ORIGINAL ARTICLE

Serodetection of anti-tissue-transglutaminase antibodies amongst children under 5 years old suffering from coeliac disease symptoms in the Omdurman Military Hospital, Khartoum

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ABSTRACT

Background: Coeliac disease is the most common disease in children less than 5 years old andmay cause many serious complications. The frequency of anti-tTG (IgA) in these children were also determined.
Aim: To detect anti-tissue-transglutaminase (anti-tTG) antibodies (i.e., IgA) in children under5 years old with signs and symptoms of coeliac disease, as well as to detect the relationship of seropositivity with signs, symptoms, age, and gender.
Methods: This work was a descriptive, cross-sectional study performed atthe Omdurman Pediatric Military Hospital (Khartoum) from April to June 2017. A total of 90children under 5 years of age, including40 (44.4%) males and 50 (55.6%) females, were enrolled. These patients suffered from coeliac disease signs and symptoms. Anti-tTG (IgA)levels were tested in serum specimens through enzyme-linked immunosorbent assay (ELISA). Data were collected using a questionnaire andanalyzed with SPSS program version 16.
Results: The overall results revealed that 5(5.6%) were positive for anti-tTG, 4 of whom were females. Statistical analysis showed significant relation between coeliac disease and gender (P = 0.055). Regarding age, all positive for coeliac disease were 2–3years old. Statistical analysis showed a significant relation between anti-tTG seropositivity and age (P = 0.000).
All infected children (positive for anti-tTG) were suffering from diarrhea, bloating, and abdominal pain.
Conclusion: Coeliac disease was prevalent inchildren, especially amongst those aged 2–3 years. Statistical analysis showed significant relation anti-tTG seropositivity,age,and gender.
Keywords: Serodetection rate, Anti-tissue transglutaminase antibodies, Coeliac disease, Children

INTRODUCTION

Coeliac disease (CD) is a condition involving an abnormal small-intestinemucosa that reverts to normal when patients are treated with a diet free from gluten (a protein found in cereals, such as wheat, rye, barely, and possibly oat) and relapses when gluten is reintroduced[1]. This disease can develop at any age, with symptoms ranging from the classic signs of malabsorption syndrome (e.g., diarrhea, weight loss, growth failure, osteoporosis, and anemia) to nonspecific symptoms (e.g., chronic constipation or abdominal pain)[2,3]. CD is a genetically based autoimmune disorder that leads to malabsorption from the small intestine, and it occurs in children and adults who are susceptible when they eat gluten[1].

CD is estimated to affect 1 in 100 people worldwide. Two and a half million Americans are undiagnosed and are at risk for long-term health complications[1].This disease can develop at any age after people start eating foods or medicine containing gluten. If left untreated, CD can lead to additional serious health problems, such as iron-deficiency anaemia, early onset osteoporosis or osteopenia, infertility and miscarriage, lactose intolerance, vitamin and mineral deficiencies, central and peripheral nervous system disorders (e.g., ataxia and leucoencephalopathy), pancreatic insufficiency, and gall bladder malfunction[4].

CD is hereditary, and people with a first-degree relative having CD (parent, child, or sibling) have a 1 in 10 risk of developing CD. This disease, also known as CD, celiac sprue, non tropicalsprue, and gluten-sensitive enteropathy[1], can lead to malignancies such as non-Hodgkin lymphoma (intestinal and extra-intestinal, T- and B-cell types), small-intestine adenocarcinoma, esophageal carcinoma, papillary thyroid cancer, and melanoma. CD is associated as well with a number of autoimmune disorders, with the most common being thyroid disease and type-1 diabetes[4].

The first report of CD in Sudan was diagnosed in seven Sudanese children. The diagnosis based on clinical and histological improvement after administering a gluten-free/sorghum-free diet[5]. The diagnosis of CD in Sudan depends only on the histological findings of small-bowel biopsy, and serological tests are not yet used. The incidence is probably higher and masked by more prevalent conditions, such as protein-energy malnutrition, diarrheal diseases, and parasitic infections[6].

Anti-tissue transglutaminase (anti-tTG) antibodies have become the accepted diagnostic indicator of CD[7]. Accordingly, this study aimed to determine frequency of anti-tTG antibodies in children less than 5 years old with CD symptoms.

MATERIALS AND METHODS

This work was a descriptive, cross-sectional study carried out at Omdurman Pediatric Military Hospital (Khartoum, Sudan) from April to June 2017.Ethical approval from the Research Ethical Review Board of Al Neelain University and verbal consents from parent/s of each patient were obtained. Data were collected using a structural questionnaire covering demographical and clinical data, e.g., age, sex, signs, and symptoms. Collected data were analysed using SPSS program. The relationship of serodetection rates with age, sex, and signs and symptoms were analysed using the chi-square test, and the p-value was calculated.

