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Research Article
Establishment of New Immunostatistical Equations for Diagnosis and Follow-Up of Gluten-Free Diet in Patients with Celiac Disease

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Abstract

Background and Objective: Celiac disease (CD) is an autoimmune condition characterized by immune responses to gluten. The reported prevalence of CD has increased globally due to improved screening and diagnostic techniques, however, there is significant global variation in methods of diagnosis and follow-up. A new immunostatistical equation that determines the values of two types of antibodies using the total IgA test. **Materials and Methods:** Only 70 blood samples were taken from people who had celiac disease or were suspected of having it, with ages ranging from 5 to 40 years and they were separated into two groups. The first group contained 40 people of both sexes who had previously been diagnosed with celiac disease, while the second group included 30 individuals of both sexes, with ages ranging from 5 to 35 years, who were suspected of having celiac disease due to the appearance of some clinical symptoms. The control group also included 30 blood samples from healthy individuals of both sexes, with ages ranging from 5 to 40 years. Total IgA antibody tTG/IgA and DGP/IgA levels were estimated for all study samples. **Results:** By using a regression coefficient test the results showed the percentage of the effect of total IgA on tTG/IgA, DGP/IgA amounted to 11 and 25%, respectively, as shown by the value of the coefficient of clarifications (R^2) in patients with CD. On the other hand, The percentage of the effect of total IgA on tTG/IgA and DGP/IgA amounted to 11, 14%, respectively, as shown by the value of the coefficient of clarifications (R^2) in patients with suspected CD in $p \leq 0.05$. The results of the study were revealed and through the regression coefficient, four new immunostatistical equations were mathematically derived that give the value of tTG/IgA and DGP/IgA based solely on the total IgA test in the laboratory. **Conclusion:** By adopting one laboratory test which determines total IgA, the study concluded four new immunostatistical equations that will help academic researchers and attending physicians to diagnose celiac disease in addition to following up on patient's adherence to a gluten-free diet.

Key words: Celiac disease, total IgA, tissue transglutaminase/IgA, deamidated gliadin peptide/IgA

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Celiac Disease (CD) is an intestinal illness induced by gluten consumption in genetically susceptible people defined by variable-severity enteropathy found in small intestine biopsy tissue[1]. It is estimated that 1-2% of the population is affected. One of the most frequent gastrointestinal illnesses is CD. Additionally, from a clinical standpoint, it affects people across multiple age groups. It has been observed through clinical follow-ups with patients, in which it usually appears during the second year of life, where the most important symptoms are characterized by the appearance of weight loss, appetite and flatulence, in addition to cases of chronic diarrhoea and malabsorption of nutrients[2]. The CD patients' genes appear as the human leukocyte antigen HLA-dominant DQ2 or DQ8. Pathological symptoms begin as the result of an adverse reaction of the immune system to gluten, as this immune response results in antibodies against Tissue Transglutaminase (tTG) present in the tissues. However, many immune responses contribute to the development of the disease. Furthermore, the glycoprotein gliadin (found in gluten) is directly harmful to intestinal cells, as it boosts IL-15 production[3]. Infections of the digestive tract in children in their early years of life are linked to the development of CD later in life. Furthermore, the CD is mostly caused by an immune function issue.

Immunoglobulin A (IgA) deficiency occurs in 10-15% of patients with CD compared with healthy people. The IgA-deficient individuals with CD may produce false-negative serotypes because available serological tests are restricted in that they can only detect the IgA subtype of antibodies (except for immunoglobulin G [IgG] gliadin assays). The tTG/IgA is one of the most preferred serological tests for the detection of CD[3]. The CD test is highly sensitive to the IgA anti-tTG antibody, up to 95%[4]. Because the anti-tTG assay is not standardized, the diagnostic accuracy of anti-tTG/IgA antibodies varies between laboratories[5].

