

# Diagnostic Yield of Duodenal Biopsy Site in Celiac Disease in Children

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## Abstract

**Background:** Histopathological lesions of celiac disease (CD) were described in the duodenal bulb, proximal and distal duodenal sites.

**Aim:** To assess the association between the IgA anti-tTG titer and histological changes in different duodenal biopsy sites in pediatric patients with CD.

**Patients and Methods:** A total of 42 symptomatic, seropositive children (21 male and 21 female) with an age range of 29 to 163 months, underwent oesophagogastroduodenoscopy (OGD). Biopsies were taken from the first part of the duodenum (bulb), proximal and distal duodenum. The association between IgA anti-tTG titer and celiac disease histopathological morphology of different duodenal biopsy sites was assessed.

**Results:** IgA anti-tTG of  $\geq 150$  U/ml had a significant relation ( $p= 0.040$ ) to positive biopsy (marsh grade 1 -3) results. A cut off value of 126.15 U/ml of IgA anti-tTG titer was significantly ( $p=0 .000$ ) associated with sensitivity of 58.6- 63 % with the presence of marsh grade 3 in studied proximal duodenal site and duodenal bulb site respectively and 1- specificity of 0.00 value in both sites. IgA tTG of  $\geq 150$  IU/mL, is significantly ( $p= 0.0001-0.002$ ) associated with 100% specificity and 100% positive predictive value of having marsh grade 3 in the examined bulb and proximal duodenal sites.

## Conclusion:

Our data revealed that high titers of IgA tissue transglutaminase of  $\geq 150$  U/mL significantly possessed 100 % positive predictive value of yielding marsh grade 3 results in the bulb and proximal duodenal sites consistent with the diagnosis of celiac disease in symptomatic children. Duodenal bulb and proximal duodenal sites react similarly concerning the IgA tTG level.

**Keywords:** Celiac disease, duodenal biopsy site, IgA tTG level, pediatrics.

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## Introduction

Celiac disease (CD) is an immunologic inflammation of the small bowel caused by sensitivity to gluten present in certain foods and occurs in individuals with genetic

susceptibility<sup>(1)</sup>. The recommendations of NASPGHAN, BSPGHAN and ESPGHAN mentioned an option to skip the duodenal biopsy in children with classic symptoms of CD, a tTG-IgA > 10 times the upper limit of

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normal (ULN), a positive EMA-IgA, and the presence of HLA DQ2/8 haplotype, in such conditions, villous atrophy (Marsh 3) of the duodenal mucosa is nearly always found<sup>(2, 3)</sup>. Reliable cutoff titers of tTG-IgA may avoid endoscopic biopsy<sup>(4, 5)</sup> and it is clearly beneficial to assess if the elevated anti tTG titer could be associated with confirmatory histological changes of CD, particularly if endoscopy is not feasible and HLA or EMA test are not available. The aim of the current study is to assess the association between anti-tTG titer and morphology of different biopsy sites of the duodenum in seropositive, symptomatic pediatric patients.

#### Methods:

This case series study included 42 children (21 male and 21 female), aged  $\geq 24$  months who were on a gluten-containing diet and presented with clinical features suggestive of celiac disease as described by NASPGHAN<sup>(2, 6)</sup>. They were tested serologically by using second-generation ELISAs IgA human recombinant tissue transglutaminase antibody (IgA anti tTG2) which was performed by using a kit brand (AESKULISA tTG-A3503/ Germany). A titer of  $\geq 15$  U/mL was considered positive. OGD was performed by a senior gastroenterologist at Al-Salam teaching hospital in Mosul city. Duodenal biopsies were taken from three sites; duodenal bulb, proximal and distal part of the duodenum. Two fragments were obtained from each site and submitted separately in 3 labeled containers. The tissue was fixed in 10% formalin and process through formalin fixed paraffin embedded sections that were stained with hematoxylin and eosin. All the biopsies were examined by an expert pathologist and the findings were graded according to Marsh criteria<sup>(4)</sup>. A patchy lesion was defined as marsh grade 0 in any duodenal site<sup>(6)</sup>.