The sampling technique used was a nonprobability, convenience-sampling type. The sample size was 90 children under 5 years of age. Venous whole blood (5 mL) was collected from each child under aseptic conditions. Serum was collectedby centrifugation and was stored at -20 °C. Anti-tTG antibodies (IgA) were tested for in-serum specimens by using the enzyme-linked immunosorbent assay (ELISA). Sandwich ELISA technique was performed using qualitative kits to detect specific IgA antibodies in human serum (AESKU DIAGNOSTIC, Germany).

Procedure: All reagents and samples were handled at room temperature. Serum samples were diluted1:101 and incubated in a microplate coated with the specific antigen. The binding of a patient's antibodies in the specimen to the antigenwas observed. Afterwards, anti-human immunoglobulins were conjugated to horseradish peroxidase (conjugate) bovine serum albumin and reacted with the antigen–antibody complex of the microplatesamples. A sufficient number of microplate modules was prepared to accommodate controls and prediluted patient samples. About 100 µLof calibrators, controls, and prediluted patient samples were pipetted into the wells. After incubationfor 30 min at room temperature (20–28 °C), the contents of the microwells were discarded and washed thrice with 300 µLof washing solution(Tris, NaCl, Tween 20, and <0.1% sodium azideas a preservative). Then, 100 µLof enzyme conjugate (anti-human immunoglobulins conjugated to horseradish peroxidase) was dispensed, and incubated for 15 min at room temperature. After discarding the microwell contents and washing thrice with 300 µLof washing solution, 100 µL of stabilize dtetremethylbenzidine (TMB) and hydrogen peroxide (TMB/H_2O_2) substrate solution was dispensed, and the solution was incubated for 15 min at room temperature. Finally, 100 µL of stop solution (1 M hydrochloric acid) was added to each well of the modules, and incubation was performed for 5 min at room temperature. Optical density was read at 450 nm (450–620) nm, and results were calculated.

Reading and interpretation: For **qualitative interpretation**, the optical density of the cutoff calibrator and patient samples were read and compared. For qualitative interpretation, the considerations were as follows:

Negative		**OD patient**	**<**		**0.8 × OD cutoff**
Equivocal	0.8×	OD cutoff	≤OD patient	≤	1.2 × OD cutoff
Positive:		OD patient	>		1.2 × OD cutoff

Data analysis: Data were analysed by SPSS software version 16.

RESULTS

Ninety children with CD signs and symptoms were enrolled in this study. Among them, 5(5.6%) were positive for anti-tTGIgA (Fig. 1), and 4 (4.4%) of them were female (Table 1).

Regarding age, all study populations under 5 years old and all children positive for CDranged within 2–3years old (Table 2).

Statistical analysis showed significant correlation between anti-tTG seropositivity and age (P = 0.000). All studied children suffered from constipation, and most suffered from bloating. All infected children (positive for tTG) had diarrhea and bloating, and most of them had abdominal pain. Table 3 shows the different clinical presentations with the serofrequency of anti-tTG IgA

Table 1: Serofrequency of anti-tTGamongst study populations in relation to gender

Anti-tTGIgA	**Gender**		**Total**
	Male	**Female**	
Positive	1(1.1%)	4(4.4%)	5(5.6%)
Negative	39(43.3%)	46(51.1%)	85(94.4%)
Total	40(44.4%)	50(55.6%)	90(100%

P = 0.055

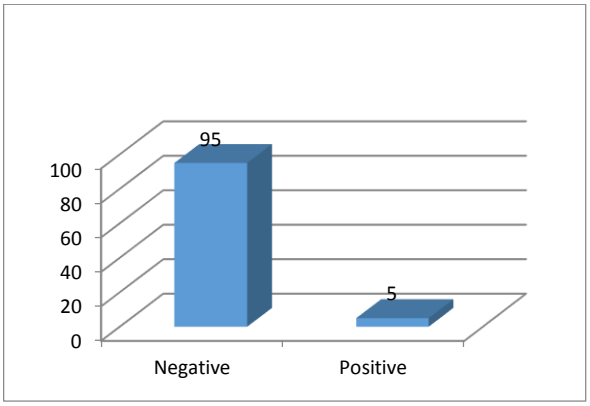

Table 2: Sero frequency of anti-tTGamongst study populations in relation to age

Age	**Anti-tTG–IgA**		**Total**
	Positive	**Negative**	
0 >1 year	0	39	39
1>2year	0	0	0
2>3 year	5	45	50
3>4 year	0	0	0
4>5 year	0	1	1
Total	5	85	90

