Clinical profile of patients with Seronegative celiac disease

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Abstract

Background

Celiac disease (1) mostly diagnosed base on positive serology and duodenal mucosal atrophy, but some patients have negative serology and their diagnosis have some limitation, it delay in diagnosis likely accompanied a poor prognosis and high risk of developing complications of CD. The aim of this study was determent clinical profile of patients with Seronegative CD (SNCD).

Methods

in this retrospective study, 1115+8 patients, that evaluated for CD with mucosal atrophy included between 2010 to2020. All patients with IgA deficiency other IgG based serology for diagnosis of celiac was done and if these antibodies were negative consider as possible SNCD. If they had positive DQ2-DQ8, and clinical symptoms or had positive challenge test after12 months of GFD were considered as SNCD.

Results

of total 1115 patients 27 (2.4%) had seronegative mucosal atrophy of duodenum and diagnosed as a SNCD (96.2% marsh3), the mean age and BMI in SNCD patients were significantly higher than other CD patients ($p<0.05$).

Conclusion

The prevalence of SNCD was 2.4% that likely related to over weighting, so clinicians should be considered high possible of seronegative CD in patients with over weighting and mucosal atrophy of duodenum.

Introduction

Celiac disease (1) is a common immune-mediated inflammatory disorder triggered by gluten's ingestion (2). In CD, an immune response to gliadin in small intestines results in villous atrophy and lymphocytes' infiltration in lamina properia and epithelium (3, 4). Based on serologic studies, the prevalence in most area is approximately 1 percent (5). CD is classically a disease of all ages (6).

Clinical manifestations of CD divide into two groups (7); gastrointestinal and extraintestinal. The gastrointestinal manifestations mostly present with classic symptoms, such as diarrhea, weight loss, abdominal pain and malabsorption (8). The more common extraintestinal manifestations are mucocutaneous (such as dermatitis herpetiform (9),(10)), metabolic bone disorders (11) (osteomalacia, osteoporosis (12)), hematologic (iron deficiency anemia (13), hyposplenism (14)), and neuropsychiatric

(15). In the diagnostic approach, in adults is tissue transglutaminase (tTG)-IgA antibody and deodenal biopsy which shows mucosal atrophy before initiating a gluten-free diet (GFD) (2, 16).

IgA deficiency is more common in celiac patients and we need to measure TGA IgA, total IgA levels for diagnosis in all patients, and if patients are IgA deficient, an IgG based testing with deaminated gliadin peptide (DGP)-IgG should be performed. (16)the majority of CD diagnosis based on a combination of clinical, serological, histological changes in duodenal biopsies and sometime genetic finding (2). However, CD antibodies can be negative in a minority of CD patients, creating a new description called SNCD (2). The reported prevalence of SNCD is 6.4-9.1% of patients with normal IgA serum concentrations (17). In these cases, CD's diagnosis is based on typical histopathology in a combination of positive genetic factors (HLA-DQ 2 and/or HLA-DQ 8). Moreover, changes in histology and clinical manifestations should be proved after 6-24 months of the gluten-free diet (GFD). Because the villous atrophy along with negative serology can be found in a variety of diseases other than CD, such as autoimmune enteropathy, small intestinal bacterial overgrowth, eosinophilic gastroenteritis, etc. Before making a firm diagnosis of SNCD, other causes of villous atrophy should be ruled out to stay away from unnecessary lifestyle GFD (18).

According to the pathogenesis of SNCD, two theory have proposed the explanation of SNCD: deposition of the anti-tTG immune complexes in the duodenal mucosa, making it unable for anti-tTG to pass into the bloodstream, or immunity of plasma cells leads to deficient antibody production (2).

Because the diagnosis of SNCD is delayed and difficult, and these patients can have a poor prognosis and high risk of developing complications, it is essential to know prevalence of SNCD and diagnose them without delay. In this study, we aim to assess the prevalence of seronegative CD and compare the clinical and laboratory findings in seronegative CD with the seropositive CD patients in the northeast of Iran.

Methods

This retrospective study was conducted on patients who were referred to the Celiac Disease Center in Mashhad (Iran) during 2010-2020. This study has been approved by the ethics committee of Mashhad University of Medical Sciences (code: IR.MUMS.MEDICAL.REC.1400.089). Furthermore, confirming that informed consent was obtained from all subjects and their legal guardian.

