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Assessment of IL-10 and TGF-β1 among Patients with Celiac Disease

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Abstract--- *Celiac disease (CD), also known as "celiac sprue," is a persistent inflammatory condition that involves the small intestine, with a incidence of 1% in the majority of population. This study aimed to investigate the role of some immune parameters such interlukin-10 (IL- 10) and Transforming growth factor-beta1 (TGF-β1) among patients with Celiac Disease. This study was conducted on a total of (75) individual in different sex and age group cases (26 males + 49 females) including (30) patients with Celiac Disease and (15) on a Gluten free diet and (30) healthy individuals. CD patients were recruited at Imam Sadiq Teaching Hospital, through the duration of the beginning of August 2019 till the end of December 2019. All patients diagnosed with CD by Anti-tTG test. The age range of the study population was from (5-75) years with mean age 32 years. Blood was withdrawn from a vein, for hematology analyzer (total WBCs count, total RBCs count and ESR) the serum was used for immunological tests including IL-10 and TGF-β1 by ELISA technique. The findings revealed that the total WBCs count and ESR were elevated significantly (p< 0.01) in CD patients as (mean= 11.56),(mean= 91) receptively, and CD patients had a significantly lower mean RBC count than healthy Group , 4.39 vs. 4.81 x103 cell/ml, respectively (p=0.01). GFD group had a significantly lower mean RBC count than healthy Group 4.49 vs. 4.81 103 cell/ml, respectively (p=0.01). Celiac Disease patients had a significantly higher mean of IL-10 than the healthy Group, 443.34 vs. 244.87, respectively. (P. value <0.01) GFD group also had a significantly higher mean of IL-10 than the healthy Group, 420.85 vs. 244.87, respectively. as (P. value < 0.01). GFD group had a significantly higher mean of TGF-β1 than CD patients, 1082.09 vs. 387.57, respectively (P. value = 0.01), GFD group also had a significantly higher mean of TGF-β1 than the healthy Group, 1082.09 vs. 72.39, respectively (P. value = 0.01), There was a significant direct (positive) correlation between IL-10 and TGF-b1 (p<0.01).*

Keywords--- *Assessment of IL-10 and TGF-β1, Patients with Celiac Disease.*

I. INTRODUCTION

Celiac disease (CD) is a chronic autoimmune-mediated enteropathy induced by the ingestion of dietary gluten which is a prolamin protein, found in wheat, rye, and barley in genetically susceptible individuals (Ludvigsson et al., 2012). Celiac disease affects about 1% of Western populations (Rubio-Tapia and Murray, 2010). and can occur at any age depending on the introduction of gluten in the diet. Nevertheless, onset of CD is more frequent in early childhood (Meresse et al., 2012). Anti-inflammatory cytokine IL-10, recognized as a significant immunomodulator in the intestinal tract, has been both up-regulated and unchanged in patients with celiac disease relative to controls in previous trials (Veenbergen and Samsom, 2012). IL-10 acts by interfering with antigen presentation and induces low responsiveness in gliadin specific T cells (Romero-Adrián, 2016). TGF-β1 expression increased have been observed

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in the lamina propria of children with intestinal villous atrophy, which refers to its significance in the pathogenesis of CD (Romero-Adrián, 2016, Forsberg et al., 2007).

II. MATERIALS AND METHODS

Patients Group

The collection of blood specimens was carried out during the period from the beginning of August 2019 till the end of December 2019 from 30 patients with Celiac disease whose ages ranged between (5-75) patients were recruited of Imam Sadiq Teaching Hospital. First, patients were interviewed directly by using an anonymous questionnaire which included the details and history of the patients. This study was in agreement with the ethics of Imam Sadiq Hospital and verbal informed consent were obtained from all participants.

GFD Group

The GFD group was composed of 15 individuals all had history of celiac disease and all of them were on a gluten free diet

Healthy Group

The Healthy Group was composed of 30 randomly healthy persons with the age ranging between (5-70) years. This Healthy Group was examined by ELISA. All Healthy Group was asked to fill a questionnaire and all had no family history of disease.

