Original Article

Celiac Disease: Biochemical and Histopathological Considerations of Local Patients

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Abstract

Background: Celiac disease (CD) is a chronic small intestinal condition caused by immune-mediated pathology, which is due to the prolonged deficiency of gluten in genetically susceptible people and induced by the ingestion of a complex protein, gluten, found in cereals such as barley, wheat, and rye. **Objectives:** This study aimed to identify the frequency of individuals affected by CD in Duhok city and the impact of certain environmental factors on the disease occurrence. **Materials and Methods:** The current study was conducted in the Duhok central public health laboratory in Duhok/Kurdistan region/Iraq. This study involved 500 intestinal biopsy samples from which 34 biopsies diagnosed as having CD characteristics by histopathological examination performed by specialists in Duhok central public health laboratory. This finding was also supported by serological testing results using the tissue transglutaminase assay (tTG-IgA). **Results:** The results revealed that female CD patients exceeded male CD patients and represented by 24 (70.6%) and 10 (29.4%), respectively. Furthermore, a total of 18 (52.9%) individuals affected by CD were born in summer and spring months, while 16 (47.1%) subjects were born in fall and winter months. Our work also showed that subjects who had clinical presentation indicating and/or referring to risk factors for developing CD and tested positive for anti-TTG antibodies had a greater probability of manifesting duodenal damage and an ultimate diagnosis of the disease. The result of this study showed that the percentage of breastfed patients was 22 (64.7%) and cow milk-fed patients were 4 (11.8%), five (14.7%) patients were breastfed for ≥ 6 months, and 3 (8.8%) patients were fed both. **Conclusion:** Histopathological and serological assays have powerful diagnostic potential, and one can potentiate the results of others. Environmental risk factors can determine the rate and intensity of the condition.

Keywords: Autoimmune disorder, celiac disease, gluten

Introduction

Celiac disease (CD) is a chronic small intestinal disorder results from immune-mediated pathology which is in turn attributed to the sustained gluten sensitivity in genetically susceptible people and initiated by the consumption of a complex protein, gluten, found in cereals such as barley, wheat, and rye.[1]

Since the early eighties of the last century, the primary approach toward the diagnosis of CD has been the estimation of serum autoantibodies, and nowadays, one of the most highly distributed antibody testing is the measurement of IgA autoantibodies specific for tissue transglutaminase (tTGA). Several approaches for proper estimation have culminated in high sensitivity (90%–95%) and specificity (99%–100%). Autoantibodies are measured utilizing either solid-phase enzyme-linked immunosorbent assays (ELISA) or liquid phase radioligand binding assays, and both procedures have revealed a reliable correlation. IgG antibodies specific for deamidated gliadin peptides (DGP), practiced the last fifteen years, have nearly similar specificity and sensitivity as IgA-tTGA and in the revised ESPGHAN diagnostic criteria for CD, IgG-DGP is preferred to be conducted for individuals with deficient IgA and children no more than 2-year-old.[2]

All IgA-based methods should be accompanied by detection of IgA serum levels because CD is more frequent in IgA deficient patients. In IgA deficient CD victims, IgG-tTGA and IgG-DGP show similar sensitivities as for IgA-tTG in IgA-sufficient people. Gluten ingestion is mandatory for the reliability of

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measurement of all the above-mentioned antibodies. Thus, the result of the tests can mark the response to treatment and monitor the dietary compliance.[3]

However, in uncertain cases with minimal concentrations of tTGA with mild or without clinical manifestations of the disease, intestinal biopsies obtained by upper endoscopy or through capsule still constitute a significant diagnostic method.[4]

This study aimed to identify the frequency of individuals affected by CD in Duhok city and the impact of certain environmental factors on the disease occurrence.

Materials and Methods

Study design

The study started with sample collection from September 2018 to December 2019. It was a cohort retrospective (back to 2011) and prospective one to diagnose CD. Enrolled individuals were those who attended Heevi Hospital laboratory and Duhok central public health laboratory.

Patient group

Five hundred intestinal biopsies were included in the current work; the ages of participants ranged from 6 months to 61 years. Each patient was provided with a paper form specifying age, sex, marital status, symptoms, and history.

Clinical presentation

Included in this study were the participants who attended the hospital, had specific medical and surgical complaints, and happened to be diagnosed as CD affected subjects. A thorough family history, age of patients at the onset of symptoms, age of patients at the time of collecting samples, and laboratory test results were taken from the medical records.

Samples collection

After obtaining informed consent from all involved subjects, 3 ml of venous blood samples were collected in 3.2% trisodium citrate tubes, processed immediately, centrifuged at 4000 rpm for ten minutes, serum then segregated and kept at −20°C.

