Original Research Paper

Volume - 13 | Issue - 12 | December - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Pathology

ROLE OF SEROLOGY AND HISTOPATHOLOGY IN CELIAC DISEASE :AN EXPERIENCE OF SUB HIMALAYAN REGION

Dr. Gauri Nakra	Senior Resident, Department of pathology, IGMC Shimla.
Dr. Sudarshan Kumar Sharma	Head of the department & Professor, Department of Pathology, IGMC, Shimla.
Dr. Brij Sharma	Head of the department & Professor, Department of Gastroentrology, IGMC , Shimla.
Dr. Digvijay Singh Dattal	Assistant Professor, Department of Pathology, IGMC, Shimla.
Dr Supriya Verma	Senior Resident, Department of pathology, IGMC Shimla.
Dr Anchana Gulati*	Professor, Department of Pathology, IGMC, Shimla. *Corresponding Author

ABSTRACT ***Objective*:** The study aimed to evaluate the degree of mucosal damage by histopathologic Modified Marsh Grading, serum anti tTG levels , hematological and clinical parameters in celiac disease. ***Setting And Design:*** The present study was a cross-sectional, observational study carried out from January 1st, 2020 to June 30th , 2021 for a period of one and a half year in the Department of Pathology and Gastroenterology, IGMC, Shimla. ***Materials & Methods:*** The present study included 88 clinically suspected patients in whom endoscopy, duodenal biopsy , complete hemogram and serum anti tTG levels were done. Mucosal damage was graded according to the Modified Marsh Grading and serum anti tTG levels were done using ELISA kit. ***Results*:** Varied clinical presentation was seen in patients of celiac disease. Serum anti tTG levels and mean baseline hemoglobin levels were found to statistically correlate with increasing severity of mucosal damage ($p<0.001$). ***Conclusion*:** Serum anti tTG level is an important serologic marker for initial evaluation and follow up in patients of celiac disease, however, duodenal biopsy still remains the gold standard for definite diagnosis.

KEYWORDS : Celiac disease, Autoimmune disease,gluten, serum anti tTG levels,endoscopy, Modified Marsh Grading, anemia.

INTRODUCTION

Celiac Disease(CD) is an immune mediated small intestinal enteropathy. Gluten and related peptides present in wheat, barley, rye incite an autoimmune response in individuals having a genetic predisposition (HLA DQ2 and HLA DQ8)[1]. The prevalence of CD in different parts of the world is estimated to be approximately 0.5% to 1% [2]. Antibodies attack intestinal tissue transglutaminase (tTG) which is responsible for deamidation of gluten peptides. Detection of IgA anti tTG antibodies in serologic screening has helped to evaluate the patients with suspected CD presenting with typical or atypical symptoms. Definitive diagnosis is based on histological changes in D2 biopsy which are graded as per the Modified Marsh classification (I-III). Though the gold standard for diagnosis of CD, endoscopic biopsy is an invasive procedure with certain limitations. Hence, less expensive , non invasive serologic screening markers like anti tTG antibodies, anti- endomysial antibodies (EMA) and deamidated gliadin antibody (DGA) have been evaluated in CD diagnosis [3]. Titres of serum anti tTG antibodies have been observed to rise with increasing severity of mucosal damage. This prompted the researchers to evaluate anti tTG levels as indicators of CD especially in clinically asymptomatic patients.

This study was undertaken to assess the role of serum anti tTG levels and evaluate the degree of mucosal damage using Modified Marsh grading.

MATERIALS AND METHODS

The present study was a cross-sectional, observational study carried out from January 1st, 2020 to June 30th , 2021 for a period of one and a half year in the Department of Pathology and Gastroenterology, IGMC, Shimla. A total of 88 patients with suspected celiac disease gave consent to undergo celiac serology(serum anti tTG levels) and duodenal biopsy. D2 biopsies were evaluated for increase in IEL (≥30/100 enterocytes), crypt villous ratio, crypt hyperplasia and inflammatory infiltrate in lamina propria. These were then graded as per the Modified Marsh grading. The serology samples were taken for determining the levels of anti-tissue transglutaminase antibodies (anti tTG ab) which were assessed using ELISA kit. A value of ≥15 IU/ml was considered positive.

