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Original Article

Correlation of Modified Marsch Grades with Serum Anti Tissue Transglutaminase Antibody Levels in Celiac Disease

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Abstract:
Objective: To correlate the histopathological grades of celiac disease using Modified Marsch grading system with serum IgA anti-tissue transglutaminase antibody levels.
Study Design Prospective Cross-sectional study
Study Place Department of Pathology, Al Nafees Medical College& Hospital
Duration of Study May 7, 2015 To December 10,2015
Methodology: Informed consent was taken from all the enrolled patients in written form depending upon inclusion and exclusion criteria. Endoscopic biopsy from all patients was processed routinely for histopathological analysis. Modified Marsch grading system was used for grading the morphological findings. Anti-Tissue transglutaminase (tTG) IgA antibodies were measured by the ELISA (Enzyme-Linked Immunosorbent Assay) technique. Both serological marker and biopsy data were correlated using Regression analysis test and Spearman rho test in SPSS version 20.
Results: In this study 77 patients were included, out of which 55 were labeled as celiac disease patients. There were38.9%males and 61.1% females. By the Modified Marsch Grading System, there were 9.1% cases in Marsch 1 and 13% in Marsch 2 whereas Marsch-3a, Marsch-3b and Marsch-3c included 15.6%, 19.5% and 14.3% cases respectively. After correlation with tTG IgA Ab titers, 27.2% patients had negative serology. Total22% patients in both Marsch 1& 2 were serologically positive and had IgA tTG Ab titers > 11 units/ml whereas majority of patients with Marsch 3a had tTG IgA Ab titers between 11 to 100 units/ml. Marsch grades 3b and 3c strongly correlated with increasing values of serology as IgA tTG Ab titers > 100 units/ml.
Conclusion: Celiac disease grades obtained by using Modified Marsch System significantly correlates with tTG IgA Ab titers
Key Words: Anti Tissue Transglutaminase, Celiac disease, Serology, Marsch, Histopathology.

Introduction

Celiac disease is a gluten sensitive autoimmune disease of the small intestine that develops in persons who are genetically susceptible individuals. Celiac disease may present with gastrointestinal symptoms, extra intestinal symptoms or maybe asymptomatic.[1] The classical gastrointestinal symptoms include diarrhea, steatorrhea and weight loss. Extra intestinal symptoms include anemia, dermatitis herpetiformis, osteoporosis and neurological problems.[2]
Various factors such as age, gender, genetics, immunology, dietary habits and extent of mucosal injury play an important role in modifying the clinical expression of the disease creating a variable picture of celiac disease.[3]
Various studies show that the prevalence of celiac disease is approximately 1% throughout the world[4]. In Western Europe and the United States, about 1% of the population is affected[5].The incidence of celiac disease in Asian countries is low whereas frequency is increasing in China[7].Individuals of South Asia have high prevalence of celiac disease[8].
Diagnosis of patients presenting with celiac disease symptomatology depends upon the histopathological findings of duodenal biopsy along with serologic tests for antibodies against tissue transglutaminase[9]. Microscopic features that help in the diagnosis of celiac disease includes increase number of intraepithelial lymphocytes and architectural changes of the villi and crypts.[10] Modified Marsh grading system is based on all these microscopic features for the diagnosis of the disease.[11]

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Modified Marsch grading system consists of the following categories: type 1 includes increased intraepithelial lymphocytes: (Figure 1) whereas type 2 is crypt hyperplasia; (Figure 2) Type 3 lesions, based on villi to crypt height ratio, is further divided into type 3a (mild atrophy) (Figure 3), type 3b (marked atrophy) and type 3c (total villous atrophy).Type 4 is complete atrophy along with crypt hypoplasia.[12,13]

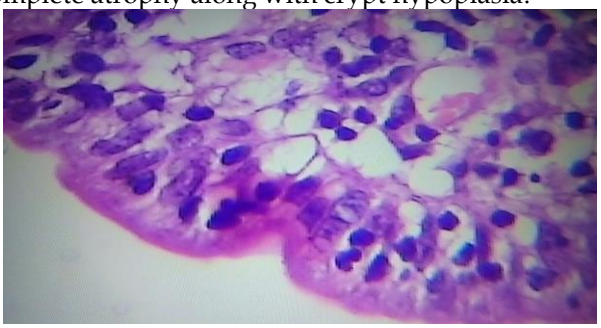


Figure 1: Intraepithelial lymphocytes along with enterocytes. Marsch-1 (H&E X400)

