Assessment of Interferon Gamma (Ifn-Γ) & Interleukin-10 (IL-10) in Patients Afflicted with Celiac Disease

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Abstract

Celiac disease (CD) is an immune mediated malabsorption syndrome that occurs in genetically susceptible individuals intolerant to dietary gluten. Although considered as a primary gastrointestinal disease, CD is now known to have widespread systemic manifestation. We attempted to define the nature and role of some systemic cytokine that possibly play a role in the pathophysiology of the disease.

The sera were collected from those patients suspected of having CD on clinical ground (Newly diagnosed) & then subjected to serologic tests namely anti – tissue transglutaminase IgA, IgG (tTG) & IgA, IgG - Endomysial antibody (EMA).

The positive sera for anti - tTG IgA and IgA EMA autoantibody above the cut-off level were then subjected to cytokines assessment namely serum interferon gamma (IFN-γ) and interleukin-10 (IL-10) level.

The participant groups comprised 50 newly diagnosed (ND) CD, 20 patients on gluten free diet (GFD) and 20 apparently healthy CD free control (These groups were subjected to the above parameters).

The results of the present study among the newly diagnosed cases reveals anti-tTG IgA and IgA - EMA seropositivity in 15.1 % of the total 330 sera examined ;with anti-tTG IgG & IgG -EMA seronegativity (below the cut-off ) in all sera tested. The highest percent distribution of anti-tTG IgA and IgA -EMA seropositivity was at the serum concentration of 20-29 (36 % & 34 % respectively).

A significant decrease (P< 0.01) was observed in the mean concentration of anti-tTG IgA and IgA- EMA between newly diagnosed CD and patients on GFD. The mean

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concentration of IFN-γ and IL-10 cytokine was significantly higher (P < 0.01) among newly diagnosed and patient on GFD when compared with health control.

The serum concentration of IFN-γ and IL-10 according to mode of clinical presentation & duration of disease revealed no significant differences (P > 0.05) except for IL – 10 (≤ 3 years & > 3 years on GFD) P < 0.05. In newly diagnosed CD patient, a correlation exist between anti – tTG IgA & IL -10 (P <0.02 ), as IL – 10 has immunostimulatory effect on B – cells.

**Keywords:** Celiac disease ; IFN-γ ; IL-10; tTG ; EMA.

**Introduction**

Celiac disease, an autoimmune-mediated systemic disorder commonly presenting as enteropathy, is provoked in genetically predisposed individuals by the ingestion of wheat, barley & rye proteins i.e. gluten [1]. The best characterized genetic factors contributing to disease predisposition are the human leukocyte antigen (HLA) molecules DQ2 & DQ8. Approximately 95% of patients carry the alleles encoding the DQ-2 & the most of the rest the DQ-8 molecule [2]. The incomplete hydrolysis of gluten during gastrointestinal digestion leads to the appearance of a large repertoire of gluten -derived gliadin peptides with a variety of characteristics [3]. The so-called toxic peptides, of which p31-43 is probably the most fully studied, modulate the small intestinal mucosal biology via an innate immune mechanism. The immunogenic gliadin peptides (such as the 33-mer & p57 - 68) then gain access into the tissue & when accumulated in the tissue, the immunogenic peptides upon deamidation bind to the DQ-2 or DQ-8 molecule on antigen -presenting cells presenting the peptides to gliadin -specific CD4+ cells in the lamina propria. These cells become activated & begin to secrete proinflammatory cytokines such as IFN-γ & IL-12, whose marked production would also contribute to the small intestinal mucosal deterioration [2].

In celiac patients the ingestion of gluten leads to small intestinal mucosal inflammation & villous atrophy together with crypt hyperplasia as well as the appearance of clinical symptoms. CD is hallmarked by increased small intestinal epithelial cell proliferation & decreased differentiation.

