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Research Article

Association between Anti-tissue Transglutaminase (Anti-tTG IgG and IgA) , Circulating membrane attack complex (MC5b-9) and interleukins (IL17 and IL21) in Celiac Disease

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Abstract:

Celiac disease is triggered by gluten -derived peptide (e.g., gliadin) in genetically susceptible HLA DQ2 and DQ8 histocompatibility antigen, and enteropathy. This study aimed to investigate the relationship Anti-tissue Transglutaminase (Anti-tTG IgA),Anti-tissue Transglutaminase (Anti-tTG IgG) with membrane attack complex (MC5b-) and IL-17 and IL-21.From 1st August to 1st January, 2024, this study was carried out at Marjan Hospital in the Babylon Governorate. This study concluded that anti- IgG and IgA concentrations increased gradually with marsh (0,1,2,3) were (12.77, 26.60, 39.45, 60.56/ 9.17,11.38,41.96, 64.7) respectively, while (MC5b-9) and . (IL-17) and IL-21 were high significantly increased with marsh 3 only . (430.50) (77.20, 217.64) respectively. This study concluded increased serum anti-tTG IgG , IgA with all marsh gradually and IL-17, IL-21 and MC5b-9 concentrations with Marsh 3.

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Introduction

Celiac disease (CD) an intestinal immune -mediated disorder triggered by the ingestion of gluten in genetically susceptible individuals. Although the mechanisms underlying gliadin-mediated activation of adaptive Immunity in CD have been well-characterized, regulation of Innate immune responses and the functions of certain immune cell populations within the epithelium and lamina propria are not [1].

Celiac disease is triggered by gluten -derived peptide (e.g., gliadin) in genetically susceptible HLA DQ2 and DQ8 histocompatibility antigen, and enteropathy [2].

Despite the fact that the processes by which gliadins in CD activate adaptive immunity have been thoroughly described, the control of innate immune responses and the roles of specific immune cell populations in the lamina propria and epithelium are not [3].

One reliable confirmatory method for a conclusive non-biopsy diagnosis of celiac disease is to combine DGP IgG and tTG IgA in a single step [4].

In celiac disease elevated membrane attack complex (MAC) levels corelate with villous atrophy in celiac disease [5].

Complement MC5b-9The membrane attack complex (MAC) or terminal complement complex (TCC) Is a complex of proteins typically formed on the surface of pathogen cell membranes as a result of the activation of the host's complement system, and as such is an effector of the Immune system.[6].

IL-17 and IL-21 are pivotal cytokines in mucosal immunity: IL-17 mediates early defense against pathogens while IL-21 regulate T Cell response [7].

This study aimed to elucidate the interplay between anti-tissue transglutaminase (Anti-tTG IgG and IgA) ,interleukins (IL17 and IL21) ,circulating membrane attack complex (MAC) in Celiac disease with in different histological marsh 3 .

Materials and Methods

Sample collection

Ninety patients (male:28 ;female:62) divided into three groups (1-15,16-30 and > 30) years (newly diagnosed / refactors) were clinically diagnosed by physician. ,for period 1st August to 1st January ,2024 ,after confirming that the patients were suffering from Celiac disease. Five milliliter blood samples were draw and serum was separated from them at 3500 rpm /5min for the purpose of conducting laboratory tests (Anti-tTG IgG and IgA) Bio source (USA) at Marjan Hospital /Babylon Governorate .

Exclusion criteria : Incomplete data ,Age less than 3 yrs. ,Patients with tumors.

Inclusion criteria : Patients with Ulcerative colitis ,Patients with celiac disease

Biopsies

For histological evaluation, biopsies of the duodenum's mucosa (D1,D2and D3) from various celiac diseases were routinely treated , stained with hematoxylin and eosin stain , and biopsies were classified by Histopathologist at Marjan Hospital /Babylon Governorate according to the modified Marsh- Oberhuber classification [8].