The histopathologic picture of intraepithelial lymphocytosis alone (Marsh type 1) is not specific for CD, but concomitant positive (TTG or EMA) test increases the likelihood of diagnosis of CD<sup>(7)</sup>. In accordance with NASPGHAN guidelines<sup>(7)</sup> studied children with marsh 1 and elevated tTG-IgA were regarded as confirmed CD cases if they become asymptomatic and their tTG-IgA declines to normal after 6 months of non-gluten diet.

Verbal and written consents were obtained from parents of all children regarding clinical, hematological testing and endoscopic duodenal biopsy. Ethical approval of the study was attested by the Committee of Mosul health directorate.

Data analysis was executed by employing SPSS version 17 software statistical packages. Chi-squared test was applied for measurement of magnitude of statistical significance. Receiver operating characteristic (ROC), used to acquire IgA anti-tTG cutoff value predictive of confirmed marsh grade 3 histopathological lesions. Sensitivity, specificity, negative predictive value, and positive predictive value were measured in relation to IgA anti-tTG titer of  $> 150$  U/ml.

#### Results:

The studied sample included 42 affected children with equal number of each gender. The age ranged from 29 to 163 months, mean age in months  $\pm$  standard error of the mean was  $65.48 \pm 5.84$  months

Among the 42 seropositive patients, 35 (83.3%) displayed positive biopsy results consistent with CD diagnosis. Biopsy results were not significantly different in relation to gender ( $p= 0.21$ ). The titer of tTG-IgA ranged between 16.10 -198.00 U/ml and its mean  $\pm$  SE was  $95.53 \pm 10.18$  U/ml. IgA anti-tTG value of  $\geq 150$  U/ml had a significant relation to positive biopsy (marsh grade 1 -3) results ( $p=.040$ )

(Table 1).

Among 7 seropositive patients with negative biopsy, all fragments from selected duodenal sites were by definition of potential CD, had uniformly marsh grade zero. All of the seven patients had IgA anti-tTG of < 150 U/ml

Duodenal lesions were patchy in 2 out of 35 cases (5.7%) of confirmed CD; both of them

had a titer of IgA anti-tTG of < 150 U/ml, and their biopsy results revealed Marsh grade zero in bulb site and were of marsh grade 1 in distal duodenal site whereas proximal site had one case with marsh zero and other with marsh with 1 pattern. Duodenal lesions were of similar marsh grading in 26 out of 35 (74.3%) of patients with confirmed CD (Table 1).

**Table 1: Characteristics of 42 symptomatic children with a positive tTG-IgA level in relation to biopsy results**

Variables			
	Negative biopsy (n=7) No (%)	Positive biopsy (n=35) No (%)	P
Sex			
Male	5 (23.8)	16 (76.2)	.214
Female	2 (9.5)	19 (90.5)	
Serology			
Less Than 150 U/ml	7 (25.0)	21 (75.0)	.040
Equal or more than 150 U/ml	0 (0.0)	14 (100.0)	
Biopsy			
Uniform	7 (17.5)	33 (82.5)	.421
Patchy	0 (0.0)	2 (100.0)	
Marsh Degree			
Same	7 (21.2)	26 (78.8)	.130
Different	0 (0.0)	9 (100.0)	

In all of the examined duodenal biopsy sites; Marsh grade 3 was the most frequently

displayed histopathological pattern (Table 2).

