

## ASSESSMENT OF THYROID DYSFUNCTION IN CHILDREN WITH CELIAC DISEASE

TOQEER BUTT,<sup>1</sup> ASIM MUMTAZ,<sup>1</sup> AKIF QASIM,<sup>1</sup> NADEEM HAMEED<sup>2</sup>  
M. IBRAHIM<sup>3</sup> AND KANWAL AZHAR<sup>1</sup>

Departments of <sup>1</sup>Pathology, <sup>2</sup>Pediatrics, University of Health Sciences, Jinnah Hospital  
Shalamar Medical and Dental College, <sup>3</sup>Department of Statistics, Govt. Dyal Singh College, Lahore – Pakistan

### ABSTRACT

*Introduction:* Celiac Disease (CD) is an immune response to ingested wheat gluten and related proteins of rye and barley that leads to inflammation, villous atrophy and intestinal crypt hyperplasia.<sup>1,2</sup> CD was considered as a rare malabsorption syndrome in the previously that can only occur in children, now it is a common condition that may be diagnosed at any age.<sup>3</sup> The aim of this study was to assess the thyroid functions in Celiac Disease patients by measuring serum levels of free thyroxine ( $fT_4$ ) and thyroid stimulating hormone (TSH). Anti thyroid peroxidase antibodies (Anti TPO) were also measured to assess the autoimmune nature of thyroid disease.

*Patients and Methods:* It was an analytical cross sectional study, on 50 patients of celiac disease and 25 apparently healthy non celiac children were included in the study. CD patients were already diagnosed by elevated levels of serum anti tissue transglutaminase (IgA class) antibodies ( $> 15X$  ULN). CD was ruled out in control group by normal levels of serum anti tissue transglutaminase (IgA class) antibodies. TSH and  $fT_4$  were performed on Vitros ECIQ immunodiagnostic system and anti-TPO Antibodies were performed by manual ELISA technique.

*Results:* Mean age of the patients included in the study was  $5.3 \pm 3.3$  (range, 1.5 – 13) years. Mean age of individuals in control group was  $5.8 \pm 2.5$  (range, 2.0 – 12) years. Statistically significant increase levels of TSH and Anti TPO Antibodies were observed in the patient group. No statistically significant difference was observed between means of  $fT_4$  in patients and control group. Subclinical hypothyroidism was noticed in 10% patients of celiac disease while anti TPO Antibodies were positive in 16% patients.

*Conclusion:* The present study showed an increased occurrence of thyroid dysfunction and serological evidence of thyroid autoimmunity in patients with CD. Assessment of thyroid function in patients of celiac disease is recommended at regular intervals.

*Keywords:* Celiac Disease, Thyroid Peroxidase Antibody, Thyroid Function Test.

### INTRODUCTION

Celiac Disease (CD) is an immune response to ingested wheat gluten and related proteins of rye and barley that leads to inflammation, villous atrophy and intestinal crypt hyperplasia.<sup>1,2</sup> CD was considered as a rare malabsorption syndrome in the previously that can only occur in children, now it is a common condition that may be diagnosed at any age.<sup>3</sup> Recent population screening studies have shown that the prevalence of CD in Western countries is 1%.<sup>4,5</sup> Several studies in Europe<sup>6</sup> and USA also confirmed this prevalence.<sup>7</sup> CD is the end result of three processes leading to mucosal damage in the intestine. These processes are genetic predisposition, environmental factors and immunologically based inflammation.<sup>8</sup> CD is an inherited condition, associated with HLA – DQ<sub>2</sub> or HLA – DQ<sub>8</sub> proteins, products of two of the HLA genes.<sup>8</sup> Recent epidemio-

logical studies have shown an increased statistical susceptibility for people with one autoimmune disease to develop another autoimmune disease.<sup>9</sup> There are some important environmental factors that can lead to the expression of CD in genetically susceptible individuals e.g. initial administration of gluten before 4 months of age is associated with an increased risk of disease development, while the introduction of gluten after seven months is associated with a marginal risk.<sup>11</sup> The overlap of gluten introduction with breast – feeding may be a more important protective factor in minimizing the risk of CD.<sup>12</sup> The occurrence of certain gastrointestinal infections, such as rotaviral infection, also increases the risk of CD in infancy.<sup>13</sup> Thyroid autoimmunity is common and is due to an apparent immune reaction directed against self antigens of the thyroid. The antigens against which the autoimmune reac-

tions are directed to produce thyroid autoimmune disease include thyroglobulin (Tg), thyroid peroxidase (TPO) and the TSH receptor. CD and autoimmune thyroid disease share a common genetic predisposition, namely, the DQ<sub>2</sub> allele. This common predisposing genetic background would explain the higher incidence of thyroid autoimmune disease in CD than in the general population.<sup>13</sup>

