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Relative Circulating Autoantibodies IgA/IgG to Tissue Transglutaminase and Deamidated Gliadin with Celiac Disease Patients

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ABSTRACT

Background: *A Celiac disease (CD) is an immune-mediated condition characterized by small intestinal enteropathy, systemic symptoms related to malabsorption or immune activation, and autoantibodies to tissue transglutaminase (TTG). The disease may present with a wide spectrum of symptoms, such as diarrhea, abdominal pain, involuntary weight loss, bloating, fatigue, anemia, vitamin deficiencies.* ***Aim of Study:*** *To evaluate the serological test (Anti-tissue transglutaminase, anti-gliadin peptides Ab IgG,IgA) in Al-Diwaniyah city.* ***Methods:*** *Method included designed on 150 patients and control (both sexes and the ages from 2 to 10 year) divided into three groups, the one group included 30 control and the other included 30 patients with CD at diagnosis, on a gluten containing diet (newly diagnosed); group 2 consisted of 30 patients on a Gluten Free Diet (GFD) for at least 1 year (GFD); group 3 consisted of 60 controls (control) and clinic private. Serological analysis was carried out on the serum samples to quantitatively measure the presence of circulating autoantibodies IgA/IgG to tissue trans glutaminase and deamidated gliadin by the indirect enzyme-linked immunosorbent assay technique using antibody-specific (ELISA) kits.* ***Results:*** *the percentage of positive results from four serological tests: Anti-gliadin IgA, Anti-gliadin IgG, Anti-tTG IgA, and Anti-tTG IgG. Anti-gliadin IgA shows the highest positivity rate (100%), indicating strong diagnostic relevance. Anti-gliadin IgG and Anti-tTG IgA have moderately lower positivity rates, while Anti-tTG IgG shows the lowest but still significant positivity. SEM error bars ensure statistical reliability. The results emphasize the importance of Anti-gliadin IgA as a key diagnostic marker for celiac disease.* ***Conclusion:*** *Estimation of Anti tissue transglutaminase,anti-gliadin peptides Ab IgG,IgA in ELISA assay was considers as a screening test.*

Keywords: CD, Anti-Gliadin IgA, GFD, Anti-Tissue Transglutaminase, Deamidated Gliadin.

Article Information

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INTRODUCTION

Celiac disease (CD) is a common immune-mediated enteropathy triggered by the interaction between environmental and genetic factors(1,2). Clinically, the disease may present with a wide spectrum of symptoms, such as diarrhea, abdominal pain, involuntary weight loss, bloating, fatigue, anemia, high transaminase levels, vitamin deficiencies, and infertility(3,4.5). Resulting in a multisystem disorder rather than isolated intestinal disease , CD diagnosis is based on the combined analyses of risk factors, clinical manifestations, serum antibodies, endoscopic findings, and histopathological duodenal lesions(6,7,8) . Although several drugs have been studied, the only available treatment strategy for CD is

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lifelong adherence to a gluten-free diet (GFD), which usually reverses symptoms and serological and intestinal alterations(9,10,11). However, GFDs are difficult to follow and may induce adverse metabolic effects, resulting in a reduction in the patient's quality of life owing to this restrictive diet (12,13,14). Serological tests may be performed in the clinical setting, and a positive result supports the diagnosis; however, no single test is 100% specific for CD, and diagnostic accuracy varies considerably between laboratories(15,16) . These tests consist of measuring tTG-IgA while on a regular gluten-containing diet and concurrent measurement of total IgA if the patient had not previously been tested for IgA deficiency (17,18,19).

Immunoglobulin A (IgA) is the principal antibody secretions in the gastrointestinal mucosal surfaces and acts as an important first line of defense against invasion of pathogenicmicro-organisms(20,21). the incidence of IgA deficiency is 10 to 15 times more common in patients with celiac disease (CD), that cause false-negative results in such cases (22,23). Recently, circulating and intracellular miRNAs have been proposed as biomarkers for the diagnosis of CD and have potential applications in gene-based therapies however, their functions are not yet fully understood. Therefore, in this study, we aimed to provide the basic concepts of CD and miRNAs and review the current data on the relationship between CD and circulating and tissue miRNAs in children patients(24,25,26).