The incidence of CD was determined using the two-step testing approach; first, the tTG-IgA to identify primarily those who had elevated serum anti-transglutaminase antibodies. Second, histopathological examination of patients' biopsies, which yielded an optimal diagnostic picture of the disease.[5]

Of the 500 biopsies, 34 were diagnosed as having CD features by histopathological examination performed by specialists in Duhok central public health laboratory. Serological testing, which aimed to assess tTGA, was also performed by the ELISA method as a preliminary test to select individuals who had CD.

The TTG IgA test

In the specific antigen coated microplates, diluted serum samples (1:101) were aspirated. If a sample contains antibodies they will eventually bind antigen forming detectable complexes. All dilutions were made as per the manufacturer's instructions (AESKULISA tTg-A New generation kit).

Steps of the TTG IgA test

One hundred microliters of calibrators (CAL. A to CAL. F) and 100 µl of each of the following: Diluted serum (P1, P2.etc.), Positive control, and negative control (NC) were pipetted into the designated wells. Samples and calibrators were incubated for 30 min at 20°C–32°C; then they were washed three times with 300 µl diluted (1:50) washing buffer. One hundred microliters of Conjugate were pipetted into each well. The microplate was incubated for thirty minutes at 20°C–32°C. The washing step repeated. Into each well, one hundred microliters TMB substrate was pipetted. The microplate was incubated in dark for 30 min at 20°C–32°C. One hundred microliters of stop solution were added into each well. The microplate was incubated for 5 min and then agitated carefully for 5 s and the absorbance read at 450 nm (recommended 450/620 nm) within 30 min.

Histopathological examination

The histopathological examination considered the gold standard for the diagnosis of CD and was carried out by obtaining and processing a tiny intestinal. Histopathological examination was performed by specialists in Duhok central public health laboratory. The histopathology report often included the description of the villus status, the degree of atrophy, and crypt elongation.

Statistical analysis

Statistically significantly different findings were considered if $P < 0.05$. Data were analyzed using SPSS version 20.0 (SPSS, IBM Company, Chicago, IL 60606, USA), specifically Chi-square of independence to find P value.

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee.

Results

This study involved 34 (6.8%) CD positive biopsies out of 500 intestinal biopsies recovered from patients from 2011 to 2020. The participants consisted of 24 (70.6%) females and 10 (29.4%) males resembling a total of 34 patients [Table 1].

Season of birth

Birth season effect on the occurrence of CD in Dohuk city was studied. A total of 18 (52.9%) individuals affected by CD were born in summer and spring months, and 16 (47.1%) subjects were born in Fall and Winter months. There was no statistically significant difference between summer and winter births as far as CD occurrence is concerned ($P > 0.05$) [Table 2].

Table 1: The risk of celiac disease in male and female

Gender	Number of celiac disease (%)
Male	10 (29.4)
Female	24 (70.6)

Table 2: Distribution of patients according to season of delivery

Number of patients	Summer (%)	Winter (%)	*P*
34	18 (52.9)	16 (47.1)	0.731601

Table 3: Correlation between anti-tissue transglutaminase ratio and histological findings with *P* value

Biopsy finding	TTG IgA level (%)		*P*
	<8 (U/ml)	>8 (U/ml)	
Frequency of patient with normal histology (%)	2 (9.5)	1 (4.8)	0.563703
Patient with subtotal villus atrophy	1 (4.8)	11 (52.4)	0.003892*
Patient with total villus atrophy	0	6 (28.6)	0.014305878

*Significant at $P < 0.05$. TTG: Tissue transglutaminase

TTg level and biopsy

The present investigation showed that subjects who had clinical presentation indicating and/or referring to risk factors for developing CD and tested positive for anti-TTG antibodies had a greater probability of manifesting duodenal damage and an ultimate diagnosis of the disease. In the current study, an anti-TTG antibody value >8 has been shown to be associated with the presence of histological lesions Marsh ≥2 (subtotal villous atrophy) and diagnosis of CD. The significant difference ($P < 0.05$) has been obtained when patients suffering subtotal villus atrophy who had TTG IgA level <8 (U/ml) were compared with those who had TTG IgA level >8 (U/ml).

A value >30 had an often maximum (100%) association with atrophic lesions (Marsh 3a, b, c) and 100% positive predictive value for CD. The results also indicated a significant difference ($P < 0.05$) when the same classification criterion was adopted but in patients showing total villous atrophy this time [Table 3].

Type of feeding

The result of this study showed the frequency and percentage of breastfed patients 22 (64.7%) and cow milk-fed patients 4 (11.8%), while there were only 5 (14.7%) patients who experienced breastfeeding for ≥6 months of age and 3 (8.8%) patients were fed both [Table 4].

