# QUANTIFICATION OF SERUM IgA OF COELIAC DISEASE PATIENTS

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**ABSTRACT**

*Background: Coeliac disease (CD) is a gluten – induced multi – organ disorder where small intestine is the primary target of inflammation. Onset of CD may occur at any age and its symptoms vary among individuals. Definitive diagnosis of CD is by intestinal biopsy but determination of anti – IgA tissue transglutaminase (tTG) and anti-gliadin antibodies has become key factors to decide for tissue biopsy. IgA – deficient CD patients may yield false – negative results, therefore total serum IgA level must be determined along with other serological markers to diagnose CD.*

*Methods: The study included 42 CD patients who were positive for anti–tTG antibodies (Group A) and 40 subjects (Group B: disease control) presented with gastrointestinal complaints but were negative for anti-tTG antibodies. On the basis of age, Group A was further divided into: Sub-group – I comprised of patients between 1 – 6 years (n = 31) and Sub-group – II consisted of patients between 7 – 15 years (n = 11). Level of serum IgA was determined by nephlometry technique.*

*Results: Total serum IgA level was 38.77 ± 31.21 mg/dl and 26.88 ± 28.27 mg/dl in CD patients and disease control group respectively and the difference in the level of serum IgA between these groups was not statistically significant (p = 0.75). Mean IgA level in sub-group – I and sub-group– II was 40.85 ± 33.29 mg/dl and 32.92 ± 24.85 mg/dl respectively and the difference in the level of serum IgA between these sub-groups was not statistically significant (p = 0.47). In Group – A, mean level of IgA in males and females was 42.38 ± 38.02 mg/dl and 34.41 ± 20.36 mg/dl respectively and the difference in the level of IgA level was not statistically significant between these groups (p = 0.41).*

*Conclusion: Selective IgA deficiency (SIgAD) was found in CD and in patients of other gastrointestinal complaints. In order to detect CD in SIgAD, total serum IgA level should also be performed with IgG – antigliadin or IgG-anti-tTG antibodies.*

*Key words: Coeliac disease, tissue transglutaminase, nephlometry, selective IgA deficiency.*

## INTRODUCTION

Coeliac disease (CD) is a gluten – induced multi-organ autoimmune disorder and small intestine is the primary target.[1] It may occur at any age[2] and its symptoms vary considerably.[1] In children under 2 years of age gastrointestinal symptoms and failures to thrive are common. In older children and adults, symptoms are often nonspecific, such as abdominal pain, anemia, osteoporosis, fatigue, and even depression. Consequently, the diagnosis may easily be delayed or even missed.[3]

Etiology of CD is multifactor where both genetic and environmental factors are involved.[4] Human leukocyte antigen (HLA) genes encoding class II molecules i.e. $DQ_2$ and $DQ_8$ are risk factors. More than 90% of patients expressed $DQ_2$ molecule while remaining carried $DQ_8$ molecule. Definitive diagnosis of CD was based on biopsy of small intestine that demonstrated increased number of intestinal lymphocytes, crypt hyperplasia and villous flattening which resolved on gluten – free diet.[1]

Histological findings and symptoms of CD should be evaluated along with history of ingesting gluten diet and there should be histological improvement on gluten – free diet. Serologic testing has become the main mode to determine suspected CD patients who should undergo intestinal biopsy. Sensitive serological tests include determination of connective tissue antibodies such as anti-reticulin (ARA), anti-endomysial (EMA) and anti-tissue transglutaminase (tTG) antibodies and IgA antigliadin antibodies (AGA) but low sensitivity and specificity of AGA (70% – 80%) have raised doubts for its use to diagnose CD.[5]

In CD auto-antibodies that are produced against tTG and other tissue antigens are of IgA in nature, therefore if IgG anti-gliadin antibodies are used, IgA – deficient CD patients may yield false-negative serology.[6] Guidelines to diagnose CD have suggested that total serum IgA level should be determined in parallel with IgA – anti – tTG antibodies. In such patients IgG anti-tTG antibodies are of diagnostic

value.[7,8] Similarly, specificity of diagnostic tests is also reduced in patients with increased IgA concentration.[9]

This study was planned to quantify total serum IgA level among CD patients and nephlometry method was adopted because this technique was suggested more sensitive than ELISA.

## MATERIALS AND METHODS

Blood samples of eighty-two (82) clinically diagnosed CD patients were collected from The Children Hospital and Institute of Child Health Lahore. On the basis of anti-tTG antibodies, subjects were divided into two groups. Group A included 42 (51.2%) CD patients that had anti-tTG antibody in their serum while Group B comprised of 40 (48%) subjects who presented with gastrointestinal complaints but were negative for anti-tTG antibodies (disease control: non-CD). On the basis of age, Group A was further divided into two sub-groups. Sub-group I comprised of patients between 1 – 6 years (n = 31) and Sub-group II consisted of patients between 7 – 15 years (n= 11). Mean age of participants in group A and group B was 5.38 ± 3.49 years and 4.60 ± 2.48 years respectively.

### Determination of Total Serum IgA Level

Level of immunoglobulin A (IgA) in the serum was determined by commercially available turbidimetric immunoassay kit from AMP Diagnostics (Austria) and test was performed according to the instructions of manufacturer (BioRad).