MATERIALS AND METHODS

A case control study was designed on 150 patients and control (both sexes and the ages from 2 to 10 year) divided into three groups, the one group included 30 control and the other included 30 patients with CD at diagnosis, on a gluten containing diet (newly diagnosed); group 2 consisted of 30 patients on a GFD for at least 1 year (GFD); group 3 consisted of 60 controls (control) and clinic private during December 2024 to March, 2025 in Al-Diwaniyah and Al-Muthanna provinces . Serological analysis was carried out on the serum samples to quantitatively measure the presence of circulating autoantibodies to tissue trans glutaminase (tTG) antigen as well as IgA/IgG antibodies to deamidated gliadin by the indirect enzyme linked immunosorbent assay technique using antibody specific ELISA kits. The general assay principle entails the interaction between antigens coated on the surface of the microwells and specific antibodies in the patients‟ serum sample. The incubation period allows strong antibody-antigen interactions as washing steps after incubation removes unbound protein component. Subsequent addition enzyme conjugate binds to the immobilized antibody - antigen- complexes, while a second washing after incubation removes unbound enzyme conjugate. Addition of substrate solution to the enzyme-bound conjugate hydrolyses the substrate forming a coloured product. The stop solution which is an acid stops the reaction generating a coloured end product which can be measured spectrophometrically; the intensity of which correlates with the concentration of the antibody-antigen complex. The positive and negative controls serve as an internal quality control to validate the results obtained.

1. Determination of Anti-tTG antibody

The Anti-tTG Ab ELISA Kit (Euroimmun, Germany) was used to measure the presence of circulating transglutaminase autoantibody as per manufacturer‟s procedures. The micro wells were labelled control, calibrator and sample before dispensing 100 µL of the appropriate fluid into each well. The wells on roll A containing no sample (reserved for blank). The plates were then covered, sealed with paraffin and incubated for 1 hour at room temperature. Subsequently, the contents of the well were discarded and the wells were washed

thrice with 300 mL wash buffer solution after which 100 µL of reconstituted Enzyme Conjugate reagent were added to the immobilized antibody -antigencomplexes and the plate was covered and incubated for 1 hour at room temperature. Afterwards, the micro wells were washed three times and 100 µL of substrate solution was added into all the wells at a rapid and steady pace void of any interruption. The plates were then covered and placed in the dark for 30 minutes which generated a blue coloured product. Afterwards, 50 µL of the stop solution was added into each well at a rapid, steady pace without interruption, during which the formation of a bright yellow product was observed. The absorbance of the plates where then read at 450 nm from (Human, Germany). A dose response curve (DRC) was plotted on a linear graph paper, plotting each calibrator value (as indicated on the calibrator vial label) on the X-axis and its corresponding absorbance value on the Y-axis. A line of best fit was drawn between the three points and the tTG value of each serum sample was determined using its absorbance value and extrapolating from the DRC on the X-axis.

2. Determination of Deamidated gliadin antibody

The quantitative measurement of IgA/IgG antibodies to deamidated gliadin antigen in the serum samples, by the indirect enzyme linked immune reaction was carried out using the DGP IgA/IgG ELISA Kit (Euroimmun, Germany) was used as per manufacturer‟s procedures. The micro wells were labelled control, calibrator and sample before dispensing 100 µL of the appropriate fluid into each well. The wells on roll A containing no sample (reserved for blank). The plates were then covered, sealed with paraffin and incubated for 1 hour at room temperature. Subsequently, the contents of the well were discarded and the wells were washed thrice with 300 mL wash buffer solution after which 100 µL of reconstituted anti- human lgA peroxidase conjugate was added and the plate was covered and incubated for 1 hour at room temperature. Subsequently, the micro wells were washed three times and 100 µL of tetramethylbenzidine (TMB) substrate solution was added into all the wells at a rapid and steady pace void of any interruption. The plates were then covered and placed in the dark for 20 minutes which generated a blue dye product. Afterwards, 50 µL of the stop solution was added into each well at a rapid, steady pace without interruption, during which the formation of a bright yellow product was observed. The absorbance of the plates where then read at 450 nm from (Human, Germany). A dose response curve (DRC) was plotted on a linear graph paper, plotting each calibrator value (as indicated on the calibrator vial label) on the X-axis and its corresponding absorbance value on the Yaxis. A line of best fit was drawn between the three points and the gliadin lgA value of each serum sample was determined using its absorbance value and extrapolating from the DRC on the X-axis.