Deamidated gliadin peptide (DGP) antibodies are another important immunological and serological indicator of CD. Gliadin removal by the tTG enzyme increases the immune response to the modified gliadin peptides as compared with the original peptides[6]. However, other data indicate a lack of dependence on IgG DGP antibodies for accurate diagnosis. The IgG DGP is very useful in detecting CD in children as young as two years old[7]. Since IgA/DGP is of great value in the diagnosis of CD[8], for optimal diagnosis in adults, serology should test for both total IgA and anti-tTG antibodies. Patients should also show a high positive anti-tTG/IgA titer and a normal total IgA level. The most important serological tests used for CD diagnosis today are total IgA, anti-tTG and gliadin DGP (D-linked antibodies), which are all members of the IgA and IgG classes. However, only those members of the highly sensitive IgA class can be considered specific to CD. Due to the high prevalence of false positives, the use of IgG markers, except DGP, should be restricted to individuals with IgA deficiency[9].

Because of the presence of antibodies tTG/IgA and IgA/DGP, in addition to the variation in total IgA globulin levels, which is a strong clinical indicator for the development of CD, the current study aims to determine a new set of equations through which researchers can determine infection rate. Focusing on CD by examining total IgA only, the current study found that the value of total IgA was linked to tTG/IgA and DGP/IgA through four statistical equations using the effect analysis relationship in patients with CD gastrointestinal disorders, as well as people suspected of having this disease.

MATERIALS AND METHODS

Samples collection: Seventy blood samples were taken from people who were diagnosed with or suspected of having CD, with ages ranging from 5 to 40 years. They were separated into two groups, the first of which contained 40 people of both sexes who had previously been diagnosed with CD, while the second group included 30 individuals of both sexes, with ages ranging from 5 to 35 years, suspected of having CD due to the appearance of some clinical symptoms, such as diarrhoea, general pallor and fatigue. Samples were collected from patients at Al-Salam Teaching Hospital in Mosul for one year from 9th April, 2022 to 25th December, 2022 and the study was carried out at Mosul University College of Sciences/Iraq. The control group also included 30 blood samples from healthy individuals of both sexes, with ages ranging from 5 to 40 years. A survey, which included name, age, gender, weight and height, was collected from all members of the study sample.

Samples: A 5 mL blood sample was withdrawn for each patient using 5 mL medical syringes and 2 mL of the blood samples were distributed into tubes containing EDTA anticoagulant and gently shaken to prevent blood clotting. Until being used for molecular tests, the remaining 3 mL blood samples were placed in gel tubes to separate the serum by centrifugation at 3,000 rpm for 15 min. After separation, the blood serum was distributed into small plastic Eppendorf tubes and frozen at a temperature of -20°C until the serological tests were conducted.

Measurement of the concentration of tTG/IgA antibodies and antigliadin antibodies (DGP/IgA): Serological analysis of serum samples was performed to measure the concentration of tTG/IgA antibodies. Proprietary test kits by the manufacturer (AESKULISA) for determination of tTG/IgA antibodies and by (DiaMetra) for DGP/IgA antibodies were used, following the company's instructions and utilizing indirect Enzyme-Linked Immunosorbent Assay (ELISA) technology. Serum samples were added to the pits of the ELISA plate and then a plate-washing process was performed to remove the unbound parts and enzyme-linked antibodies that interact with the antibody-antigen complex were added followed by a plate-washing process to remove the unbound paired antibodies. Then, the base material was added, followed by a solution to stop the reaction, which leads to a colour change-the intensity of which depends on the concentration of the target antibodies. The optical absorbance was measured at the wavelength of 450 nm[10].

Estimation of total IgA level in serum: The level of total IgA in the body was estimated using the sandwich ELISA technique, where the principle of the assay is based on the use of identical pairs of antibodies, in which each antibody recognizes a distinct, non-overlapping area of the antigen molecule. The first antibody, known as capture, is coated on the pits. Then the samples are added to the etching. A second antibody known as detection, which is bound to the enzyme, is added. Then the base material is added, which allows the formation of a colour reaction. Then, a solution to stop the reaction is added and the absorbance is measured[11].