Discussion

CD is an autoimmune disorder (a state where the body's immune system mistakenly directs an attack against the host tissue), and women notably have a greater opportunity for autoimmune disease than men. As with CD, researchers have not been capable of comprehensively clarify the total higher risk of autoimmune disorders in women either.[6]

The findings of the present study were nearly comparable to the results of Llorente-Alonso *et al*.,[7] who stated in the findings of their cross-sectional study that the CD group involved 87 females (69.6%) and 38 males (30.4%). In addition, in a study conducted in Saudi Arabia, it was found that 17 females (3.1%) and 9 males (1.5%) were affected by CD and Al-Qaseem district had the highest CD prevalence among the three intended areas (Aseer, Madinah and Al-Qaseem region) of the country.[8]

The month of birth could presumably influence both the normal microbial flora and the innate immune response of individuals at risk of CD during multiple encounters of infectious agents. It was hypothesized that summer birth babies would be linked to subsequent CD. This observation was derived from the rationale that offspring born in summer period are more likely to have primary exposure to gluten during winter months when a concordant viral infection is highly likely.[9]

The finding of the current study was in line with the results of Tanpowpong *et al*.[10] who indicated that the CD group included 382 subjects with biopsy-diagnosed CD. 56% were born in spring/summer compared to 44% in fall/winter. In addition, in a finding conducted in Sweden, it was found that 54.10% of individuals with CD were born in the summer months. Summer birth was hence connected to a minor exaggerated hazard of later CD emergence.[9]

On the other hand, in another cohort study, it was found that CD risk was greater for children born during spring, summer and autumn as compared to children born during winter.[11]

In the primary stage of the disease, CD-associated mucosal injury and leanness may take place intermittently; thus, at least four endoscopic biopsies should be obtained from varying locations of the duodenal mucosa. The specimens must be accurately processed. It is general notion that an improper processing of the sample can induce both pseudo shortening of the villi and a high lymphocytic count in epithelium and lamina propria, which lead to a mistaken diagnosis of CD.[12] Occasionally, particularly in quite young offspring, the amount of material acquired may be insufficient to attain a decisive decision; a European multicenter research revealed that >10% of histological biopsies recovered for suspected CD were inadequate for conclusive final judgment.

Another determining element of histological investigation is the variability among observers and the poor reproducibility of the pathological findings. Corazza *et al*.[13] stated that the extent of agreement among pathologists with analogous expertise did not surpass 75%, and was even less, approximately 25%, for the primary stage lesions (modified Marsh types 1 and 2), which may result in suspicions or diagnostic inaccuracy.

Finally, in early life, esophagogastroduodenoscopy is often practiced under anesthesia, with elevated jeopardy and

Table 4: Percentage of type of feeding with celiac disease

Frequency and percentage of Breastfed subjects	Frequency and percentage of Bottle fed subjects	Frequency and percentage of Breastfed babies ≥ 6 months	Both feeding methods
22 (64.7)	4 (11.8)	5 (14.7)	3 (8.8)

decreased compliance of parents to present the child to such a harsh examination technique.

Several researches have attempted to establish a relationship between autoantibody concentrations and the amount of mucosal impairment, to limit the number of endoscopies needed for the diagnosis of CD.[12]

The current work supports the conclusions of other investigators and ascertain that in a particular group of people, elevated levels of autoantibodies are associated with a higher grade of mucosal injury and existence of CD in virtually all of the situations.[12]

Hansson *et al*.[14] showed a connection between anti-TTG antibody concentrations and mucosal destruction in a cohort of 57 infants with a definitive diagnosis of CD; similar findings were attained by Tursi *et al*.[15] in mature individuals by means of a guinea pig anti-TTG assay; they similarly proposed a conceivable role for anti-TTG antibodies in expecting mucosal injury, irrespective of the source of the TTG. Diamanti *et al*.[16] presumed that in subjects with clinical signs, there was a robust association between anti-TTG antibody levels and the grade of mucosal damage, and evidenced that an anti-TTG cutoff value of 20 U/mL (5 times the cutoff) was appropriate for anticipating mucosal degenerate.

Conversely, a study occurs in the Czech Republic; found that there have been negative antibodies with Marsh IIIC, indicating that serological screening is not 100% precise for the diagnosis of CD.[17]

Genetic predisposition is not the only factor that determines the development of CD; therefore, other environmental risk aspects, such as how gluten is presented in the infant's regime, have been speculated.[18]

The results of this investigation were nearly analogous to the results of Størdal *et al*.,[19] which described an amplified risk of CD in newborns breastfed for >12 months compared to individuals breastfed for <6 months.

Contrary to our results, Ivarsson *et al*.[20] compared breastfeeding period in people born in 1993 and 1997. They suggested that the people with minor CD risk, that born in 1997, were breastfed for a further extended duration than those born in 1993. Thus, lengthy breastfeeding beyond diet allowance could lessen the risk of CD. Moreover, in Sweden, no statistically significant differences were found in breastfeeding duration, age at initiation of cows' milk products, frequency of breastfeeding after gluten introduction, and gluten consumption.[21]

Conclusion

Circumstantial risk factors have a significant impact on disease development.