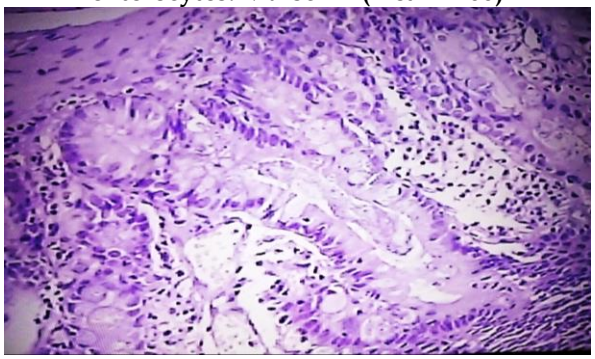


Figure 2: Crypt hyperplasia Marsch 2 (H&E X400)

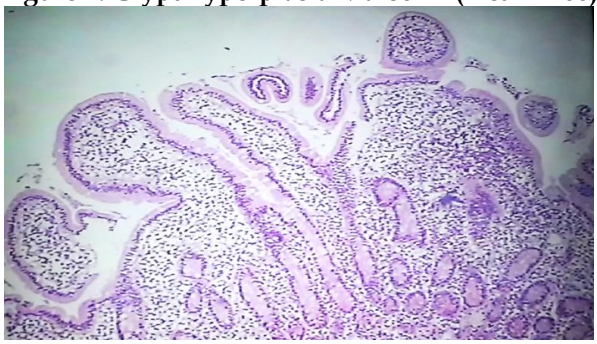


Figure-3: Moderate villous atrophy Marsch 3a (H&E X200)

Serological diagnosis has been introduced because of asymptomatology of patients of celiac disease. Commonly used antibodies are antigliadin antibodies (AGA), endomysial antibodies (EMA) and tissue transglutaminase antibodies (tTG).[14] Serological assays of immunoglobulin A (IgA) antibodies against tissue transglutaminase (tTG) have better diagnostic characteristics as compared to others[1,5] Various studies shows that IgA-tTG antibodies have a higher sensitivity and specificity as compared to others and considered as an deal test for screening patients of all ages.[16,17]

Patients & Methods

It is across sectional study conducted at Pathology Department, Al Nafees Medical College & Hospital, Islamabad over a period of nine months from May 7,2015 to December 10, 2015. Endoscopic duodenal biopsy specimens and the sera for serological testing were taken from both males and females of all age groups having symptoms of malabsorption. Improperly processed samples and stained slides were not included in the study.

The biopsy specimens were collected in formalin and processed in automated tissue processor. Paraffin embedded sections were stained with the hematoxylin and eosin stain. Slides were examined by consultant histopathologists under light microscope. All the macroscopic and light microscopic findings of duodenal biopsies were noted in systemic manner in the proforma. The final diagnosis was written at the end in each case. Endoscopic biopsy findings were recorded and grouped into different grades as per Modified Marsch grading system. Serological marker of celiac disease of each patient i.e. anti-tTG antibodies was measured by the ELISA technique. SPSS20 was used for the analysis of collected data. Regression analysis test and Spearman rho test was applied for statistical evaluation.

Results

A total of 77 patients were enrolled in the study having a clinical diagnosis of suspected celiac disease on symptomatology. Among these there were 71.4%(n=55) cases of celiac disease confirmed both by histopathology and serology. In Modified Marsch Grading System, Marsch-0 included 28.6% (n=22) patients, Marsch-1 consisted of 9.1% (n=07) patients and Marsch-2 included 13% (n=10) patients. Whereas, Marsch-3a, Marsch-3b and Marsch-3c comprised of 15.6% (n=12), 19.5%(n=15) and 14.3%(n=11) patients respectively

Serological test for celiac disease was done in all patients using serum IgA anti tissue transglutaminase antibodies. Serological IgA anti-tissue transglutaminase antibodies titers were divided into three groups according to the serological kit manufacturer guidelines. Stratification of Marsch grades into quantitative anti-tissue transglutaminase tTG Ab groups (negative 0-10 units, positive 11 to 99

units, strongly positive ≥ 100 units) was done which is shown in **Table -1**.

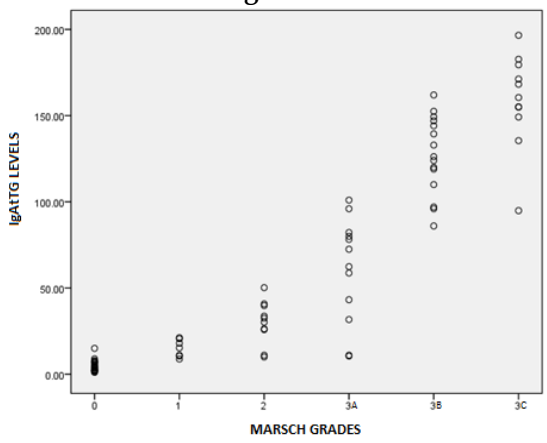
In Marsch grade 0, anti-tTG (IgA Ab) value <11 U/ml was identified in twenty one patients while 1 patient had IgA value between 11 to 100 units/ml. Seventeen patients in Marsch grade 1 and 2 were IgA tTG Ab positive but none had values greater than 100 units/ml. Twelve patients with Marsch 3a grade had IgA tTG Ab values between 11 to 100 units/ml. Twenty six patients with Marsch 3b and 3chad IgA tTG Ab values more than 100 units/ ml as shown in **Table -1.**

