Potential Coeliac Disease among First Relatives of Iraqi Patients

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Abstract: Background: Coeliac disease (CD) is a common diagnosis among children and adults in Iraq; however to the best of our knowledge, no documented data is available about its familial prevalence in Iraq yet. This study was carried out to determine the prevalence of potential coeliac disease in a group of first degree relatives of Iraqi coeliac patients. Methods: 106 first degree relatives of coeliac patients attending Gastrointestinal Hospital at Medical City in Baghdad, Iraq. Their sera were underwent serological screening for coeliac disease using the IgA anti-endomysium antibody test (EMA), in addition to human leukocyte antigen class II typing. Duodenal biopsies were performed in all subjects positive to EMA. Coeliac disease diagnosis was established according to modified Marsh criteria. All family members were on a gluten-containing diet when serological tests and HLA typing were performed. Results: Fifteen (14.1%) were positive EMA among 106 relatives and thirteen (12.2%) were found as new cases of coeliac disease depending on histology results (Marsh III). However, the DQ2 antigens ratio was 39.6%, DQ8 antigens ratio was 35.8%, meanwhile, DR3 ratio was 16% and the DR5/7 ratio was 8.5%. Conclusion: Silent CD cases were more than expected in Iraq, therefore, serological testing is recommended for all first-degree relatives of CD patients. Moreover, they should undergo HLA typing to detect those whose HLA phenotype is consistent with CD.

Keywords: Coeliac disease, Endomysial antibodies, screening, Human leukocytes antigens, Iraq

1. Introduction

Coeliac disease (CD) is a common, familial, autoimmune, multifactorial gastrointestinal disease defined as a permanent gluten-sensitive enteropathy characterized by malabsorption and mucosal injury of the small bowel. It is related to permanent gluten intolerance that develops in genetically predisposed individuals any time during their life. Clinical presentations of CD vary widely from absolute absence of symptoms to pronounced clinical, gastrointestinal, or extraintestinal manifestations [1]. There are three different forms of coeliac disease according to the ESPGHAN criteria [2, 3]; (1) the latent (or potential) form defined only by the presence of specific antibodies; (2) the silent form defined by the presence of specific antibodies and the observation of a villous atrophy of the small intestine; (4) and the symptomatic form defined by the presence of specific antibodies, the observation of a villous atrophy and the observation of clinical symptoms.

In the last 3 decades the emergence of progressively more reliable serological markers, such as anti-gliadin (AGA), anti-endomysium (EMA), and anti-transglutaminase (tTG) antibodies allowed the precocious diagnosis of atypical or even silent form of the disease [4] and greatly contributed to the widespread acknowledging that the prevalence of CD was higher than previously thought.

Concordance for coeliac disease in first-degree relatives ranges between 8% and 18% and reaches 70% in monozygotic twins. This has been demonstrated by several studies [4-11]. A large American study that recruited relatives during CD support group meetings identified 5% of first-degree relatives with CD [5]. This was less than in Europe [6-7] and might reflect different case selection, recruitment, and testing that the prevalence was higher in relatives of affected sibling pairs (17.2%) , in monozygotic twins (75%), and HLA identical siblings (40%) [8]. The prevalence of biopsy-proven CD among first degree relatives varies between 5.5% [9] and 8.3% [10], reaching a value of 18% in siblings [13]. Similar results have been reported from non-European populations, including a report from the Punjab region of India [10]. In addition, more extensive studies in biopsy-defined celiac disease have further confirmed these findings, particularly in “at-risk” first-degree [13]. This risk appears to be especially increased in families with at least 2 siblings diagnosed as CD [14], and in this setting, more males were detected compared to females[15].

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The main risk factor for development of the condition is the presence of HLA DQ2 or DQ8. Most Northern European patients express the DQ2 heterodimer HLA-DQA1*0501 and DQB1*0201. Those who do not express this heterodimer, most commonly, have the HLA-DR4, DQ8 haplotype [16]. Further susceptibility genes, such as the CTLA-4 gene on chromosome 2q33 [17], are thought to reside both inside and outside the HLA region and are currently being evaluated, although the disease is not expressed in the absence of the HLA genes. However, the prevalence of HLA-DQ2 is high in the normal population (25%-30%), suggesting the involvement of additional, probably non-HLA- linked genes in coeliac disease pathogenesis [17].

