International Journal of Contemporary Pediatrics
Grotra R et al. Int J Contemp Pediatr. 2024 May;11(5):545-548
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: https://dx.doi.org/10.18203/2349-3291.ijcp20241034

Original Research Article

Positive association of serum IgG4 levels with modified marsh classification system in children with gluten enteropathy: a single centre experience

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Received: 30 January 2024
Revised: 01 March 2024
Accepted: 26 March 2024

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ABSTRACT

Background: Celiac disease or gluten enteropathy is an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals and is characterized by the presence of gluten enteropathy, celiac disease-specific antibodies, HLA-DQ2/DQ8 haplotypes. IgG4-related diseases is an increasingly recognized immune-mediated condition in autoimmune disorders such as primary sclerosing cholangitis, autoimmune hepatitis, and autoimmune thyroiditis, characterized by tissue fibrosclerosis and infiltration by IgG4-positive plasma cells and increased serum IgG4 concentrations.
Methods: A total of 33 children with newly diagnosed celiac disease and 31 control subjects were included in this study. All suspected celiac disease children underwent duodenal biopsy and were diagnosed based on Marsh grading. Serum IgG4 level estimations were performed using an enzyme-linked immune sorbet assay method with a cut-off of 135 mg/dl for diagnosis.
Results: A significant positive association between serum IgG4 levels and Marsh classification was found, the higher the levels of IgG4 in serum, the higher the grade on Marsh staging. Mean serum IgG4 levels in Marsh 3a was 126.32 mg/dl, in 3b was 171.35 mg/dl and in 3c was 209.24 mg/dl (p value=0.004)
Conclusions: With increasing serum IgG4 levels, increased severity of damage was seen on biopsy specimens based on higher Marsh grade. To the best of found knowledge, this is the first study to establish the relation between IgG4 and mucosal damage in children with celiac disease.

Keywords: Celiac disease, IgG4-related diseases, Marsh grading, Endoscopy, Biopsy

INTRODUCTION

Celiac disease (CD) is an immune-mediated multisystem disorder that is frequently seen among genetically predisposed individuals upon exposure to gluten-containing foods and environmental factors.[1] It is worth noting that celiac disease presents a unique model of autoimmunity due to the well-known and studied involvement of genes, target auto-antigens and environmental factors.[2] IgG4-related diseases (IgG4-RD) is a newly identified fibro-inflammatory condition characterized by infiltration of plasma cells positive for IgG4 in tissues, causing fibrotic abnormalities along with elevated serum IgG4 levels.[3] The role of autoimmunity in IgG4-RD was known by its associations with auto-immune pancreatitis (AIP).[4] Given the discovery of elevated IgG4 and AIP, Engelhart et al conducted a study to investigate potential disease associations with IgG4. This led to a discovery of the potential link between CD and isolated elevation of IgG4.[5] Serum levels of IgG4

were studied in adult patients with CD and were found to be elevated and positively correlated with Marsh grading.[6] There is a paucity of studies involving CD and IgG4. Hence the present study aims to fill the existing research gap by investigating the relationship between serum IgG4 levels and severity of duodenal damage in children with CD based on Marsh grading.

METHODS

This hospital-based cross-sectional study included 64 subjects (aged 1-18 years) recruited from paediatric OPD/IPD of SMS Medical College and was carried out from October 2020 to January 2022. Informed written consent was taken from attendants and the study commenced after approval from the Institutional Ethics Committee. 33 participants who were newly diagnosed with CD were labelled as cases while 31 healthy control participants without any chronic illness or gastrointestinal disorder were labelled as controls. Basic demographic details, anthropometry, and clinical features were obtained on a predesigned proforma. All participants were investigated for routine haematological and biochemical parameters including serology for CD. Subjects with serum TTG-IgA antibody >15 AU/ml underwent endoscopic biopsy followed by histopathological examination and were diagnosed with CD as per modified ESPGHAN criteria.[7] All participants aged between 1-18 years who were newly diagnosed with biopsy-proven celiac disease on a gluten diet were enrolled as cases in the study. Children with any chronic disease, gastrointestinal disorder, immunosuppression or autoimmune disease were excluded from the study. Blood samples were taken from all subjects for estimation of serum IgG4 and preserved at -80 degrees Celsius. In this study, serum IgG4 levels were measured in preserved samples using a commercial ELISA kit (Cayman Chemical Company, Ann Arbor, Michigan, USA), with a cut-off value of 135 mg/dl for diagnosis. Merilyzer Eiaquant spectrophotometer was used to read the microplates. Endoscopic Biopsy was performed by a single endoscopist using the Paediatric Gastroscope GIF–0150. Biopsy specimens were graded according to Modified Oberhuber Marsh grading.[8]

Statistical analysis

Analysis done using SPSS Inc., Chicago, IL, version 22.0 for Windows. Means of two groups were compared using Student's t-test. Categorical data was compared using the Chi-square test or the Fisher exact test whichever was applicable. A two-sided $p<0.05$ was considered statistically significant.

