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Cross-sectional Study

Relationship of citrulline and tissue transglutaminase antibody with duodenal histopathology among children with celiac disease☆

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ABSTRACT

Objectives: Non-invasive biomarkers, for the diagnosis of celiac disease, can reduce the need for biopsy, particularly in pediatric patients. The aim of this study was to investigate the levels tissue transglutaminase antibody (tTG) and plasma citrulline and its correlation with intestinal biopsy.
Methods: In this cross-sectional descriptive study, Pediatric patients with celiac disease referred to (XXX)were included. The patients underwent tTG antibody test along with plasma citrulline measurements using HPLC ((high performance liquid chromatography). Biopsy was performed in all the patients and clinical and demographic findings were recorded in a patient form. The data were statistically analyzed using SPSSv22.
Results: Of 118 patients with celiac disease, the mean level of citrulline in patients was 17.48 ± 6.92 and the mean tTG titer was 183.17 ± 41.25. The two variables were inversely correlated with each other, $p < 0.01$. With an increase in Marsh levels, a significant reduction in citrulline levels and an increase in plasma tTG levels were seen, $p < 0.01$, respectively. The mean citrulline and tTG titer was not associated with gender and the age of the patients.
Conclusion: Our findings indicate that citrulline and tTG antibody titer are significant biomarkers for the diagnosis of celiac disease and the severity of intestinal atrophy among pediatric patients.

1. Introduction

Celiac disease is an auto-immune disease that is characterized by chronic inflammation of the small intestine seen by the changes in serological and immunological markers, triggered by the ingestion of gluten [1,2]. The annual incidence of celiac disease is 2–51 per 100,000 children. Type 1 diabetes, autoimmune thyroid disease and the history of the disease in first-degree relative are important risk factors of celiac disease [3]. The prevalence of celiac disease is lower in the Middle East and North Africa, as compared to the American and European regions [4].

Human leukocyte antigen (HLA) and the auto-antigen tissue transglutaminase (tTG) are involved in the pathogenesis of celiac disease, in addition to the gluten. The symptoms of the disease are heterogenous, that can make the diagnosis challenging. In the absence of serological diagnosis, celiac disease can remain undiagnosed [5]. Diagnosis of celiac disease is made by the means of serological markers (tTG antibodies, anti-endomysium antibodies, and deamidated gliadin peptide antibodies) and histopathological findings from the biopsy showing mild villous atrophy and intraepithelial leukocytes. However, recent studies have shown that the use of biomarkers can eradicate the need of biopsy, particularly in pediatric patients [4,6]. Marsh classification divides the intestinal lesions into 5 different categories, which are used for the diagnosis of CD by duodenal biopsy [6].

A number of studies have shown that citrulline, a non-protein amino acid, is synthesized in enterocytes of the epithelial of small intestine. Damage to intestinal epithelial can alter the synthesis of citrulline and therefore, decrease in plasma levels of citrulline can correspond to the

Abbreviations: HLA, Human leukocyte antigen; tTG, tissue transglutaminase; GFD, gluten-free diet; HPLC, high-performance liquid chromatography.

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degree of intestinal atrophy [7].

The aim of this study is to investigate the relationship between citrulline and tTG antibody levels and Marsh pathology from biopsy among celiac disease pediatric patients.

2. Methods

In this cross-sectional descriptive study, children referred to the (XXX), who had already been diagnosed with celiac disease, were included. These patients had antibodies against tTG-IgA and pathological changes in histology with at least 2 Marsh. Samples were obtained for the evaluation of serum citrulline levels from patients who have been referred for follow-up and endoscopy performed by a gastroenterologist and duodenal biopsy. Parental consent was obtained before the participation in the study. The inclusion criteria for the study were all children with celiac disease referred to our center within a year, 2018–2019. Patients under gluten-free diet (GFD), IgA deficiency and autoimmune disease, diseases that increase the risk of damage to intestinal villi such as diarrhea and those with renal dysfunction were excluded from the study.