CD is a common diagnosis among children and adults in Iraq [18-21]; however to the best of our knowledge, no documented data is available about its familial prevalence in Iraq yet. The aim of the present study was to determine the prevalence of CD in a group of first relatives of biopsy-proven patients, to know the disease prevalence and incidence among first-degree relatives as the first study done in Iraq. Thus, a special attention to follow the sibs with higher risks will be made as soon as possible.

2. Material and Methods

Study group
A total of 106 subjects were recruited in this study. They were first-degree relatives of patients with proved coeliac disease, attending Gastrointestinal Hospital at Medical City in Baghdad, Iraq. Age, sex, and family relation to patients had been taken. Their sera tested for EMA in addition to HLA class II typing. All family members were on a gluten-containing diet when serological tests and HLA typing were performed.

Serologic method:
A ready kits of EMA by using monkey esophagus substrate (supplied from Medic Company, Italy 1X8: tests) was used. All sera subjected to the test using indirect immunofluorescent (IIF) method. Negative sera, for IgA EMA were subjected to the test with IgG monoclonal conjugate by IIF to exclude IgA deficiency disease associated with coeliac disease. EMA was considered positive if it corresponds with the pattern described by Chorzelski TP, et al 1984 [22] in coeliac disease. The tests were performed as follows: 5 micrometer sections of fresh frozen monkey esophagus were air-dried for 20 min. Five microliters of undiluted serum or of twofold dilutions with an initial dilution of 1:2.5 were applied on the sections and incubated for 30 min. The slides were washed in phosphate-buffered saline PBS (From: Sanofi Diagnostic Pasteur., France) for two 10-min periods, followed by 30 min of incubation with Fluorescein labeled anti-human globulin (F.I.T.C.) conjugate (Sanofi diagnostic pasteur kallested-chaska, France). The conjugate characteristics were as follows: F/P ratio, 2.3; antibody concentration, 100 pg/ml; protein, 0.8g/l; and working dilution, 1:64. After being washed twice in PBS the samples were mounted in buffered glycerin. The slides were examined in a Fluorescence microscope (orthanol-Leitz laborutx, D-Leitz, Wetzler; Germany) with an exciting primary filter K 500 and supplied with a fully automatic camera. IgA specific for the endomysial lining of myofibrils were identified by their reticulin like staining of smooth muscle bundles. Sera samples containing antibodies at any titer were considered positive.

Histology method
Duodenal biopsy was recommended to subjects showing positive results to EMA. Sections of the duodenal mucosa were cut at a thickness of 5 micrometer and stained with haematoxylin and eosin (H&E). Slides were interpreted by two pathologists who were not informed about the clinical status of the subjects and interpreted small intestinal histological features, according to the Marsh classification and modified Marsh criteria: [23-24] Marsh I consists of raised intraepithelial lymphocytes (IELs) with >40 lymphocytes per 100 enterocytes, Marsh II consists of raised IELs and crypt hyperplasia, Marsh IIIa partial villous atrophy, Marsh IIIb subtotal villous atrophy, and Marsh IIIc total villous atrophy. Diagnosis of coeliac disease was dependant on the presence of Marsh III only. Any report, which did not include the features of Marsh III was considered as non-coeliac patient.

HLA typing class II
Typing of HLA DR and DQ antigens were carried out in Immunology laboratory of Al-Karama hospital, Baghdad. The microlymphocytotoxicity test which has been modified by Bender 1984 [25] was followed, using various lyophilized antisera for HLA typing, include class II (DR, DQ), (Biotest, Germany), Class II positive and negative control antisera, (Biotest, Germany).

Briefly; Ab-mediated complement dependant cytotoxicity assay was done by treating sample of patient's leukocyte with a panel of anti-HLA antisera and complement. Anti-HLA sera react with the corresponding lymphocyte antigens without visible cell alteration. The addition of rabbit complement, result in a structural alteration of the lymphocyte cell membrane, so that the indicator vital dyes are able to penetrate into the cell. Staining of lymphocytes occurred in the positive reaction. The lysed and vital lymphocytes are assessed using an inverse phase contrast microscope.

