

## EVALUATION OF SERUM LEVELS OF IL-12 AND IL-17A IN IRAQI PATIENTS INFECTED WITH CELIAC DISEASE

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**ABSTRACT :** Celiac disease (CD) is a common intestinal disease among adults and children in Iraq. However, a little documented data about its prevalence in Iraq. This study was included 40 samples of Iraqi Arab CD patients in Baghdad, their sera with 20 sample of apparently healthy controls, were investigated for Interleukin-12 (IL-12) and Interleukine-17A (IL-17A) by ELISA method, to determine the potential relationship between this disease with cytokines in Iraqi CD patients. Results showed significant variations between IL-12 and IL-17A in CD patients. Where the serum level of IL-17A in the patients (88.25 pg/ml) comparing with control group (37.07 pg/ml), while the serum level of IL-12 is significantly lower in the patients (24.07 pg/ml), comparing with control group (46.35 pg/ml). There was no found a significant effect of sex on the level of serum IL-12 in patients and control, males (25.27 and 49.68 pg/ml) respectively, females (22.87 and 43.01 pg/ml) respectively. There also was not a clear significantly difference between serum level of IL-17A in patients and control, males (92.28 and 42.83 pg/ml) respectively, females (84.21 and 31.33 pg/ml), respectively. Interestingly, the level of IL-17A was higher in the male of control group (42.83 pg/ml) than in females (31.33 pg/ml).

**Key words :** IL-12, IL-17A, Celiac Disease, gender.

### INTRODUCTION

Cytokines play essential role in the pathogenesis of autoimmune diseases. The precise triggers for the breakdown of self-tolerance and the subsequent events leading to the induction of pathogenic autoimmune remain unclear for most of the autoimmune diseases. However, proinflammatory cytokines contribute to the initiation and propagation of autoimmune inflammation, whereas anti-inflammatory cytokines facilitate the regression of inflammation and recovery from acute phase of the disease. This mechanism is materialized by the Th1/Th2 interaction, which has had a major influence on most of studies about the role of cytokines in autoimmunity (Shachar I and Karin N, 2013). Indeed, the IL-17/Th17 axis has emerged as the major cytokine-driven immune events in the pathogenesis and treatment of autoimmunity (Lahdenpera A *et al*, 2011). In CD hence play a significant role in the development of an acute or chronic inflammatory response in several autoimmune diseases (Biancheri P *et al*, 2016).

### MATERIALS AND METHODS

This study included a total of 40 Iraqi Arab CD patients, their ages ranged between (2-55) years. They were attended the consultant clinic of Pediatric department in Pediatric Welfare Teaching Hospital/ Medical city and Al-Kadhimia Teaching Hospital, during October 2015 to March 2016. Studied cases were previously diagnosed by serological and histological tests. Also, as a control group, 20 apparently healthy persons were included in this study.

#### Serological testing included

Enzyme Immunoassay Kits, (Komabiotech, South Korea): intended to quantification IL-12 & IL-17A in serum of CD patients and healthy controls. Statistical tables including observed frequencies with their percentages, and summary statistic of the readings distribution (mean, SD, SEM, minimum & maximum). Student test (t-test) was used and also one way ANOVA in addition to post hoc test (Duncan test) were used to accept or reject the statistical hypotheses.  $P < 0.05$  is considered a Significant difference.

## RESULTS AND DISCUSSION

In current study, the serum levels of two cytokines (IL-12 and IL-17A) were assessed in celiac patients and healthy control, Table (1). Results reveal that serum level of IL-12 in CD patients ( $24.07 \pm 1.82$ ) pg/ml, are decreased significantly ( $p \leq 0.001$ ) as compared with healthy control group ( $46.35 \pm 2.34$ ) pg/ml. In contrast, this study recorded a significant increase of IL-17 levels in CD patients ( $88.25 \pm 6.44$ ) pg/ml, compared with healthy controls ( $37.08 \pm 2.36$ ) pg/ml.

Moreover, the connection between cytokines and gender among celiac patients and controls is obtainable in table (2). It has been found that there are no significant differences in levels of IL-12 among patients, between males (mean of  $25.27 \pm 3.15$ ) and females (mean of  $22.87 \pm 1.87$ ) compared with controls (mean of males  $49.68 \pm 3.36$ ) (mean of females  $43.01 \pm 3.06$ ), while the levels of IL-17A are presented a modest significant between males (mean of  $92.28 \pm 9.71$ ) and females (mean of  $84.21 \pm 8.63$ ) in category of patients and category of controls (mean of males  $42.83 \pm 2.44$ ) or (mean of females  $31.33 \pm 3.19$ ).

