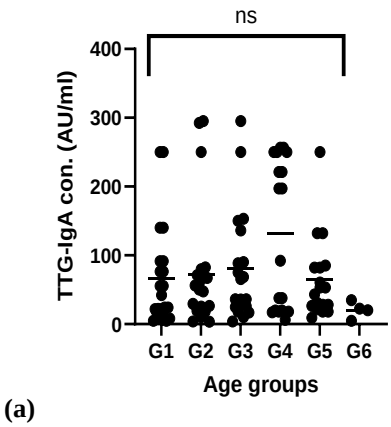
The anti-TTG markers of celiac disease (CD), as both anti-gluten and anti-tTG antibodies vanish from the bloodstream within a few months after patients adopt a gluten-free diet (GFD) (20). However, IgG anti-tTG measurement demonstrates poor predictive value for CD in IgA-competent children when anti-TG results are present (20).

In this study, the analysis focused on the level of anti-tTG IgA, as it is widely recognized as a potent diagnostic tool for CD as confirmed by (21). The data revealed that 75 out of 85 (88.23%) were tested positive for anti-tTG IgA, while 11.76% (10 out of the total 85) were tested negative. These findings align with previous Iraqi study conducted by (13). (22) reported that positive values were observed in the control group. Notably, the positive results (88.23%) in the CD patient was higher than that of the negative results (11.76%) with a mean 72.06±91.45. The absence of positive results in this study may be linked to IgA deficiency, as suggested by (23), or to seronegative villous atrophy CD patients, as proposed by (24).

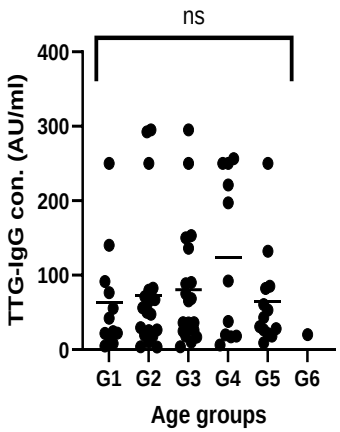
In this study, the absence of significant differences at diagnosis between IgA anti-tTG and IgG anti-tTG levels in CD patients was observed. This finding is consistent with a previous study (20) involving IgA-deficient individuals, which recommends performing an IgG-based test when total IgA levels are low, given its high sensitivity and specificity (84-97% and 91-93%, respectively). Furthermore, (20) the study discovered a resemblance at diagnosis between IgA anti-tTG levels in IgA-competent CD patients and IgG anti-tTG levels in selective IgA deficiency (SIgAD) CD patients. This suggests that in cases where IgA antibodies are not produced at all, compensatory IgG production could be stimulated by the typical T-helper 1 response in celiac disease (CD).

The age group G2 (15-24) and G3 (25-34) appeared to had high level of anti-tTG IgA (64.5±74.81) and anti- tTG IgG (48.11±51.23) than others group with no significant differences ($p > 0.05$) than others groups as in figure (2a) and (2b) .

According to sex,females appeared to had high level of anti-TTG IgA (58.50±62.34) and anti-TTG IgG (45.98±53.13) than males (64.91±78.63, 61.25±63.94, respectively) with no significant difference ($p > 0.05$) between (female & male) in both types as in figure (4.3 a&b).



(a)



(b)

Figure (3): the distribiution of CD patients according to (a) TTG-IgA (b) TTG-IgG concentration and age groups.

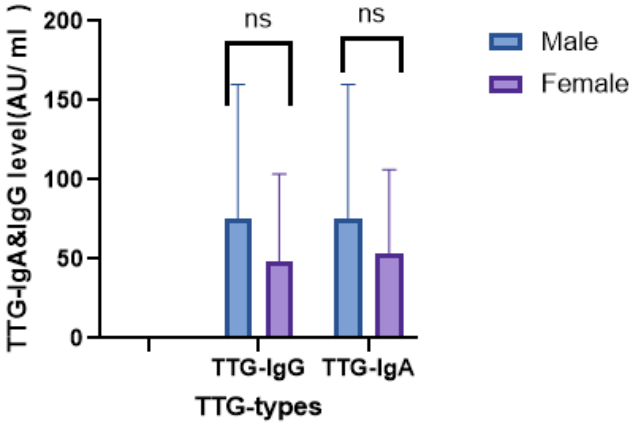


Figure (4): The distribution of CD patients according to the level of anti-TTG and sex**.**

Serum level of anti-AGA (IgA& IgG) in the CD patients

According to anti-AGA IgA kit, the level between 12 and 18 AU/ml considered as a equivocal point to differentiate between the (positive and negative) results. In this study, the positive anti-AGA IgA the patients group was 72 out 85(68.9%) while cases with negative results recorded 13 out 85(31.1%). The (mean ±SD) of the patients sample was 47.62 ±52.55 as seen in the table (6), with no significant difference table (2).