The study was approved by Ethics Committee of the College of Medicine, University of Baghdad. All
subjects were informed about the objectives of the study and the eventual necessity of small intestinal biopsy. Informed consent was obtained from adults and from the parents for their children.

Statistics

Analysis comprised of summary statistics for gender and age. Data were analyzed using SPSS v16 for Windows and paired t-tests were used to compare the change in histopathology findings (Marsh grade). Analyses where the P-value was <0.05 were considered to be statistically significant. HLA DR and DQ phenotype frequencies were calculated by direct counting method, using following formula: \( A = \frac{n}{N} \), where \( n \) is number of persons with a given antigen and \( N \) is total number of persons studied [26]. Relative risk (RR) for measuring strength of association with each II class HLA antigen was calculated by the Woolfe’s method. [27]

3. Results

Since children with coeliac disease differ from adults in certain aspects, associations presented in this study was grouped into two categories; first, children (<18 years) second, adults (≥18 years).

Table 1 shows the frequency distribution of 106 first-degree relatives of proved coeliac patients and their family relation to them, while table 2 shows the EMA positivity rates and biopsy results among these relatives according to age group. Among 38 male relatives children there were 4 positive EMA (3.8%), 2 of them showed Marsh II and another 2 showed Marsh IIIa compared with one positive from 15 (0.9%) in male adults who showed Marsh IIIa, meanwhile, among female relatives children there were 6 positive EMA (5.6%) who were all showed Marsh IIIa compared with 4 positive from 30 (3.8%) in female adults. 2 of them showed histological results of Marsh IIIa, one with Marsh IIIb and another showed Marsh IIIc. This gave total of 15 (14.1%) positive EMA among 106 relatives and 13 (12.2%) proven CD depending on histology results.

Table 1: Frequency distribution of first-degree relatives of coeliac patients according to gender, age group and family relation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>%</th>
<th>Family relation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>38</td>
<td>62.3</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
<td>37.7</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>33.3</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>66.6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>100</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

F = father; M = Mother; B = Brother; S = Sister; NS = Not significant statistically

Table 2: EMA positivity rates and biopsy results among first-degree relatives of coeliac patients according to age group.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Positive EMA</th>
<th>Biopsy result</th>
<th>Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>Marsh IIIa</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>3.8</td>
<td>2</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>5.6</td>
<td>0</td>
</tr>
<tr>
<td>Adult</td>
<td>1</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>3.8</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>14.1</td>
<td>2</td>
</tr>
</tbody>
</table>

The frequency distribution of the HLA class II antigens among first-degree relatives is shown in table 3. The DQ2 antigens ratio was 39.6%, DQ8 antigens ratio was 35.8%, meanwhile, DR3 ratio was 16% and the DR5/7 ratio was 8.5%.

Table 3: Frequency distribution of HLA class II antigens within first-degree relatives of coeliac patients according to gender and age group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Children</th>
<th>Adults</th>
<th>Total No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>M</td>
<td>No.</td>
<td>%</td>
<td>F</td>
</tr>
<tr>
<td>DQ2</td>
<td>8</td>
<td>18</td>
<td>26</td>
<td>24.5</td>
</tr>
<tr>
<td>DQ8</td>
<td>9</td>
<td>14</td>
<td>23</td>
<td>21.7</td>
</tr>
<tr>
<td>DR3</td>
<td>6</td>
<td>4</td>
<td>10</td>
<td>9.4</td>
</tr>
<tr>
<td>DR5/7</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1.9</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>61</td>
<td>77</td>
<td>100</td>
</tr>
</tbody>
</table>

4. Discussions

Two parameters had been investigated among 106 first-degree relatives of our coeliac patients, they were; presence of EMA and HLA-typing in order to know the family association of CD as a first study in Iraq to the best of our knowledge, and to discover the latent or potential coeliac disease in the families, then the diagnosis of CD could be confirmed by histology examination.