To determine citrulline using HPLC (high-performance liquid chromatography), serum was deproteinized using an ultrafiltration system, and ion-exchange chromatography was performed using 4 buffer lithium citrate system on Beckman system gold HPLC, as indicated in other studies(8). Biopsy results were categorized using Marsh scores, Marsh 0, 1, 2, 3a, 3b and 3c [4].

According to the percentage of prevalence of the disease in Iran and the number of visits to the Children's Medical Center Hospital, using the Cochran's formula and calculating the error rate of 9%, the sample size was calculated to be 118.

$$N = \frac{Nz2pq}{Nd2 + z2pq}$$

Patients' information including sex, age, height, weight, tTG-IgA antibody levels and familial history of infection were recorded in one form, and serum citrulline levels with tTG antibodies against duodenal pathology results were evaluated. Patients were further divided into different age groups, and sub-groups based on the levels of tTG and citrulline.

The data were computerized and statistically analyzed using SPSSv22. Demographic outcomes of the study were presented in the form of percentage, mean and standard deviation. The relationship between demographic data and antibody and citrulline was assessed using one-way ANOVA test. A Spearman's correlation and Friedman's test was used to evaluate the correlation between biopsy and tTG and citrulline. P-value < 0.05 was considered to be statistically significant.

The methods are stated in accordance with STROCSS 2021 criteria for reported cohort, cross-sectional and case-control study [9].

Unique identifying number is: researchregistry7276.

3. Results

In this study, 118 children with a mean age of 7.0 ± 2.8 years (2–15) years were examined. The mean weight of the children was 20.9 ± 9.6 kg (7–57 kg). Overall, 58.4% children were female and 41.6% were male (Table 1).

The mean tTG antibody level in patients was 183.70 ± 41.25 IU/ml, which was 181.80 ± 43.42 IU/ml in males and 187.71 ± 33.32 IU/ml in females. The mean tTG antibody level was not significantly different in the two groups, $p = 0.41$. The mean citrulline levels were 17.48 ± 6.92 μM (9.5–36 μM), which was 16.82 ± 6.03 μM in females and 18.04 ± 7.67 μM in males (Table2). The mean citrulline levels were not significantly different in the two groups $p = 0.35$. We divided the tTG values into three groups: 0–100, 101–199, and above 199, and the average citrulline in groups 1, 2, and 3 which was 32.24 μM, 26.02 μM, and 14.80 μM, respectively. ANOVA and Pearson's test showed that the serum levels of tTG were significantly associated with citrulline, $P < 0.001$, respectively(Fig. 1).

Children were divided into three different age groups, to evaluate the correlation of citrulline levels with age: 0–5, 6–10 and greater than 10 years. The average in the first group was 17.61, in the second group 17.49, and the third group was 17.85. ANOVA test showed no significant association between age and citrulline levels ($p = 0.97$)(Fig. 2). Independent Samples T Test showed no significant association between sex and citrulline levels ($p = 0.35$), Table 2.

Serum tTG levels among these three age groups were 182.02 IU/ml, 184.92 IU/ml, and 178.32 IU/ml, respectively. ANOVA test showed no significant association between age and tTG antibody ($p = 0.31$)(Fig. 3). Independent Sample T-Test showed no significant association between sex and tTG antibody ($p = 0.41$), Table 4.

Friedman's test was showed that the association between citrulline and tTG antibody and duodenum histology, was statistically significant, $p < 0.001$.

Spearman's correlation coefficient showed that patients under Marsh classification of histologic findings in celiac disease 1–3B had a negative correlation with citrulline levels. In this study, the march above 2 was considered positive.

The outcomes of the biopsy showed that 9.6% (12 patients) were under class 2 of Marsh, 16% (20 patients) in Marsh 3a, 36% (45 patients) in Marsh 3b and 25.6% (32 patients) in Marsh 3c(Table 3) (see Table 5).