Microsoft excel spread sheet was used to enter the encoded data. Analysis was carried out on STATA software, version 15.1. P-value of < 0.05 was considered significant. Quantitative values are expressed as means and standard deviations, and qualitative values are expressed in percentages. Serum anti-tTG titre and degree of mucosal damage in duodenal biopsy were compared among different groups was made by Student t test. The receiver operated characteristics (ROC) curve was used to determine the anti-tTG titre cutoff which could predict severe mucosal damage. Sensitivity and specificity with a 95% confidence interval (CI) was calculated.

RESULTS

Out of 88 patients included in the study 20 patients had normal serum anti tTG levels and were hence taken as controls. Sixty eight patients with high serum anti tTG level(>15 IU/ml) formed our study group.

The mean age of presentation was 41.23 years (range-1 to 71 years). The majority of patients were in the 5th decade . Out of 68 patients, 36 were females and 32 were males with a female to male ratio of 1.12:1. Diarrhea was the most common presenting symptom seen in 26 patients followed by easy fatiguability, weight loss, dyspepsia, pain abdomen and vomiting in decreasing order of frequency.

All patients underwent duodenal endoscopy. Normal study was observed in majority of the patients (54.5%). Scalloping and grooving were observed in 23.5 % & 11.8 % patients respectively. Nine patients had both scalloping and grooving. Less common findings on endoscopy were decreased number and height of duodenal folds seen in 3 patients , deformed duodenal bulb and nodularity and ulcers in 2 cases each.

In our study majority of patients (47 %) had moderate anaemia. Microcytic hypochromic anemia was most common followed by normocytic normochromic anemia observed in 36.8 % patients and macrocytic anemia in 14.7% patients. Increasing grade of mucosal damage was associated with lower Hb levels(<0.001). The serology samples were taken for anti-tissue transglutaminase antibodies (anti tTG ab) which were assessed using ELISA kit. A value of ≥15 IU/ml was considered positive. Serum anti tTG levels ranged from 16.2 IU/ml to 199.5 IU/ml in the cases with a mean serum anti tTG level of 55.5 ± 7.4 SD IU/ml. (TABLE I) D2 biopsy was assessed for intraepithelial lymphocytes, crypt hyperplasia and varying degrees of villous atrophy (Figure I,II,III).Out of 68 patients, 1 patient included in type 1 category was a sibling of CD patient and 3 patients in type 2

category had presented with dermatitis herpetiformis. Sixty two patients were included in type 3 category and 2 patients in potential CD category had normal morphology on duodenal biopsy.

An increase in serum anti tTG levels was noted with increasing severity of mucosal damage and found to be statistically significant using student t test (p value <0.001). No statistical significance was observed between different clinical and endoscopic parameters and Modified Marsh grading (TABLE II).

The Receiver operating characteristic (ROC) curve was plotted to determine the cut off value of anti tTG Levels which could predict severe mucosal damage (Marsh grade 3). For this purpose, cases with type 1 &2 morphology were clubbed and compared with cases having type 3 histology. Sensitivity and 1-Specificity were plotted at different values of anti tTG The area under the ROC curve (AUROC) was 0.742 with 0.068 standard error. Optimal sensitivity (74.19%) and optimal specificity (75%) was seen at a cut off point of ≥29.7 IU/mL. ROC curve(FIGURE IV)

Table I: Median Serum Anti tTG Levels And Modified Marsh Grading

Modified Marsh Grade	No. of patients	Range of serum anti tTG levels*	Median Serum anti tTG levels
Control group (Type 0)	20	1.19 - 11.3 IU/ml	6.45 IU/ml
Type 1	1	21.1 IU/ml	25.3 IU/ml
Type 2	3	16.2 -72 IU/ml	44.1 IU/ml
Type 3A	39	16.8- 199.5 IU/ml	34.61 IU/ml
Type 3B	17	31-81 IU/ml	66.12 IU/ml
Type 3C	6	74.2- 181.1 IU/ml	104.97 IU/ml
Potential category	2	25.13-32 IU/ml	28.57 IU/ml