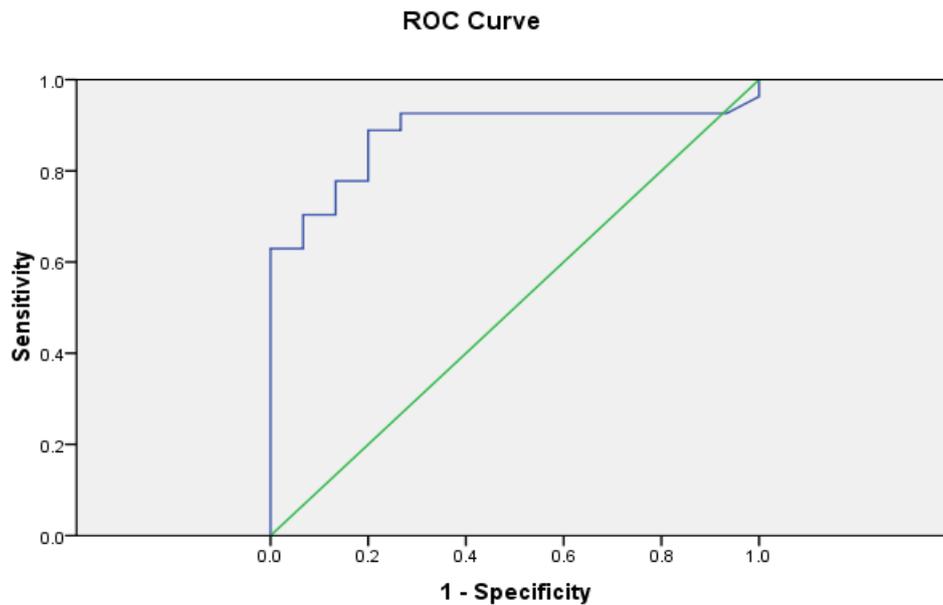
**Table 2: Marsh grading results in different duodenal sites**

Marsh grade	Site of duodenal biopsy					
	bulb		Proximal duodenum		Distal duodenum	
	No	%	No	%	No	%
<b>0</b>	9	21.4	9	21.4	8	19.0
<b>1</b>	1	2.4	1	2.4	3	7.1
<b>2</b>	5	11.9	3	7.1	4	9.5
<b>3</b>	27	64.3	29	69.0	27	64.3

In ROC curve with area under the curve of 0.880 ± SE 0.056, a cut off value of 126.15 U/ml

of IgA anti-tTG titer was significantly (p= .000) associated with sensitivity of 63 % with the

presence of marsh grade 3 in duodenal bulb site and 1- specificity of 0.00 value (Figure 1).

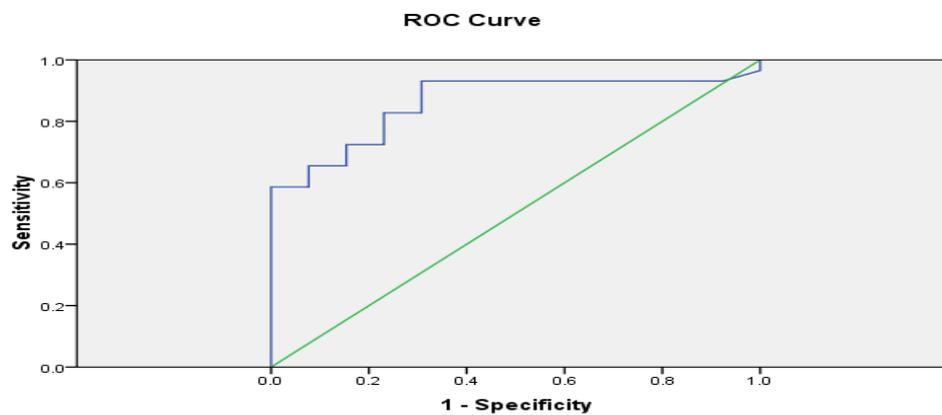


Diagonal segments are produced by ties.

**Figure 1: Relation between IgA anti-tTG titer and marsh grade 3 in duodenal bulb in ROC Curve**

In ROC curve with area under the curve of  $0.861 \pm SE 0.058$ , a cut off value of 126.15 U/ml of IgA anti-tTG titer was significantly ( $p=0.000$ ) associated with sensitivity of 58.6 % with the

presence of marsh grade 3 in the proximal duodenal site and 1- specificity of 0.00 value (Figure 2).

