The significance of first part duodenal biopsy in diagnosis of celiac disease in Kirkuk city

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ABSTRACT

Introduction: Celiac disease (CD) is a common immune-mediated intestinal intolerance to gluten protein. It has a wide range of symptoms and subsequent grief complications. Treatment involve gluten free diet for life, therefore accurate diagnosis is essential which relies,essentially, on duodenal biopsy. **Objective:**Identify the part of duodenum from which a biopsy can be taken which can accurately reflect the underlying celiac disease pathology. **Material and methods:**In this prospective study we included one hundred twenty four patients who are expected to have celiac disease due to various clinical reasons who were referred to the GastroenterologyCentre at Azadi Teaching Hospital over a three year period between January 2014-January2017. Blood samples were taken from all the patients and investigated for CD serology markers(Anti-tissue Transglutaminase Antibody; tTG; tTGA and tTGG; Total IgA). Patients who were positive for CD serology or those who are categorised as high risk candidates(even if failed to show positive serology) underwent upper gastrointestinal tract (GIT) endoscopy. Twobiopsy samples were taken from both; the first part or bulb(D1) and second part of the duodenum (D2). The samples were sent for histopathology and graded for celiac disease according to modified Marsh classification,Oberhuber. SPSS software was used to calculate the statistical significance of difference between the two sites of sampling. **Results:** This studyincluded 94 females and 30 males with age range ٤-60, those patients were referred to the gastroenterology centre due to suspension of CD.Almost all (97.5%) the patients were CD serology positive apart from three cases who did not show positive serology(2.5%). The three serology negative cases were high risk cases, thus underwent endoscopy and biopsy. All the biopsies from the first part of the duodenum showed the diagnostic changes of CD according the modified marsh grading;Oberhuber(124/124,100%) while biopsies from the second part expressed these changes in almost half of the patients (65/124,52.5%), the difference between the two groups was statistically significant($p<0.005$).Additionally, most of the D 2 cases were grade 1 in contrast to D1 which were grade 2 and 3**Conclusion:** Biopsy from the duodenal bulb is enough for histopathological diagnosis of CDand additional samples from other parts of duodenum are not necessary. Less number of biopsies means lower burden on the histopathology units and reduction of complications that may face the patient.

Keywords: Celiac disease, biopsy, bulb, histopathology

Introduction:

Coeliac disease (CD)is a common life-long disease that is caused by intestinal intolerance to gluten protein in individuals who are genetically susceptible(1). Epidemiological studies have shown that it affects 1-4 %of the world population with some population seems to be more vulnerable than others due to genetic and environmental factors (2, 3).For instance, it is four times more common in the United States of America than Brazil (3). Due to genetic and environmental factors, it was suggested that it has low prevalence in Iraq, however the exact incidence is yet to be known(2).The prevalence also increases by age; for example, in Finland; it is 1.5% in children, increases to 2% in adults and reaches 2.7% in the elderly (4).Celiac disease has a strong hereditary component, and epidemiological studies showed that up to 20% of first-degree relatives are affected by the disease with concordance rates of 75–80% in monozygotic twins and 10% in dizygotic twins (5, 6).The manifestation of the disease are very wide, depending on; the age of the patient, the duration and scope of disease, and the presence of extra-intestinal pathology (2).It may stay for long years without signs and symptoms or diagnosed due to complications such as osteoporosis or present in a shock-like condition called “celiac crisis". The typical presentation occurs in children between the 6 to 18 months and characterised by chronic diarrhoea, failure to thrive and abdominal distension (7, 8).The pathophysiology of coeliac disease isintestinalT cell immune-mediated inflammatory disorder which involves hypersensitivity to dietary gluten(9).Gluten is a protein found in wheat, rye , barley (malt) and

