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Clinicopathological Study of Seronegative Celiac Disease in Adults in Pakistan: A Pilot Study

Farina Mohammad Hanif[1,*], Rajesh Kumar Mandhwani[1], Nasir Hassan Luck[1], Zaigham Abbas[1], Muhammed Mubarak[1], S.Mudassir Laeeq[1], Abbas Ali Tasneem[1]

1. Departments of Hepatogastroenterology and Histopathology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan

* **Corresponding Author:**
Farina M. Hanif, MD
Sindh Institute of Urology and Transplantation, Chand Bibi Rd, Karachi 74200
Tel: + 9221 99215752
Fax: + 9221 99215469
Email: farinahanif@hotmail.com

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ABSTRACT

BACKGROUND

Celiac disease (CD) is usually missed, if the serology is negative. We aimed to evaluate the clinicopathological characteristics of seronegative CD (SNCD) and its response to gluten-free diet (GFD) in adult patients.

METHODS

This observational study was carried out at the Department of Hepatogastroenterology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan from 2009 to 2015. All consecutive adult patients (≥17 years) with features of marked villous atrophy (Marsh class≥III) on duodenal biopsy, negative tissue transglutaminase IgA and IgG antibodies (anti-tTg IgA and IgG) and human leukocyte antigen (HLA) DQ2 or DQ8 serotypes were studied. Clinical characteristics, laboratory parameters, and response to GFD were analyzed by SPSS software version 20. Median and interquartile range (IQR) were used for summarizing quantitative data. Frequency (percentages) was used for qualitative data.

RESULTS

A total of 12 patients with median age of 31.5 years (IQR: 19.75-46.75 years), of whom five (41.6%) were men were studied. The presenting complaints were: weight loss in 11 (91.6%) and abdominal pain in 9 (75%) patients. Anemia was observed in 10 (83.3%) patients with median hemoglobin of 9.5 g/dL (IQR: 6.3-13.25 g/dL). Median alanine transaminase (ALT) was 21 U/L (IQR: 13–27 U/L) and median albumin was 3 g/dL (IQR: 2.4-3.6 g/dL). Anti-tTg IgA and IgG were negative in all patients. HLA DQ serotyping showed homozygous DQ2 and DQ8 in four and one patients, respectively; while heterozygous DQ2 and DQ8 in five and two patients, respectively. All patients were advised to receive GFD. Nine (75%) patients showed complete clinical response. Two patients were non-compliant and one with non-alcoholic fatty liver disease (NAFLD)-related cirrhosis had partial clinical response. Out of the nine responders, two patients showed response within 6 months while the remaining showed improvement over a year period.

CONCLUSION

The diagnosis of SNCD is rewarding as it responds favorably to GFD in most patients. HLA serology provides an important tool for diagnosis of this entity.

KEYWORDS:
Celiac disease, Weight loss, Gluten free diet, Tissue transglutaminase IgA antibodies, HLA DQ2, HLA DQ8.

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INTRODUCTION

Seronegative celiac disease (SNCD) is characterized by negative anti-tissue

transglutaminase antibodies (anti-tTG) in the presence of histological findings consistent with celiac disease (CD) and associated human leukocyte antigen (HLA) haplotype, DQ2 and/or DQ8.[1] Although CD as a cause of duodenal villous atrophy (DVA) is commonly seen, but the presence of DVA with negative serology leads to diagnostic dilemma. Various etiologies associated with DVA and absence of celiac serology include small intestinal bacterial overgrowth, Crohn's disease, intestinal lymphoma, collagenous sprue, and tropical sprue. As a lifelong commitment to gluten free diet (GFD) is required for the treatment of CD, these diseases are needed to be excluded prior to ultimate diagnosis.[2]

It has been proposed that seronegativity in these patients occurs due to deposition of immune complex deep into intestinal lamina propria, which does not reach the blood vessels. Immaturity of plasma cells is another hypothesis in the mechanism of SNCD. The treatment of SNCD is debatable. Some authors advocate the use of GFD while others refute it.[1]

No study in Pakistan is available regarding SNCD. The aim of this study was to evaluate the clinical characteristics of SNCD and its response to GFD in patients who presented at a single tertiary care hospital of Karachi, Pakistan.

