Abstract

**Background:** Celiac disease is a systemic disease due to immunological reaction of intestinal mucosa and submucosa on exposure to gluten leading to villous atrophy. The clinical statistic refers to increase the magnitude of this problem in Iraq.

**Objective:** Diagnosis of celiac disease by serological and histological tests in patients clinically suspected and comparison accuracy of these tests.

**Methods:** 161 blood samples out patients were collected in a labeled 5ml tubes. aged (2 years to 40 years) were tested by Enzyme Linked Immuno Sorbant Assay to detect anti-gliadin Immunoglobulin G and Immunoglobulin A, and anti-Tissue transglutaminase Immunoglobulin G and Immunoglobulin A Antibodies, and 161 biopsies of same patients with celiac disease were taken from small intestinal histological features were interpreted according to the revised Marsh were carried in Al-Husien Hospital in Karbala, during the period from January 2006 to December 2007.

**Results:** The levels of anti-gliadin immunoglobulins (Immunoglobulin G, Immunoglobulin A) were elevated. The following serum antibody levels were elevated in 32.91 % (53 out of 161) 21.11 % (34 out of 161) respectively, whereas Anti-tissue transglutaminase immunoglobulins (Immunoglobulin G, Immunoglobulin A) were elevated in 6.83 % (11 out of 161), and 27.32 % (44 out of 161) respectively. One hundred forty four (89.44%) patients had biopsy result consistent with celiac disease.

**Conclusions:** Importance using of histological findings in diagnosis celiac disease and prove its superiority to the serological tests in clinically suspected patients.

**Key words:** Celiac disease diagnosis, histological and serological tests.

Introduction

Celiac disease (CD) is an immune-mediated disorder caused by intolerance to gluten, which is found in wheat and barley (1). It is characterized by the presence of chronic inflammation of the small bowel's mucosa and submucosa, and is clinically characterized by the presence of systemic manifestations. It may start at any age, both during childhood and adolescence, and is also relatively common in adulthood. It is being increasingly diagnosed even in elderly patients (1,2). CD may be associated with extra-gastrointestinal tract conditions such as anemia, osteoporosis, or neurological disorders, often without the presence of gastrointestinal tract symptoms (3). It affects only predisposed individuals, whose most susceptible genetic feature are related to human leukocytes antigens from class two (HLA-II) (4). A strong genetic susceptibility is present with about 75% concordance rate, among monozygotic twins. Certain populations have an increased prevalence of CD. For instance, the first-degree relatives of individuals with biopsy-proven CD, have a prevalence between 4%-12% of those suffering from this disease. Second-degree relatives also appear to have an increased prevalence. Patients with type 1 diabetes mellitus (IDDM) have a prevalence of CD ranging to 3%-8%. In Down's syndrome, the presence of CD is found between 5%-12%. Also, CD is associated with Turner's and William’s syndromes, IgA deficiency and several autoimmune disorders (5,6)
In the 1960s, it became clear that CD is associated with autoantibodies against tissue (loose connective tissue surrounding smooth muscle fibers)(7,8). These antibodies became known as anti-endomysial and anti-reticulin antibodies and were generally detected by immunofluorescence assays. In 1997, TG2 was identified as the main autoantigen for the anti-endomysial antibody(9) Autoantibody production is an important feature of many autoimmune disorders, signifying a breakdown of immune tolerance to self-antigens. In CD, an autoimmune enteropathy with multiple extra-intestinal manifestations, autoantibody reactivity to transglutaminase 2 (TG2) has been shown to closely correlate with the acute phase of the disease. It serves as a specific and sensitive marker of CD, and is highly useful in aiding diagnosis and follow-up.

Immune reactivity to other autoantigens, including transglutaminase, actin, ganglioside, collagen, calreticulin and zonulin, among others, has also been reported in CD. The clinical significance of these antibodies is not known, although some may be associated with specific clinical presentations or extra-intestinal manifestations of CD. The presence of anti-transglutaminase and other autoantibodies in CD, were discussed their diagnostic value, their potential role in disease pathogenesis and current hypotheses that explain how their release may be triggered(10).

Prevalence of celiac disease is belong to genetic factors, most importantly linked to the HLA region of chromosome 6. The anti-TG2 antibody response is believed to be driven by the intestinal immune reaction to gluten, although the mechanism of its release and its role in the pathogenesis of celiac disease are not entirely clear yet(11).

**Methods**

This study was conducted in Al-Hussein General Hospital during the period from January 2006 to December, 2007. 161 blood samples were collected from out patients (majority of patients) and in patient (few cases). The patients studied were aged (2 years to 40 years) during the period. The samples were collected in a labeled 5ml tubes then stored and delivered at 4°C cool containers. The sera were stored in 0.2 ml aliquots at -20°C till testing.