The mean hemoglobin level was 11.74.

4. Discussion

Anti-tTG antibody is known as one of the sensitive biomarkers for the diagnosis and initial screening of celiac disease. It also corresponds to the degree of intestinal damage and villous abnormality [10]. The present study was performed on 118 celiac disease patients in the largest children's hospital of (XXX) that is a Tertiary Referral Hospital.

The study found that the tTG antibodies levels was negatively associated to the citrulline levels ($p < 0.001$).

The main finding of the study was that the average level of citrulline and the mean tTG antibody level was negatively correlated to each other, $p < 0.001$. These variables didn't have any correlation with the age groups and gender. The findings of our study reported that an increase in Marsh classification level was associated with reduced levels of citrulline and increased levels of tTG antibody. Glutaminase deamides bond between glutamine and lysine in gluten. Anti-tissue glutaminase has been reported to have high sensitivity for CD screening [11].

Our findings are in parallel with those presented by Ioannou, Fotoulaki [12] reporting that the decrease in plasma citrulline levels is associated with the severity of villous atrophy, which corresponds to an increased Marsh score. Citrulline is a biomarker for enterocyte function and mass [13]. It is known to be associated with the length of residual intestine and villous anomality presented with Marsh grade >2 [11].

Table 1
Patient demographic status.

Age	7 ± 2.8
Weight	9.6 ± 20.9
Sex	49 male (41.6%)
	69female (58.4)%

Table 2
The mean tTG antibody level and citrulline based on sex and correlation. ($p < 0.05$ was statistically significant).

Citrulline	Male	18.04 ± 7.67 μM	P = 0.605
	Female	16.82 ± 6.03 μM	
tTG antibody	Male	181.80 ± 43.42 IU/ml	P = 0.829
	Female	187.71 ± 33.32 IU/ml	