MATERIALS AND METHODS

This prospective, observational study was conducted at the Department of Hepatogastroenterology, Sindh Institute of Urology and Transplantation (SIUT), Karachi, Pakistan. Ethical approval was sought from the institutional ethics committee.

SNCD was defined as having all of the following features; marked DVA on duodenal biopsy, negative anti-tTg IgA and IgG, and HLA DQ2 or DQ8 serotype in the presence of compatible clinical features.[1] Response to GFD was not used as a diagnostic criterion.

DVA was confirmed and classified according to Marsh classification by histopathological evaluation on duodenal biopsy sample. Patients with giardiasis, small intestinal bacterial overgrowth, human immunodeficiency virus (HIV), collagenous sprue, and tropical sprue were excluded.

All patients fulfilling the criteria of SNCD from January 2009 to December 2015 were included in the study. All the patients' demographics, clinical characteristics, and laboratory parameters were recorded. All the patients were counseled by nutritionist for GFD, and diet charts were also provided. The patients were followed up by the primary researcher and nutritionist to check compliance. Response was assessed clinically by improvement in symptoms and signs and labeled as complete when the patients' presenting symptoms improved or settled completely on GFD (>70 % improvement) and partial when some of the symptoms improved (eg. diarrhea settled without weight gain). The duration at which the response was achieved was also noted.

The patients' demographics, clinical, and laboratory features and response to GFD were analyzed. Statistical analysis was performed using SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). All continuous variables were represented by median±interquartile range (IQR). Categorical variables were represented by frequencies and percentages. No inferential statistics were applied.

RESULTS

A total of 12 patients were included in this study with median age of 31.5 years (IQR: 19.75-46.75 years), out of whom five (41.6%) were men. The clinical and laboratory parameters of individual patients are shown in table 1. The presenting complaints were: weight loss in 11 (91.6%), abdominal pain in 9 (75%), diarrhea in 7 (58.3%), and amenorrhea in 2 (16.6%) patients. Median weight at presentation was 39 kg (IQR: 32.50–57.50kg).

On physical examination, pedal edema was observed in five (41.6%) patients. Anemia was found in 10 (83.3%) patients with median hemoglobin of 9.5 g/dL (IQR: 6.3-13.25 g/dL) and median mean corpuscular volume (MCV) was 85 fL (IQR: 65.25–86.65 fL). One patient presented with jaundice and had gastroesophageal varices associated with extrahepatic portal vein obstruction (EHPVO) and portal biliopathy. One patient had associated non-alcoholic fatty liver disease (NAFLD)-related cirrhosis.

On laboratory analysis, median creatinine was 1.6 mg/dL (IQR: 0.64-1.01), median alanine transaminase (ALT) was 21 U/L (IQR: 13–27 U/L), and median albumin was 3 g/dL (IQR: 2.4-3.6 g/dL). HLA DQ serotyping showed homozygous DQ2 and DQ8 in four and

Table 1: Main demographic, clinical, and laboratory features of individual patients with seronegative celiac disease

