

## Prevalence of Tissue Transglutaminase Antibodies in cases of Celiac Disease

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### ABSTRACT

Celiac disease is a human leukocyte antigen-DQ2 (or DQ8) associated autoimmune disorder of the small intestine induced by dietary exposure to wheat gliadin, barley hordein, rye secalin and possibly oat avenin. It is characterized by mucosal damage, loss of absorptive villi and hyperplasia of the crypts leading to malabsorption. In addition, to nutrient deficiencies and growth failure, prolonged untreated celiac disease is associated with an increased risk of malignancy, especially intestinal T cell lymphoma. Tissue transglutaminase antibody tTG antibodies of isotype IgA and IgG is a simple, sensitive and specific noninvasive screening test for diagnosis of celiac disease, particularly in developing countries like Pakistan. In current study from September 2010 to May 2014, samples from 3643 cases referred to Dow Diagnostic Research and Reference lab were screened for serum IgA and IgG for tissue transglutaminase by ELISA. Results showed that about 1290 patients were anti tTG IgA positive including male and female with a ratio of 1:1.4 respectively, indicating an active stage of the disease. Children (age group between 0 to 16) showed a greater prevalence i.e 37% as compared to other age group. However IgG against tissue transglutaminase didn't show any significant prevalence pattern.

**Key words:** Celiac disease, anti-tissue transglutaminase, anti- gliadin, gastroenteritis.

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### INTRODUCTION

Celiac disease (CD) is a chronic autoimmune enteropathy of the small intestine in genetically predisposed children and adults. It is precipitated by the ingestion of gluten-containing foods<sup>1</sup>. Gluten is a rubbery protein mass that remains when wheat dough is washed to remove starch<sup>2</sup>. The major storage proteins in wheat are glutenin, gluten and gliadin. Infact, gluten component of wheat is used as a major ingredient in food processing industries, it is widely used in bakeries because it gives desired baking properties to the dough. Consuming gluten is involved in human diseases, the well known of which is celiac disease<sup>3</sup>. Early epidemiological studies regarded celiac disease as a disease of individuals with Caucasian ancestry, mainly located in Europe and North America<sup>4</sup>. Recent studies have shown that celiac disease is a common disorder in North Africa<sup>5</sup>, the Middle East<sup>6</sup>, India<sup>7</sup>, and Pakistan<sup>8</sup>. In order to diagnose untreated celiac disease, serological markers have been identified with higher sensitivity

and specificity. The target antigens, are divided into two groups<sup>9-10</sup> Ist group includes autoantibodies [Anti endomysial (EMA) and anti-tissue transglutaminase (tTG)] antibody tests, and second group comprise antibodies targeting the offending agent (gliadin). Clinical key symptoms of the disease in adults include chronic diarrhea (formerly considered the most common symptom), abdominal distension, weight loss, lassitude, anemia and malaise. In children with classic celiac disease symptoms could be failure to thrive, vomiting, muscle wasting, weight loss, diarrhea, recurrent abdominal pain, Irritable bowel and unhappiness. Gluten-free diet (GFD) is very helpful in improving the symptoms, abnormal biochemical markers, and morbidity in patients with celiac disease. Recent studies have also suggested that patients who were screened for the disease, most of them were asymptomatic, could be able to improve their quality of life in the longer term with a Gluten Free Diet<sup>11</sup>. Patients with long-term untreated CD are at higher risk for benign and malignant complications<sup>13,15</sup>. Cancer, malignant lymphomas, oropharyngeal tumors, small-bowel neoplasia, and unexplained infertility<sup>16</sup>. The study is based on observing auto antibodies (tTG) detected in cases referred to the lab and its collection points that cover a large area of Karachi, Pakistan.

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## METHODOLOGY

### Sample collection:

In current study from September 2010 to May 2014, samples from 3643 cases referred to Dow Diagnostic Research and Reference lab Karachi, Pakistan were included. Blood samples were collected from different age groups of patients reported to Dow. Age, gender, and disease severity were recorded. A 3-5ml of venous blood was drawn from each patient. Blood was allowed to clot for 1hr at room temperature, centrifuged at 3500 rpm for 5 minutes, and then serum collected and stored at -20°C until further use. The study was approved by the ethical committee of Dow University of health sciences.