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oats (10).Human leukocyte antigen (HLA) class II genes known as HLA-DQ2 and HLA-DQ8 are the strongest predictor to celiac disease risk, these molecules are in charge for presentation of antigens to immune cells(11). CD immunology is manifested by the appearance of high titre of antibodies against different antigens (12). Patients with CD who are untreated typically have high titres of antibodies against the endomysium antigen (EMA test) and exposure to gluten stimulates plasma cells to generate antibodies to tissue transglutaminase (IgA-TG2) and deamidated gliadin peptides (IgA-DGP and IgG-DGP)(12).Significance of these antibodies are considered when the antibodies are five times their normal range (13).This immunological intolerance to gluten leads to the golden diagnostic trait of small intestinal; villous hypertrophy, intra-epithelia lymphocytosis (IEL>30/100 epithelial cells) and lamina properia inflammation.However, duodenal biopsies can vary widely with changes in villous can lead to complete atrophy or be normal (10). Mrash stagingsystem was devised to categorise celiac disease severity according to the histopathology of the duodenal biopsy, the commonly utilised system is a modified one called Oberhuber(10).The diagnosis of coeliac disease have evolved over time from merely depending on clinical signs and symptoms to a modern approach depending on the presence of the classic antibodies and supported by biopsy(14). To confirm a diagnosis of CD biopsies of the duodenum must be taken when patients are on a gluten-containing diet (14, 15).Despite being a pillar in the diagnosis of coeliac disease, there is no general agreement on; the number of the duodenal biopsies, their orientation and place. There is a hot debate whether duodenal biopsy is to be taken from lower duodenum or from its bulb. Each country has its own guidelines, the American college of gastroenterology has its costume recommendation with regard to biopsy (15) The British Society of Gastroenterology has developed their guidelines(16)

Since each community has its own genetic makeup and environmentalstatus; both influencecoeliac disease pathology (17) and consequently the diagnostic strategy. Inthis study we tried to examine the ability of each biopsy place to be the accurate sample to represent coeliac disease pathology, this will help to establish our local guidelines.

Material and Methods:

This observational study was carried out at Kirkuk Azadi University Hospital; Gastroetrology Centre and the Histopathology Units over three years period, between January 2014 till January 2017. We included 160 patients with age range of 4-60 years from both sexes. The patients were referred to the Gastroenterology centre due to suspicion of coeliac disease. We divided patients into: high risk, medium and low risk patients as described before(18). High risk patients are those with (1)chronic gastrointestinal symptoms with a family history of celiac disease or a personal history of autoimmune disease or IgA deficiency (2) dermatitis herpetiformis confirmed by biopsy (3) chronic diarrhoea (4) growth failure in children (5) iron deficiency anemia refractory to enteral treatment. Other presentation are considered medium and low risk. Table 1 gives numerical details about the mode of presentation.Blood sample were obtained from the patients for immunology studies, we used Aligra machine (Germany) to measure the serum concentration of tissue transglutaminases(TTGA, TTGG) along with the measurements of IgA. The serology was considered positive when it is five times the upper normal limit Endoscopy using Olympus endoscope(Olympus, Japan) was done for all high risk patients even if the serology was negative. Medium and low risk patients underwent endoscopy only when their celiac serology is positive. General contraindications for endoscopy were : those who were physically unfit, oesophageal diseaseor being on gluten free diet (GFD)for more than 3-6 months, possible perforation, medically unstable patients, unwilling patientson anticoagulation, pharyngeal diverticulum, or head and neck surgery (relative contraindications). Informed consent was taken from the patients or their guardian, the procedure; its steps and risk were explained.Four biopsies were taken, two from thesecond part of the duodenum and the other two from the first part (duodenal bulb) from 9- or 12-o'clock position; All were sent to the hospital Histopathology Unit. The histopathological severity of the coeliac disease was assessed according to the modifiedMarsh grading system (Oberhuber). SPSS version 21 was used to calculate sensitivity and specificity of the study.

Results:

This study encompassed, 160 cases who were referred due to suspicion of celiac disease, serology was done for all the patients. While endoscopy was done for 124 patents and the remaining 36 patients refused the procedure or been illegible for it. Of the 124 patients nearly three quarters were female and the remaining quarter was male.The study population has a wide age range (6-60 years) with the age group 10-29 years makes about two third of the study population as detailed in Table 1. Childhood and adolescence presented for screening mainly due to diarrhoea and growth failure while adults were investigated for celiac disease due to,primarily, weight loss. Anaemia was common reason for referral in both age groups as shown in Table 2.