161 Serum specimens were tested by ELISA to detect anti-gliadin IgG and IgA antibodies using Biohit IgG and IgA (Biohit, Finland) according to manufacturer’s instructions, so anti-Tissue transglutaminase IgG and IgA antibodies were tested by ELISA using Biohit IgG and IgA (Biohit, Finland) according to manufacturer’s instructions.

Endoscopy of gastro intestinal tract is done by expert endoscopists under local anaesthesia for adults while general anaesthesia is mandatory for children (including more than two years age children). 161 biopsies of same patients with CD were taken from small intestinal histologic features were interpreted according to the revised Marsh were carried in Al-Husin Hospital in Karbala.

Marsh 0 is described as normal mucosal architecture, without significant intraepithelial lymphocytic infiltration. Marsh I (lymphocytic enteritis) is normal mucosal architecture with a marked infiltration of villous epithelium by lymphocytes; marked is defined as more than 30 lymphocytes per 100 enterocytes. Marsh-II (lymphocytic enteritis with crypt hyperplasia) consists of intraepithelial lymphocytes and elongation and branching of crypts in which there is an increased proliferation of epithelial cells. Marsh-III comprises intraepithelial lymphocytosis, crypt hyperplasia, and villous atrophy. There are three distinct stages of villous atrophy. In Marsh IIIA, partial villous atrophy, the villi are blunt and shortened. Arbitrarily, samples classified as partial villous atrophy if the villus-crypt ratio was less than 1:1. In Marsh IIIB, subtotal villous atrophy, villi are clearly atrophic, but still recognizable, and in Marsh IIIC, total villous atrophy, villi are rudimentary or absent, and the mucosa resemble colonic mucosa(12).
Biopsy is the gold standard in diagnosis of many diseases but it is not a routine medical practice because it needs cooperation between patients and medical team-work specialists. So, biopsy has certain indications, celiac disease among them.

Statistical analysis

A descriptive statistical analysis of the serological data used in the study was done. T-test was used and appropriate p values of <0.05 were considered significant. Data were analyzed using SPSS version 14 software.

Positive predictive value = True positive/(True positive + False positive) X 100
Negative predictive value = True negative/(True negative + False negative) X 100

Results:

Out of 161 sera tested, 53 samples anti-gliadin IgG were positive (32.91%) at ages (2-10) (11-20) (21-30) (31-40) were 8 (36.89%), 9 (34.61%), 4 (19.04%), 2 (18.18%) respectively while 34 were IgA positive (21.11%) were 24 (23.07%), 5 (19.23%), 2 (9.52%), 3 (27.27%) respectively (Table 1), (Figure.1, 2), on the other hand 11 were IgG positive (6.83%) at ages (2-10) (11-20) (21-30) (31-40) were 8 (7.69%), 2 (7.69%), 1 (5%), 0 (0%) respectively, while 44 were IgA positive (27.32%) were 27 (25.96%), 11 (42.30%), 4 (20%), 2 (18.18%) respectively were regarding anti-tissue transglutaminase (Table 2), (Figure.3, 4).

Our results were indicated that rise of anti-gliadin IgG ratio at children (2-10), years were (36.89%), and IgA at (31-40) years (27.27%), while anti-tissue transglutaminase IgG ratio was (7.69%) at both (2-10) (11-20) years, and IgA at (11-20), (2-10) years, was (42.30%), (25.96%) respectively. There was a statistical significant correlation between ages groups anti-gliadin IgG and IgA, so that anti–tissue Transglutaminase IgG and IgA in all Ages (2-40) years at p ≤ 0.05.

One hundred forty four (89.44%) patients had biopsy result consistent with CD (Table- 4). Out of 144 (89%) CD patients, 72 (44.72%) had Marsh I, 18 (11.18%) Marsh II, 25 (15.52%) Marsh IIIA, 16 (9.37%) Marsh IIIB, 13 (8.07%) Marsh IIIC.

Negative predictive value of histological diagnosis and Anti-gliadin IgG = TN/FN + TN X 100
True negative = 161 _ 144 = 17
False negative for anti-gliadin IgG = 144 _ 53 = 91
17/17 + 91 = 15.74 %

Negative predictive value of histological diagnosis and Anti-gliadin IgA
TN = 161 _ 144 = 17
FN (for Antigliadin IgA) = 144 _ 34 = 110
17/17 + 110 = 13.38 %

Discussion:

The results of our study were indicated that gluten exposure should be sufficient to cause sensitization. Foods containing gluten are major components of the daily diet in our country.

Our results agreed with a study done by Murry Gastroenterologists should
Consider performing a biopsy of the duodenum in patients who undergo gastroscopy but do not have an obvious endoscopic cause for their symptoms. Occasionally, celiac disease will be suspected by an astute endoscopist as a result of the suggestive appearance of the duodenal mucosa (13).