The prevalence of CD among relatives observed in our subjects was higher than expected in comparison with other studies in the world. About 14.1 % of first-degree relatives of Iraqi CD patients according to the present study had silent coeliac disease depend on the presence of EMA (which has 100% specificity) in addition to presence of DQ2 alleles. This finding was much more than results came from USA, Italy, and Brazil that reported a prevalence of 4.5% [5], 9.5% [10] and 4.8% [28], respectively, while this prevalence was less than the result documented in Bosnia which was 20%. [29]. These differences may be due to the variability of the genetic background of the distinct population evaluated rather than the variability of total number of cases that have been studied in different societies.

We used a one-tier serological screening system for coeliac disease, that was the EMA test since it is a highly sensitive, cheap and simple
screening test, rather than as a more specific diagnostic test among other tests like anti gliadin antibodies and anti tissue transglutaminase. We found it was useful for rapid large scale screening test especially in developing countries like Iraq since a lot of people are poor and low educated so it is difficult to do the costly two- tier screening system that has been suggested by Jocelyn, et al 2006. [30]

The gold standard for CD diagnosis is the intestinal biopsy, but its invasiveness and cost preclude the submission of all CD relatives to this examination [31]. For this reason, serologic test was used to select subjects who need intestinal biopsy. Histopathologic examination of thirteen biopsy samples with positive EMA in the present study, revealed typical abnormalities of CD classified as Marsh III, however, another two positive serology samples related to male children subjects showed increased number of IELs and crypt hyperplasia, without villous atrophy (Marsh II). Increased number of intraepithelial lymphocytes is not necessarily specific for CD since it can be found in other disorders as, for example, small intestinal bacterial overgrowth. [32]

Although the specificity of HLA-genotype DQ2, DQ8 for coeliac disease is rather low, they are highly sensitive and independent of disease activity or diet, and therefore a very suitable component in screening tests for the diagnosis latent/potential coeliac disease. Thus, among all tests investigated, the presence of the HLA-DQ2, DQ8 alleles and positive EMA may represent markers of latent/potential coeliac disease. These ideas were consistent with other studies. [9-10, 14-15]

Individuals with a specific genetic background, together with positive coeliac serology are not "healthy" [13]. This is true among our (healthy group relatives), where there were 15 individual had silent coeliac disease or may be latent coeliac disease among them there were 13 confirmed by histology exam. A healthy person, who initially has a normal clinical and normal biopsy but had HLA-DQ2, DQ8 and positive coeliac serology, may later develop signs and symptoms of the disease. Enteropathy might not present, but other gluten-induced diseases such as; dermatitis herpetiformis or enamel lesion in permanent teeth of individuals may develop. These findings consistent with many studies. [2, 13-14, 33-34]

The occurrence of coeliac disease in more than one member of a family was reported over 50 years ago [35]. Subsequent studies of small intestine biopsy specimens from first-degree relatives of patients with coeliac disease provided complete evidence that genetic factors may influence susceptibility to this disease. Precisely how this HLA association predisposes persons to CD is far from clear [33-34]. The vast majority of subjects who have DQ2 haplotypes (39.6% in present study) common with coeliac patients remain healthy, however, because (25%) of the general population also express DQ2, it is likely that other gene are involved in CD. This can also be concluded from the results of studies that tried to determine the contribution of the HLA-region to the pathogenesis of various diseases. [13, 33,36]

According to the results of the present study that were done for the first time in Iraq; CD is a significant health issue in this country and worldwide as well, since the prevalence of CD among first-degree relatives is much higher than the prevalence of the disease in the general population. Most of these patients have an atypical or silent form of the disease and would therefore be overlooked without an active search. This issue requires a deeper understanding of this multifaceted condition, and a multidisciplinary approach that increasingly relies on close collaboration among all practitioners involved in support of an important response of patient's family members to be tested for antibodies to coeliac disease. This may be conducted by educational discussions about the importance of this disease and the frequency of its occurrence among first degree relatives.

As conclusion; the presence of EMA, and HLA-class II typing are good protocol to study the family association in the first-degree relatives of Iraqi coeliac patients, since 14.1% of them had latent/potential CD. This was more than expected in our society, therefore, serological testing is recommended for all first-degree relatives of CD patients. Moreover, they should undergo HLA typing to detect those whose HLA phenotype is consistent with CD.

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