RESULTS

The (Table 1) summarizes the baseline characteristics of the study population which consisted of 64 subjects including 33 individuals with newly diagnosed CD and 31 healthy control participants. Among 33 CD patients, 12 (36.4%) were males and 21 (63.6%) were females. No significant differences were observed between participants with CD and healthy individuals in terms of age and sex ($p>0.05$).

Table 1: Baseline characteristics of study population.

Parameters	CD (n=33)	Controls (n=31)	P value
Age (years); Mean (SD)	5.2 (2.9)	5.5 (2.9)	0.687
Males (%)	36.4	32.3	0.729
Females (%)	63.6	67.7	0.729
BMI (kg/m^2); Mean (SD)	13.62 (1.5)	16.48 (2.9)	<0.001
Haemoglobin (g/dl); Mean (SD)	9.3 (2.1)	11.7 (2.0)	<0.001
MCV (um^3); (SD)	67.3 (9.6)	78.2 (8.9)	<0.001
Albumin (mg/dl); Mean (SD)	4.0 (0.4)	3.8 (0.5)	0.097
TTG-IgA (AU/ml)	137.45	-	-
IgG4 (mg/dl); Mean (SD)	164.8 (47.2)	78.5 (34.7)	0.005
High IgG4 (%)	81.8	25.8	0.01

SD=Standard Deviation; CD=Celiac Disease; BMI=Body Mass Index; MCV=Mean Corpuscular Volume Ig= Immunoglobulin; TTG-IgA=tissue transglutaminase IgA Antibody; IgG4=Immunoglobulin G4

Lower body mass index (BMI) and Haemoglobin (Hb) levels were seen in patients with CD in comparison to healthy controls. IgG4 levels were significantly higher in children with CD as compared to healthy controls. Mean (SD) IgG4 levels among cases were 164.792 (47.28) mg/dl while 78.47 (34.71) mg/dl in controls ($p=0.005$). the study revealed a significant number of CD patients, specifically 82 %, had elevated serum IgG4 levels (>135 mg/dl) and in 25 % healthy controls ($p<0.01$) (Figure 1). The study found that among CD patients, 24 % (8/33) had Marsh 3a, 58 % (19/33) had Marsh 3b and 18% (6/33) had Marsh 3 c lesions. In correlation analysis, it was found that IgG4 levels were not associated with age, sex, BMI. This study found that there was a positive correlation between the grade of mucosal damage to the duodenum, as determined by Marsh grading, and the serum levels of IgG4. Mean (SD) IgG4 levels were 126.32 (49.4) mg/dl in Marsh grade 3a, 171.35 (27.07) mg/dl in Marsh grade 3b and 209.24 (24.36) mg/dl in Marsh grade 3c (Figure 2). These findings suggest that as the severity of duodenal damage increases, there is a corresponding increase in IgG4 levels. No significant

correlation existed between serum IgG4 and TTG-IgA levels (r value =0.003, p=0.986) (Figure 3).

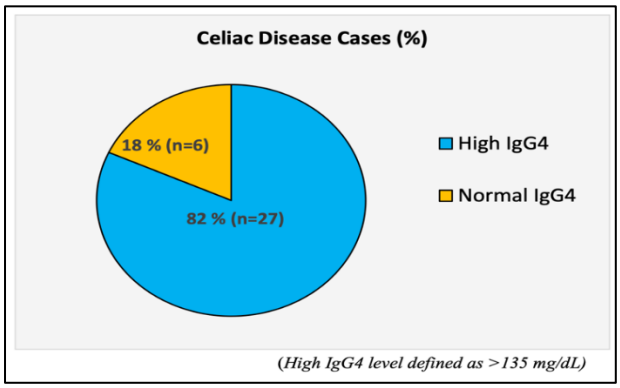

Figure 1: Pie chart showing the distribution of CD cases with high vs. normal IgG4 levels.

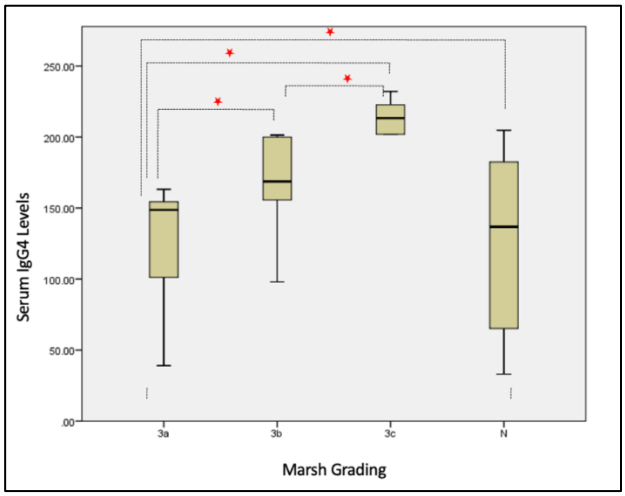

Figure 2: Box-and-Whisker-Plot shows the correlation between Marsh grading and serum IgG4 levels. The higher the serum IgG4 levels, the higher is the grade of Marsh grading, thus indicating that increased IgG4 levels are seen in severe CD.