Table 1.The demographic characteristics of the study population

Age (year)	Female	Male	Total
4-10	9	3	12
10-19	30	6	36
20-29	21	12	33
30-39	15	6	21
40-49	10	3	13
50-59	9		9
Total	**94 (76%)**	**30(24%)**	**124**

Table 2.The reasons for referral according to age groups. Diarrhoea and failure to thrive were the main causes in children and adolescent. In adults weight loss was the main reason. Anaemia was common in both age groups.

Age (year)	Diarrhea	Failure to thrive	Anemia	Weight loss
4-10	4	6	2	-
10-19	8	9	11	8
20-29	9	-	11	13
30-39	5	-	12	4
40-49	-	-	6	7
50-59	2		3	4
Total	28	15	45	36

We sent the blood samples of all our patients forceliac serology (TTG IgA& IgG and IgA) before endoscopy, all of them were positive for celiac diseases except three cases. Thelater cases were high risk (two patients with chronic and one with anaemiarefractory to iron therapy), therefore we proceeded to endoscopy with biopsy.

The main goal of our study is to find the best representative biopsy that can accurately reflect the pathological status of duodenum due to presence of celiac disease which is known to have patchy lesions. We took two biopsies from part one orbulb (D1) and twofrom the second part of duodenum (D2) and sent them to histopathology. Examples are shown in **Figure 1.**It is clear that more cases were histopathologically confirmed by biopsies from the duodenal bulb (firs part) than from the second part (p<0.005),almost half of the patients would have been miss-diagnosed as celiac free according to the samples from D2.More details are expressed numerically in **Table 3.**Fifty nine more cases showed grade two and three simplified Marsh scoring system from biopsies taken from D1 than from D2. The greatest differences was in the age group 10-19 years, here D1 can identify three times more cases than other parts. Double number of cases were seen in D1 at the age group 30-39 years .

Those below ten years showed no difference with regard to the histopathological changes seen in biopsies taken from different places of duodenum.Other age groups showed higher changes in D1 than other sites but to lesser extent.Most of the second part lesions had lower grade of severity compared to D1. Half of the cases were grade 3 and 4 while in D2 most of the positive cases were grade 2 as exemplified in Figure 1