Several studies have shown an increased incidence of thyroid diseases among patients with CD. Thyroid dysfunction is autoimmune in origin and anti TPO and anti Tg antibodies have been detected in CD patients.<sup>14,15</sup> There is no definite pattern of thyroid hormones levels in CD patients; hypothyroidism, hyperthyroidism or euthyroidism have been reported.<sup>16</sup> The prevalence of autoimmune diseases was reported to be 17% in Celiac Disease patients, when frequency of autoimmune disorders was evaluated in 64 patients with celiac disease by Kechaou et al.<sup>17</sup> Significantly higher frequencies (range 21 – 43%) of positive thyroid antibodies has been reported by different workers.<sup>15,18,19</sup>

The aim of this study was to assess Thyroid function in Celiac Disease patients and non celiac controls by measuring free thyroxine (fT<sub>4</sub>) and thyroid stimulating hormone (TSH) and then find out any statistically significant difference between the corresponding means. Thyroid peroxidase antibodies (Anti-TPO) were also measured to assess the presence of autoimmune thyroid disease.

## PATIENTS AND METHODS

It was an analytical cross sectional study. Fifty patients of CD and 25 non celiac apparently healthy children were included in the study. CD patients were already diagnosed by elevated levels of serum anti tissue transglutaminase (IgA class) antibodies (> 15X ULN). CD was ruled out in control group by normal levels of serum anti tissue transglutaminase (IgA class) antibodies. These patients were selected from the department of Pediatrics at Shalamar Hospital Lahore. The study was performed at University of Health Sciences, Lahore. Thyroid function test including thyroid stimulating hormone (TSH) and free thyroxine (fT<sub>4</sub>) were performed. Anti-TPO Abs were also performed to assess autoimmune nature of thyroid dysfunction. TSH and fT<sub>4</sub> were performed on Vitros ECIQ immunodiagnostic system and anti-TPO Abs were performed by manual ELISA technique. TSH kit by Ortho-Clinical Diagnostics was used to measure TSH (Reference range: 0.4–4.2 mIU/L) fT<sub>4</sub> kit by Ortho-Clinical Diagnostics was used (Reference range 10 – 30 pmol/L). Anti-TPO Elisa kit by Eskulisa was used to measure anti-TPO: (Levels more than 60 IU/mL were considered as positive).

## Statistical Analyses

The data was entered and analyzed by using standard SPSS software version – 18 (SPSS Inc, Chicago) for statistical analysis. Mean ± SD is given for quantitative variables like fT<sub>4</sub>, TSH, anti-TPO and age. Frequency and percentages are given for qualitative variables. Two independent sample *t* tests were applied to observe group mean differences. Pearson chi square was applied to observe association between qualitative variables. A *p*-value < 0.05 was considered as statistically significant.

## RESULTS

A population of 50 CD patients and 25 non CD apparently healthy children as controls were included in the study. Among patients, 30 (60%) were female and 20 (40%) were male, while 12 (48%) were male and 13 (52%) were females in control group. Mean age of the patients included in the study was 5.9 ± 3.3 (range, 1.5 – 13) years. Mean age of individuals in control group was 5.8 ± 2.5 (range, 2.1 – 12) years. A total of 27 (54%) patients were older than 5 years of age, 14 (28%) were more than 5 to 10 years of age and 9 (18%) patients were of age more than 10 years. Mean weight of individuals in patient and control groups were 14.6 ± 7.05 Kg and 10.7 ± 6.6 kg, respectively. Mean BMI was 22.05 ± 8.39 kg/m<sup>2</sup> in patient group and 21.30 ± 1.78 kg/m<sup>2</sup> in control group. Family history of CD and thyroid dysfunction was found in 3 (6%) and 1 (2%) in patient group, respectively. Dermatitis Herpetiformis and aphthous stomatitis were observed in 14 (28%) and 11 (22%) cases, respectively. Four (8%) CD patients were known diabetic and they were taking insulin regularly.

In the patient group, 3 (6%) individuals had their 1<sup>st</sup> exposure to gluten in less than 4 month of age, 31 (62%) in less than 6 month of age, 12 (24%) in less than 8 month of age and 4 (8%) had their 1<sup>st</sup> exposure to gluten after 8 month of age.