Table (1) Kit of celiac strip.

Component		Quantity
Strip s	**Strip s**	**16**
Sol DN	**Denaturation solution**	**1ml**
BUF HYB	**Hybridization buffer**	**60 ml**
BUF wash1	**Washing buffer 1**	**100ml**
CONJ HRP	**Conjugate**	**60ml**
BUF wash2	**Washing buffer 2**	**130 ml**
SUBS TMB	**Chromogen substrate**	**30 ml**

Table (2) Elisa kit TtG antibody IgA.

Reagent	Quantity
SORB MT 1 divisible micro plate	
Calibrator A-F	
Controls positive ,negative	2x1.5
Sample buffer P	5x20
Enzyme conjugate	15ml
TMB substrate	15ml
Stop solution	15ml
Wash buffer	50x20
1 instruction for use	
1 certificate of analysis	

Table (3) Elisa kit content of Anti gliadin antibody.

Symbol	Components	Quantity
SORB Mt	Galidin antigen coated microliter strips	12 ml
Cal A	Calibrator A(negative control)	2 ml
Cal B	Calibrator B(cut -off standard)	2 ml
Cal C	Calibrator c (weak positive control)	2 ml
Cal D	Calibrator D(positive control)	2ml
ENZ CONJ	Enzyme conjugate	15 ml
SUB TMB	Substrate solution	15 ml
Stop SOLN	Stop solution	15 ml
SAM DIL	Sample diluent	60ml
WASH SOLN 10	Washing Buffer (10x)	60 ml

Statistical analysis

Descriptive statistics were used to summarize the data. Frequencies and percentages were used for categorical variables. Mean and standard deviation were calculated for continuous variables. Comparisons between groups were done using independent t-test or ANOVA. The chi-square test was used for categorical data. Correlations were assessed using Pearson or Spearman coefficients. Partial correlation was used to control confounders. K-means clustering and PCA were applied for pattern detection. Random Forest, Gradient Boosting, and K-Nearest Neighbors were used for classification. Feature importance was determined from model outputs. All analyses were performed using SPSS version 26 and GraphPad Prism version 9.

RESULTS

1.Age Group Distribution in Study Population

This bar chart illustrates the percentage distribution of age groups among total participants, celiac patients, and controls. The x-axis categorizes age groups, while the y-axis represents percentages. The 2–4-year age group is most represented, while under 2 years and 8–10 years have the lowest representation. Celiac patients and controls show similar age distributions, confirming a balanced study population. SEM error bars indicate statistical reliability. This visualization highlights how celiac disease impacts various age groups, emphasizing the importance of early diagnosis **(Figure 1)**.