Blood Collection

Five milliliters of venous blood sample was taken from all study groups. Then the blood samples were divided into two portions. The first portion (2 ml) was transferred into an anticoagulant tube from both groups and immediately stored for use in the hematological analysis. The other portion (3ml) was transferred into a Gel tube for serum separation, the blood was left for about 30 minutes in room temperature for clotting and then centrifuged at 3000 g for 2 minutes. Then the serum was collected in a sterile eppendrofe tube in three repeaters and kept frozen at -80 C for the determination of Interlukin-10 (IL-10) and Transforming growth factor-beta1(TGF-β1) (Bonetti et al., 2010).

Anti-Tissue Transglutaminase (Anti-tTg) Antibody

Serum samples diluted 1:101 was incubated in the microplates coated with the specific antigen. Patient´s antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) was incubated and react with the antigen-antibody complex of the samples in the micro-plates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, that was stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample (Abrams et al.,2006).

Interleukin-10

The used kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IL-10 antibody. IL-10 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human IL-10 Antibody is added and binds to IL-10 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IL-10 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human IL-10. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm. The concentration of Human IL-10 is calculated by comparing the OD of the samples to the standard curve (Natarajan and Remick, 2008).

TGF-β1 Principle

The used kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human TGF-β1 antibody. TGF-β1 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human TGF-β1 Antibody is added and binds to TGF-β1 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated TGF-β1 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human TGF-β1. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm. The concentration of Human TGF-β1 is calculated by comparing the OD of the samples to the standard curve (Natarajan and Remick, 2008).

Total (WBCs) Count Procedure

- (Twenty dilution) of blood was done by adding (20µl) of blood to (0 38 ml) of diluting fluid in a (75×10 mm) glass or plastic tube.
- After tightly corking the tube, the suspension was mixed by rotating in a cell-suspension mixer for at least (1 min.).
- The Neubauer's counting chamber was filled by means of a Pasteur pipette or stout glass capillary.
- At least (100 cells) were counted in as many as (1 mm^3) areas (0 1 µl in volume). The leukocytes were counted in each of the four large (1sq mm.) corner squares (Al-Rasheid *et al.*, 2010).

ESR procedure

- (Forty ml) of sodium citrate mix genital with (160i ml) from whole blood in test tube.
- Aspirate the mixture of tube by Western green pipette and put in (ESR) rack.
- Waiting for one hour and read the result. (Bray et al., 2016).

Total (RBCs) Count Procedure

- RBCs counting solution is isotonic saline.
- Dilution of 1:200 with diluting solution and wait for 2 minutes to settle the cells.
- 40X was used to count the RBCs, the center square which has 25 smaller squares was used.
- The 4 corners of squares and one central square were counting.

- Repeat the count twice and divide by 2 to get the average Multiply factor = 10 x 200 / 0.2 = 10,000 (Pagana, 2017).

Statistical Analysis

Data of the study participants, CD patients, GFD group and Healthy group, were entered , managed and analyzed using the Statistical Package For Social Sciences (SPSS) version 25 software for windows, IBM, US, 2017. All variables were checked for errors or inconsistency prior to the analysis process.

Continuous variables included t, WBCs count, RBCs count, ESR, interleukin-10, and interleukin – 15 were tested for statistical normality distribution using histogram and normal distribution curves and they all appeared to follow the statistical normal distribution. (ANOVA) F test used to compare mean levels of these parameters. Level of significance (P. value) of 0.05 or less considered significant. Finally, results and findings presented in tables and or figures accordingly, using the Microsoft Word application 2010 for windows (Al-Rawi, 2000).

III. RESULTS AND DISCUSSION

Total White Blood Cell Count

The mean value of WBCs count of Celiac Disease patients and GFD Group and healthy Group revealed that Celiac Disease patients had a significantly higher mean of WBCs count when compared to the healthy Group , 11.56 x103 cells /ml vs. 8.37 x 103 cells/ml, respectively, (P-value<0.01). Celiac Disease patients also had a significantly higher mean of WBCs count when compared to the GFD Group, 11.56 x103 cells /ml vs. 9.36 x103 cells /ml, respectively, (P-value <0.01). as shown in table 1 and figure 1.