No.	Age (y)/Sex	Weight	Associated Diagnosis	Presenting features	HLA DQ2/8	Biopsy Marsh classification	Villous atrophy (VA)	Response to GFD	VA follow up Biopsy
1	40/F	30	Nil	Diarrhea	heterozygous DQ8	3b	Subtotal	Diarrhea improved Wt gain	Mild
2	20/F	35	Nil	Weight loss, abdominal pain	homozygous DQ8	3a	Partial	Wt gain	Normal
3	23/F	39	NASH CLD	Weight loss, abdominal pain, diarrhea	homozygous DQ2	3c	Total	Diarrhea improved	Not done
4	19/F	35	ESRD+HTN	Weight loss, abdominal pain	homozygous DQ2	3a	Partial	Wt gain	Not done
5	22/M	39	SMA Syndrome	Diarrhea, weight loss, abdominal pain	heterozygous DQ8	3c	Total	Not improved	Total
6	17/F	30	Nil	Weight loss, abdominal pain	heterozygous DQ2	3a	Partial	Wt gain	Not done
7	40/M	65	Nil	Diarrhea, weight loss	heterozygous DQ2	3a	Partial	Diarrhea improved Wt gain	Partial
8	60/M	50	IBD/Pulmonary TB	Diarrhea, weight loss	heterozygous DQ2	3a	Partial	Diarrhea improved Wt gain	Not done
9	45/F	50	EHPVO	Weight loss, abdominal pain`	heterozygous DQ2	3b	Subtotal	Wt gain	Normal
10	52/M	65	Nil	Diarrhea, weight loss, abdominal pain	homozygous DQ2	3b	Subtotal	Diarrhea improved	Not done
11	32/F	36	Nil	Weight loss, abdominal pain	Homozygous DQ2	3b	Subtotal	Wt not improved	Subtotal
12	31/M	40	Nil	Weight loss, abdominal pain	heterozygous DQ2	3a	Partial	Wt not improved	Subtotal

CLD, chronic liver disease: EHPVO, extrahepatic portal vein obstruction; ESRD, endstage renal disease; HTN, hypertension; SMA, superior mesenteric artery; IBD, inflammatory bowel disease, NASH, non-alcoholic steatohepatitis; TB, tuberculosis; VA, villous atrophy

one patients, respectively while heterozygous DQ2 and DQ8 in five and two patients, respectively (figure 1). All the patients had negative tests for anti-tTg IgA and IgG. Duodenal biopsies showed marked villous atrophy. Among these, six patients had partial (Marsh class IIIa), four had subtotal (Marsh class IIIb), and two had total villous atrophy (Marsh class IIIc), as shown in table 1 and illustrated in figures 2 and 3. Repeat biopsies were performed in seven patients. Among them, three showed improvement in villous architecture, three showed no change in histology, and one showed worsening of villous stunting, as shown in table 1.

All patients were advised to receive GFD; out of them, nine (75%) patients showed complete clinical response. Among the remaining three patients, two were non-compliant, while one patient with NAFLD-related cirrhosis had partial clinical response with improvement in diarrhea. Out of the nine responders, two patients showed response within 6 months while the remaining showed clinical improvement over a year.

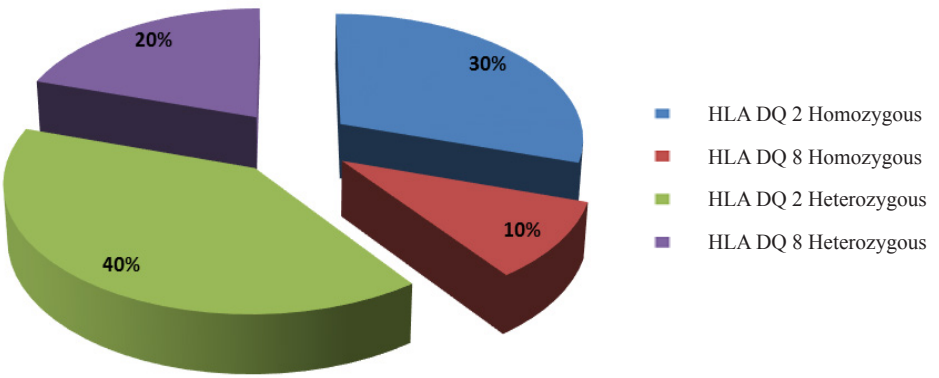

Fig.1: **HLA DQ distribution in patients with seronegative celiac disease (n=12)**