The present study showed the mesurmeant of anti-AGA IgG. the mean of (anti tTG antibody) level was 72.06 AU/ml in the patient groups with SD difference was 91.45 AU/ml. Patients with negative results below the 12 AU/ml constituted 11.76% (10 out of the total 85) while those with positive values (>18 AU/ml) were 75 out of 85 (88.23%). Table (2), with no significant difference.

There was a significant difference observed (p <0.05) between level of anti-AGA IgA& anti-AGA IgG in CD patients as shown in figure (5).

Table (2): The distribution of CD patients according to the level of anti-AGA IgA& IgG.

Anti-AGA IgA	**Patient**		**Mean ± SD**
	No.	**%**	47.62±52.55
< 12 AU/ml	13	15.29%	
>18 AU/ml	72	84.70%	
Total	85(100)		
Anti-AGA IgG	**Patient**		**Mean ± SD**
	No.	**%**	32±37.27
< 12 AU/ml	22	25.88%	
>18 AU/ml	63	74.11%	
Total	85(100)		

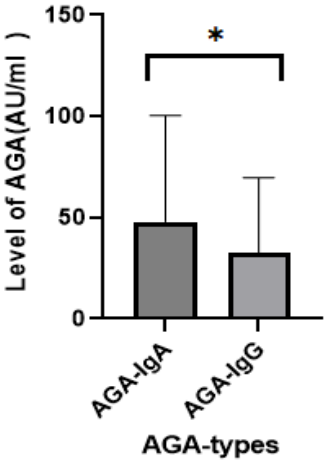


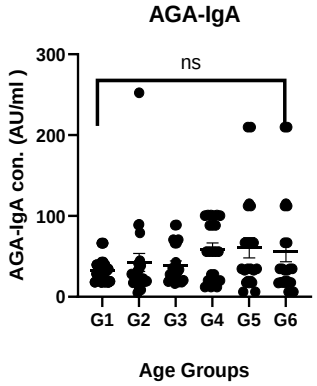
Figure (5): Level of anti-AGA IgA&IgG in CD patients**.**

Anti-AGA It served as a sensitive predictive marker for detecting and monitoring CD patients, as it is extensively present in the serum of CD patients, particularly those with villous atrophy (25).

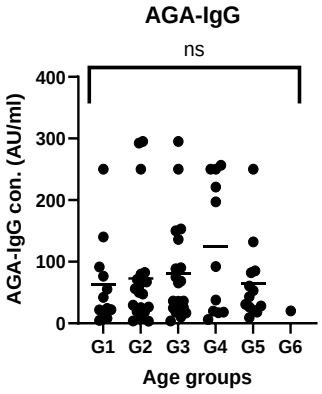
The results in this study non compatible with results of (12) who reported that the anti DGP-IgG demonstrates a sensitivity of 98% and specificity ranging from 90.3% to 100% as repoted by other previous studies (Majsiak *et al*., 2022), also, the anti-DGP-IgA test was not performed due to its lower sensitivity (87.8%) compared to anti-tTG-IgA (93%), with no significant differences observed between the two tests. Additionally, anti-AGA-IgG was conducted to identify CD in patients with negative anti-tTG-IgA results. (26).

According to the level of anti-AGA IgA, the age group G2 (15-24) years appeared to had high level of antibodies (44.5±52.64) than others group with non significant differences as in figure (4.4a), while anti-AGA IgG presented in a high level (28.91±15.68) at G3 (25-34) age group with non-significant difference (p>0.05) than others figure (6 b).

According to sex, females appeared to had high level of anti-AGA IgA (38.25±29.99) and anti-AGA IgG (30.59±37.93) than males (53.72±57.11, 35.7±37.12, respectively) with no significant difference (p > 0.05) between (female & male) in both types as in figure (6 a&b).



(a)



(b)

Figure (6): The distribiution of CD patients according to (a) AGA-IgA (b) AGA-IgG concentration and age groups.

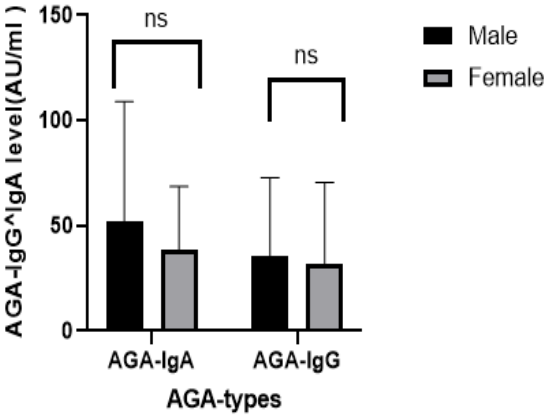


Figure (7): The distribution of CD patients according to the level of anti-AGA and sex.

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