Cohort Criteria:

All patients who had endoscopy and mucosal atrophy in duodenum and referred as CD to celiac clinic were included. Patients who had TGA IgA negative and normal IgA serum level was sent for stool exam and evaluation for WBC-RBC and ova and parasite. Any history of infection, giardia, IBD, HIV and medication use antecedent to biopsy were gathered.

The modified Marsh classification was used in the classification of mucosal lesions. Patients with negative TGA -IgA level and normal IgA total and Modified Marsh Classification grade > 1 were send for

reviewing the biopsy specimen by expert pathologist in this field and repeat serology too. For patients with IgA deficiency other IgG based serology for diagnosis of celiac was done and if these antibodies were negative consider as possible SNCD. By confirming pathology and serology, if they had positive DQ2-DQ8, and clinical symptoms or had positive challenge test by 12 months of GFD were considered as SNCD. In patients who didn't have genetic testing we rely on challenge test by GFD for at least 12 months and re biopsy. In case of mucosal healing by GFD we consider as SNCD too.

Overall 1123 patients that had Villus atrophy were enrolled in this study, although statically analysis conducted on 1115 cases, and 3 Crohn's disease and 2 were common variable immunodeficiency and two were drug user and one had pepetic deodenits, which were exited of statically analysis.

Statistical analysis

Data were analyzed using STATA package. We evaluated the prevalence of SNCD in this cohort, and comparing laboratory tests and pathology and clinical findings between seropositive and seronegative CD. The qualitative characteristics of participants are described by the frequency and percentage, and quantitative variable, by mean and standard deviation. T-test and Chi-square were also used for comparisons. The significant level for total tests was 0.05.

1088 were CD with positive serology and 27 patients were diagnosed SNCD by histology and clinical feature and negative for disease associated with villus atrophy of duodenum and confirmed by genetic and challenge test after 12 months,

Results

This study evaluated 1115+8 patient's referred to Celiac Disease Center for diagnosis of CD during ten years. 27 (2.4%) of cases had seronegative mucosal atrophy of duodenum (SNCD) and 1088(97.5%) were seropositive patients (CD patients) and only four of them had IgA defficeiency. Also there are 8 patients (plus), who had mucosal atrophy of duodenum. The more further differential assessments in these patients displayed that 3 had Crohn's disease and 2 were common variable immunodeficiency and two were drug user and one had pepetic deodenits.

Of total 1115 patients that evaluated, 788 (70.7%) were female, that 19 (2.4%) of them were SNCD. Sex ratio (f/m) SNCD patents were 2.0, in both groups female was the predominant gender. The overall mean age was 29.7 ± 15.7 (1 to 76 year), and mean age of SNCD was 37.1±16.3 (6 to 63 years), the compared of two mean age shows, age of patients significantly related to SNCD and seropositive patents (p= 0.01). Mean BMI were 23.9 and 21.4 in SNCD and CD patients respectively, that is higher in SNC patients (p=0.02). Of 15 SNCD patients with recorded family history, only 2 (13%) of them had at least one seropositive celiac disease relative in their family. The patients with seropositive CD have a more positive family history than SNCD, but it is not significantly different between them (p=0.38) (table1).

95.7% of total patients were marsh 2 and 3, all SNCD patients (100%) had villus atrophy (marsh 2&3), and 95.6% of seropositive patents had villus atrophy (p=0.88). 60% of SNCD and 84.5% of CD patients histopathologic samples showed scalloping and fissuring in the duodenal biopsy (P=0.17) (table1). Most SNCD patients (73.1%) and 65.1% of CD patients had gastrointestinal presentation of celiac disease (p=0.65). Common clinical symptoms were chronic diarrhea 44% and dyspepsia 28.6% in SNCD patients (p>0.05), also 76% of SNCD and 60.1% of CD patients had flatulence (p=0.08). Overall both groups have almost the same clinical presenting features and there was no significant difference between intestinal and extra-intestinal manifestations. The more complication were 50% anemia, 55.6%, neurological manifestations and 13% AFT experience in SNCD patients (p>0.05), dermatitis herpetiformis was seen in 13% of CD patients although any SNCD patients hadn't dermatitis herpetiformis .

