

## Screening of Celiac Disease Using Anti-Tissue Transglutaminase-IgG Test Among Helminths-Infected School Children in Ziway, Southeast Ethiopia

Mekdes Alem<sup>1\*</sup>, Kassu Desta<sup>1</sup>, Bineyam Taye<sup>2</sup>, Mussie Girma<sup>3</sup>, Mistire Wolde<sup>1</sup>, Aster Tsegaye<sup>1</sup>

### Abstract

**Background:** Celiac disease is a major health problem worldwide. It is a gluten-induced inflammation of the small intestine, marked by elevated levels of serum auto anti-bodies against tissue transglutaminase, including anti-tissue transglutaminase IgG (Anti-tTG). Anti-tTG anti-bodies are auto anti-bodies, highly sensitive and specific for the diagnosis of Celiac disease (CD). Celiac disease is an overlooked disease among school-aged children that account for a missed diagnosis in children's morbidity and mortality.

**Objective:** To screen the prevalence of celiac disease - using an anti-tissue transglutaminase IgG anti-body among Helminths-infected school children in Ziway, South East Ethiopia.

**Methods:** A cross-sectional study was conducted in Ziway, South-Eastern Ethiopia. Socio-demographic data and medical records were taken from a previous study. Left-over samples from that study that were stored at -80°C were analyzed for anti-tissue transglutaminase IgG antibodies. The anti-tissue transglutaminase IgG measurement used the principle of Enzyme immunoassay (ELISA). Children samples positive for anti-tissue transglutaminase were further assessed for any differences between the Helminths infected and non-infected children by using Binary logistic regression. Data was entered and analyzed by using SPSS version 23, and descriptive analysis, Chi-square, and logistic regression were used to see any associations, and P-value <0.05 was considered statistically significant.

**Result:** This cross-sectional study involved 97 left-over samples of children between 6 and 14 years old. Among these, 18 (18.6%) of study participants tested positive for anti-tissue transglutaminase IgG, which suggests a positive celiac disease diagnosis. Females had a higher prevalence of anti-tTG IgG than males, 13/18 (72.2%). A significant association was found between Helminthes infection and anti-tTG IgG [COR 3.45 (95% CI:1.057,11.265), P= 0.033].

**Conclusion:** This study showed that prevalence of celiac disease as screened by anti-tissue transglutaminase IgG to be 18.6% and was 3.45 times higher among Helminthes infected school children than non-infected ones. This screening test might suggest that children who acquired helminths infections had a high chance of developing Celiac Disease. To gain a better insight, further large-scale studies are strongly recommended. [*Ethiop. J. Health Dev.* 2024; 38(2): 00-00]

**Keywords:** Anti-tissue transglutaminase IgG, celiac disease, Helminthes

### Introduction

Celiac disease (CD) is a gluten-induced inflammation of the small intestine. Celiac disease in its active phase is accompanied by elevated levels of serum immunoglobulin IgA auto anti-bodies against tissue transglutaminase. However, the specificity of IgG-tTG is comparable to that of IgA-tTG (1).

The development of celiac disease is believed to be initiated by proteins found in wheat, barley, and rye, that cause damage to the intestinal epithelium releasing the enzyme tissue transglutaminase (tTG). Cross-linking by the enzyme produces gliadin-gliadin or gliadin-enzyme complexes, which trigger an immune reaction to cells in the intestine in genetically susceptible individuals. The characteristic enteropathy is then induced by the release of interferon- $\gamma$  and other pro-inflammatory cytokines (2).

The poor diagnosis of celiac disease often contributes to more than 20 associated symptoms and conditions, including growth failure, infant malnutrition, gastrointestinal disease, liver conditions, and mortality (3).

Celiac disease is a major health problem worldwide. However, it is not diagnosed routinely, which leads to the missed burden it contributes to childhood morbidity and mortality (4). The prevalence of celiac disease varies among age, sex, and geographic locations (5).

Parasitic Helminths have matured within different mammals while promoting their survival by changing the host's immune responses. This immune response by these Helminths activates tissue repair that brings about a predominant anti-inflammatory response; minimized infection experience in early childhood may lead to a high risk of hypersensitivity later in life (6).