Immunology tests :

Two milliliters of serum were divided into four Eppendorf tubes. They were then frozen at -20°C until they were needed. Immunological tests were conducted using the ELISA technique Germany/ Human Reader HS). These tests included the use of the Human Terminal Complement Complex MC5b-9 ELISA, IL-17, and IL-21 ELISA kits, manufactured by Jiaxing Korean Biotch (China).

Ethical Approval

This study was approved by the ethics committee of Department of Clinical Laboratories/College of Applied Medical Sciences /University of Kerbala . After the Ministry of Health and Environment granted permission to conduct the study, samples were collected and starting the study. The study participants gave their permission to collect socio-demographic data as well as undertake experiments on the selected samples with respecting patient confidentiality.

Statistical Analysis

Statistical Analysis

The statistical analyses of the data were conducted using IBM SPSS statistics software version 23. Descriptive statistics have been conducted to describe the analysis findings. The results of the experiment have been evaluated by employing a p-value threshold of 0.05 for determining statistical significance. The mean and standard deviation were recorded. The Shapiro-Wilk test was utilized to determine data normality, while the Levene test was adopted to examine variance homogeneity. Nonetheless, chi-square as well as Pearson's correlation tests have been performed to investigate the relationship between category and numerical data. Furthermore, analysis of variance (ANOVA) was utilized to conduct multiple comparisons among groups, while Schaeffer's post-hoc tests were carried out for multiple comparisons within groups. Data having a P value below 0.05 are indicated by asterisks. Finally, GraphPad Prism 9 was utilized for generating all visualization

Results and Discussion

Table (1): Distribution of celiac disease according to the age groups

The result revealed to the age group 16-30 was 39 more than other age groups (**3-15, <30**) (33, 18) respectively and more of them in Marsh 3.

The reason for the percentage of patients who suffer with in this age group may be due to psychological reasons and perhaps also due to genetic factors.

Table (1) Distribution of celiac disease according to the age groups

Age	Marsh0	Marsh1	Marsh2	Marsh3a	Marsh3b	Marsh 3c	Total marsh 3	Total
3-15	8	4	3	6	5	7	18	33
16-30	7	7	8	4	6	7	17	39
<30	0	4	4	5	4	1	10	18
Total	15	15	15	15	15	15	44	90

Celiac disease identified in early childhood was found to have a higher risk of autoimmune disease, indicating the need for a high level of autoimmune disease awareness in this population [9].
Recent study indicated that evidence regarding celiac diseases, with special emphasis on clinical implications, diagnosis, dietary management, socioeconomical aspects, and future perspectives [10].

table (2) Distribution of celiac disease, According to sex , the result are indicated that female are more than male to have the condition. The proportion of the disease was 68.88% of female 31.11% of male.

Table (2) The Distribution of celiac disease According to sex

ON	Marsh 0	Marsh1	Marsh2	Marsh3	Ratio
Female	**9**	**4**	**12**	**37**	**62(68.88)%**
Male	**6**	**11**	**3**	**8**	**28(31.11)%**
Total	**15**	**15**	**15**	**45**	**90(100)%**

This results were similar to Lima et al that noticed women were more likely than men to have celiac disease [11].
In contrast Tan conducted that men are more likely than women to present with non-classical celiac disease, which is linked to a delayed diagnosis. Early diagnosis of celiac disease is crucial since a protracted diagnostic delay is linked to a slower rate of symptom recovery following the implementation of a gluten-free diet (GFD) [12].

Table 3 illustrated that the results of the tests Anti-tTG IgG were (12.77,26.60,39.45,60.56) and Anti-tTG IgA were (9.17,11.38,41.96,64.7) to the marsh (0,1,2,3) .
A section of the duodenum from a patient with celiac disease revealed histological changes in all three areas: Marsh 3 showed villous atrophy and increased lymphocytes and IELs >30/100.