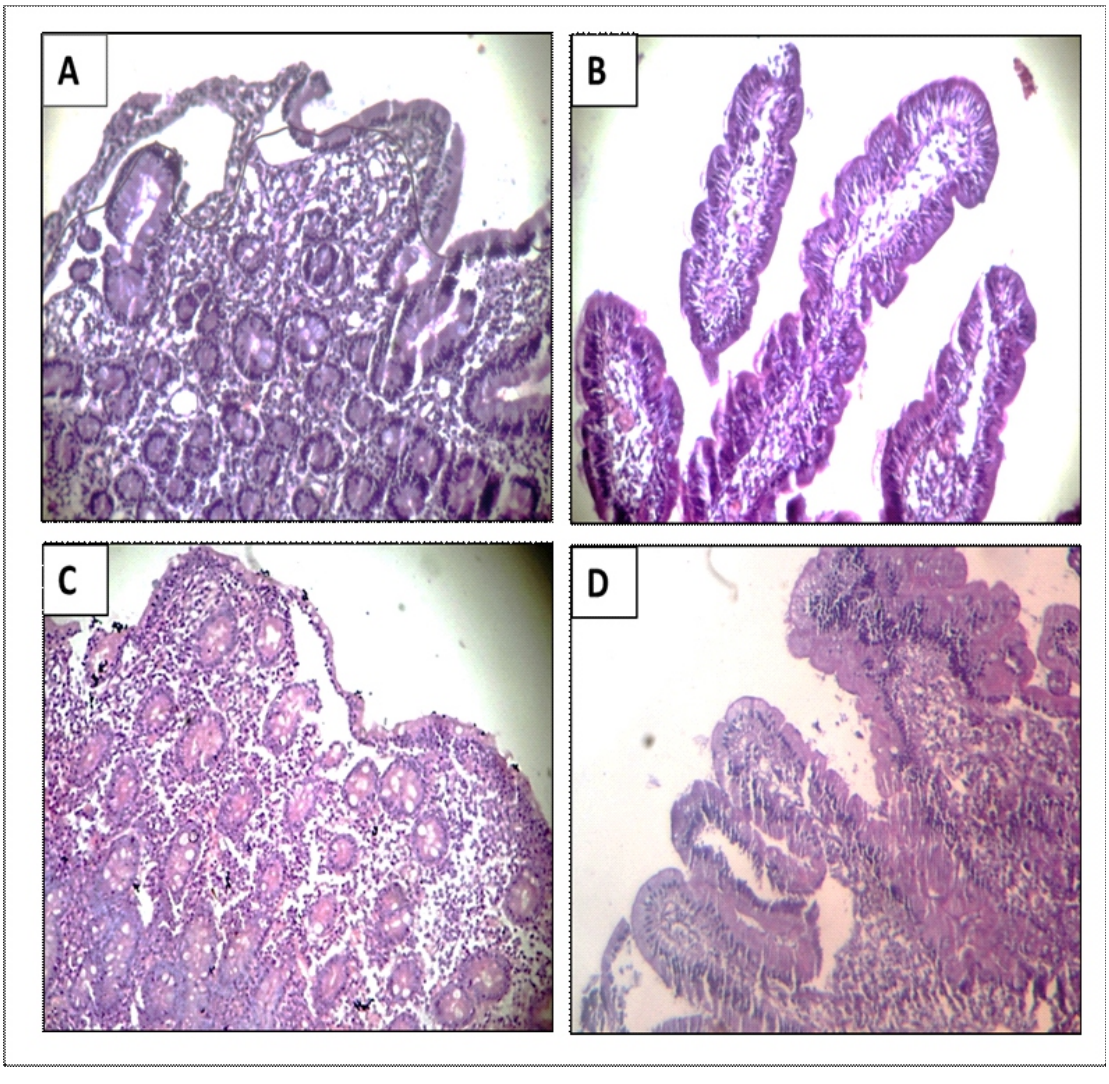

Figure 1. Histopathological examples of normal and different grades of duodenal celiac lesions. Samples A and B were taken from a patient first (bulb) and second parts of the duodenum respectively. Pictures C and D were taken from another patient's first and second parts sequentially. A shows grade 2 while B shows biopsy from second part that has normal histology. C is grade 3 while D is one both taken from the same patient.

Table 3.Histopathological outcome of biopsies taken from two different sites of duodenum, D1 from the first part while D2 referrers to a sample obtained from the second part. The frequency of positive histology is much higher in biopsies taken from the first part compared to the second part

Age (year)	D2	D1	P value
4-10	12	12	
10-19	10	36	
20-29	14	33	
30-39	14	21	**<0.005**
40-49	8	13	
50-59	7	9	
Total	**65**	**124**	

Discussion:

Proper diagnosis of diseases that have long term implication on patients' health and life style is essential to avoid unnecessary limitation on sufferer activity and feeding. Coeliac disease is a common disease with increasing prevalence (11). The diagnosis of celiac disease means a long life commitment to gluten free diet with its influence on the family eating habit and budget.The diagnosis of celiac disease is multi-layer approach, it is a combination of suggestive signs and symptoms and celiac serology confirmed by duodenal biopsy.

The American celiac society issued its guidelines on diagnosis of CD (15).They insist that proper diagnosis of celiac diseases requires duodenal biopsy; multiple biopsies including one or two biopsies of the duodenal bulb (either 9- or 12- o'clock position) and at least four biopsies of post-bulbar duodenum (13).The British Society of Gastroenterologydiagnosis criteria of CD is by serology and duodenal biopsy, ideally with the patient on a regular, that is, gluten-containing diet, biopsy remains essential for the diagnosis of adult CD and cannot be exchanged by serology (12).Adequate (more than four) biopsies should be taken, from the distal duodenum and the duodenal bulb to maximise diagnosis (12). Joint European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (BSPGHAN) and Coeliac UK guidelines for the diagnosis and management of coeliac disease in children endoscopy, take four biopsies from D2 or lower and 1–2 from duodenal bulb (since patchy changes do characterise CD)(19). It is clear from the above guidelines that there is some sort of consensus on how many and from where to take biopsy in Western population