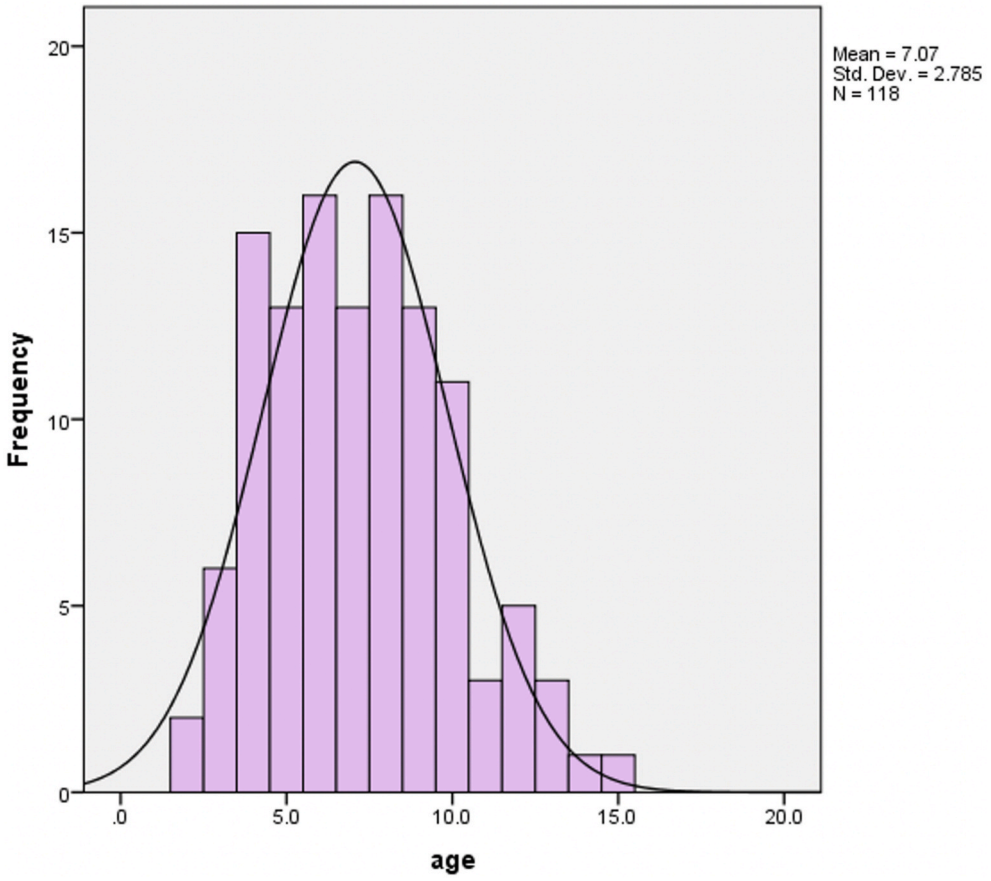

Fig. 1. Distribution of age histograms of children

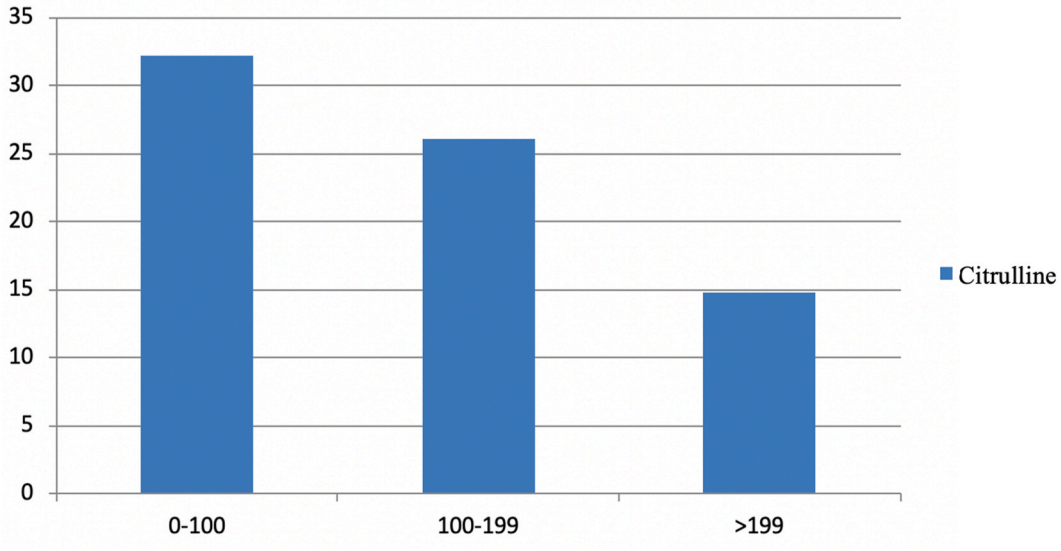

Fig. 2. Correlation between two variables of serum citrulline level and GTT level.

The study also reported that GFD is associated with a significant increase in citrulline levels. Similarly, Basso, Capriati [14] reported that serum citrulline indicates the structural and functional integrity of the small intestine. Blasco Alonso, Serrano Nieto [15] presented a study enrolling 48 untreated celiac disease and 9 GFD patients, to determine a correlation between the severity of intestinal villous atrophy and citrulline level. The outcomes of the study reported that citrulline levels are lower among the patients with severe atrophy as compared to mild atrophy. They concluded that citrulline is an effective marker to determine the intensity of enterocyte mass reduction. Similarly, Ioannou, Fotoulaki [8] also reported that reduced citrulline levels correspond to the severity of villous atrophy, whereas, GFD markedly increased citrulline levels among pediatric patients.