A statistically significant difference was observed between mean TSH concentration in patient

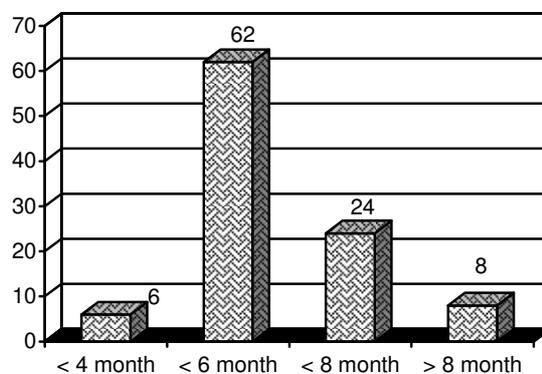


Figure 1: Bar graph of patients according to their first exposure to gluten.

and control group (*P*-value 0.028). Mean TSH concentration was  $2.40 \pm 2.30$  (range, 0.4-10.95) mIU/L in patients and  $1.35 \pm 0.56$  (range, 0.59 – 2.4) mIU/L in controls. Thyroid dysfunction (raised serum TSH) was found in 5 (10%) patients whereas remaining 45 (90%) had normal TSH levels. All the

**Table 1:** Mean  $\pm$  SD of TSH, *fT*<sub>4</sub> and anti TPO Abs in control and patient group.

Parameter	Control	Patients	95% CI for difference	<i>P</i> -value
TSH	$1.35 \pm 0.56$	$2.40 \pm 2.30$	1.11 – 1.98	0.028
<i>fT</i> <sub>4</sub>	$18.47 \pm 2.93$	$18.66 \pm 4.00$	-1.61 – 1.986	0.834
Anti TPO	$7.66 \pm 15.44$	$29.74 \pm 30.35$	9.20 – 34.98	0.001

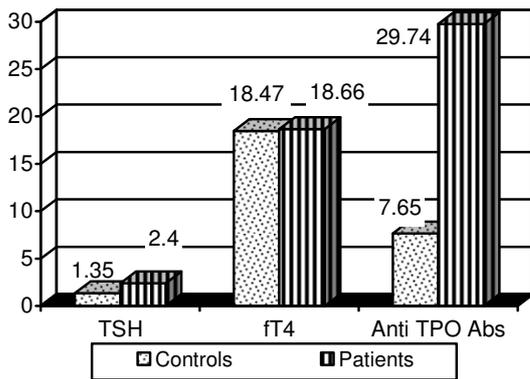


Figure 2: Bar graph of comparison of means of TSH, *fT*<sub>4</sub> and anti TPO Abs between control and patient group.

**Table 2:** Levels of anti TPO Abs in patients and controls group.

	Patients N (%)	Control N (%)
Normal ( $\leq 40$ IU/mL)	32 (64)	24 (96)
Boderline (40 – 60 IU/ML)	10 (20)	0 (0)
Positive ( $> 60$ IU/mL)	8 (16)	1 (4)

*P*-value = 0.001

subjects of control group showed normal levels of TSH. No statistically significant difference was observed between mean *fT*<sub>4</sub> concentration in patient and control groups (*P*-value 0.834). The *fT*<sub>4</sub> concentration in patient and control population was  $18.66 \pm 4.00$  (range, 9.2 – 27.3) pmol/L and  $18.47 \pm 2.93$  (range, 14.4 – 23.4) pmol/L respectively. All the subjects of the study had normal levels of *fT*<sub>4</sub>. A highly statistically significant difference was observed between mean anti TPO concentration in patient and control groups (*P*-value 0.001). The mean value of anti TPO Abs in patient and control groups was  $29.75 \pm 30.35$  (range, 2.12 – 124.5) IU/mL and

$7.66 \pm 15.44$  (range, 2.48 – 81.20) IU/mL respectively. Among patient group, 32 (64%) subjects showed normal levels, 10 (20%) subjects remained in borderline zone and 8 (16%) subjects showed elevated levels of anti TPO Abs. One member of control group showed elevated levels of anti TPO Abs.

**DISCUSSION**

The occurrence of autoimmune thyroid disease in association with Celiac Disease has been reported by many workers. The prevalence of subclinical hypothyroidism and overt hypothyroidism is reported to be 2.27% and 4.54% respectively by Toscano et al.<sup>20</sup> Elfstrom et al.<sup>21</sup> reported hypothyroidism in 0.90 by hyperthyroidism in 0.34% and celiac patients. The reported incidence of hypothyroidism of CD was 1.85% as reported by Meloni et al.<sup>22</sup>

CD is an inherited condition but in present study family history of CD was found only in 6% of CD patients included in the study. The decreased evidence of family history in CD patients may be due to lack of availability of diagnostic facilities in the country during the past decades. A highly specific and sensitive test i.e. ELISA was not available and small bowel biopsy was the only method for screening and diagnosis of CD. Biopsy testing was not available in all parts of the country and it was too expensive procedure, so in the past most of the CD patients remained undiagnosed. Another possible reason may be the fact that the CD requires environmental trigger e.g. administration of gluten before six month of age or rotaviral infection, besides genetic predisposition for expression of clinical symptoms, so that trigger may not be there in previous generations of patients included in the study.