Statistical analysis: To statistically compare the means, which included comparisons between the levels of tTG/IgA and DGP/IgA gliadin in patients with CD and those suspected of having CD in addition to the control sample, a test was used with a probability of $p<0.05$. To investigate the association between the variables, a regression coefficient analysis was carried out. The SPSS 24.0 software was used for statistical analysis.

RESULTS AND DISCUSSION

Several statistical indicators, such as sample size, arithmetic mean and standard deviation, for assessing tTG/IgA concentrations for the study groups, which were comprised of CD patients, those suspected of having CD and controls as shown in Table 1.

The results of the Duncan's Test appeared in Table 2. The results of the test measure the concentration of tTG/IgA among the three groups as follows. There was a significant increase in the concentration of tTG/IgA in CD patients compared with both those suspected of having CD and the controls. There were no significant differences in the concentration of tTG/IgA between those suspected of having CD and the controls in terms of the probability value associated with Duncan's Test, which amounted to 0.874, which is greater than 0.05.

Some statistical variables for evaluating the DGP/IgA concentration of the study groups, which included CD patients, those suspected of having CD and observers compared with the control sample, as indicated by sample size, mean and standard deviation as shown in Table 3.

The results of Duncan's Test, which measured the concentration of DGP/IgG among the three groups, appeared in Table 4. There was a significant increase in the concentration of DGP/IgA in CD patients compared with both those suspected of having CD and the controls. There were no significant differences in the concentrations of DGP/IgA between those suspected of having CD and the controls in terms of the probability value associated with Duncan's Test, which amounted to 0.945, the given number is more than 0.05.

Effect relationship analysis in patients with CD

Analysis of the effect of total IgA on tTG/IgA in patients with CD: The best model for the effect of total IgA on tTG/IgA is the Exponential model, as it represents the best relationship between these two variables in terms of the probability value accompanying the F test, which amounted to 0.001, which is less than 0.05. The percentage of the effect of total IgA on tTG/IgA amounted to 11%, as indicated by the value of the clarifications coefficient (R^2), meaning that 11% of the changes that occurred in tTG/IgA were caused by total IgA, or the remaining percentage of 89% is for other variables that were not included in the model. There was a significant effect of total IgA on tTG/IgA in terms of the value of the regression coefficient, which amounted to -3.131 and this effect was significant in terms of the probability value associated with the t test, which amounted to 0.038, which is less than 0.05. The mathematical relationship between these two variables, according to the Exponential model, was shown in Table 5 and Fig. 1.

Effect analysis of total IgA on DGP/IgA in patients with CD: The best model for the effect of total IgA on DGP/IgA is the Power model, as it represents the best relationship between these two variables in terms of the probability value

Table 1: Estimation of tTG/IgA concentration in the study groups compared with the control sample

Case summaries					
Factor	Number	Mean	±SD	Minimum	Maximum
tTG/IgA					
Control	30	7.6905	5.66316	0.64	19.90
Patients	40	108.9639	148.23372	0.56	375.25
Suspected	30	12.2683	15.53951	1.22	67.50

Table 2: Duncan's Test for pairs of comparisons of the tTG/IgA variant

Duncan's Test (tTG/IgA)			
		Subset for alpha = 0.05	
Factor	Number	1	2
Control	30	7.6905	-
Suspected	30	12.2683	-
Patients	40	-	108.9639
p-value	-	0.874	1.000

*p≤0.05

Table 3: Estimation of DGP/IgA concentration in the study groups compared with the control sample

Case summaries					
Factor	Number	Mean	±SD	Minimum	Maximum
DGP/IgA					
Control	30	9.7673	5.39454	2.17	25.46
Patients	40	67.1345	124.15024	0.14	479.39
Suspected	30	11.4458	17.26035	1.77	91.60