Normal vitamin D serology level was present in 4 (21.1%) of SNCD patients. Analysis of femoral bone densitometry showed 3 with osteoporosis and 6 with osteopenia, and retrobulbar spine bone densitometry showed 5osteoporosis and 3 osteopenia. TPO levels were normal in 58.3% of SNCD, and 41.7% were higher than the normal range. Moreover, SGOT and SGPT levels are higher in the seropositive group than SNCD, although this difference was not statistically significant (p>0.05). SNCD displayed co-association with thyroid diseases more frequently than SPCD (11.1% in SNCDs versus 9.1% in SPCDs, (P= 0.50) and SPCDs had more co-association with diabetes mellitus than SNCDs (5.9% in SPCDs versus 3.7% in SNCDs, although these differences did not reach statistical significance (P-=0.5) (table1).

Table 1. the characterize of patients related to SNCD and SPCD.

Variable	Total	SNCD (n=27)	CD (n=1080)	P-value
Age mean ± SD, years (min, max)	29.7± 15.7, (1 -76)	37.1 ± 16.3, (6 -63)	29.5 ± 15.7, (1-76)	0.01*
BMI mean ± SD, (min, max)	21.4±5.2, (9.2 -42.8)	23.9±5.3, (12.8-33.3)	21.4±5.2, (9.21-42.8)	0.02*
SGOT mean rank, (min, max)	35.5± 57.4, (0-1112)	314.5, (13-1112)	356.3, (0- 585)	0.33
SGPT mean rank, (min, max)	33.6±43.7,(0-452)	288.1 ,(11- 156)	356.7 ,(0- 452)	0.11
Sex n (%)				
female	785 (70.7)	19 (70.4)	766 (70.7)	0.96
male	325 (29.3)	8(29.6)	317 (29.3)	
Familial history of CD n (%)				
Yes	179 (24.1)	2 (13.3)	177 (24.3)	0.38
No	453 (60.9)	9 (60.0)	444 (60.9)	
Undetermined	112 (15.1)	4 (26.7)	108 (14.8)	
Marsh n (%)				0.88
I	42 (4.1)	0	42 (4.2)	
II	55 (5.4)	1 (3.8)	54 (5.4)	
III	927 (90.3)	25 (96.2)	902 (90.1)	
Pathology n (%)				
Scalloping & fissuring	363 (84.2)	3 (60.0)	360 (84.5)	0.17
others	68 (15.8)	2 (40.0)	66 (15.5)	
Main symptoms n (%)				
Gastrointestinal	702 (65.3)	19 (73.1)	683 (65.1)	0.65
Diarrhea	425 (41.9)	11 (44.0)	414 (41.9)	0.85
Flatulence	574 (60.5)	19 (76.0)	555 (60.1)	0.08
Dyspepsia	248 (23.2)	2 (28.6)	146 (42.8)	0.70
Main complication n (%)				
Anemia	475 (53.1)	10 (50.0)	465 (53.1)	0.70
LFT abnormality	295 (32.3)	3 (13.0)	292 (32.8)	0.10

Dermatitis herpetiform	32 (13.0)	0	32 (13.1)	0.99
Neurologic disorders	436 (57.7)	10 (55.6)	426 (57.7)	0.84
Vitamin D levels n (%)				
Normal	243 (35.7)	4 (21.1)	239 (36.2)	
Low	214 (31.5)	8 (42.1)	206 (31.2)	0.37
Insufficient	223 (32.8)	7 (36.8)	217 (32.7)	
Metabolic bone disorder (Femur) n (%)				
Normal	251(48.6)	7 (43.8)	244 (48.8)	
Osteoporosis	75 (14.5)	3 (18.8)	72 (14.4)	0.83
Osteopenia	190 (36.8)	6 (37.5)	184 (36.8)	
Metabolic bone disorder (Spine) n (%)				
Normal	257 (50.0)	8 (50.0)	249 (50.0	
Osteoporosis	84 (16.3)	5 (31.3)	79 (15.9)	0.22
Osteopenia	173 (33.7)	3 (1.8)	170 (34.1)	
TPO levels n (%)				0.80
Normal	244 (63.0)	7 (58.3)	237 (63.2)	
High	137 (35.4)	5 (41.7)	132 (35.2)	
Low	6 (1.6)	0	6 (1.6)	
Autoimmune Diseases n (%)				
no	944 (85.0)	23 (85.2)	921 (85.0)	0.50
Thyroid diseases	102 (9.2)	3 (11.1)	99 (9.1)	
Diabetes Mellitus	65 (17.7)	1 (3.7)	64 (5.9)	