Table-1: IgA anti-tTG Ab Titers in different Marsch Grades

Marsch grade	No. of patients		IgA tTG titers < 11 U/ml (Negative)		IgA tTG titer 11–99 U/ml (Positive)		IgA tTG ≥ 100 U/ml (Strongly Positive)	
	n	%	n	%	n	%	N	%
0	22	28.6	21	95	01	5.0	00	00
1	07	9.1	02	29	05	71.0	00	00
2	10	13.0	02	20	08	80.0	00	00
3a	12	15.6	01	8	09	75.0	02	17
3b	15	19.5	00	00	03	20.0	12	80
3c	11	14.3	00	00	01	9.0	10	90
4	00	00	00	00	00	00	00	00

Analysis of IgA tTG with Marsch lesions by Spearman rho correlation revealed a statistically higher probability of Celiac disease with increasing IgA anti-tissue transglutaminase antibody titers. Each unit of increase of IgA tTGtiters increased the risk of having Marsch grade 3a, 3b or 3c lesions as evident in scatter plot in **Figure-4**.

Figure 4: Scatter plot of tTG IgA Ab titers by Marsch grades



IgA tTG Ab titers ware significantly correlated with Marsch grades (Spearman rho =0.948, p < 0.01) as shown in **Table-2**.

Table-2: Spearman's rho Correlation for Marsch Grades Versus Serology

n=77			MARSCH	SEROLOGY
Spearman's rho	MARSCH	Correlation Coefficient	1.000	.948**
		Sig. (2-tailed)	.	.000
	SEROLOGY	Correlation Coefficient	.948**	1.000
		Sig. (2-tailed)	.000	.

Discussion

Celiac disease is one of the most important gastrointestinal diseases. Because of variable presentation of both typical and atypical symptoms and signs in majority of patients, diagnosis of celiac disease is difficult.[18] The assessment of patients suspected to have celiac disease is usually based on positive serum antibody titers and morphological findings of the endoscopic biopsy. Modified Marsch grading system is actively used for diagnostic purpose as well as for follow-up of celiac disease patients.[19]Anti-tTG IgA antibody titer is used to screen and monitor symptomatic and asymptomatic individuals for celiac disease.[20]

Recent studies show that patients having celiac disease have a significant correlation between endoscopic biopsy findings and serum anti-tissue transglutaminase IgA Ab titers.[21]On the Contrary, *Aldaghi M et al* showed that higher serological titers have lower sensitivity and specificity with the positive pathologic biopsy findings.[22]

In the current study, a statistically significant change is recorded in anti-tissue transglutaminase IgA tTG Ab levels from mild grade disease to highly severe grade disease for Modified Marsch grading systems. *Rahmati A*, in his study in 2014, also showed similar results of positive correlation between serum antibodies and modified Marsch grading system.[23]

Different statistical tests are applied in current study to verify the correlation i-e Spearman rho and regression R2 methods yielded a significant positive correlation with Marsch grading system with p value < 0.01.In 2016, *Banotto M*, in his study, had similar statistically results of positive correlation.[24]

Marsch grading system correlates with serological marker because it has a wide range of levels describing the ascending morphological severity of celiac disease in patients. Results show that the raised positive prognostic titers of serology become more significant in Marsch grade 3 as compared to Marsch 1 and 2.Our finding is in agreement with previous study done in2014 by *Bhattacharya M*.[25]

TG antibody has been used as a sensitive and specific ELISA based test in celiac disease and also there is a positive correlation between titers of tTG antibody and Marsh grading in current study and in various studies mentioned previously. We propose that repeated endoscopic biopsy should be discouraged in celiac patients having strongly positive tTG antibody titers.

Vivas and colleagues in 2015 also proposed that duodenal biopsy might be avoided in celiac patients with strongly positive tTG antibody titers.[26]This diagnostic approach has been confirmed by *Mubarak and co-workers*, in 2013, that small intestinal biopsy can be avoided in symptomatic patients with tTGA≥100 U/ml.[27]

Conclusion

Conclusion of current study is that histopathological grades of modified Marsch grading system has a positive correlation with serum IgA anti-tissue transglutaminase antibodies levels.

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C. Analysis/Interpretation/Discussion
D. Manuscript Writing
E. Critical Review
F. Facilitated for Reagents/Material/Analysis