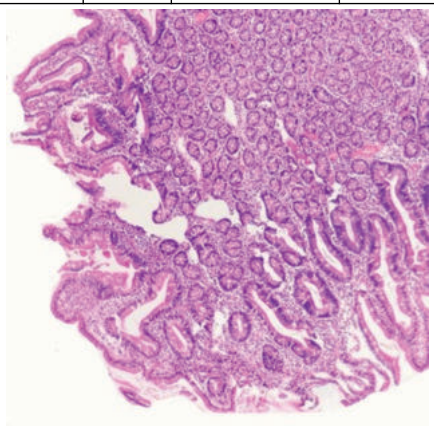

Figure I : Low Power View Of Duodenal Biopsy Showing Blunted And Shortened Villi (modified Marsh Grade Type 3a) 100x H&E

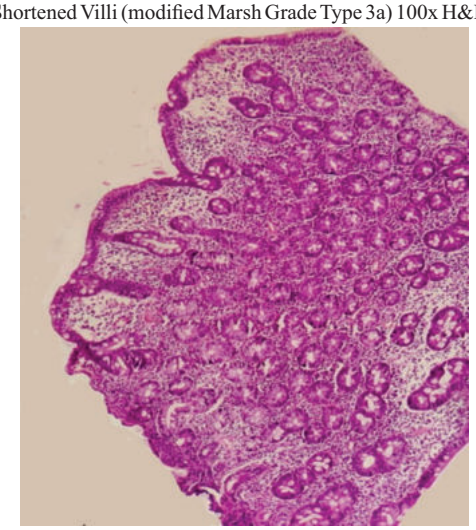

Figure II: Low power view of D2 biopsy showing blunted and shortened villi with subtotal villous atrophy (Modified Marsh grade Type 3b) 100x H&E.

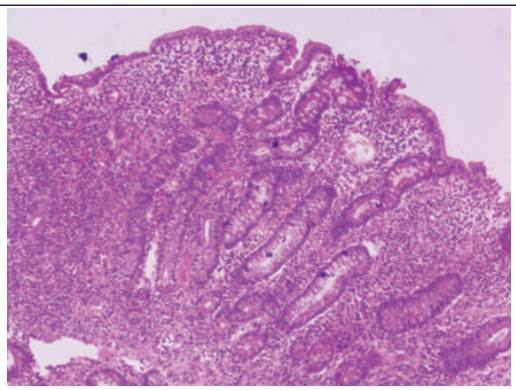

Figure III: Low power view of D2 biopsy showing complete villous atrophy (Modified Marsh grade type 3c) 100x H&E

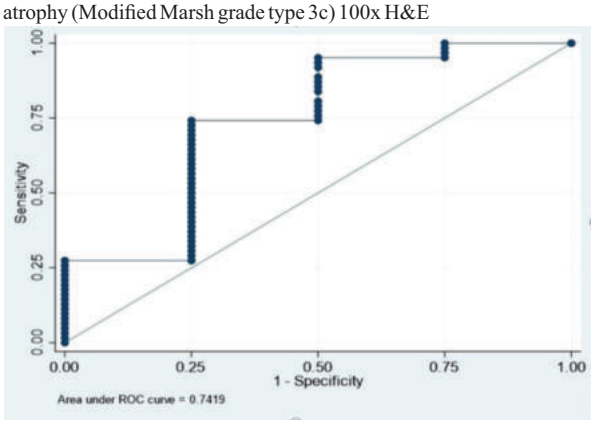

Figure IV: Receiver operating characteristic curve (ROC) showing maximum area under curve (AUC) for Modified Marsh grade 3 histopathology at an anti tTG value of 29.7 IU/ml.