Table 4: Duncan's Test for pairs of comparisons of the DGP/IgA variant

Duncan's Test (DGP/IgA)			
		Subset for alpha = 0.05	
Factor	Number	1	2
Control	30	9.7673	-
Suspected	30	11.4458	-
Patients	40	-	67.1345
p-value	-	0.945	1.000

*p≤0.05

Table 5: Effect of total IgA on tTG/IgA in patients with CD

Coefficients							
	Unstandardized coefficients		Standardized coefficients	ANOVA			
Model (Exponential)	B	Std. Error	Beta	F (p-value)	R^2	T	p-value
B_0	73.385	44.754	-	4.598 (0.038)	0.11	1.640	0.109
B_1	-3.131	1.460	-0.329	-	-	-2.144	0.038

B_0: Constant, B_1: Regression coefficient, F: Calculated F value, R^2: Coefficient of clarifications, Dependent variable (tTG /IgA) = Y, Independent (total IgA) = X and e = 2.71828, Equation 1: $Y = B_0 \times (e^{B1 \times X})$

accompanying the F test, which amounted to 0.001, which is less than 0.05. The percentage of the effect of total IgA on DGP/IgA amounted to 25%, as indicated by the value of the clarification coefficient (R^2), meaning that 25% of the changes that occurred in DGP/IgA were caused by total IgA, or the remaining percentage of 75% is for other variables that were not included in the model. There was a significant effect of total IgA on DGP/IgA in terms of the value of the regression coefficient, which amounted to -1.818 and this effect was significant in terms of the probability value