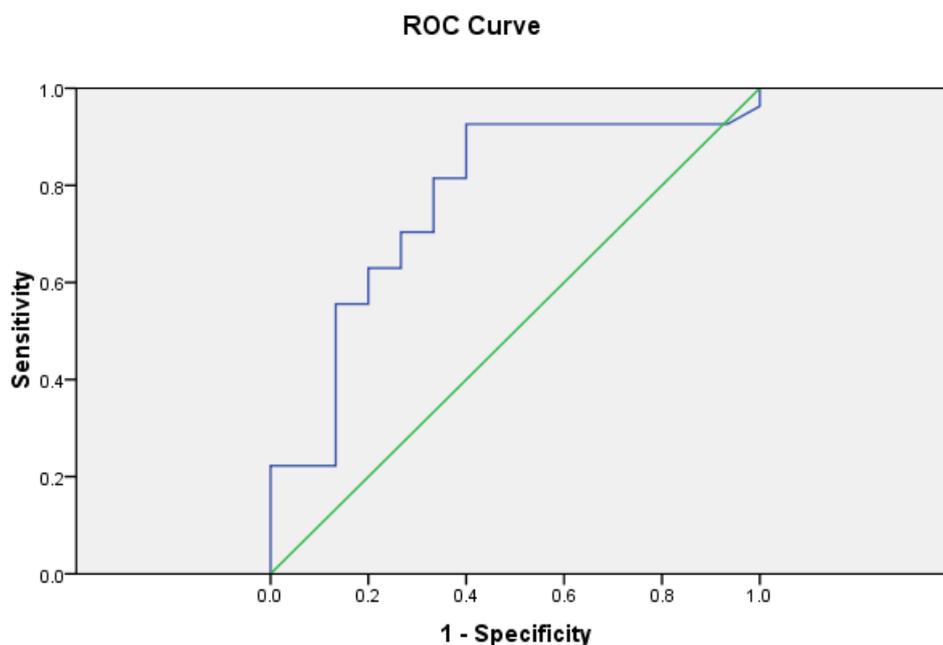


Diagonal segments are produced by ties.

**Figure 2: Relation between IgA anti-tTG titer and marsh grade 3 in the proximal duodenal site in ROC Curve**

In ROC curve with area under the curve of  $0.767 \pm SE 0.080$ , a cut off value of  $169.7 \text{ U/ml}$  of IgA anti-tTG titer was significantly ( $p = .000$ ) associated with sensitivity of  $22.2\%$  with the

presence of marsh grade 3 in the distal duodenal site and 1-specificity of  $0.00$  value (Figure 3).



Diagonal segments are produced by ties.

**Figure 3: Relation between IgA anti-tTG titer and marsh grade 3 in the distal duodenal site in ROC Curve**

**Table 3: Sensitivity, specificity, predictive values, accuracy and significance of IgA tTG titer in relation to marsh grade 3 in different duodenal sites of biopsy**

Variable	Biopsy results		Sensitivity	Specificity	PPV*	NPV**	Accuracy	P	
	Marsh 3	< marsh 3							
<b>Bulb site</b>									
IgA tTGA IU/mL	$\geq 150$	14 (100)	0 (0.00)	51.85	100	100	53.57	69.04	.001
	< 150	13 (46.4)	15 (53.6)						
<b>Proximal duodenal site</b>									
	$\geq 150$	14 (100)	0 (0.00)	48.27	100	100	46.42	64.28	.002
	< 150	15 (53.6)	13 (46.4)						
<b>Distal duodenal site</b>									
	$\geq 150$	12 (85.7)	2 (14.3)	44.44	86.66	85.71	46.42	59.52	.040
	< 150	15 (53.6)	13 (53.7)						

\*PPV: Positive predictive value

\*\*NPV: Negative predictive value

As shown in Table 3, IgA tTG value of  $\geq 150$  U/mL was significantly ( $p=.0001-.002$ ) associated with 100% specificity of having marsh grade 3 and 100% positive predictive value in the bulb and proximal duodenal sites.

CD-related histological lesions had uniformly marsh grade 3 in all analyzed 14 patients with IgA tTG of  $\geq 150$  IU/mL in all examined fragments from the bulb, proximal duodenum and among 12 out of 14 (85.71) fragments from distal duodenum.