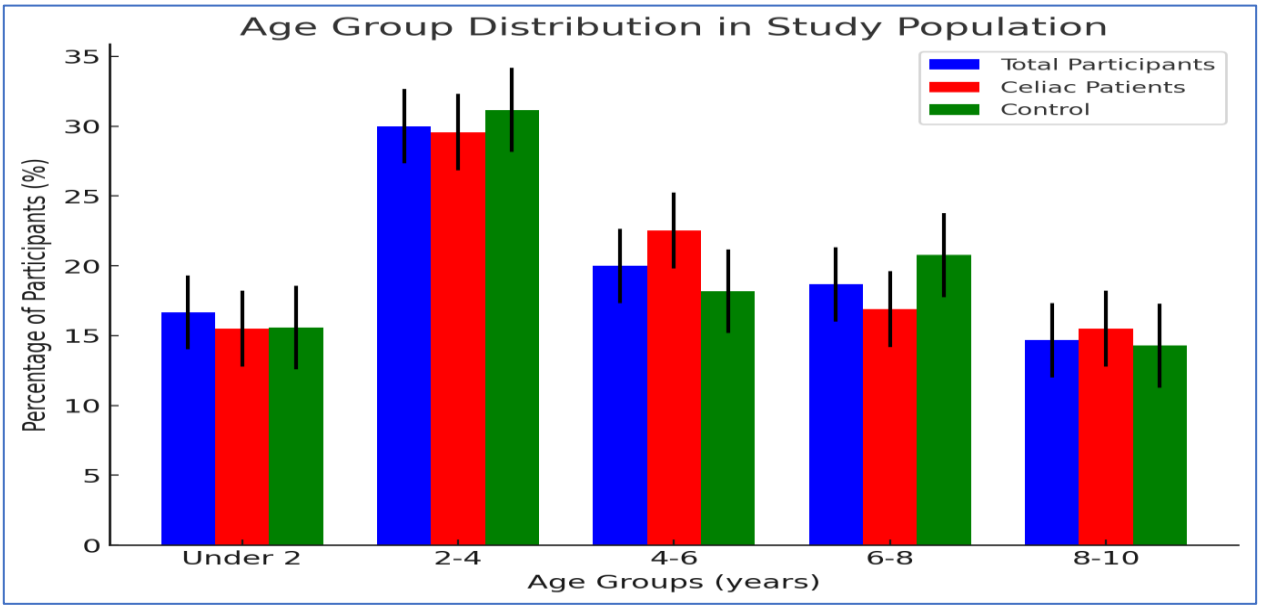

Figure 1: Age Group Distribution in Study Population: The figure shows the percentage of participants in different age groups among total participants, celiac patients, and controls. Error bars represent the standard error of the mean (SEM).

Also bar chart appears Celiac disease may develop any time after wheat or other gluten containing foods are introduced into the diet, typically after 6-9 months of age. This is agree with other studies that have shown that some children have Celiac disease early in life and others infected after years of exposure. It is very important to test your child at the very first signs, or if celiac disease runs in your family. First-degree relatives (parent, sibling, child) have a 1 in 10 chance of developing celiac disease themselves.(26). Also the bar chart shows Celiac disease is higher in the age group 2-4 years compared to other age groups because symptoms of celiac disease may be appear in this age by interferes with nutrient absorption and it can also lead to growth problems in children , and this is agree with several studies.(27,28).

2. Percentage of Positive Cases in Serological Tests for Celiac Disease

The bar chart presents the percentage of positive results from four serological tests: Anti-gliadin IgA, Anti-gliadin IgG, Anti-tTG IgA, and Anti-tTG IgG. Anti-gliadin IgA shows the highest positivity rate (100%), indicating strong diagnostic relevance. Anti-gliadin IgG and Anti-tTG IgA have moderately lower positivity rates, while Anti-tTG IgG shows the lowest but still significant positivity. SEM error bars ensure statistical reliability. The results emphasize the importance of Anti-gliadin IgA as a key diagnostic marker for celiac disease.