Another study agreed with our results, intestinal biopsy is recommended to confirm the diagnosis and the necessity of lifelong gluten restriction. (14).

Third study accepted our results said the small intestinal abnormalities characterized by histological changes are essential diagnosis of celiac disease (15).

There are studies disagree with our results e.g biopsy is not always necessary for diagnosis. (16)

Increased antigliadin IgG at ages (2-10) years more than another ages may be referred to lack of breast feeding and weaning with great amount of gluten intake, gliadin peptide uptake, processing and presentation by antigen-presenting cells, leading to B cell clonal expansion and release of antibodies to gliadin (10).

The clinical significance of anti-tissue transglutaminase antibodies is not known although some may be associated with specific clinical presentation or extra-intestinal manifestations of CD (10).

However, they can play a role in assessing young children in whom anti-tissue transglutaminase assays are less sensitive and in following some celiacs on a gluten free diet then the anti-tissue transglutaminase levels are slow to decline. These antibodies assays are useful in monitoring compliance since levels will decrease on a gluten-free diet and increase after the ingestion of gluten, it can also play a role in the timing of endoscopy and biopsy during a gluten challenge (17).

Gluten is the proteins in specific cereal grains that are harmful to persons with celiac disease. These proteins are found in all forms of wheat, and related grains: rye, barley are lead to revealing CD symptoms. The genes (HLA class II) are involved in the regulation of the body’s immune response to the gluten protein fractions (18). The therapy for the disease is a gluten-free diet; however, the response to therapy is poor in up to 30% of patients, and dietary nonadherence is the chief cause of persistent or recurrent symptoms (19).

Studies in both Europe and North America estimate the seroprevalence to be 1 of every 120 to 300 persons (20). Researchers do not know what causes discernible disease to develop in adulthood after existing subclinically for years, although studies suggest that signs and symptoms may become noticeable after physically or emotionally stressful events. In the United States, CD is most often diagnosed in adults, which is probably a result of the lack of a specific screening protocol in children and the limited knowledge of CD and underestimation of its prevalence among clinicians (21).

Recent studies show that CD also increases the risk for squamous cell esophageal cancer, primary small bowel adenocarcinoma, non-Hodgkin’s lymphoma, lymphocytic gastritis, and lymphocytic colitis. Indeed, the risk of these cancers seems to be eliminated in patients who remain gluten-free for more than 5 years (22). Additionally, the rate of autoimmune diseases in patients with CD is increased and is related to the duration of exposure to gluten (23). The mechanisms responsible for the development of malignancies in CD patients are not known. The following explanations have been suggested: increased intestinal permeability of environmental carcinogens, chronic inflammation, chronic antigen stimulation, release of pro-inflammatory cytokines, immune surveillance problems and nutritional deficiencies caused by the disease (24).

Armin and Peter were studied a role of gliadin peptides are presented in complex with DQ2 or DQ8 molecules of antigen presenting cells gliadin-specific B cells receive help from the activated gliadin-specific T cells, leading to release of antibodies to gliadin. Transglutaminase-specific B cells receive help from the gliadin-specific T cells, lead to release anti-transglutaminase antibodies in the absence of Transglutaminase-specific T cells (10).
A change in the normal columnar appearance of the absorptive epithelium with crypt hyperplasia and increased numbers of intraepithelial lymphocytes IEL and lamina propria mononuclear cells. More minor degrees of histopathology may be missed on routine examination since the earliest changes are an increase in IEL. These features are not specific for celiac disease in that some or all of the histological findings can be found in tropical sprue, small intestinal bacterial overgrowth, viral gastroenteritis, intestinal lymphoma and severe acid-induced injury associated with a gastrinoma. Duodenal biopsies obtained at endoscopy are usually sufficient to make the diagnosis, but occasionally additional samples from the more distal jejunum are needed. Appearance of the positive histological findings rather than serological tests were give accurate diagnosis of CD.

**Conclusion:**
This study focused alight on diagnosis of CD which need following parameters:
2. Histological tests which are most accurate way in diagnosis and monitoring.
3. Serological tests when histological tests cant be done.

**Recommendation:**
This study revealed that histological test is best method in diagnosis of CD so the physicians should depend whenever its possible so this study encourage more endoscopic and biopsies practice when dealing with patients clinically suspect to have CD.

**References**


**Table (1). The percentage of anti-gliadin IgG and IgA antibodies.**

<table>
<thead>
<tr>
<th>Ages groups (years)</th>
<th>N</th>
<th>IgG</th>
<th>IgA</th>
</tr>
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<tbody>
<tr>
<td>2-10</td>
<td>104</td>
<td>38(36.89%)</td>
<td>24(23.07%)</td>
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<tr>
<td>11-20</td>
<td>26</td>
<td>9(34.61%)</td>
<td>5(19.23%)</td>
</tr>
<tr>
<td>21-30</td>
<td>20</td>
<td>4(19.04%)</td>
<td>2(9.52%)</td>
</tr>
<tr>
<td>31-40</td>
<td>11</td>
<td>2(18.18%)</td>
<td>3(27.27%)</td>
</tr>
<tr>
<td></td>
<td>161</td>
<td>53(32.91%)</td>
<td>34(21.11%)</td>
</tr>
</tbody>
</table>

**Figure (1). The correlation between anti-gliadin IgG and ages.**
Figure (2). The correlation between anti-gliadin IgA and ages.