Due to historical reasons, it was suggested that middle east (including Iraq) has lower incidence of celiac disease due to early exposure of the area population to wheat from the era of farming as human moved from hunting to settlement which made people tolerant to gluten(20). However, recent studies suggest that the prevalence in Middle Eastern and North African countries is similar to the western countries, unfortunately there was no parallel increase in research in this field (21, 22). Over a period of 30 years (1980 till 2010), there were only 120 publications that focused on descriptive aspects of the disease (21). Searching the pubmed.com database with the key words(Iraq, celiac disease) did not show more than a dozen of research articles (23).To our best knowledge no study was done so far, neither in Iraq nor in Middle East, to find the proper method to diagnose celiac disease using biopsy with histopathology. In this study we tried to identify the best way to take duodenal biopsy from patients suspected of having celiac disease. We found that biopsies taken from the second part of the duodenum,only, would show normal histology in patients who are really celiac disease victims by biopsy from the bulb area, which shows histological changes consistent with celiac disease. Indeed, no sample that was positive by second part samples showed no change in the bulb counterpart. Accordingly bulb biopsy is more sensitive and equally specific to the second part biopsy.Another merit is that part one biopsies showed more sever lesion than the second part which reflects the real disease status and avoid misleading simplification of the pathology.

Our study augments the finding of others' work which showed that celiac disease changes are always found in duodenal bulb of adult and paediatric celiac patients and in some cases it is the only affected place as we observed in our work(24, 25).To add more insult to the injury, another study showed that bulb and second part duodenal biopsies are equally reflective of underlying celiac disease, this study was carried out in Sub Indian continent(26).

In the current work we included a wide range of age groups, which means that only bulb biopsy is enough to diagnose or exclude celiac disease in children and adults.

Caution must be exercised when histopathology results are interpreted, other GIT disease such as Helicobacter pylori infection, tropical sprue, Giardia lamblia infection, prolonged viral gastroenteritis, food allergies, autoimmune enteropathy, IgA deficiency, Crohn's disease and ulcerative colitis can give akin histology, however other clinical signs and symptoms might help to distinguish between them (27). Additionally, serology of celiac disease will boost the decision to diagnose celiac disease.Similar to others finding, we showed that there is a strong association the Tissue transglutaminase are highly specific and all the serology positive cases were histology confirmed CD(28, 29)

However, in some cases the diagnosis of CD may not be straightforward, for example, patients are already on a GFD and therefore antibodies are negative, biopsies were not oriented correctly (this could lead to false-negative or false-positive villous atrophy) or show solely intraepithelial lymphocytosis (lymphocytic duodenosis) without architectural changes. In these situations, the patient needs to be maintained on a gluten containing diet and further evaluated with additional testing and, if necessary, referred to a centre or clinician with a specific interest in CD. Additionally, the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) proposed that it may be possible to avoid any intestinal biopsy in children who meet the following criteria: characteristic

symptoms of CD, TTG IgA levels > 10 × upper limit of normal (confirmed with a positive EMA in a different blood sample), and positive HLA-DQ2.

In our study we screened all our patients for celiac disease by serology and found that three of them had negative test, but because they were high risk patients we proceeded to endoscopy and our prediction was right and CD was confirmed. It is no wonder to have false negative serology, it is estimated that anti-gliadin antibodies have sensitivity and specificity of 75–95% and 80–95% respectively, on the other hand, despite being much accurate, the tissue transgultaminases are still not 100 % sensitive and specific (Sensetivity:85–98% and specificity 95–99%)(4).Causes of negative CD serology include: very young patients (<2 years), GFD, IgA deficiency and use of corticosteroids or immune-modulator drugs(30)

Indeed, a large international study found that laboratory sensitivity ranged from 63 to 93 % and specificity ranged from 96 to 100 % when comparing TTG assays among various research and clinical laboratories (13). Thus, a negative CD-specific serology in patients with villous atrophy does not completely exclude the diagnosis of CD though it does make it much less likely.

The significance of his study stems from its clear recommendation to take biopsy from the duodenal bulb only and avoiding patients the exposure to unnecessary risk of multiple biopsies such as haematoma and perforation (31, 32). The other value of the work is the place where it was carried out, it is a fact that Kirkuk is cosmo-politic city and we found no difference among the people of different ethnic background, thus the result can be extended to countries with similar demographic fabric.

In conclusion, bulb biopsy may be considered as the place of choice for diagnosis of CD and can replace other parts and reduce the number of biopsies.

Conflict of interest:

Authors declare no conflict of interest related to this work.

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