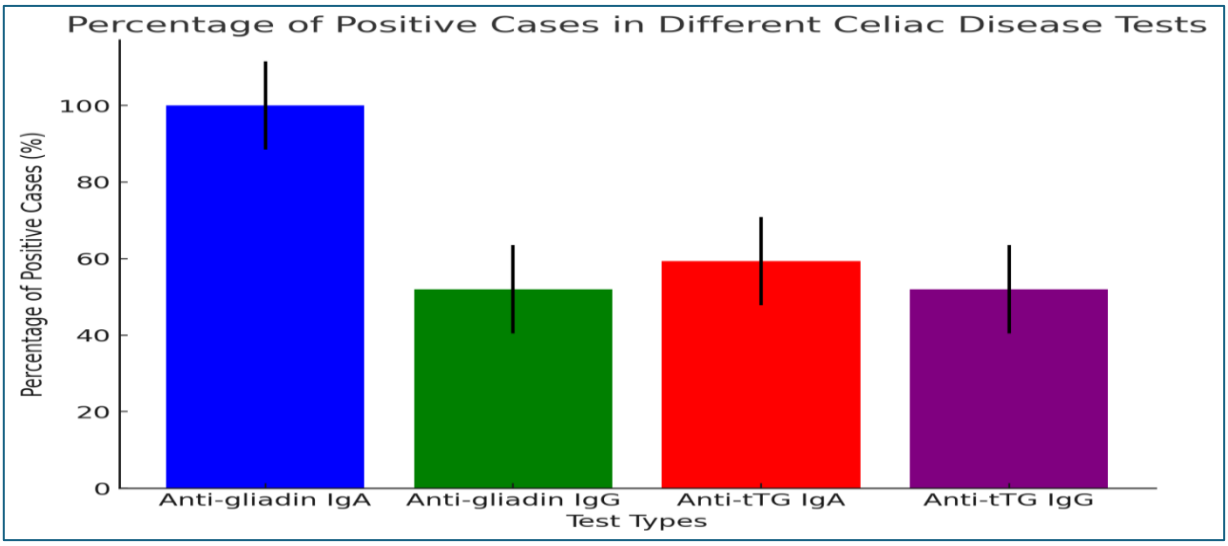

Figure 2: Percentage of Positive Cases in Different Celiac Disease Tests: The figure shows the proportion of positive cases across four celiac disease serological tests. Error bars represent the standard error of the mean (SEM).

3. Effect of Gluten-Free Diet on Celiac Patients

The bar chart displays the impact of a gluten-free diet on celiac disease patients, showing the percentage of positive and negative cases after dietary intervention across three groups: newly diagnosed celiac patients, celiac patients following a gluten-free diet for at least six months, and healthy controls.

All newly diagnosed patients tested positive for celiac disease. Among those on a gluten-free diet, 38% tested negative while 62% remained positive, suggesting dietary intervention reduces but does not completely eliminate serological markers. Controls had 100% negative results. SEM error bars provide statistical confidence in these trends.