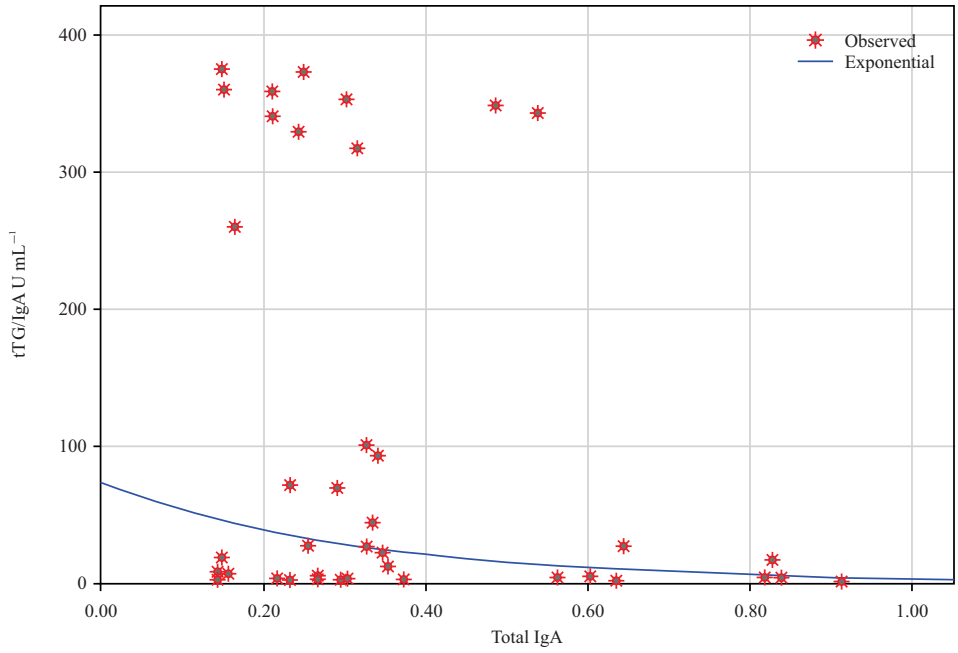

Fig. 1: Effect of total IgA on tTG/IgA in patients with CD

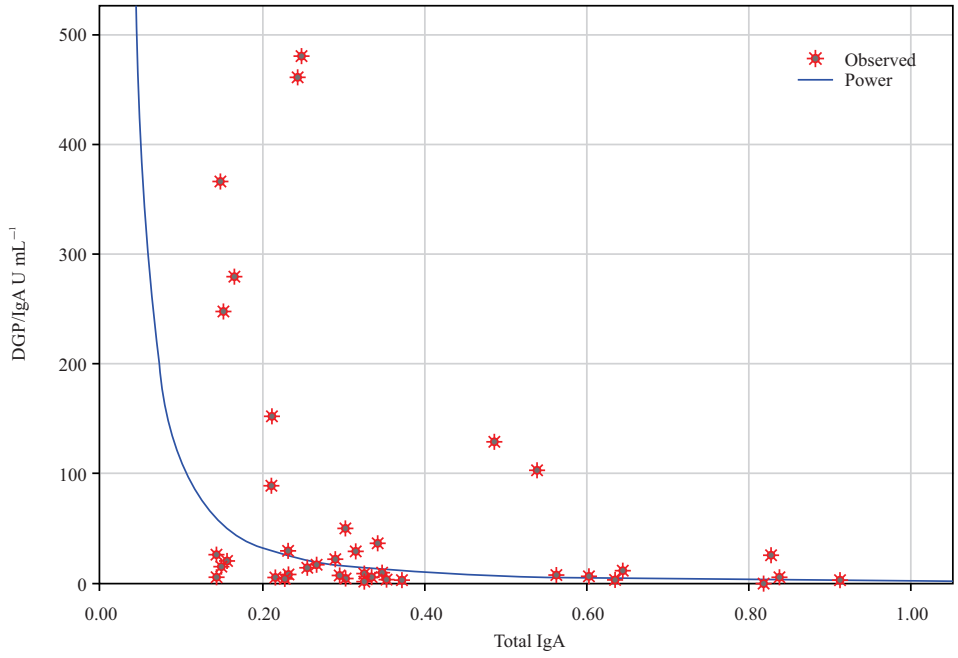

Fig. 2: Effect of total IgA on DGP/IgA in patients with CD

associated with the t test, which amounted to 0.001, which is less than 0.05. The mathematical relationship between the two variables according to the Power model was shown in Table 6 and Fig. 2.

Effect relationship analysis for patients with suspected CD

Effect analysis of total IgA on tTG/IgA: The best model for the effect of total IgA on tTG/IgA is the Compound model, as it represents the best relationship between these two

Table 6: Effect of total IgA on DGP/IgA in patients with CD

	Coefficients						
	Unstandardized coefficients		Standardized coefficients	ANOVA			
Model (Power)	B	Std. Error	Beta	F (p-value)	R^2	T	p-value
B_0	1.714	1.135	-	12.315 (0.001)	0.25	1.510	0.139
B_1	-1.818	0.518	-0.495	-	-	-3.509	0.001

B_0: Constant, B_1: Regression coefficient, F: Calculated F value, R^2: Coefficient of clarifications, Dependent variable (DGP/IgA) = Y, Independent (total IgA) = X and Equation 2: $Y = B_0 \times X^{B1}$

Table 7: Effect of total IgA on tTG/IgA in patients with suspected CD

	Coefficients						
	Unstandardized coefficients		Standardized coefficients	ANOVA			
Model (Compound)	B	Std. Error	Beta	F (p-value)	R^2	T	p-value
B_0	10.554	3.874	-	2.963 (0.049)	0.11	2.944	0.008
B_1	0.540	0.332	0.654	-	-	2.070	0.044

B_0: Constant, B_1: Regression coefficient, F: Calculated F value, R^2: Coefficient of clarifications, Dependent variable (tTG/IgA) = Y, Independent (total IgA) = X and Equation 3: $Y = B_0 \times B_1^X$

Table 8: Effect of total IgA on DGP/IgA in patients with suspected CD

	Coefficients						
	Unstandardized coefficients		Standardized coefficients	ANOVA			
Model (Compound)	B	Std. Error	Beta	F (p-value)	R^2	T	p-value
B_0	9.906	2.745	-	4.663 (0.017)	0.14	3.609	0.001
B_1	0.597	0.239	0.779	-	-	2.501	0.019