P = 0.000

Table 3: Serofrequency of anti-tTG IgA amongst study populations in relation to patient signs and symptoms (n=90)

Signs and symptoms		anti-tTG IgA		Total n=90)
		Positive (5)	Negative (n=85)	
Abdominal pain	Yes	5	40	45
	No	0	45	45
Bloating	Yes	5	85	90
	No	0	0	0
Diarrhea	Yes	5	40	45
	No	0	45	45
Constipation	Yes	1	78	79
	No	4	7	11
Fatigue	Yes	3	40	43
	No	2	45	47
Bone or joint pain	Yes	3	2	5
	No	0	85	85
Anaemia	Yes	1	36	37
	No	4	49	53
Weight loss	Yes	3	23	26
	No	2	62	64
Delay growth	Yes	3	35	38
	No	2	50	52
Recurrent mouth canker	Yes	1	53	54
	No	4	32	36

DISCUSSION

CD is a serious genetic autoimmune disorder where the ingestion of gluten inflicts damage to the small intestine. If undiagnosed, CD may lead to serious health complications[8]. CD was first reported in Sudan by Dr. GaafarIbn Aofin (1978) when seven children were diagnosed by intestinal biopsy[9].

In the present study, the overall frequency of anti-tTG IgA amongst the studied children was 5(5.6%). This finding was lower than that of Mokhtar and her coauthors (2013), who reported a seropositivity of about 41.6%[10]. Conversely, Hussien and his colleagues reported a value of 27.3% in 2013[11], whereas Ageep reported a finding of 74.4%seropositivity ratein2012[12].

In 2002, Ilham studied 80children and reported that the frequency of CD was 55%. A total of 18 children (22.5%) were confirmed using at least two markers of CD and duodenal biopsy confirmatory for CD. A total of 26 children (32.5%) were probable CD (with at least one marker positive and no confirmatory biopsy). A total of 36 children (45.0%) were non-coeliacs (with negative AgA test for IgA and IgG classes)[6].

The differences between the results of the present research and previous Sudanese studies were due to their use of other diagnostic methods and specimens (i.e., small-intestine biopsy, anti-gliadin antibody test for IgA and IgG classes, and anti-tTGfor IgG and IgA).

Compared with regional studies, the present results were found to be lower than those obtained by Al-Twaty and Albargathy, who studied 243 Libyan children in 1998 and diagnosed 77% of them as coeliacs[13].

In the current work, all positive patients belonged to the 2–3 years age group, and no association existed between CD and age. However, a significant relation of CD with gender was observed.

Meanwhile, infants <4 months old were initially protected to some extent by maternal antibodies against diarrhea owing to CD. They apparently acquired adequate immunity between 12–16 months of age. A greater risk of infants and young children was found in the interim period of 6–12 months when the levels of maternal antibodies to CDdeclined[14].

Furthermore, children between 2–3 years of age had the highest incidence of admission and were more likely to be ill with CD than all other age groups. This result emphasized the importance of CD as an etiological agent of severe childhood diarrhea requiring hospital admission throughout the period of severity with regard to the gender.

Regarding presenting symptoms, bloating and diarrhea were the most common amongst the studied population, with 100% of positive cases suffering from them. In Iran, Lebanon, Iraq, Saudi Arabia, and Kuwait, CDis reportedly one of the most common causes of chronic diarrhea[15], with 5 out of 5 positive cases suffering from abdominal pain, 3 out of 5 suffering from fatigue, bone or joint pain, and weight loss. In Egypt, 4.7% of children presenting with diarrhea and weight loss had CD[15]. The variation in the prevalence of clinical manifestations of CD across different studies may be due to the low number of patients evaluated[12].

In the present study, delayed growth was observed amongst 3 out of five (60%) positive cases, which was higher than that reported from Jordan where 26% of children had CD[15].

Anemia was noted in 1out of 5CD patients and was due to iron deficiency in most of the cases. The worldwide prevalence of CD amongst patients with iron deficiency anemia was 2.8%–8.7% and may be as high as 15%[16,17]. Folate and B12 deficiency may contribute to anemia in CD, and surprisingly, anemia due to chronic disease is relatively common[18].

CONCLUSION

In conclusion**,** CD was prevalent amongst children aged 2–3 years. Confirmation of CD by laboratory testing of blood samples was necessary for reliable CD surveillance and can be useful in the clinical setting.

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Authors' contributions: RAI designed and conducted the study and drafted the paper. MHM participated in practical work. WIE supervised all study starting from proposal writing, coordinated with the laboratory quality-control team, and revised and approved the manuscript particularly the critical contents. All authors read and approved the final manuscript.

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