DISCUSSION

Celiac disease (CD) is the most common genetically related food intolerance, worldwide. In individuals with genetic susceptibility, gluten consumption acts as a trigger for an inflammatory reaction. This brings about morphological changes in the small bowel mucosa characterised by lymphocytic infiltration in the epithelium(>30 IEL/100 enterocytes), variable villous atrophy and crypt hyperplasia[1]. These histologic changes have been categorised in Modified Marsh classification and are evaluated in duodenal biopsies of the patients who present with symptoms of malabsorption, anaemia, abdominal pain and non-classical features. Though histopathology is the gold standard in the diagnosis of celiac disease, it is stressful, time consuming and expensive. Patchy nature of disease and improper orientation of biopsy may be responsible for it being inconclusive.

Therefore, disease associated autoantibodies especially EMA & anti tTG levels have proved to be highly sensitive and specific serologic tests in non invasive evaluation of clinically suspected patients of CD. Various studies done across the world have shown that tTG antibody levels rise with increasing degree of mucosal damage assessed using Modified Marsh grading.

Diagnosis of celiac disease is made by recognizing its clinical features, and evaluating with serological tests including serum IgA tTG and serum IgA EMA levels. An automated enzyme-linked immunosorbent test used to detect IgA tTG antibodies is considered the best as its less expensive and easier to perform than is the test used to detect IgA endomysial antibodies. IgA tTG has a sensitivity of 95% to 98% and a specificity of 94% to 95%[3].

The age of patients in our study cohort ranged from 1-71 years with majority of patients in 5th decade which was concordant with Paul B et al[4]. Slight female preponderance was observed as reported by other authors[5-10]. Diarrhea was the most common symptom at presentation (38.2 %) similar to most studies [6,8,11,12]. Endoscopy was normal in majority of our patients while some authors have reported grooving and scalloping as most common finding [6,10]. Alaaragy et al also reported normal endoscopic findings in 47% patients [10].

In our study , no statistically significant correlation was observed

between clinical presentation and endoscopic findings vis a vis Modified Marsh grading. This is concordant with the observations of other authors[6,9,10]. However, significant correlation has been observed by Kalhan et al[6].

Majority of our patients had microcytic hypochromic anaemia with a mean Hb levels of 9.1 g/dl.Lower mean Hb levels were found to correlate significantly with increasing grade of mucosal damage in our study. Similar observation has been made by most other authors [6,11,12].

The value of serum anti TG levels in our study cohort ranged from 16.2 IU/ml to 199.5 IU/ml with a mean of 55.5 IU/ml which was found to be lower than most studies.This discordance can be explained by relatively small sample size in our study possibly due to COVID-19 pandemic and lesser number of cases(n=6) in type 3c category.We had 39 cases in type 3a category and 17 cases in type 3b category. Intake of both rice and wheat as staple food in the diet of our population may also be a contributing factor. Lower anti tTG levels have been observed in studies conducted in South India where rice is the staple food. One such study conducted in South India by Paul B et al[4] found mean anti tTG levels of 30.50 IU/ml in patients with grade 3 histology.

Statistically significant correlation was observed between rising levels of serum anti tTG levels and increasing severity of celiac disease (grade 1 to grade 3c). This is concordant with the observations by most authors[6,8,9,11,12,13].

In our study 2 patients had positive serology i.e raised serum anti tTG levels with normal histology on D2 biopsy and were included in the *potential CD category* Kalhan et al[6]have also reported patients in latent Category. In the present study, a cut off value of anti tTG level obtained by ROC curve was 29.7 IU/ml with 74.19 % optimal sensitivity and 75 % optimal specificity. This is concordant with cut off values observed by Vivas et al [5] though lower than most other studies. Lower sensitivty and specificity in our study is possibly due to less number of cases in type 1 and type 2 category.

CONCLUSION

This study draws attention to Celiac Disease in sub-himalayan region of North India.

In this study, rise in anti tTG levels and decrease in mean baseline Hb levels have shown to statistically correlate with higher Marsh grade indicative of increasing severity of mucosal damage.A high degree of clinical suspicion followed by evaluation of serum anti tTG levels as screening tool help in selecting the cases for endoscopic D2 biopsies. Though serum anti tTG levels is a cost effective reliable serologic marker the importance of duodenal biopsies cannot be overlooked as it is the gold standard for definitive diagnosis and follow up in these patients.