B_0: Constant, B_1: Regression coefficient, F: Calculated F value, R^2: Coefficient of clarifications, Dependent variable (DGP/IgA) = Y, Independent (total IgA) = X and Equation 4: $Y = B_0 \times B_1^X$

variables in terms of the probability value accompanying the F test, which amounted to 0.049, which is less than 0.05. The percentage of the effect of total IgA on tTG/IgA amounted to 11%, as indicated by the value of the clarifications coefficient (R^2), meaning that 11% of the changes that occurred in tTG/IgA were caused by total IgA, or the remaining percentage of 89% is for other variables that were not included in the model. There was a significant effect of total IgA on tTG/IgA in terms of the value of the regression coefficient, which amounted to 0.540 and this effect was significant in terms of the probability value accompanying the t test, which amounted to 0.044, which is less than 0.05. The mathematical relationship between the two variables according to the combined model was shown in Table 7 and Fig. 3.

Analysis of the effect of total IgA on DGP/IgA in patients with suspected CD: The best model for the effect of total IgA on DGP/IgA is the combined model compound, as it represents the best relationship between these two variables in terms of the probability value accompanying the F test, which amounted to 0.017, which is less than 0.05. The percentage of the effect of total IgA on DGP/IgA amounted to 14%, as indicated by the value of the clarification coefficient (R^2), meaning that 14% of the changes that occurred in tTG/IgA were caused by total IgA, or the remaining percentage of 86% was for other variables that were not included in the model. There was a significant effect of total IgA on DGP/IgA in terms of the value of the regression coefficient, which amounted to 0.597 and this effect was significant in terms of the probability value associated with the t test, which amounted to 0.019, which is less than 0.05. The mathematical relationship between the two variables was shown in Table 8 and Fig. 4.

Total IgA, anti-tTG and gliadin DGP (D-linked antibodies) are the most significant serological tests for CD diagnosis nowadays. They are all members of the IgA and IgG classes. Only members of the extremely sensitive IgA class, however, may be termed CD-specific. Except for DGP, the use of IgG markers should be limited to people with IgA deficiency due to the high occurrence of false positives[9]. When patients maintain a gluten-free diet (GFD), these types of antibodies can be utilized alone or in combination to provide a trustworthy diagnosis in virtually all cases of CD. They can also