**Aim of the study**-

✓ To define serum cytokine concentration of IFN-γ & IL-10 in CD patients.
✓ To evaluate the effect of gluten free diet (GFD) on serum concentration of IFN-γ & IL-10.
✓ To correlate serum cytokine levels with anti - tTG IgA & IgA -EMA serum concentration.
**Material and Methods**

*A. Patients and sample collection*

The study is a case control & the enrolled suspected patients were (330) diagnosed on a clinical ground as celiac disease. The enrolled patients particularly children on one hand are those attending to the pediatric department at Raparin teaching hospital complaining of diarrhea, loss of weight, failure to thrive and abdominal pain & their blood samples were send to private laboratories.

In adults, the sera were collected from those attending private laboratories in Erbil city referred from private clinic. In addition, 20 CD patient on gluten free diet were chosen in which they referred from private clinic to private laboratory for the purpose of follow-up.

The sera of the suspected CD patients & those on GFD & control group were subjected to serologic tests for anti- tTG IgA, IgG & IgA, IgG –EMA level using ELISA technique.

Thus sera with anti- tTG & EMA antibodies ≤ 10 I.U/ml. were labeled as negative and those with values >10 I.U/ml. were regarded as positive based on the manufacturers’ protocol.

**Results**

Table 1. revealed 15.1% seropositivity for both anti-tTG IgA & IgA-EMA among the total 330 cases suspected of having CD.

<table>
<thead>
<tr>
<th>Tested parameters</th>
<th>Suspected CD (No. = 330)</th>
<th>Healthy Control (No. = 20)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Anti - tTG IgA seropositive</td>
<td>50</td>
<td>15.1</td>
</tr>
<tr>
<td>Anti - tTG IgG seropositive</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IgA - EMA seropositive</td>
<td>50</td>
<td>15.1</td>
</tr>
<tr>
<td>IgG – EMA seropositive</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
In Table 2, 36% & 34% of the tested sera of CD patients was positive for IgA anti-tTG & EMA, at sera concentration of 20-29 I.U/ml. respectively.

Figure 1 show a highly significant differences (P< 0.01) between mean level of anti-tTG & EMA (IgA & IgG classes) among ND, GFD & control groups.

Table 2. Distribution of anti-tTG IgA & IgA - EMA serum concentration among newly diagnosed CD patients.

<table>
<thead>
<tr>
<th>Range of serum Concentration</th>
<th>Newly diagnosed (ND) = 50</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Anti- tTG (IgA)</td>
</tr>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>10.1-19</td>
<td>11</td>
</tr>
<tr>
<td>20-29</td>
<td>18</td>
</tr>
<tr>
<td>30-39</td>
<td>11</td>
</tr>
<tr>
<td>40-49</td>
<td>5</td>
</tr>
<tr>
<td>50-59</td>
<td>2</td>
</tr>
<tr>
<td>60-69</td>
<td>2</td>
</tr>
<tr>
<td>70-79</td>
<td>1</td>
</tr>
<tr>
<td>80-89</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1: Mean serum concentration of anti-tTG & EMA (IgA, IgG) among CD & control group.
Figure 2 delineate a highly significant (P< 0.01) differences between mean levels of IFN-γ & IL-10 in CD patients when compared with control group.

Figure 2. Mean serum concentration of IFN - γ & IL -10 among CD & control group.

Regarding Table (3.3), no significant differences (P> 0.05) was observed between mean level of IFN-γ & IL-10 according to gender in ND,GFD & control groups.

Table 3: Mean serum concentration of IFN - γ & IL – 10 among CD subset & control group according to gender

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>IFN - γ</th>
<th>IL - 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>ND = 50</td>
<td>11.250</td>
<td>3.07365</td>
</tr>
<tr>
<td>GFD = 20</td>
<td>7.2333</td>
<td>1.92735</td>
</tr>
<tr>
<td>Control = 20</td>
<td>2.1100</td>
<td>.94687</td>
</tr>
</tbody>
</table>

According to mode of clinical presentation in newly diagnosed CD(Figure 3.3), no significant differences was seen between levels of IFN-γ & IL- 10(P> 0.05).
Figure 3. Serum concentration of IFN-γ & IL-10 according to mode of clinical presentation in newly diagnosed CD patients.