Table (3) the relationships between serological tests and histological changes

	Histological changes			
Serological tests	**Marsh 0 (Normal villi) Normal Lymphocytes**	**Marsh 1(Infiltrative Lesion) Increase Lymphocytes**	**Marsh 2 (Crypt hyperplasia)Increase Lymphocytes**	**Marsh 3 (Villous Atrophy) Increase Lymphocytes >30/100**
Anti -tissue transglutaminase- IgG ng/L	**12.46**	**26.60**	**40.00**	**60.51**
Anti -tissue transglutaminase- IgA ng/L	**8.20**	**11.38**	**41.95**	**59.00**

Another study indicated a gradual and considerable increase with the histological categorization a stronger correlation with duodenal histological alterations [13].

Genetic predisposition and exposure to dietary gluten, with immune system involvement . The incidence is increasing globally, and the societal economic burden of celiac disease stretches beyond the cost of gluten-free food. This enteropathy that affects the small intestine has been related to different disorders and comorbidities [10].

Marsh 3 acute inflammation was (430.505), exhibited increased significantly a s (p-0.01) in concertation of (MC5b-9) whereas other marshes exhibited no significant that illustrated in figure (1).

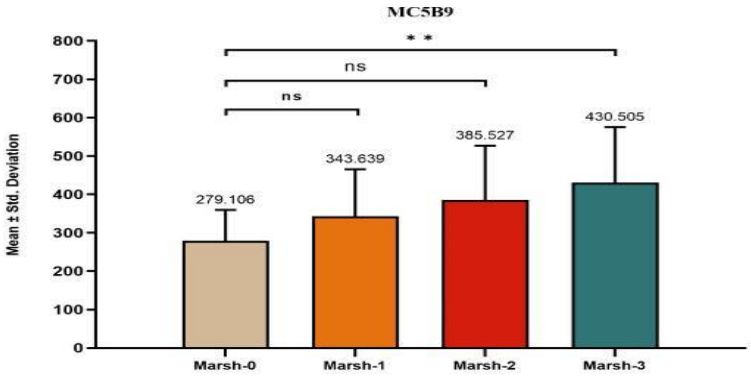

Figure 1: The concertation Levels comparison of MC5b9 marker with Marsh Classification Stages in Pre-Celiac Disease.

Other study showed a substantial association between anti-tissue transglutaminase-IgA and Marsh Grade 3c[14].

According to Rampersad *et al*. [15], the C5b-9 membrane attack complex (MAC) localizes to villous damage sites and alters trophoblast activity.

An additional study demonstrated sMAC's diagnostic potential for prognostic and diagnostic applications, as well as its prospective use as a companion diagnostic [16].

This current study showed Marsh3, the concertation of IL-17 in acute inflammation was 77.205, which was highly significant (p-0.001), however in the other marshes, it was not significant (figure 2).

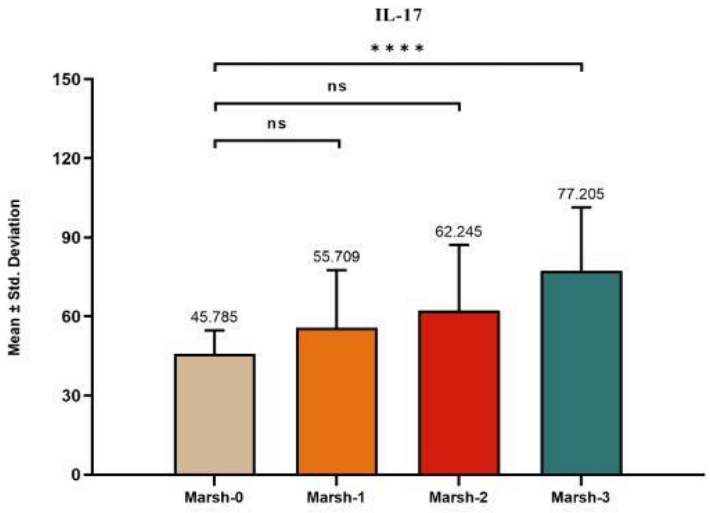

Figure 2: The concertation Levels comparison of IL-17 marker with Marsh Classification Stages in Celiac Disease.