A study Papadia, Sherwood [16] presented that citrulline is not

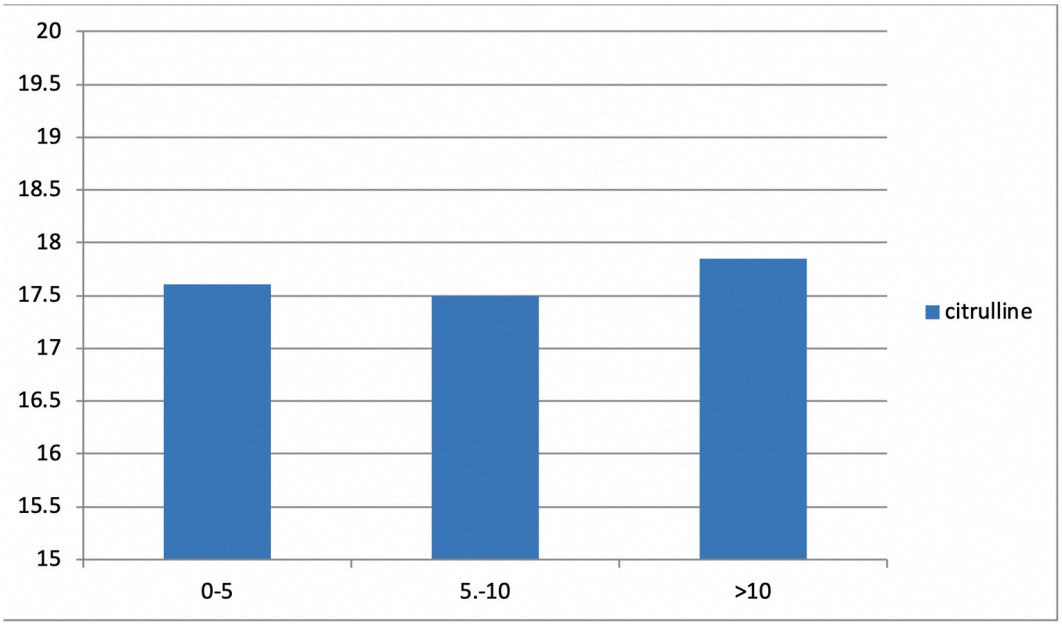

Fig. 3. The relationship of mean citrulline levels in different age groups.