### Statistical Analysis

The data was entered and analyzed using Predictive Analytics Software (PASW) 18.0. Mean ± SD was given for quantitative variables. Frequencies, percentages and graphs were given for qualitative variables. Two independent sample $t$ tests was applied to observe mean differences between two levels of qualitative variables. A $p$-value of <0.05 was considered statistically significant.

## RESULTS

In Group A, mean level of IgA was 38.77 ± 31.21 mg/dl and in Group B it was 26.88 ± 28.27 mg/dl. The difference in the levels of IgA between these two groups was not statistically significant (p=0.75) (Table 1). The mean level of IgA was 40.85 ± 33.29 mg/dl in subgroup I and it was 32.92 ± 24.85 mg/dl in subgroup II. The difference in the level of IgA between these two subgroups was not statistically significant (p= 0.47) (Table 2). In Group – A, the mean level of IgA in males and females was 42.38 ± 38.02 mg/dl and 34.41 ± 20.36 mg/dl respectively and the difference in the level of IgA level was not

statistically significant between these two groups (p = 0.41) (Table 3).

Table 1: *Mean, number and comparison of IgA in Coeliac Disease and non-Coeliac Disease patients.*

| Mean ± SD Group A (n = 42) | Mean ± SD Group B (n = 40) | p-value |
|---|---|---|
| 32.67 ± 45.19 mg/dl | 35.62 ± 44.88 mg/dl | 0.75 |

$n$ = number of patients
$p$ value significant < 0.05
Group A = patients of anti-tTG antibodies
Group B = patients without anti-tTG antibodies

Table 2: *Mean, number and comparison of IgA level between two sub-groups.*

| Age groups | Mean ± SD IgA | p-value |
|---|---|---|
| Total positive patients (n = 42) | | |
| Sub-group I (n = 31) | 40.85 ± 33.29 mg/dl | 0.47 |
| Sub-group II (n = 11) | 32.92 ± 24.85 mg/dl | |

$n$ = number of patients
p value significant < 0.05
Sub-group I = 1 – 6 years
Sub-group II = 7 – 15 years

Table 3: *Mean, number and comparison of IgA level in male and female of coeliac disease patients.*

| | IgA Mean ± SD | p-value |
|---|---|---|
| Males (n = 23) | 42.38 ± 38.02 mg/dl | 0.41 |
| Females (n = 19) | 34.41 ± 20.36 mg/dl | |

$n$ = number of patients
$p$ value significant < 0.05

## DISCUSSION

In this study, among 82 clinically diagnosed CD patients, 76 (92%) patients had low level of IgA when compared to normal reference range of Caucasians.[10] Our findings did not match the findings of McGowan et al, who suggested IgA deficiency in general population as 1 : 400. It was suggested that although large number of individuals with selective IgA deficiency were relatively healthy but detailed history could point out their recurrent infections, allergies and autoimmune disorders.[11,12] Similarly Sinclair et al. documented prevalence of IgA defici-

ency as 1 in 152 patients tested for CD.[13] Another study suggested that patients with IgA deficiency should be considered as risk group for CD.[14]

In our study a significant positive correlation was observed between the level of IgA and the level of anti–TG antibodies (p = .006, r = 0.299, n = 82). It showed that level of anti-TG antibodies increased along with increased level of IgA in CD patients.

In this study among 82 patients, 76 (92.6%) patients had low levels of IgA and 35 (87.5%) patients did not have anti-tTG antibody in the serum. Mc-Gowan *et al* observed that the number of IgA deficient patients without IgA anti-tTG antibodies who were appropriately evaluated and treated were significantly less (54%) than patients with normal levels of IgA who had anti-IgA-tTG- antibodies (77%, p = 0.004). Beutner *et al* reported the first case of IgA – deficient CD patient who was positive for EMA, ARA, AGA IgG antibodies. The level of these antibodies disappeared when the patient was put on gluten free diet and these antibodies reappeared on a gluten challenge, suggesting that IgG EMA antibody levels could be used to monitor IgA deficient CD patients for their compliance to gluten free diet (GFD).

In our study, low level of IgA in CD patients was observed in both the genders and there was no association between the level of IgA and age groups (p = 0.47), thus the possibility of low IgA level is same at different age groups. In our study detection of low IgA level might be the result of selection of patients with younger age group (mean age 4 – 5 years). Regarding age groups Litzman *et al* concluded increased prevalence of selective IgA deficiency (SIgAD) in both children and adults[15] but Castano *et al* and other studies documented increased prevalence of IgA deficiency in children.[16] In a study, it was found that at least 80% of CD patients diagnosed before the age of 4 years were advised GFD as compared to 36% of CD patients diagnosed after the age of 4 years. Thus it is important to diagnose childhood CD at an early age to minimize the risk for serious complications, otherwise they would be advised an inadequate diet.[17]

It is *concluded* that SIgAD was found in both CD and patients of other gastrointestinal complaints. There is an increased risk of CD with IgA deficiency; therefore clinician should go far IgG-anti tTG antibodies test along with total serum IgA level. Presence of IgG-anti tTG antibody is suggestive of CD and patient should be referred for intestinal biopsy.

## ACKNOWLEDGMENT

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