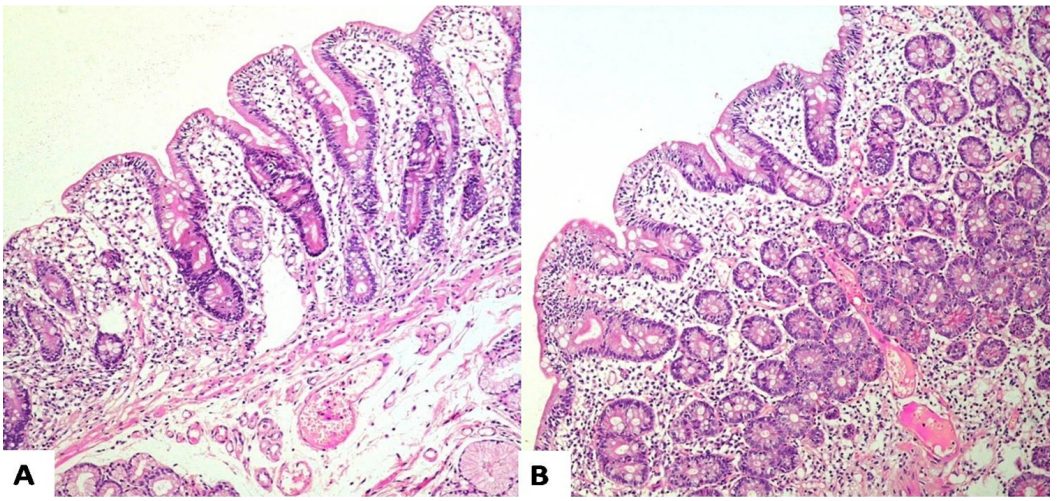

Fig.2: **Histology of representative cases. A: Duodenal biopsy showing partial villous atrophy, Marsh class IIIa (H&E, ×200). B: Duodenal biopsy showing subtotal villous atrophy, Marsh class IIIb (H&E, ×200).**

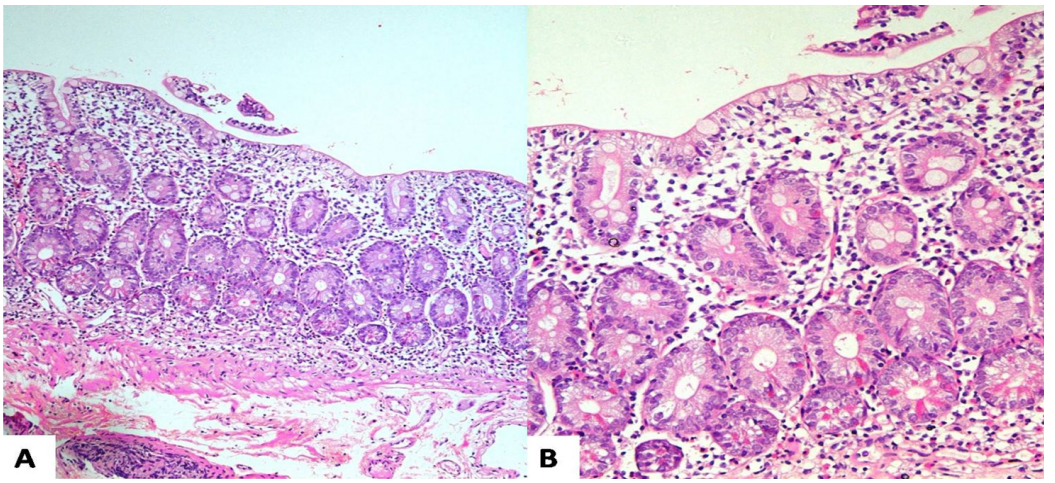

Fig.3: **Histology of representative cases. A: Duodenal biopsy showing total villous atrophy, Marsh class IIIc (H&E, ×200). B: High-power view of duodenal biopsy showing markedly increased intraepithelial lymphocytes and Paneth cell hyperplasia (H&E, ×400).**