Table 1: Comparison of Mean Values of WBCs Count, RBC Count and ESR of CD Patients, GFD Group and Healthy Group

		Groups		
Parameter		**CD Patients (n=30)**	**GFD (n=15)**	**Healthy Group (n=30)**
WBCs count (x 103 cell/ml)	Mean	11.56	9.36	8.37
	SD	3.50	2.90	2.68
	F		8.21	
	P.Value	0.00 *Sig*		
	LSD	1.90		
ESR mm/hr	Mean	91.00	8.13	6.13
	SD	17.64	3.76	2.54
	F		488.62	
	P.Value	0.00 *Sig*		
	LSD	7.08		
RBCs count (x 106 cell/ml)	Mean	4.39	4.49	4.81
	SD	0.42	0.47	0.59
	F	5.43		
	P.Value	0.01 *Sig*		
	LSD	0.31		

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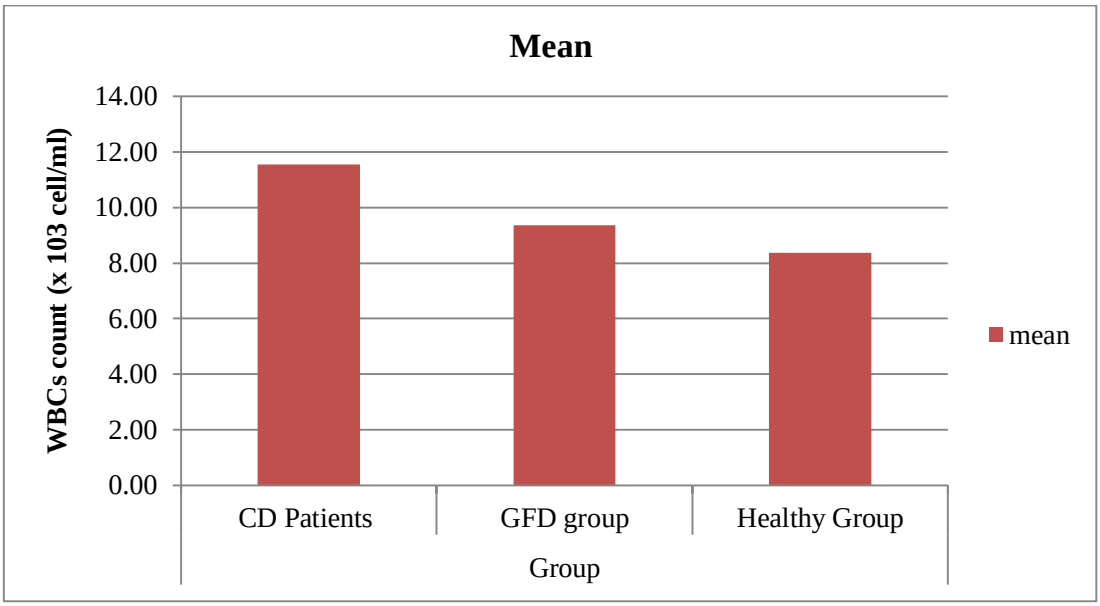

Figure 1: Graphical Comparison of the Mean WBCs Count of CD Patients and GFD Group and Healthy Group

Erythrocyte Sedimentation Rate

Celiac Disease patients had a significantly higher mean ESR level than healthy Group 91 vs. 6.13 mm/hr, respectively (P<0.01). Celiac Disease patients also had a significantly higher mean ESR level than GFD group 91 vs. 8.13 mm/hr, respectively (P<0.01). as shown in table 1 and figure 2. No significant difference was found between healthy Group and GFD group as shown in table 1. Rampertab et al., 2004. conducted a study revealed that CD is an inflammatory bowel disorder with chronic inflammatory effects as demonstrated by elevation of ESR, including values > 100. The ESR declines with the concomitant GFD. CD will also be considered for the differential reatment of elevated ESR.