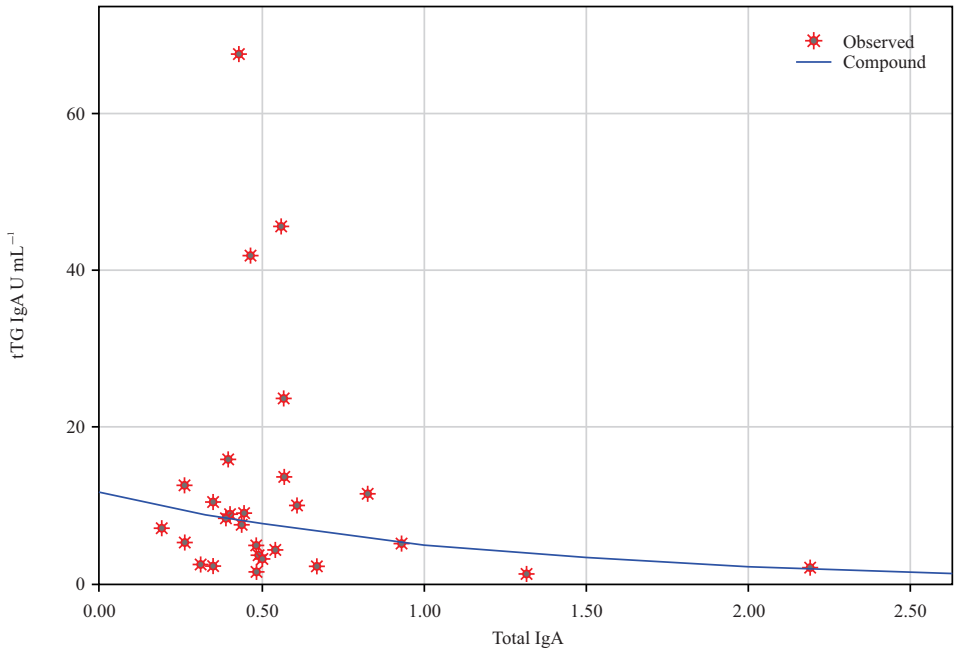

Fig. 3: Effect of total IgA on tTG/IgA in patients with suspected CD

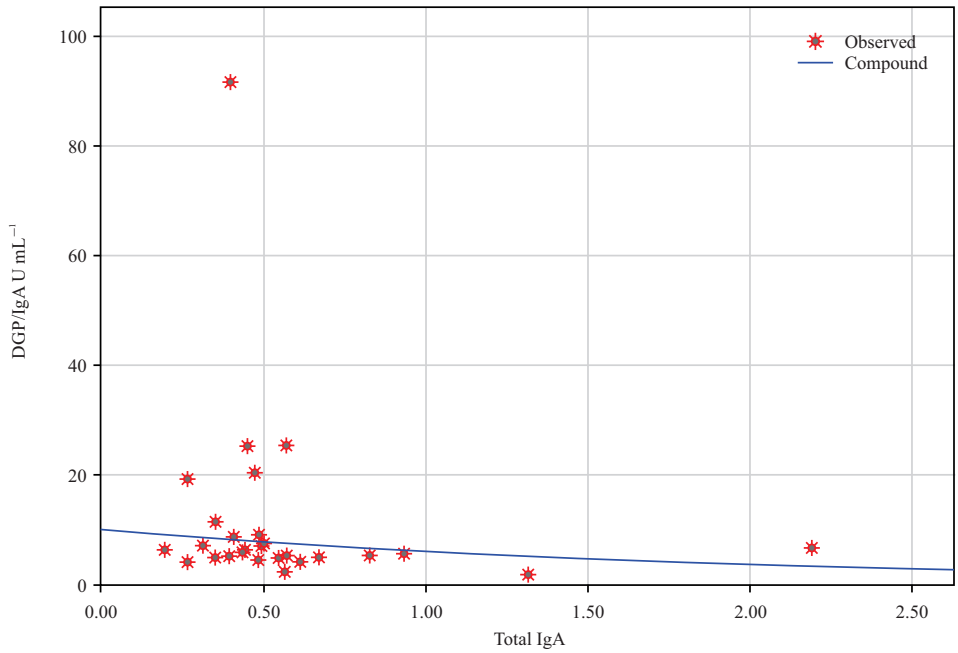

Fig. 4: Effect of total IgA on DGP/IgA in patients suspected of CD

be used to track a patient's reaction to a GFD, as levels of these antibodies drop when gluten is removed from the diet. Therefore, these serologic selections have a dual role in monitoring GFD efficacy and patient adherence after diagnosis, but first, they precisely and reliably identify patients with CD.

Over the past few decades, IgG and IgA gliadin antibody tests have been replaced by the more sensitive and specific IgA anti-tTG test[12]. The use of a tissue biopsy is mandated by the guidelines of gastrointestinal facilities in North America and Europe for the diagnosis of CD[13]. Furthermore, due to the huge variety of preparations for intestinal tissue biopsy, the high financial expense of performing the biopsy and the high frequency of CD in the general population[14], the search for autoantibodies is currently an important initial serological test for individuals who may need a duodenal biopsy. During the past 10 years, very significant advances have taken place in the field of serological testing and the wide availability of these tests has allowed any clinician to rapidly diagnose the pathogenesis of patients with CD[15].