Different auto anti-bodies and anti-bodies are used

<sup>1</sup> Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, Ethiopia

<sup>2</sup> Department of Biology, Colgate University Division of Natural Sciences and Mathematics, Hamilton, New York, USA

<sup>3</sup> Aklilu Lema Institute of Pathobiology, Addis Ababa University

\*Corresponding author email: mk.alem12@gmail.com

in the diagnosis of celiac disease, but anti-tTG antibodies are highly sensitive and specific for the diagnosis of CD. IgG-based tests are one of the most useful autoantibody tests for celiac disease (7).

Ethiopia, like many sub-Saharan African countries, grapples with a relatively high prevalence of helminths infections. These parasitic infections have been a significant public health concern in Ethiopia, particularly among rural school children. Although the exact mechanism is yet unknown, Helminths infection is believed to induce immunologic tolerance, preventing autoimmune diseases like celiac disease by modulation of bacterial communities in the intestine (6).

Recently, different research performed on simple, sensitive, and specific screening tests like the anti-tissue transglutaminase anti-body assays in the developing areas of the world, where there is a

## Materials and Methods

**Study design and setting:** A cross-sectional study was conducted on elementary school children in Ziway, southeast of Addis Ababa, Ethiopia. This study used a stored sample that was collected for a study on the association between *H.pylori* infection and P platelet indices, Baxendell K *et al.*, 2018 (10). Socio-demographic data and Helminths infection status of these study participants were taken from the previous study in retrospective. Results of wet mount preparations from the collected data were taken to assess the Helminths infection and those positive for any intestinal parasites were labeled as Positive, and those with No finding of Intestinal parasite as negative for ease of analysis.

### Sample selection criteria

Sample availability was cross-checked by taking the recorded information about the Helminthes infection status with the sample ID on serum-containing microtubes. Then, samples with sufficient serum volume (1 ml) were taken, and those samples with no Hemolysis were selected for this study as part of the prospective part of the analysis, which is a measurement of anti-tissue transglutaminase IgG. Accordingly, a total of 97 samples with no Hemolysis and sufficient volume were selected and analyzed from May 2020 to June 2020.

## Laboratory Testing

**ELISA to detect Anti-tissue transglutaminase IgG anti-body:** Anti-tTG-IgG is an enzyme immunoassay technique to determine IgG autoantibodies to tissue transglutaminase in human serum. Auto anti-bodies react with human tissue transglutaminase immobilized on the solid phase microtiter plate; after incubation and washing step bound, auto anti-bodies react specifically with anti-human IgG anti-bodies conjugated to horseradish peroxidase(HRP). HRP converts the colorless substrate 3, 3' 5, 5'-tetrametylenebenzidine into the blue product. Then, this enzyme reaction is stopped by adding an acidic solution( $H_2SO_4$ ) that converts

growing usage of wheat, shows that gluten intolerance has been more than the expected rate, showing its' under-diagnosis (8). On the other hand, it has been hypothesized that microbial exposure like Helminthes may affect the risk of CD. Studies have shown that Helminths stimulate gluten tolerance among CD patients (9). CD is not considered as a disease of priority and hence, studies are very limited in resource-constrained settings. It was with this background that the current study aimed to determine anti-tissue transglutaminase IgG levels in children. Therefore, the objective of the present study was to screen the prevalence of celiac disease by using anti-tissue transglutaminase IgG anti-body among Helminths-infected school children in Ziway, South East Ethiopia.

the solution from blue to yellow. The absorbance of the solution at 450nm is directly proportional to specific anti-bodies bound (commercially available Kit- Anti-tTG IgG by Generic Assay, Germany).

### Quality control and Quality assurance

All the tests performed were assessed by using a control that was run accordingly with the sample. Calibrators were used for the determination of Anti-tissue transglutaminase Ig-G, and tests were assumed valid when the mean OD of standard 1 was  $< 0.7$  and the mean OD of Standard 4 was  $> 1.2$ . The pre-analytical, analytical, and post-analytical factors that could interfere with the sample analysis were thoroughly controlled in the lab. Before using the statistical tool for analysis, results were recorded appropriately, and data was checked for completion.

### Data analysis

Data was entered and analyzed by using SPSS version 23, and descriptive analysis, Chi-square, and logistic regression were used to see any associations, and a P-value  $< 0.05$  was considered statistically significant. Finally, the results were presented in figures and tables.