Table (2) The ratio of anti-tissue transglutaminase IgG and IgA.

<table>
<thead>
<tr>
<th>Ages groups(years)</th>
<th>N</th>
<th>IgG</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-10</td>
<td>104</td>
<td>8(7.69%)</td>
<td>27(25.96%)</td>
</tr>
<tr>
<td>11-20</td>
<td>26</td>
<td>2(7.69%)</td>
<td>11(42.30%)</td>
</tr>
<tr>
<td>21-30</td>
<td>20</td>
<td>1(5%)</td>
<td>4(20%)</td>
</tr>
<tr>
<td>31-40</td>
<td>11</td>
<td>(0%)</td>
<td>2(18.18%)</td>
</tr>
<tr>
<td></td>
<td>161</td>
<td>11(6.83%)</td>
<td>44(27.32%)</td>
</tr>
</tbody>
</table>

Figure (3). The correlation between anti-transglutaminase IgG and ages.
Figure(4). The correlation between anti-transglutaminase IgA and ages.

Table (3). The Sensitivity and Specificity of immunologic tests.

<table>
<thead>
<tr>
<th>Test</th>
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<th>IgA</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>Sensitivity</td>
</tr>
<tr>
<td>Anti-gliadin</td>
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<tr>
<td>anti-transglutaminase</td>
<td>11</td>
<td>40.74%</td>
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Table (4): Histological findings in the studied patients.

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>%</th>
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<tr>
<td>Normal</td>
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</tr>
<tr>
<td>Marsh I</td>
<td>72</td>
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</tr>
<tr>
<td>Marsh II</td>
<td>18</td>
<td>11.180</td>
</tr>
<tr>
<td>Marsh IIIA</td>
<td>25</td>
<td>15.527</td>
</tr>
<tr>
<td>Marsh IIIB</td>
<td>16</td>
<td>9.937</td>
</tr>
<tr>
<td>Marsh IIIC</td>
<td>7</td>
<td>4.347</td>
</tr>
<tr>
<td>Total</td>
<td>161</td>
<td>100%</td>
</tr>
<tr>
<td>Tests</td>
<td>Anti-gliadin IgG</td>
<td>Anti-gliadin IgA</td>
</tr>
<tr>
<td>---------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Total</td>
<td>161</td>
<td>53(32.91%)</td>
</tr>
</tbody>
</table>

**Figure(5) .The comparism between Serological and histological tests in CD**
Figure 1: The small intestine biopsy.

Type 0: Normal

Type I: Preserved villi with mild chronic

Type II: Duodenal mucosa with preserved no moderate crypt

Type IIIa: Partial villous atrophy

Type IIIb: Subtotal villi atrophy with crypt hyperplasia.
الخلفية:

بعد مرور الجوع من الأمراض المناعية التي تنشأ نتيجة التعرض للكلوتين والاسم الشائع له هو حساسية الحلقة. تشير الإحصائيات السريرية إلى زيادة عدد المرضى في العراق في السنوات الأخيرة.

الهدف:

تشخيص الدقيق للمريض باستخدام الفحوصات السنية والمصلية في المرضى المشتبه بهم ومقارنة اسباب التشخيص

المرضى وطرق العمل:

تم جمع 161 نموذج دم من المرضى المراجعين لمستشفى الحسين (ع) العام في مدينة كربلاء تراوحت اعمارهم بين (سنتين-40) سنة للتحري عن وجود الأجسام المضادة أنواع (ج، أ) ضد الكليتين والاجسام المضادة أنواع (ج، أ) ضد الكولتامين السنيجي في مصطلح المرضى باستخدام تقنية الألبارا فضلا عن فحص خصاص (161) خزعة نسيجية مأخوذة من الامعاء الدقيقة للمرضى المذكورين.

النتائج:

كانت نسبة الأجسام المضادة أنواع (ج، أ) ضد الكليتين 91و32٪ (53 من مجموع 161)، 151و21٪ (34 من مجموع 161) على التوالي. وكانت نسبة التحري عن مرض الجوع في الخزع السنيجي عند المرضى المذكورين (4و98٪).

الاستنتاجات:

أثبتت الدراسة أهمية استخدام الفحوص السنية في تشخيص المرض كما أثبتت فائئالية تلك الفحوص مقارنة بالفحوص المصلية المتبقية.