Cytokines play varied roles in CD by mediated in a complex Immunological processes encompasses dynamic interactions that leading to the break down of self-tolerance and the subsequent events in the induction of pathogenic responses of disease which still unclear like most other autoimmune diseases (Shachar I and Karin N, 2013 and Holdsworth S and Gan P 2015).

Some of previous studies had shown that CD is characterized by a strong Th1 response (Lahat N *et al*, 1999 and Hansson T *et al*, 1999). On the other hand, IL-12 which is a key cytokine in the Th1 response, has been revealed decreased levels in the in test in biopsies of CD patients (Nilsen E M *et al*, 1998 and DiSabatino A

*et al*, 2007), while other studies report elevated values in children with CD which decreased significantly after adapted of GFD (Bjorck S *et al*, 2015). Reduction in the serum level of IL-12 among CD patients may indicate that the Th1 bias of CD4+ T cells in CD immunity is probably depending on complex immunological-molecular mechanisms and different components (Carter L and Murphy K, 1999, Oppmann B *et al*, 2000, Szabo S *et al*, 2003 and El Kasmi K *et al*, 2007) This may be characterized by both elevated and down-regulated cytokine levels in serum within the specific type of Th cytokine group. In this study, elevated serum levels of IL-17A have been revealed in CD patients when compared with healthy controls similar to other studies were demonstrated significantly elevated of IL-17A in CD and reported that the T-cells in mucosa of untreated CD patients be gliadin-specific Th17 cells, like (Lahdenpera A *et al*, 2011; Monteleone I *et al*, 2010 and Fernandez S *et al*, 2011). Other workers had found that the T cells reactive to deaminated gliadin do not induce IL-17A secretion (Bodd M *et al*, 2010). These findings reinforced the vital role of IL-17A in disease pathogenesis, suggesting a systemically immunological-inflammatory response reflected as an increased response of proinflammatory cytokines in serum blood samples belong to CD patients. Differences in the results may due to stages of the disease, sample size of studied patients and ethnicity, where the levels of a particular immunological component in one population can be very different from that in another population due to ethnic differences (Pawelec G *et al*, 2014). Generally, difference between IL-12 and IL-17A concerning CD patients may reflect the cross-regulation between Th1, Th2 and Th17 by various immunomodulatory manners that has been found to restoring the cytokine balance by employing strategies to skew the cytokine response (immune deviation) to Th2

**Table 1 :** Serum level mean of IL-12 and IL-17A in celiac disease patients and controls

Interleukins	Celiac Disease Patients (No. = 40)			Healthy controls (No. = 20)			P-Value
	Mean $\pm$ S.E.	Min.	Max.	Mean $\pm$ S.E.	Min.	Max.	
IL-12	$24.07 \pm 1.82$	8.9	74.7	$46.35 \pm 2.34$	32.0	67.8	0.001
IL-17A	$88.25 \pm 6.44$	21.6	75.8	$37.08 \pm 2.36$	21.0	55.0	0.001

**Table 2 :** Serum level mean of IL-12 and IL-17A in celiac disease patients and controls distributed by gender

Interleukins	Mean $\pm$ Standard Error			
	Celiac Disease Patients (No.=40)		Controls (No.=20)	
	Males	Females	Males	Females
IL-12	$25.27 \pm 3.15$ B	$22.87 \pm 1.87$ B	$49.68 \pm 3.36$ A	$43.01 \pm 3.06$ A
IL-17A	$92.28 \pm 9.71$ A	$84.21 \pm 8.63$ A	$42.83 \pm 2.44$ B	$31.33 \pm 3.19$ C

Different letters represent significant difference ( $p \leq 0.05$ ) between means of rows (Duncan test)

in the case of a Th1-mediated disease. It seems relates to the relative roles of Th1 versus Th17 to immune pathology through contribution in the induction, progression and regression of auto immune disease that may vary in different approaches of the same disease. Further, the Th1 lineage is antagonistic for both Th2 and Th17, also both Th1 and Th2 can inhibit Th17 and Th17 in turn can control the activity of Th1 (Basso A S *et al*, 2009; Peck A and Mellins E D, 2010). Thus, the cytokine response in CD patients of this study were detected in peripheral blood was showed the complexity in immunity of CD which characterized by a combination of Th2 and Th17 as well as Th1 patterns.

Moreover, the studies have presented the correlation of gender and risk of autoimmune disease. Although this correlation is not fully understood, but there is increased prevalence of CD amongst women compared to men, with male: female ratio of 1:2.8 (Hin H *et al*, 1999). This could be due to the finding that men with CD were diagnosed at an older age (Hin H *et al*, 1999). Indeed, might be the production of sex hormones which influence immune responses (Muenchhoff M and Goulder P, 2014). Females seems probably to be Th1 response compared to the males which seem to be more Th2 response (Chen W *et al*, 2008).

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