Figure 4. Delineate no significant differences (P> 0.05) between mean levels of IFN-γ & IL-10 according to duration on GFD in CD patients.

Figure 4. Mean serum concentration of IFN-γ & IL-10 in patients on GFD according to duration.
Table 4. Correlation between anti - tTG IgA, IgA - EMA & IFN -γ, IL -10 in newly diagnosed CD patients.

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>IFN -γ</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r- value</td>
<td>p- value</td>
</tr>
<tr>
<td>Anti- tTG IgA (LU/ml.)</td>
<td>0.0003</td>
<td>0.90</td>
</tr>
<tr>
<td>IgA - EMA (LU/ml.)</td>
<td>0.007</td>
<td>0.576</td>
</tr>
</tbody>
</table>

This table revealed a significant concentration between anti – tTG IgA & independent variable IL-10 cytokine (r =0.1 ; P = 0.025).

**Discussion**

A. *Impact of anti –tTG & EMA (IgA, IgG) in CD serodiagnosis.*

In this study 50/330 (15.1 %) of the tested sera were seropositive for anti-tTG IgA & IgA -EMA among clinically suspected CD patients (Table,3.1). Meanwhile none of the tested sera were seropositive for anti- tTG IgG or IgG - EMA.

Systematic reviews have concluded that assays for the detection of serum IgA -tTG antibodies have a high sensitivity & specificity in both children & adult, for Identifying patients with celiac disease [4]. It has been suggested that high titers of anti -tTG are associated with elevated positive predictive values (PPV) for CD [5]. In this situation the requirement for small bowl biopsy to establish the diagnosis of CD in every case has been questioned [5].Thus having all the following, strongly positive IgA tTG, positive IgA - EMA, high baseline risk for CD and positive celiac genetics make the probability of CD almost certain in these cases [6].

B. *IFN -γ in CD.*

Among CD subsets namely newly diagnosed & patients on GFD, IFN –γ concentration was significantly higher than CD free control ( P < 0.01) (Figure, 3.2 ). A correlation between the average level of IFN -γ expression and the extent of tissue restructuring in the intestinal mucosa is a fact [7]. Thus in biopsy with complete atrophy of villi (MIIIc), this expression is about 240 – fold higher than that measured in the averaged controls [7].

Also IFN–γ increase epithelial permeability which in turn will increase the passage of gluten peptides & peptide binding to DQ2 & DQ8 molecules on antigen presenting cells [8].
The result of our study agree with other studies who reported a significant higher level of IFN-γ among active CD & those on GFD [9; 10; 11].

C. IL-10 in CD.

The anti-inflammatory cytokine IL-10 concentration among CD subset & CD free control reveal a statistically higher level when compared with control (P < 0.01).

Increase in IL-10 mRNA level in intestine of CD patients is regarded as a counter regulatory mechanism triggered by the disease but not the cause of the pathology [12]. This is supported by the fact that after gluten removal from the diet, IL-10 levels return to normal level [10]. Production of IL-10 may be a common feature of IELs producing pro-inflammatory cytokines, thereby attempting to limit inflammation in an autocrine fashion [13]. The result of our study agrees with other study (10; 11) who reported a constantly higher level of IL-10 with a dramatic and significant decrease in response to a GFD.

D. Correlation between IgA-tTG, IgA-EMA & cytokines.

A statistically significant correlation was observed only between anti-tTG IgA & IL-10. Our result agree with other [12]. At the mucosal level, IL-10 cytokine favor the generation of IgA [13; 14]. Beside the fact that IL-10 has immunostimulatory effect on B-cells [15].

References