TtG-IgA and IL-17 had a negative association [17].
role of IL-17the IL-17A producing cells play a major role in the pathogenesis of CD, and that both gluten and bacteria provoke an IL-17A response in the intestinal mucosa of CD patients. The upregulation of IL-17 immunity is associated with untreated CD and especially villous atrophy[18].

The expression levels of *IL-8* and *IL-17 A* were higher in biopsies of IBD (UC and CD) and CeD patients compared to the control group [19].

Figure (3) revealed that Marsh3 had increased a high significantly (p-0.001) in IL-21 in duodenum with acute inflammation was (217.643), whereas the other marshes did not exhibited any significant increase.

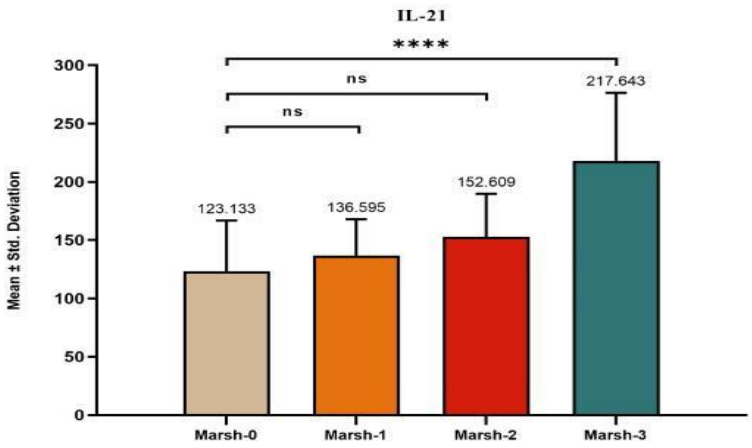

Figure 3: The concertation Levels comparison of IL-21 marker Across Marsh Classification Stages in Pre-Celiac Disease.

Other results showed that investigated IL-21 in biopsies from pediatric Celiac disease patients with different histopathological scores. High numbers of IL-21-producing cells were observed in pediatric Celiac diseae lesions, even Marsh 1–2 lesions [20].

Immune cells' production of IL-21 causes villous atrophy, which in turn causes lymphocytes to release mediators and autoantibodies, which in turn causes celiac disease and autoimmune gastritis [21].

The table 4 demonstrated the association between IL-21 alone and anti-tissue IgA was (0.016). MC5b9 had a strong correlation with IL-17 was (0.010) and a weak correlation with IL-21. Only IL-17 and IL-21 showed a correlation

.

Table 4: Correlation Coefficient Among Parameters According to Research Parameters

Parameters	Value	IgG	MC5B9	IL-21	IL-17
IgA	R. value	.412**	.081	.235*	.145
	P. Value	.000	.415	.016	.141
IgG	R. value	1.000	.104	.136	.119
	P. Value		.294	.167	.230
MC5B9	R. value		1.000	.428**	.253**
	P. Value			.000	.010
IL-21	R. value			1.000	.219*
	P. Value				.025
IL-17	R. value				1.000
	P. Value				

Anti-TG2 IgA antibodies and duodenal mucosal injury were observed to be correlated with elevated serum levels of IL-21 [22].

According to a recent study, a patient's levels of IL-21 may be useful indicators for both prognosticating the onset of celiac disease and predicting its early identification [23].

Conclusion

This study illustrated that MC5b9 ,IL17 and IL21 were increased significantly and correlated with Marsh 3 . and concluded gradually significant increased in serum anti-tTG IgG , IgA with all marsh and highly increased significantly and correlated with Marsh 3 .

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