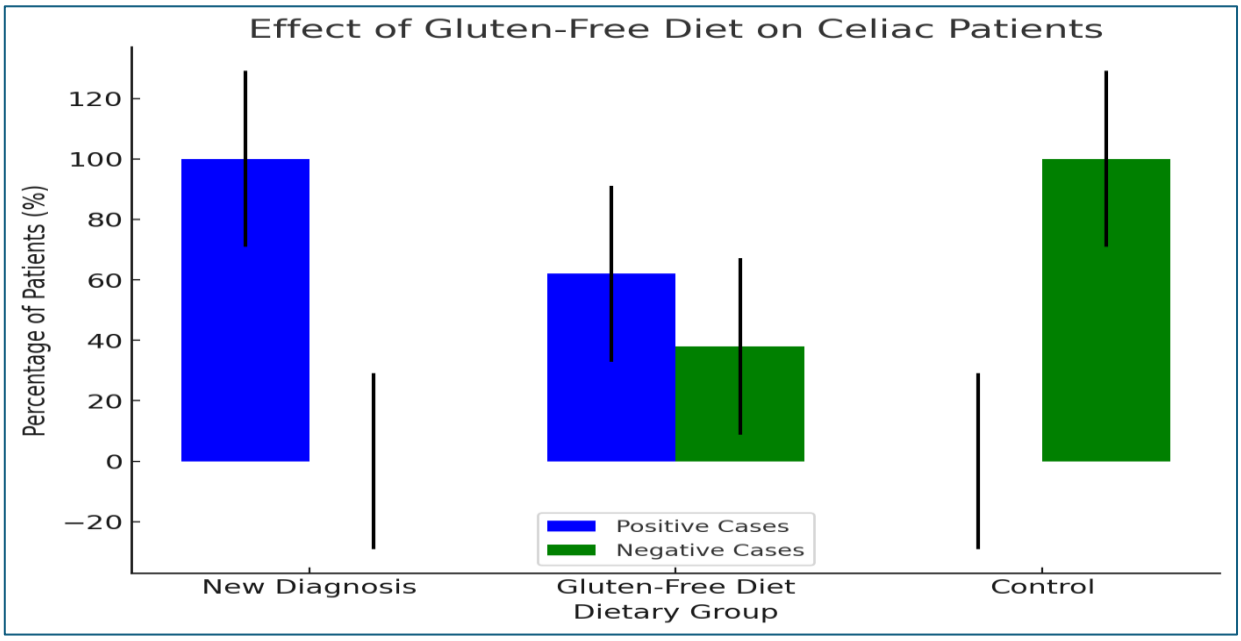

Figure 3-: Effect of Gluten-Free Diet on Celiac Patients: The figure presents the percentage of positive and negative celiac disease markers in newly diagnosed patients, those following a gluten-free diet, and controls. Error bars represent the standard error of the mean (SEM).

A decrease in the percentage positive tests to patients with celiac disease does not mean a cure for the disease, but rather a decrease in the level of immunoglobulin due to the diet, and the level may increase again if the patient resumes taking gluten. This raises questions about the effectiveness of serological tests in diagnosing the disease in children suffering from immunoglobulin deficiency, especially those under two years of age.

This is agree with many studies that have shown a very low level, or absence, of IgA in the blood is very common children. low IgA level or absence of IgA may be picked up when blood tests are undertaken for other reasons for example where the test is based on an IgA antibody, so the level of total IgA is also checked, such as to test if a person has coeliac disease. In the majority of cases (85%) there are no symptoms and the low IgA level does not cause any problems as the rest of the immune system can compensate. celiac disease also have selective immunoglobulin A (IgA) deficiency. If someone has IgA deficiency and celiac disease, the IgA deficiency can cause a false negative on a celiac disease antibody test.(29). At birth, there is very little IgA produced by the immune system, this gradually increases over the first months of life but takes several years to rise to adult levels. Therefore it is common for children under four years old to have a level which the laboratory will highlight as low. It takes time for the body's immune system to develop the IgA to a normal level so a permanent low IgA cannot be diagnosed in very young children. (30).

DISCUSSION

A decrease in the percentage positive tests to patients with celiac disease does not mean a cure for the disease, but rather a decrease in the level of immunoglobulin due to the diet, and the level may increase again if the patient resumes taking gluten. This raises questions about the effectiveness of serological tests in diagnosing the disease in children suffering from immunoglobulin deficiency, especially those under two years of age. This is agree with many studies that have shown a very low level, or absence, of IgA in the blood is very common children. low IgA level or absence of IgA may be picked up when blood tests are undertaken for other reasons for example where the test is based on an IgA antibody, so the level of total IgA is also checked, such as to test if a person has coeliac disease. In the majority of cases (85%) there are no symptoms and the low IgA level does not cause any problems as the rest of the immune system can compensate. celiac disease also have selective immunoglobulin A (IgA) deficiency. If someone has IgA deficiency and celiac disease, the IgA deficiency can cause a false negative on a celiac disease antibody test.(31). At birth, there is very little IgA produced by the immune system, this gradually increases over the first months of life but takes several years to rise to adult levels. Therefore it is common for children under four years old to have a level which the laboratory will highlight as low. It takes time for the body's immune system to develop the IgA to a normal level so a permanent low IgA cannot be diagnosed in very young children. (32).

CONCLUSION

Estimation of Anti tissue transglutaminase ,anti-gliadin peptides Ab IgG,IgA in ELISA assay was considers as screening test.

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