Since CD symptoms can affect people of any age, it is important to note that the first diagnostic step in diagnosing CD is a serological diagnosis. The detection of tTG/IgA and total IgA antibodies is advised by the American Gastroenterology Association. The antibody evaluation of total IgA type, especially in adults, along with the tTG/IgA test and the DGP/IgA test, are some of the most crucial serological tests that attending physicians must utilize throughout the process of diagnosing CD. Additionally, once a discrepancy in the aforementioned antibody readings has been confirmed, oesophageal endoscopy and bowel biopsy cannot be ignored. However, there has already been some discussion about cancelling the biopsy process in children, especially when there is a high concentration of tTG/IgA antibodies as well as a variation in total IgA levels[16].

Because of their limited sensitivity and specificity, the use of natural Antigliadin Antibodies (anti-AGA) antibodies in the diagnosis of CD is no longer recommended[17]. The tTG Test, particularly the IgA form, is 99% more sensitive than the other serological assays. In addition to diagnosis, serological testing can be used to track therapy progress[18]. While anti-DGP antibodies are a good marker to monitor patients when they are put on a GFD, anti-tTG2 antibodies persist in patients' sera for a long time, up to several years. Negative test results attest to patients' adherence to doctors' dietary suggestions[19].

The most prevalent primary immunodeficiency is IgA antibody deficiency, which is characterized by a lack of IgA antibodies (0.07g L^{-1}) and normal IgG and antibody levels in the blood and IgM in individuals who are at least four years old. To prevent IgA deficiency in children under the age of four, which is a temporary disease due to the delay in the development of the IgA system after birth, screening for total IgA antibodies should be done after the age of four. Epidemiological studies have already demonstrated that CD is one of the conditions directly linked to IgA deficiency[19-21]. In addition to accuracy in acquiring the results, medical laboratories are always searching for the most efficient approaches in terms of diagnostic and economic considerations[22]. Among the most important disorders that require serological detection of antibodies are primary biliary cirrhosis, or Miller Fisher syndrome, autoimmune gastritis, pernicious anaemia and CD-especially CD[23]. Simultaneous detection of serum CD markers using tTG/IgA may reduce the cost of serological CD diagnosis compared with the detection of CD-specific antibodies alone, as well as shorten the time needed to reach the correct result. The determination of tTG/IgA and DGP/IgA concentrations in a patient's serum celiac IgA plus total IgA takes less than two hours, so this procedure may take a long time in addition to the effort of performing the three tests. It should also be highlighted that the test requires just 250 µL of serum, which is especially essential in young patients[24,25].

Given the importance of tTG/IgA and DGP/IgA in early diagnosis of CD, in addition to the role of serum globulin IgA in shaping the immune response within the small intestine to inflammatory conditions, a regression coefficient test was performed to determine the relationship between tTG/IgA, DGP/IgA and serum globulin IgA. Moreover, statistical immunological equations were extracted that allow treating physicians and laboratories, which may only be able to perform one test, to estimate the level of IgA in the serum and extract the values of both tTG/IgA and DGP/IgA in each patient undertaking a GFD as well as those suspected of having CD due to clinical symptoms without the need for laboratory tests for either tTG/IgA or DGP/IgA.

CONCLUSION

There is a significant increase in the levels of both tTG/IgA and DGP/IgA in CD patients compared to patients suspected of having CD and control. As part of the effect coefficient test, a significant relationship was observed for the effect of total IgA antibodies on each of the tTG/IgA and DGP/IgA antibodies in patients with CD by 11 and 25%, respectively and a significant relationship appeared for the effect of total IgA antibodies in each of antibodies type tTg/IgA and DGP/IgA in suspected cases of CD by 11 and 14%, respectively.

SIGNIFICANCE STATEMENT

The importance of the current study lies in following up on some immunological indicators and molecular manifestations of patients with celiac disease in the city of Mosul, in addition to finding a new diagnostic method that helps researchers, doctors and laboratories to diagnose celiac

disease in patients suspected of having the disease, in addition to following up the extent of patients' commitment to an empty diet of gluten in an easy, fast and inexpensive way and it requires a small amount of blood to conduct the examination, especially for children under the age of four years.

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