### Study population

The distribution of subjects which were screened, divided on the basis of categorical data, patients were grouped according to their gender, age and seropositivity. According to age, group 1 comprise children upto 16 years, group 2, 17-50 years, while group 3 included cases of more than 50 years of age.

### Detection of anti-transglutaminase IgG and IgA

Detection of anti tTG (tissue transglutaminase) IgG and IgA was performed by ELISA kit, purchased from biosystem Barcelona, Spain. The assay was performed and the cutoff value was used according to manufacturer's instruction. Briefly, 100 µL of the control sera for positive and negative, sample, diluent and diluted samples (1:100) were placed into microwells and the plate was incubated for 30 min, after contents were aspirated, and washing of wells was done 3 times. 100 µL of the conjugate was placed into all well and incubated for 15 min, the wells were washed. Finally, the substrate was added in all wells and again incubated for 15 min. Stop solution 0.5 mole/L sulfuric acid was added to cease the reaction. All the incubations were done in a moist chamber at room temperature. Color development was read at 450 nm.

### Statistical analysis

Data was analyzed using SPSS version 16.0 and Chi-square tests were used as appropriate for comparison between gender and age groups. P-values <0.001 were considered as statistically significant for the positive tests.

## RESULTS

The general demographic characteristic of the subjects screened and the number of patients with CD identified in this study are summarized in figure 1, 2, 3 and 4. In this study 3643 samples were received in the laboratory with an average age of 20 years and 1290 were found positive for anti-tTG IgA. The antibodies tTG refers to anti-tissue transglutaminase, that are autoimmune antibodies, which are generated because either auto reactive T-cells are not suppressed or antigens escape

the protective process. The population consisted of 1725 males among which 554 were positive and 150 were borderline, while 1918 were females among whom 736 were positive and 110 were borderline with all ages. Table 1, figure 1.

Table 1: Gender-wise distribution of subjects suspected with celiac disease. Results showing a total number and % age of patients with anti-tTG IgA and anti-tTG positive cases.

Test	Male		Female	
	anti-tTG IgA	anti-tTG IgG	anti-tTG IgA	anti-tTG IgG
Total(n)	1725	1240	1918	1413
Positive	554 (32.11 %)	170 (13.71%)	736 (38.44 %)	232 (16.41%)
Borderline	150 (8.62 %)	20 (1.61%)	110 (5.72%)	30 (2.11%)

anti-tTG (tissue-transglutaminase) IgA (N=3643)  
anti-tTG IgG (N=2267)

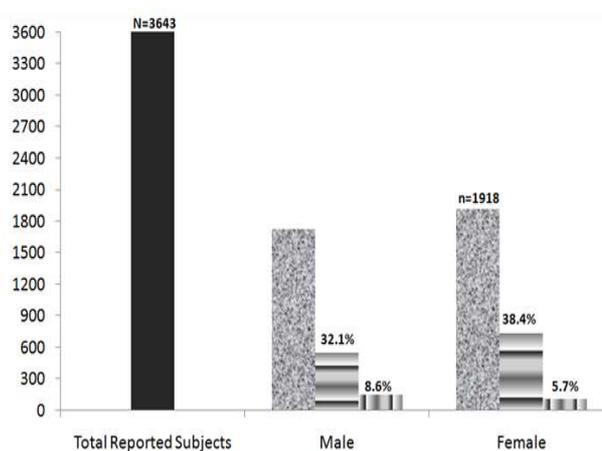


Figure 1: Gender wise distribution of anti-tTG (transglutaminase) IgA, total registered male or female population to be shown as and positive patient among the group as and borderline patients among the group as . Level of significance\*\* P = 0.001 was calculated using chi-square test.

Additionally, 3643 patients were also distributed in different age group. Among them 1724 subjects were up to 16 years and positive outcome was 651 patients, 1736 included in 17-50 years group and 584 were positive while about 183 patients were grouped in > 50 years age group and 55 were positive. Table 1, figure 2.