Discussion

In our study on 1123 patients, the prevalence of SNCD was 2.4% in CD. Mean age of SNCD had significantly higher than SPCD patients (P=0.01). Gender of patients not related to SNCD status. Moreover, SNCD is described as the most frequent cause of seronegative villous atrophy that is similar to report of Volta et al (18), also SNCDs mostly had total or partial villous atrophy and one had decrease in villous high, intra epitalial lymphocyte and crypt hyperplasia (Marsh 2) which is in line with other studies

(19). Since the diagnosis of SNCD patients was based on duodenal biopsy pathology, their histological analysis showed compatible features to celiac disease, and the most common stage was March 3 (96.4%). Also, scalloping and fissuring had been found in duodenal biopsies of 66.7% of patients with SNCD. Moreover, more than 90% of all SNCD and SPCD had villous atrophy (Marsh 3).The prevalence of SNCD in our study was 2.4%, therefore, Abram et al. reported that 15% of patients with villous atrophy had negative serology (20), and in a similar study of Rostami et al., they showed a co-occurrence of 70% between partial villous atrophy and negative serology. Moreover, their study showed similar clinical presentations and improvement following GFD in both SNCD and SPCD groups (21).

Clinical presentations of SNCD patients were similar to SPCD (22), the most common symptoms were dyspepsia followed by diarrhea, anemia, neurological manifestations. Most common associated symptom in dyspeptic patients was flatulence (74%). According to previous studies, dermatitis herpetiformis has a strong association with seropositive CD; none of the SNCD patients presents this symptom in our study too. Autoimmune diseases represented with no statistically significant difference between two groups, but clinically thyroid diseases and diabetes coexisted more in SNCDs and SPCDs respectively.

The mean age In SNCD patients was significantly higher than SPCD patients in our study, similar to our study the retrospective study by Volta et al. shows, 1.7% of patients were SNCD, and median age in these patients had significantly higher than SPCDs at the time of diagnosis, which is probably due to a later diagnosis than SPCDs. Therefore, they observed a strong association of SNCD with more severe degrees of villous atrophies; in contrast, in two other studies by (19) and (23), the greater degree of villous atrophy associated with SPCDs than SNCDs. Also, they found SNCDs had more classic clinical manifestations than the SPCD group, (18), but clinical manifestations were same in both groups of our study.

Most common cause of villous atrophy in duodenum after CD (97%), is SNCD (2.4%) and then IBD (0.2%) and CVID (0.2%), drug and deodenitis (0.2%). Prevalence of IgA deficiency was 0.3% in our CD population.

This study is set out to compare the prevalence, clinical and pathologic features of SNCD and SPCD. To our knowledge this is the first study in the northeast of Iran on the prevalence, pathological, and clinical profile of SNCD.

Conclusion

The results of this investigation showed that although SNCD has a low prevalence, it is the most common cause of villous atrophy with prevalence of 2.4% in this area.

Declarations

Ethics approval and consent to participate: This study has been approved by the ethics committee of Mashhad University of Medical Sciences (code: IR.MUMS.MEDICAL.REC.1400.089). Furthermore, confirming that informed consent was obtained from all subjects and their legal guardian.

Consent to publish: Not applicable

Availability of data and materials: All data generated or analyzed during this study are included in this published article as supplementary file.

Competing interests: There are no financial conflicts of interest to disclose for any authors.

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Author contributions

- Conceptualization; A.G
- Data curation; E.R, M.GH,
- Funding acquisition; A.G.
- Investigation; E.R, A.G, F.A, B.SH.
- Methodology; F.A, V.GH.
- Project administration; A.G.
- Resources; A.G.
- Software; F.A, V.GH.
- Supervision; A.G.
- Validation; A.G, E.R, F.A, B.SH.
- Visualization; F.A, A.G, B.SH, E.R, M.GH, V.GH.
- Roles/Writing original draft; F.A, A.G, B.SH, E.R, M.GH, V.GH.
- Writing - review & editing; F.A, A.G, B.SH, E.R, M.GH, V.GH.

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