Limitations of our study include a small sample size due to the ongoing Covid -19pandemic and inability to perform repeat D2 biopsies in patients after instituting gluten free diet. EMA antibody levels were not done and we could also not assess patients with IgA deficiency.

Acknowlegements:
We thank Dr Vineet Lakhanpal for helping us in the statistical data analysis.

Conflict Of Interest:
The authors declare no conflict of interest.

Funding: None

REFERENCES

1. Glickman J. The Nonneoplastic small intestine. In:Noffsinger, Amy, editors. Fenoglio-Preiser's Gastrointestinal Pathology. 4th ed. Philadelphia : Wolters Kluwer; 2017p. 1218-1232.
2. Gujral N, Freeman HJ, Thomson ABR. Celiac disease: Prevalence, diagnosis, pathogenesis and treatment. World J Gastroenterol 2012; 18(42): 6036-6059.
3. Rostami K, Kerckhaert JP6, Tiemessen R, Meijer JW, Mulder CJ. The relationship between anti-endomysium antibodies and villous atrophy in celiac disease using both monkey and human substrate. Eur J Gastroenterol Hepatol 1999;11(4):439–42.
4. Paul B, Kinra P, Nandi B, Prasad AS. Correlation of Serum Tissue Transglutaminase Antibody Levels and Marsh Grading of Duodenal Biopsy in Patients of Celiac Disease. Annals of Pathology and Laboratory Medicine. 2019 Mar;6(3):142A-8A.
5. Vivas S, de Morales JG, Riestra S, Arias L, Fuentes D, Alvarez N, Calleja S, Hernando M, Herrero B, Casqueiro J, Rodrigo L. Duodenal biopsy may be avoided when high transglutaminase antibody titers are present. World journal of gastroenterology: WJG. 2009 Oct 14;15(38):4775.
6. Kalhan S, Joseph P, Sharma S, Dubey S, Dudani S, Dixit M. Comparative study of histopathological Marsh grading with clinical and serological parameters in celiac iceberg of north India. Indian Journal of Pathology and Microbiology. 2011 Apr 1;54(2):279.
7. Alessio MG, Tonutti E, Brusca I, Radice A, Licini L, Sonzogni A et.al. Correlation between IgA tissue transglutaminase antibody ratio and histological findings in celiac disease. Journal of pediatric gastroenterology and nutrition. 2012 Jul 1;55(1):44-9.
8. Singh P, Arora A, Strand TA, Leffler DA, Catassi C, Green PH. Global Prevalence of Celiac Disease: Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol 2018;16(6):823–836.e2.
9. Ganji A, Esmaeilzadeh A, Bahari A, Ghafarzadegan K, Aghayee MA, Mozafari HM et.al. Correlation Between Cut-off Level of Tissue Transglutaminase Antibody and Marsh Classification. Middle East journal of digestive diseases. 2016 Oct;8(4):318.
10. Alaaragy BA, Altemimi I, Shubbar YA, Al-janabi A. The correlation of modified Marsh grading system in relation to clinico pathological and serological assessment in early diagnosis of celiac disease. Annals of Tropical Medicine and Public Health. 2020 Oct;23:231-436.
11. Madhu MP, Ashdhir P, Sharma G, Rai GP, Pokharna RK, Ramrakhiani D. Correlation of serum levels of IgA anti-tissue transglutaminase (IgA tTG) with the histological severity in celiac disease. Tropical Gastroenterology. 2017 Jun 22;38(2):102-7.
12. Taneja K, Mahajan N, Rai A, Malik S, Khatri A. Association of Anti-tissue Transglutaminase Antibody Titers and Duodenal Biopsy Findings in Pediatric Patients of Celiac Disease. Cureus. 2021 Mar;13(3).
13. Bansal E, Kaur N, Mittal N. Can High Titres of Anti Tissue Transglutaminase Antibodies Reduce the Need for Intestinal Biopsy for Diagnosis of Celiac Disease. Indian Journal of Clinical Biochemistry. 2018 Oct 1;33(4):456-60.