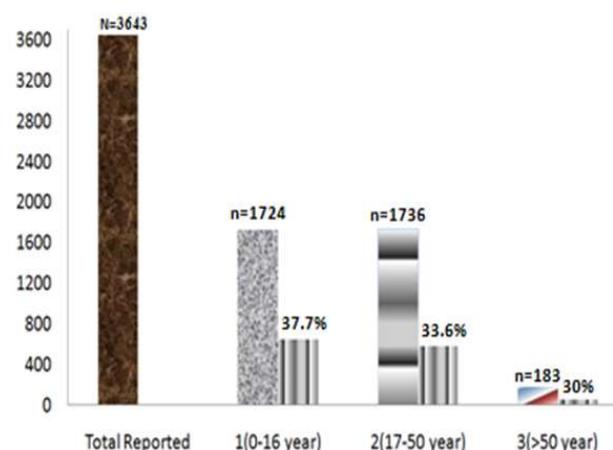


Figure 2: Distribution of seropositive anti tTG (transglutaminase) IgA patients with different age groups. The patients were subjected to group wise distribution. The age group between 0 to 16 years, in 17-50 years while is indicator of > 50 years. Results are total number of patients with positive anti tTG IgA.

On the other hand, 2267 were registered for anti-tTG IgG screening. The 1240 were male among whom positive were 170 and 20 were borderline, whereas, 1413 were women among whom 232 were positive and 30 were borderline. Table 1, figure 3.

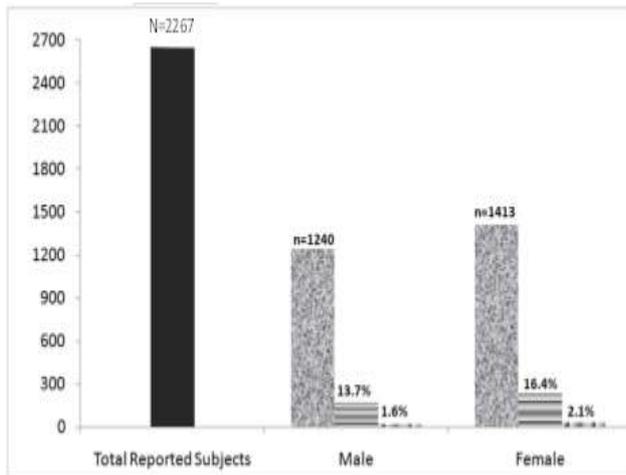


Figure 3: Gender wise distribution of anti-tTG (transglutaminase) IgG, total registered male or female population, and positive patient among the group shows borderline patients among the group. Results are representative of mean values of two independent experiments.

In addition, 2267 patients were distributed in different age group. The 1950 patient included between 0 to 16 years age group and positive patient were 90, 309 in 17-50 years age group positive patients were 48 and 394 in > 50 years age group positive were only 02 patients. Table 2, figure 4.

Table 2: Age wise distribution of subjects suspected with celiac disease. Results showing a total number and % age of patients with anti-tTG IgA and anti-tTG IgG positive cases

Test	Group 1 (up to 16 years)		Group 2 (17-50years group)		Group3 (> 50 years)	
	anti-tTG IgA	anti-tTG IgG	anti-tTG IgA	anti-tTG IgG	anti-tTG IgA	anti-tTG IgG
Total(n)	1724	1950	1736	309	183	394
Positive	651 (37.73%)	90 (4.61%)	584 (33.62%)	48 (15.52%)	55 (30%)	02 (0.54%)

anti-tTG (tissue-transglutaminase) IgA (N=3643)  
anti-tTG IgG (N=2267)

## DISCUSSION

In celiac disease immune response involves the production of antibodies against the intestinal enzyme Tissue Transglutaminase to establish an autoimmune disease. These auto antibodies are of two types immunoglobulin G [IgG] and immunoglobulin A [IgA]. In order to detect celiac disease, level of anti tTG IgA in the blood is more reliable because it is formed in the small intestine, where gluten is responsible to cause inflammation and irritation in sensitive people. Clearly there is a need for a simple, sensitive and specific non-invasive screening test for celiac disease<sup>17</sup>. In 1979 Signer et al<sup>18</sup> reported that serum antibodies to gliadin constitute a possible diagnostic tool. Since then, anti

gliadin antibodies of isotypes IgG and IgA and anti-tissue transglutaminase antibodies of isotype IgA were used as screening tests for celiac disease<sup>19-21</sup>.