In the present study majority (68%) of CD patients had their first exposure to gluten before 6 months of age. According to Norris et al.<sup>10</sup> initial administration of gluten before 4 months of age is associated with an increased risk of disease development, while the introduction of gluten after seven months is associated with a marginal risk of CD in genetically susceptible individuals.

Dermatitis Herpiformis (DH) in the present study was present in 28% population while Ciclitira et al.<sup>6</sup> reported DH in 10 to 20% of CD patients. The

increased incidence of DH in patients was seen because all patients were on regular diet (non GFD) and had active disease. In this study Aphthous Stomatitis (AS) was found in 22% CD patients. The reported prevalence of AS in CD patients by Campisi et al.<sup>23</sup> ranges widely from 3.7 to 33.3%. The prevalence of 33% has been reported by Bucci et al.<sup>24</sup> while 16.8% by Majorana et al.<sup>25</sup> In the current study 8% CD patients were known Diabetic, similar pattern (8%) was reported by Kumar et al.<sup>2</sup>

In this study 10% of CD patients (5 out of 50) showed subclinical hypothyroidism i.e. normal  $fT_4$  levels despite increased TSH levels. Similar results have been reported by Carta et al.<sup>13</sup> who studied 36 adult celiac patients for thyroid dysfunction. All celiac patients remained euthyroid, only 8.6% showed slightly increased serum TSH levels, suggesting primary subclinical hypothyroidism.

Hakanan et al.<sup>26</sup> recruited 79 CD patients and reported 10.1% subclinical and 3.8% overt hypothyroidism. Guidetti et al.<sup>27</sup> included 241 CD patients and reported 12.03% subclinical and 0.8% overt hypothyroidism. The finding of only subclinical hypothyroidism in the present study may be due to the fact that all the patients of this study were newly diagnosed. Ventura et al.<sup>28</sup> investigated 90 CD children for thyroid dysfunction but found no abnormality in TSH and thyroid hormone concentration in CD patients. The variation in results between our study and by Ventura et al. may be due to difference in the ethnic origin of the patients.

In the present study overt hypothyroidism and hyperthyroidism were not seen but different findings have been reported by some authors. Knowalska et al.<sup>29</sup> reported 34 CD children and found hypothyroidism in 8.8% patients and hyperthyroidism in 2.9% patients. Ansaldi et al.<sup>30</sup> conducted a study on 343 pediatric patients. Hypothyroidism was reported to be 8.1% in patients with CD and hyperthyroidism was observed in 1.16% patients with CD. Selimoglu<sup>31</sup> observed hypothyroidism in 5.2% children with CD. Hyperthyroidism was not observed in any patient. These differences in results may be possibly due to differences in study population sizes and different rates and stages of progression of diseases.

In our study, autoimmune thyroid disease (AITD) was observed in 16% patients of CD by using anti TPO Abs as a marker of AITD. 20% of CD patients (10 out of 50) showed borderline elevation while 4% (1 out of 25) subjects in control group showed elevated levels. Similar TPO Abs positivity has been reported previously.<sup>20,28,29</sup> Selimoglu<sup>31</sup> performed a study with 77 children of CD (aged 1 to 18 years). They documented the finding that 9.1% patients were positive for thyroid microsome antibodies.

In the present study levels of anti TPO were not well correlated with TSH levels. This could be explained

by the fact that anti TPO Ab is an early marker of thyroid dysfunction and it is elevated before the development of thyroid abnormality. The levels of anti TPO Abs were not elevated too much in the patient population in this study, only mild elevation was seen. It might be the indication of start of thyroid dysfunction.

## CONCLUSION AND RECOMMENDATIONS

The results of the present study show a high prevalence (10%) of subclinical hypothyroidism (normal  $fT_4$ , raised serum TSH levels) in our pediatric patients with CD. The children with CD also had higher incidence of anti TPO Abs. Regular screening of thyroid functions is recommended as thyroid disorders may remain unrecognized clinically in CD patients.

The thyroid antibodies were positive in some of our euthyroid patients with CD. Therefore, even if the clinical significance of these antibodies in patients with CD has not yet been clarified, the results suggest that there may be a higher susceptibility for thyroid involvement. In most patients, endocrine gland is progressively destroyed by the immune response to the target cell, leading to hypofunction. Thus, a longitudinal follow-up study of euthyroid patients with positive autoimmune thyroid serology would be advisable to establish whether these findings should be considered only an epiphenomenon or if they could be of prognostic help.

Thyroid function should also be tested more frequently in euthyroid patients with mildly raised serum TSH or with the presence of antibodies as they are at increased risk of developing overt hypothyroidism. This can ensure early detection of thyroid problems and eliminate its negative contribution to growth of these children.

All the Celiac disease patients were newly diagnosed and were on regular diet (non GFD) so a longitudinal follow-up evaluation of our patients with CD would seem advisable to understand the impact of gluten withdrawal on the evolution of autoimmune thyroid disease.

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