DISCUSSION

CD is a small bowel disease characterized by villous atrophy, epithelial lymphocytic inflammation, and crypt hyperplasia in genetically susceptible patients in response to dietary gluten. Its diagnosis is dependent on the combination of clinical features, serological positivity, and genetic and histopathological findings.[3] Although, duodenal biopsy remains as the "gold standard", but the histopathological findings are non-specific for the diagnosis of CD.[4,5] DVA with negative celiac serology is seen in various diseases and SNCD is one of such novel entities. The presence of HLA DQ2 and or DQ8 is required for the diagnosis of SNCD.[1] Even with scanty data, the reported prevalence of SNCD ranges from 1.03% to 28%.[6] There is no data in Pakistan regarding its prevalence. We are reporting 12 patients with SNCD over a period of 6 years at one tertiary care center in Pakistan. However, it must be clear that the prevalence of this disease cannot be estimated from this small case series from a single center in Pakistan.

The exact pathogenesis of SNCD is not known. It is however hypothesized that in patients with seropositive CD, the tTG and anti-tTG immune complexes are deposited in the mucosa and crossover to enter the blood stream. On the other hand, in SNCD, the antigen-antibody complexes are deposited deep into the lamina propria and are unable to reach the blood stream, thus, explaining the possible reason of seronegativity in such patients. Another proposed mechanism of seronegativity is the immaturity of plasma cells, which fail to produce antibodies.[1] Anti-tTG IgA can be negative in the presence of serum IgA deficiency, which occurs in 2- 3% of patients with CD.[3] However, most of the patients with such IgA deficiency can produce positive IgG based serological test.[5] In our study population, neither any patient was serum IgA deficient nor there was an anti-tTG IgG positivity.

The positivity of anti-tTG has been directly associated with the degree of DVA in various studies. Abrams and colleagues[7] described SNCD and reported that although anti-tTG IgA was performed in only 14 patients, negative serology was associated with partial villous atrophy in contrast to total villous atrophy in seropositive patients. We also encountered higher prevalence of partial villous atrophy (six patients). However, subtotal (four patients) and total (two patients) villous atrophy were also detected in our patients. Treatment of SNCD is controversial. Leffler and co-workers [8] had argued against the use of GFD in patients with SNCD. However, Tursi and others [9] reported histopathological improvement in 23 patients and concluded that prescription of gluten restriction is warranted in such patients. In our study, nine patients showed clinical response to GFD over the duration of 6 to 12 months; however, histological response could be assessed in seven patients only as repeat endoscopy and biopsy was not performed in five patients. Among the seven patients, three showed histological improvement in villous architecture, three showed no change in histology, while one showed worsening of villous atrophy, as shown in table 1.

SNCD is an uncommon disease and to the best of our knowledge, the largest number of patients reported was 20 over a period of 10 years.[5] This is the first report from Pakistan, incorporating 12 patients over a period of 6 years, in which the clinicopathological characteristics of SNCD and its response to GFD have been addressed. We have also reported SNCD in patients with subtotal and total villous atrophy, which has not been described before.

This study has some limitations, too. The sample size is small. It has originated from a single center. Repeat duodenal biopsy was not performed in all patients to document histological improvement. Responses to GFD and gluten challenge tests with histological confirmation were not used as diagnostic criteria. And the follow-up is short. Despite the above limitations, we believe that this study will help in understanding the full spectrum of SNCD.

DVA with negative celiac serology requires exclusion of a list of diseases, which requires a battery of tests. Like DeGaetani and colleagues[5], we also propose that HLA DQ2 and/or HLA DQ8 should be performed as the first test in such patients to reduce mental stress of undergoing many negative results and to decrease the economical burden of the country.

In conclusion, the diagnosis of SNCD is rewarding as it responds favorably to GFD in most patients. HLA serology provides an important tool for the diagnosis of this entity.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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