### Ethical Approval

Ethical clearance was obtained from the Department of Research and Ethical Review Committee of the Department of Medical Laboratory Science, College of Health Science, Addis Ababa University, to use the stored serum sample for further analysis. A sample collected for a project that has institutional and national ethical approval was used for this study.

## Result

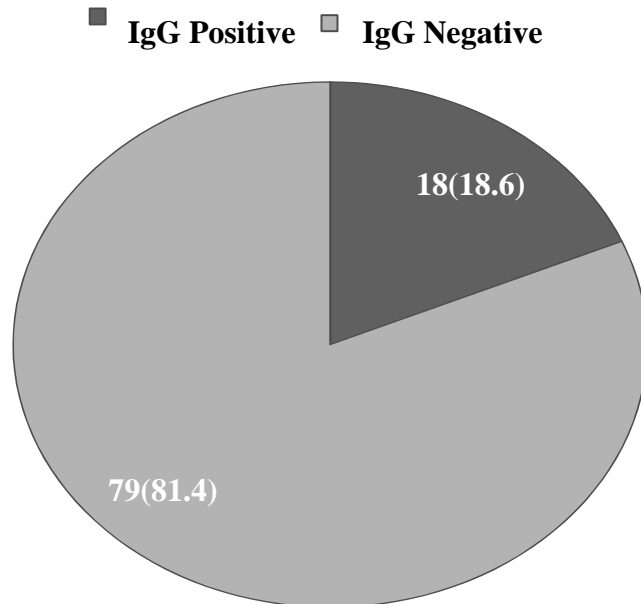
Totally 97 samples of children in age group between 6-14 years old were involved in this cross-sectional study. On the socio-demographic features, the majority of the study participants were females (54.6%) with age group between 6-9 years (63.9%). On the other hand, clinical features of study participants indicated that 16.5% (16/97) participants had a positive Helminths result, as shown in Table 1.

**Table 1.** Socio-demographic data and clinical features of study participants at Ziway, South East Ethiopia (n=97), 2020

Variable	Category	Number	Percent
<b>Sex</b>	Male	44	45.4
	Female	53	54.6
<b>Age in Years</b>	6-9	62	63.9
	10-14	35	33.1
<b>Helminths</b>	Negative	81	83.5
	Positive	16	16.5

Figure 1 below summarizes the overall anti-tissue transglutaminase IgG magnitude among the study participants, with those who tested positive for the anti-body, accounting for 18.6% of the total study

participants. Although more confirmatory tests like a biopsy are required, this finding shows that 18.6% of study participants are assumed to be positive for celiac disease.



**Figure 1.** The Magnitude of anti-tissue transglutaminase IgG among study participants at Ziway, South East Ethiopia (n=97), 2020

On the distributions of anti-tTG IgG between different sex and age groups, females had a higher magnitude of anti-tTG IgG than males 13/18(72.2%): whereas both age categories showed a similar magnitude of anti-tTG IgG. This study also showed that there was a significant association between anti-tTG IgG and helminths, a p-value of 0.033. Our study indicated that

those children infected with helminthes held a 3.45 times [OR 3.45 (95% CI: 1.057, 11.265). “P=0.033”] greater likelihood of developing a positive anti-tTG IgG (Table). However we have only done a binary logistic regression, so it is not to be forgotten that there might be a possibility of confounding factors associated.

**Table 2.** Association between variables and anti-tissue transglutaminase IgG among school children at Ziway, South East Ethiopia (n=97), 2020

Variables	Anti-tissue transglutaminase IgG (anti-tTG IgG)		Crude OR(95%CI)	P-value	
	Negative	Positive			
<b>Age</b>	4-9	53(67.1%)	9(50%)	2.038(0.723,5.746)	0.173
	10-14	26(32.9%)	9(50%)		
<b>Sex</b>	Male	39(49.4%)	5(27.8%)	2.535(0.826,7.783)	0.097
	Female	40(50.6%)	13(72.2%)		
<b>Helminths</b>	Negative	69(87.3%)	12(66.7%)	3.45(1.057,11.265)	<b>0.033</b>
	Positive	10(12.7%)	6(33.3%)		

P-value <0.05% shows a significant difference.