### Discussion

Patchy villous atrophy denotes absence of villous atrophy in any site of the duodenum is found in a minority of children afflicted by CD, indicating that, although it is infrequent, the possibility of patchy duodenal lesion should be taken into consideration<sup>(5, 6, 8, 9)</sup>. Duodenal lesions may be confined only to the bulb site, so it is recommended that 1 or 2 biopsies be taken from the bulb and  $\geq 4$  from the distal parts of the duodenum, and as the biopsies should be also adequate in number<sup>(2)</sup> so six fragments were obtained in total, Two fragments were obtained from each selected site. Duodenal lesions were patchy in 2 out of 35 studied cases (5.7%) of confirmed CD children; both of the studied cases had a normal biopsy in bulb site. In a multicenter Italian study and other smaller series<sup>(10)</sup>. Villous atrophy confined to the bulb has been described in 16 of 665 (2.4%) CD children. Other investigators have found 10 out of 53 patients had patchy villous atrophy<sup>(11)</sup>. Patchy lesions were more frequent in patients with low IgA tTG titer than in patients with high titer<sup>(6)</sup>, likewise, both analyzed cases in this research had a titer of IgA anti-tTG of  $< 150$  U/ml. Biopsy results were not significantly ( $p=.21$ ) different in relation to gender as displayed in other studies<sup>(1, 6, 12)</sup>.

Duodenal lesions of enrolled patients with confirmed CD were of similar marsh grading in 26 (74.3%) out of 35 (Table 1). In another study the majority of children also showed similar degree of villous atrophy<sup>(11)</sup>. Variability in the grade of villous atrophy in (35.4%) of patients is observed by another researcher<sup>(13)</sup>, in harmony with our results. Other studies also have described the variability of the severity of villous atrophy in relation to the duodenal biopsy site<sup>(5, 14)</sup>. Potential celiac disease presented in 7 (16.7%) out of 42 investigated patients. All of them had IgA anti-tTG of  $< 150$  U/ml which is in agreement with other studies<sup>(5, 12)</sup>.

In this study, 63 % of all who has a level of IgA tTG  $\geq 126.15$  U/ml would be correctly identified as celiac by the finding of marsh grade 3 in duodenal bulb biopsy site and none of those with IgA anti-tTG less than 126.15 U/ml would be wrongly identified as marsh grade 3 in the same site (Figure 1). Similarly, 58.6 % of evaluated children having the same IgA anti-tTG level of  $\geq 126.15$  U/ml would be appropriately identified as celiac by the finding of marsh grade 3 in the proximal duodenal site and none of those with IgA anti-tTG less than 126.15 U/ml would be mistakenly identified as marsh 3 in the same site (Figure 2). Hence duodenal biopsy could be avoided in 58.6 - 63 % of pediatric patients with a level of IgA tTG  $\geq 126.15$  U/ml.

In 22.2% of enrolled patients with IgA anti-tTG titer of  $\geq 169.7$  U/ml would be significantly identified as celiac by the finding of marsh grade 3 in the distal duodenal site and none of those with IgA anti-tTG less than 169.7 U/ml would be falsely identified as marsh 3 in the same site (Figure 3). Accordingly, Intestinal biopsy could be avoided in 22.2% with a level of IgA tTG  $\geq 169.7$  U/ml depending on marsh grade 3 results of the distal duodenal biopsy.

It has been shown that gluten affects mainly the proximal duodenum which is exposed to higher bulk of gluten load so villous injury should also be more severe proximally<sup>(11)</sup>. It seems that from the aforementioned findings that bulb and proximal sites had a lower threshold (requirement of a lower level of IgA tTG) to produce marsh grade 3 than distal site; and that bulb and proximal site are more sensitive to the effect of IgA tTG antibody production than distal sites and also implies that bulb and proximal duodenum react similarly in relation to the level of IgA tTG. This is in accordance with the finding that tissue sampling from the duodenal bulb and second part of the duodenum in CD can represent equal burden of the culprit disease<sup>(14)</sup>. Moreover, in the current study, CD-related histological lesions had uniformly marsh grade 3 in all analyzed 14 patients whose IgA tTG was  $\geq 150$  IU/mL in all examined fragments from the bulb, proximal duodenum and among 12 out of 14(85.71) fragments from the distal duodenum.