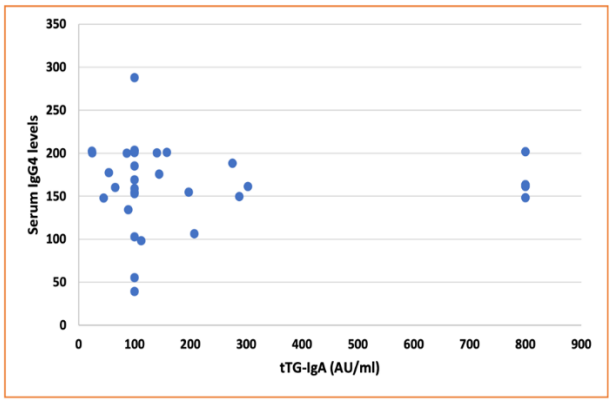

Figure 3: Shows the Correlation analysis between TTG-IgA titres and serum IgG4 levels. No significant correlation was found between them.

DISCUSSION

In this study, we showed that children with newly diagnosed CD had higher levels of IgG4 in serum as compared to healthy children. Furthermore, it was observed that the serum IgG4 levels were associated with increasing severity of damage on Marsh grading. As the serum IgG4 levels increased, higher was the grade on Marsh grading[8]. To the best of our knowledge, this is the first study to be conducted in the pediatric age group studying the association of serum IgG4 and CD. CD presents as a unique model of autoimmunity wherein there is the involvement of genetic factors (HLA DQ-2/8), environmental triggers (gluten) and host susceptibility. A pro-inflammatory innate immune response triggered by gluten, an inappropriate adaptive immune response, and an imbalanced gut microbiome have been identified as the key components autoimmune recipe[9]. Since the identification of IgG4-RD, IgG4 has been in the spotlight due to its association with various conditions and its potential as a diagnostic and prognostic marker. Hamano et al were the first to report increased serum IgG4 levels in patients with AIP.[10] Subsequently many other conditions like sclerosing cholangitis, retroperitoneal fibrosis, Mikulicz's disease, Riedel's thyroiditis, Kuttner's tumour, mediastinal fibrosis, interstitial nephritis, inflammatory pseudotumor were found to be associated with increased IgG4 levels.[11] Cebe et al studied 54 cases of duodenal biopsies, all of which were stained for IgG4. Their findings demonstrated that there is an increased presence of IgG4+ cells in the duodenal biopsy of 7 out of 18 CD patients. This discovery suggested a previously unrecognized link between CD and elevated levels of IgG4. This provided new insight into the pathogenesis of CD and suggests IgG4 may play a role in the development or progression of CD.[12] Demirci et al conducted a study in Turkey for the first time to study the correlation between CD and IgG4-RD in adult patients. They found that out of 41 patients with CD, 65.8 % had increased IgG4 levels with a mean value (SD) of 283.2 (39.02) mg/dl as compared to 28 healthy controls with mean IgG4 levels of 68.9 (15.89) mg/dl ($p<0.001$).[6] Moreover, they also correlated the serum IgG4 levels with Marsh grading and found a positive association. The correlation was similar to our study. In the present study, we found that serum IgG4 plays a significant role in CD. We also found that serum IgG4 levels significantly correlate with the degree of mucosal damage on Marsh grading as evidenced on D2 biopsy specimens. Our study was the first to correlate serum IgG4 with CD in the paediatric age group. In order to study the occurrence of IgG4-RD in these children with CD in the future, long-term, larger, multi-centric prospective studies are advised to bring more insight into CD and IgG4-RD.

Limitations

One limitation of the present study is that duodenal biopsy specimens could not be stained for IgG4.

Therefore, we were unable to determine the presence of possibly increased IgG4 + cells (>10/ high-power field) in duodenal biopsy with CD patients. The small sample size and cross-sectional nature were a few other limitations.

CONCLUSION

This study concludes that, as serum IgG4 levels increase, the severity of mucosal damage increases as evidenced by higher Marsh grading on biopsy specimens. Further studies which include biopsy specimen staining for IgG4 and prospective nature to study the effects of gluten-free diet on levels of IgG4 should be undertaken for better understanding of the disease.

ACKNOWLEDGEMENTS

The authors thank the team at SMS Medical College and Hospital for support, and the children and families for participation. Authors would also like to thank ICMR, New Delhi; Ministry of Health and Family Welfare, Govt. of India and the State government of the participating institution for support.

Funding: Indian Medical Council Research (ICMR), New Delhi, India
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Grotra R, Vijay P, Gupta RK, Malhotra B. Positive association of serum IgG4 levels with modified marsh classification system in children with gluten enteropathy: a single centre experience. Int J Contemp Pediatr 2024;11:545-8.