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Conflicts of interest

There are no conflicts of interest.

References

1. Kratzer W, Kibele M, Akinli A, Porzner M, Boehm BO, Koenig W, *et al*. Prevalence of celiac disease in Germany: A prospective follow-up study. World J Gastroenterol 2013;19:2612-20.
2. Frulio G, Polimeno A, Palmieri D, Fumi M, Auricchio R, Piccolo E, *et al*. Evaluating diagnostic accuracy of anti-tissue Transglutaminase IgA antibodies as first screening for Celiac Disease in very young children. Clinica Chimica Acta 2015;446:237-40.
3. Giersiepen K, Lelgemann M, Stuhldreher N, Ronfani L, Husby S, Koletzko S, *et al*. Accuracy of diagnostic antibody tests for coeliac disease in children: Summary of an evidence report. J Pediatr Gastroenterol Nutr 2012;54:229-41.
4. Korponay-Szabó IR, Troncone R, Discepolo V. Adaptive diagnosis of coeliac disease. Best Pract Res Clin Gastroenterol 2015;29:381-98.
5. Borsuk AM. Doctoral dissertation, The Ohio State University; 2013.
6. Jansson-Knodell CL, King KS, Larson JJ, Van Dyke CT, Murray JA, Rubio-Tapia A. Gender-based differences in a population-based cohort with celiac disease: More alike than unalike. Dig Dis Sci 2018;63:184-92.
7. Llorente-Alonso MJ, Fernandez-Acenero MJ, Sebastian M. Gluten intolerance: Sex and age-related features. Can J Gastroenterol 2006;20:719-22.
8. Aljebreen AM, Almadi MA, Alhammad A, Al Faleh FZ. Seroprevalence of celiac disease among healthy adolescents in Saudi Arabia. World J Gastroenterol 2013;19:2374-8.
9. Lebwohl B, Green PH, Murray JA, Ludvigsson JF. Season of birth in a nationwide cohort of coeliac disease patients. Arch Dis Child 2013;98:48-51.
10. Tanpowpong P, Vassallo M, Katz AJ, Camargo CA. Season of Birth and Celiac Disease in Massachusetts Children. Gastroenterology 2011;140:S-442.
11. Namatovu F, Lindkvist M, Olsson C, Ivarsson A, Sandström O. Season and region of birth as risk factors for coeliacdisease a key to the aetiology?. Arch Dis Child 2016;101:1114-8.
12. Alessio MG, Tonutti E, Brusca I, Radice A, Licini L, Sonzogni A, *et al*. Correlation between IgA tissue transglutaminase antibody ratio and histological finding in celiac disease. J Pediatr Gastroenterol Nutr 2012;55:44-9.
13. Corazza GR, Villanacci V, Zambelli C, Milione M, Luinetti O, Vindigni C, *et al*. Comparison of the interobserver reproducibility with different histologic criteria used in celiac disease. Clin Gastroenterol Hepatol 2007;5:838-43.
14. Hansson T, Dahlbom I, Rogberg S, Dannæus A, Höpfl P, Gut H, *et al*. Recombinant human tissue transglutaminase for diagnosis and follow-up of childhood coeliac disease. Pediatr Res 2002;51:700-5.

15. Tursi A, Brandimarte G, Giorgetti GM. Prevalence of antitissue transglutaminase antibodies in different degrees of intestinal damage in celiac disease. J Clin Gastroenterol 2003;36:219-21.
16. Diamanti A, Colistro F, Calce A, Devit R, Ferretti F, Minozzi A, *et al*. Clinical value of immunoglobulin A antitransglutaminase assay in the diagnosis of celiac disease. Pediatrics 2006;118:e1696-700.
17. Makovicky P, Rimarova K, Boor A, Makovicky P, Vodicka P, Samasca G, *et al*. Correlation between antibodies and histology in celiac disease: Incidence of celiac disease is higher than expected in the pediatric population. Mol Med Rep 2013;8:1079-83.
18. Pinto-Sánchez MI, Verdu EF, Liu E, Bercik P, Green PH, Murray JA, *et al*. Gluten introduction to infant feeding and risk of celiac disease: Systematic review and meta-analysis. J Pediatr 2016;168:132-43. e3.
19. Størdal K, White RA, Eggesbø M. Early feeding and risk of celiac disease in a prospective birth cohort. Pediatrics 2013;132:e1202-9.
20. Ivarsson A, Hernell O, Stenlund H, Persson LA. Breast-feeding protects against celiac disease. Am J Clin Nutr 2002;75:914-21.
21. Ascher H, Krantz I, Rydberg L, Nordin P, Kristiansson B. Influence of infant feeding and gluten intake on coeliac disease. Arch Dis Child 1997;76:113-7.