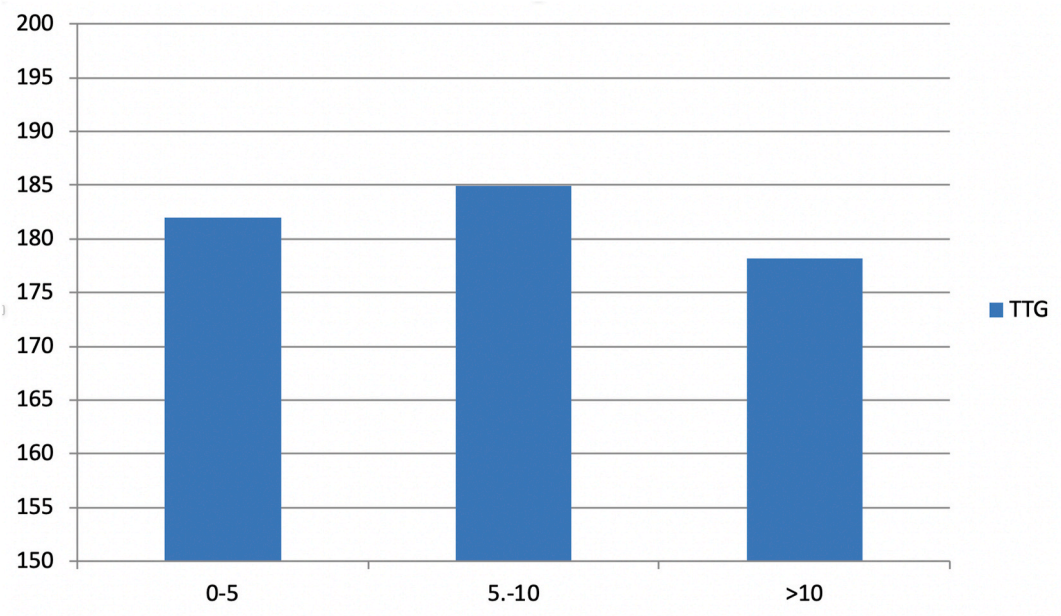

Fig. 4. Mean levels and antibodies against TTG at different ages

Table 3
Frequency of pathology in biopsy of the intestine based on Marsh score (patients with Marsh 2 and more were considered positive).

Biopsy	
Pathology	1 < 5 patients(4%) 1 = 2 patients (1.6%) 2 = 12 patients (9.6%) 3 = 3 patients (2.4%) 3a = 20 patients (16%) 3b = 45 patients (36%) 3c = 32 patients (25.6%)

Table 4
Correlation of age with tTG, citrulline, Marsh score and hemoglobin.

Age	Variable	P-value
	Hemoglobin	0.447
	Citrulline	0.232
	tTG	0.205
	Marsh Score	0.747

significantly correlated with intestinal inflammation among celiac disease and mesenteric infarction patients. However, this conclusion was made from a very small number of patients, (n = 6). Additionally, the study does not incorporate data regarding the Marsh score and the severity of the disease.

Alessio, Tonutti [17], and Singh, Kurray [18] reported that an

Table 5
Analysis of correlation between Marsh score and tTG and citrulline.

Marsh	Frequency	tTG (IU/ml)	Citrulline (μM)
Positive	14	21.86	35.35
Negative	22	145.36	28.53
P-value	–	0.05	0.015

increase in tTG immunoglobin A antibody is significantly associated with villous atrophy and increased Marsh score. Similar findings have been supported in a recent study on pediatric celiac disease patients Meena, Akunuri [19]. These outcomes suggest that a higher titer of tTG antibody can reduce the need for intestinal biopsy [4,20]. However, a study conducted in North America by Halcox, Banegas [21] concluded that a high titer of tTG is not an effective tool for the diagnosis of celiac disease without biopsy. Alharbi, Sweid [4] reported that tTG greater (5–10 times) than baseline in symptomatic patients can predict CD without duodenal biopsy however, a biopsy would still be required for confirmation among patients with a lesser increase in tTG titer and no improvement in symptoms following GFD, since all patients with Marsh >2 were not tTG positive in the study.

5. Conclusion

The findings of our study conclude that increased tTG antibody titer is associated with the reduction in citrulline level among pediatric celiac disease patients. Additionally, intestinal atrophy, seen from Marsh levels, is significantly correlated with an increase in tTG and a reduction in citrulline.

This requires further long-term follow-up studies to predict the prognosis of these two factors on the outcome of disease and mortality and possible morbidities caused by celiac disease. The sensitivity and specificity of these two tests were measured in these patients. According to the hospital's laboratory kits, these two tests had acceptable sensitivity for diagnosing the disease, but the specificity was very low, compared to other studies. This could be because the results of more false positives in our lab.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional Tehran University of Medical Sciences research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Contributors' statement page

Dr. Parisa Rahmani: conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript.

Dr. Fatemeh Farahmand: Designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript.

Dr. Alireza Moradzadeh: and Dr. Ghobad Heidari: Coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.

Consent for publication

Not applicable.

Registration of research studies

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Hyperlink to the registration (must be publicly accessible): https://ethics.research.ac.ir/ProposalCertificateEn.php?id=94352&Print=true&NoPrintHeader=true&NoPrintFooter=true&NoPrintPageBorder=true&LetterPrint=true.

Guarantor

Parisa Rahmani.

Availability of data and material

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Provenance and peer review

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Declaration of competing interest

The authors deny any conflict of interest in any terms or by any means during the study.

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