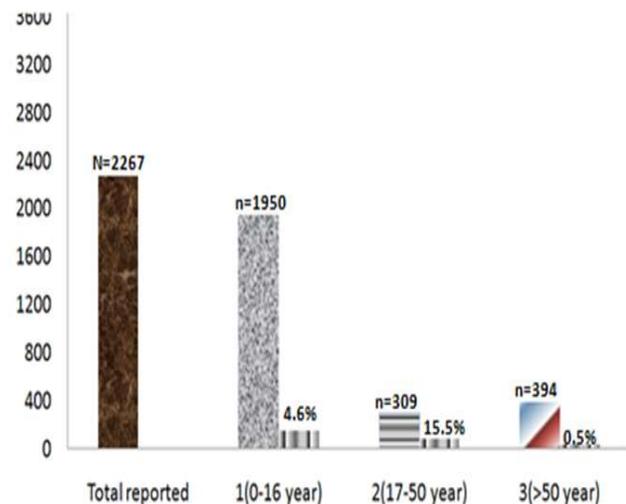


Figure 4: Distribution of seropositive anti-tTG (transglutaminase) IgG patients with different age groups. Results showed total number of positive patients in different age group, indicative of age between 0 to 16 years, 17-50 years and > 50 years. Results were compared with total registered cases with unknown gastric problems.

A recent study from Pakistan demonstrated that detection of anti-TtG IgA among patients with unknown gastrointestinal problem, evaluated almost 35.4% cases suffering from celiac disease based on current diagnosis with the male to female ratio of 1:1.4, showing females more prone to the disease (Table-1 and Figure-1). In addition, among different age groups children aged up to 16 years seemed to be more affected with the disease with a percentage prevalence of 37.7% (Table 2 and Figure 2). It is therefore suggested that screening strategies to detect such uncommon autoimmune disease, that tends to remain undiagnosed for years and can be a major cause of morbidity and mortality should be addressed.

In contrast, levels of the IgG form of tTG antibody, are less specific to celiac disease, however people who usually don't make normal amounts of IgA antibodies their IgG level could be useful for diagnosis. Our data showed that IgG antibodies against tissue transglutaminase are more common in female patient's sera as compare to male, i.e 16.4% case were positive. Additionally, younger population aged between 18 to 50 seems to show higher percentage if IgG antibodies in comparison with other age groups.

The data related to IgA antibodies shows a significant prevalence against Tissue Transglutaminase Abs in our population which is clinically important because of prevalence % age of positive patients that is 35.4%. Due to lack of awareness about the disease many patients remain untreated for years which could be major cause of morbidity and mortality. However, if patients are diagnosed properly then there are higher

chances of getting completely cure by preventions. Since strictly gluten-free diet for life is the only treatment for celiac disease<sup>4,22-24</sup>. No foods containing gluten from any sources like wheat, barley, and rye or their derivatives can be taken, as even little quantities of gluten may trigger the immune reaction. Some studies also showed 95% of patients with celiac disease can take Oats, but < 5% of population in whom oats are not safe<sup>25-29</sup>. Corn and rice can be taken as gluten-free diet. Complete removal of gluten from the diet would eventually result in betterment of symptoms, serological markers, and histological remission in most patients<sup>4,11</sup>. Current study suggested that celiac disease is prevalent in our settings, so those patients with gastrointestinal abnormalities who are yet undiagnosed should go for auto antibodies detection against tissue transglutaminase for the correct diagnosis to prevent morbidity and mortality.

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