High serological titers reflect more positive biopsy results for celiac disease<sup>(1, 4, 5, 12, 15, 16)</sup>. IgA tTG level  $\geq 100$  U/mL was associated with PPV of 100% of having duodenal lesions confirming CD<sup>(17)</sup>. IgA tTG titer more than 9 folds higher than the kit's cut-off value was 97.2% sensitive for Marsh grade 2 and is associated with more degree of villous damage

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<sup>(18)</sup>. Table (3) revealed that level of IgA tTG of  $\geq 150$  IU/mL was significantly ( $p=.0001-.002$ ) associated with 100% specificity and 100% positive predictive value of having marsh 3 grade in the examined bulb and proximal duodenal sites respectively, compared to IgA tTG of  $\geq 150$  IU/mL, which was significantly ( $p= 0. 040$ ) associated with 86.7 % specificity of having marsh 3 grade and 85.7 % positive predictive value of having marsh 3 grade in the examined distal duodenal sites. Accordingly, invasive endoscopic duodenal biopsy could be avoided in 100% of investigated children with a level of IgA tTG  $\geq 150$  U/ml-

Certainly increasing sample size would have a high impact on the validity of the study results, which was a limitation to this study, since Mosul city is lacking a GIT center.

## Conclusion:

This study demonstrated a statistically significant relation between the serological titer and positive biopsy results in symptomatic seropositive children with suspected CD. The displayed data clarified that titers of tissue transglutaminase of  $\geq 150$  U/mL significantly possessed 100 % positive predictive value of yielding positive biopsy results in the bulb and proximal duodenal sites consistent with the diagnosis of celiac disease in symptomatic children. Bulb and proximal duodenal sites respond similarly to the level of IgA tTG.

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## العائد التشخيصي لموقع خزعة الاثني عشر في مرض الجوفاء عند الأطفال

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### الملخص

**الهدف من الدراسة:** تم وصف الآفات النسيجية المرضية لمرض الجوفاء في بصلة الاثني عشر، ومواقع الاثني عشر القريبة والبعيدة، والهدف من الدراسة تقييم الارتباط بين عيار IgA المضاد لـ tTG مع التغيرات النسيجية في مواقع خزعة الاثني عشر المختلفة في الأطفال المصابين بالجوفاء.

**منهجية البحث:** خضع 42 من الأطفال الذين لديهم أعراض الجوفاء، ولديهم فحص مصلي موجب (21 ذكر و 21 أنثى) وتراوح أعمارهم من 29 إلى 163 شهراً، إلى تنظير المريء والمعدة والاثني عشري. وتم أخذ الخزعات من الجزء الأول من الاثني عشر (البصلة)، الاثني عشر القريبة والبعيدة، وتم تقييم العلاقة بين عيار IgA المضاد لـ tTG والتغيرات النسيجية لمرض الجوفاء في مواقع خزعة الاثني عشر المختلفة.

**النتائج:** كان لدى عيار IgA المضاد لـ tTG  $150 \leq$  وحده/ مل علاقة ذات أهمية إحصائية ( $p = 0.040$ ) بنتائج الخزعة الإيجابية (درجة مارش 1-3). كانت قيمة القطع البالغة 126.15 وحدة / مل من عيار IgA المضاد لـ tTG مرتبطة بأهمية إحصائية ( $p = 0.000$ ). وبحساسية 58.6-63% مع وجود درجة مارش 3 في موقع الاثني عشر القريبة وموقع بصلة الاثني عشر على التوالي و 1- خصوصية بقيمة 0.00 في كلا الموقعين، عيار IgA المضاد لـ tTG  $150 \leq$  وحده/ مل، كان له ارتباط ذو أهمية إحصائية ( $p = 0.0001$ ) ( $0.002$ ) وبخصوصية 100% وقيمة تنبؤية إيجابية بنسبة 100% لوجود درجة مارش 3 في بصلة الاثني عشر المفحوصة، ومواقع الاثني عشر القريبة.

**الاستنتاج:** كشفت بياناتنا أن العيار المرتفع من IgA المضاد لـ tTG  $150 \geq$  وحده/ مل يمتلك بأهمية إحصائية قيمة تنبؤية إيجابية بنسبة 100% مع الدرجة 3 من درجات مارش في البصلة ومواقع الاثني عشر القريبة بما يتوافق مع تشخيص مرض الجوفاء عند الأطفال الذين يعانون من أعراض الجوفاء، وتتفاعل بصلة الاثني عشر، ومواقع الاثني عشر القريبة بشكل مشابه فيما يتعلق بمستوى IgA المضاد لـ tTG. الكلمات الدالة: الجوفاء، موقع خزعة الاثني عشر، عيار IgA المضاد لـ tTG، أطفال.