\*percent within anti-tTG IgG

**Discussion**

The current study showed celiac affected females more than males (72.8% (13/18); however, a study by Fayed SB *et al.*, 2007 (11) found males were more highly affected by celiac disease than females 72.7% (8/25) although not significant clinically. This might be due to the geographical differences as well as the sample size being so small in the compared study.

This study found the prevalence of celiac disease among children of southeast Ethiopia, Ziway, to be 18.6%. However, this finding is against another cross-sectional study by Gudeta *et al.* 2019, on Celiac Disease Autoimmunity in Ethiopian Pregnant Women that found the celiac disease is rare compared with the expected prevalence (0.05%). This can be due to the small sample size in this study as well as the difference in study participants on the compared study, which focused on pregnant women, but it still opens more opportunities to have thorough research done on the area (12).

The present study found that children infected with Helminths infection had 3.45 times more chance of developing anti-tissue transglutaminase IgG antibody that is mainly found in celiac patients. However, a clinical trial by McSorley *et al.* 2011(13), on the effect of Hookworm on suppressing celiac disease showed that individuals purposely infected with different Helminthes (Hookworm) showed lower levels of cytokines in response to gluten administration that supports the idea that Helminths have a protective effect against celiac disease and additional clinical trial by Croese *et al.* (14) that followed a purposeful infection of celiac patients with Ubiquitous *Necator americanus* showed a modified host immune response to gluten. However, both these studies focus on Hookworm infection and the association it has with celiac disease; our study did not include the specific Helminths infection involved also whether the larvae or eggs cause this association with an additional lack of evaluation before and after gluten administration that can be responsible for this difference.

Taken together, the current cross-sectional study, for the first time, demonstrated screening of celiac disease by measuring the magnitude of anti-tissue transglutaminase IgG anti-body level and its association with some variables in school children. With the limited number of children involved, it was possible to demonstrate an association with Helminthes infection where children positive for helminths were 3.45 times more likely to be positive for the anti-body, suggesting a direct association between celiac disease and anti-tissue transglutaminase IgG.

It was not possible to perform more tests like tissue transglutaminase IgA and tissue biopsy to confirm celiac disease, so it was difficult to show a clear association, which was one of the limitations of this study; the sample size is also limited to a small group of children. However, the study still shades some light as a screening test for celiac disease, and it demonstrated an association between helminths

and anti-tissue transglutaminase IgG levels that can be used as baseline data considering a limited study done in Ethiopia concerning the celiac disease.

**Conclusion**

This study tried to show the screening of celiac disease by measuring anti-tissue transglutaminase IgG in school-aged children. This study was able to show the strong association between Helminthes infection and anti-tTG IgG, where children infected with Helminths had a 3.45 times greater risk of developing a positive anti-tTG IgG, which can be used as baseline data for future researchers on the area.

**Recommendation**

More additional confirmatory tests for celiac disease Large-scale case-control study to understand the exact relationship between celiac disease and other co-morbidities. More trial based studies to understand the specific associations of celiac disease with other co-morbidities.

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**Competing Interests**

The authors declared they have no competing interests.

**Author contributions**

**MA:** Select the appropriate serum sample for further analysis with data entry and analysis and result interpretation and then manuscript write-up.

**MG:** Performed ELISA procedure to identify anti-tissue transglutaminase IgG based on the instruction on the Kit

**KD:** Data analysis and document review

**MW:** Research document review, manuscript write-up, and as an advisor, giving different important comments.

**BT:** Sent the anti-tissue transglutaminase IgG kit for analysis of tissue Transglutaminase IgG from Colgate University, New York.

**AT:** Gave the research title idea with document review and as an advisor by giving different comment

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**Acronyms and abbreviations**

**Anti-tTG IgG:** Anti-tissue transglutaminase Immunoglobulin Gamma

**CD:** Celiac disease

**CI:** Confidence Interval

**COR:** Crude Odds Ratio

**ELISA:** Enzyme-Linked Immuno-sorbent Assay

**OD:** Optical density

**P-value:** Probability value

**SPSS:** Statistical Package for the Social Sciences

**TTG:** Tissue transglutaminase .

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