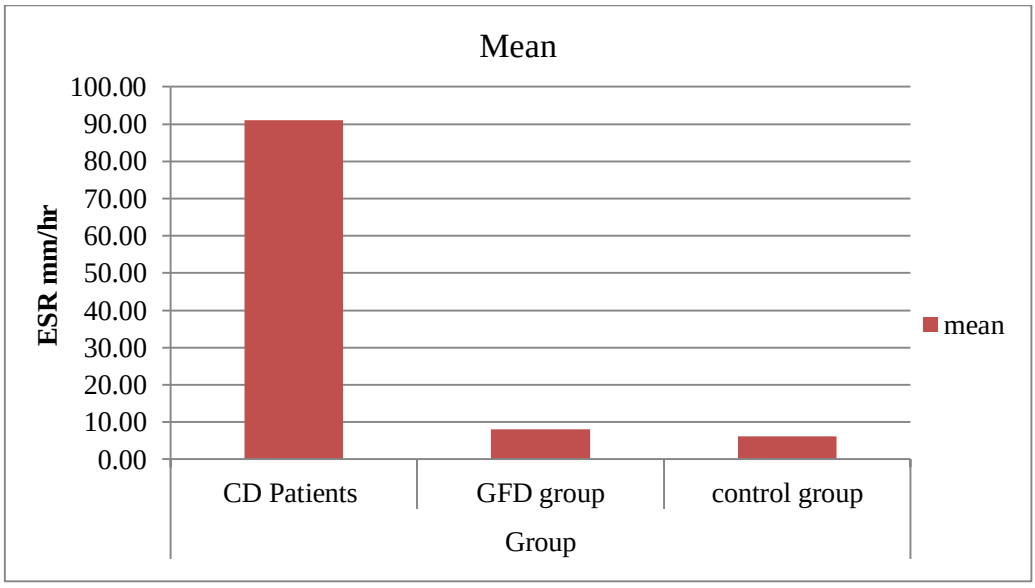

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Figure 2: Graphical Comparison of the Mean ESR Level of CD Patients, GFD Group and Healthy Group

Total Red Blood Cell Count

Celiac disease patients had a significantly lower mean RBC count than healthy Group , 4.39 vs. 4.81 x103 cell/ml, respectively (p=0.01). GFD group had a significantly lower mean RBC count than healthy Group 4.49 vs. 4.81 103 cell/ml, respectively (p=0.01). as shown in table 1. Anemia is a common occurrence in CD patients and can be a presenting characteristic.

Anemia may be the only abnormality found. Anemia has been particularly common in patients with uncontrolled CDs in the past but is still normal in non-diagnosed adults. Anemia is typically hypoproliferative, indicating poor processing of essential nutrients such as iron and multiple vitamins.

The incidence of anemia ranges significantly from report to report and was observed in 12 to 69 percent of newly diagnosed CD patients (Unsworth, Lock and Harvey et al., 1999).

Interleukin-10 (IL-10)

Celiac disease patients had a significantly higher mean of IL-10 than the healthy Group, 443.34 vs. 244.87, respectively. (P. value <0.01) GFD group also had a significantly higher mean of IL-10 than the healthy Group, 420.85 vs. 244.87, respectively. as (P. value < 0.01), There was no significant difference in the mean of IL-10 between Celiac disease patients and GFD group as shown in Table 2

Production of IL-10 in duodenum during Active celiac disease (ACD) has been confirmed, suggesting that post-challenge development of IL-10 could be part of a typical celiac reaction (Forsberg et al., 2007)

Table 2: Comparison of Mean Values of IL-10 and TGF-β1 of CD Patients, GFD Group and Healthy Group

		Groups		
Parameter		**CD Patients (n=30)**	**GFD group (n=30)**	**Healthy group (n=30)**
IL-10	Mean	443.34	420.85	244.87
	SD	384.22	208.14	172.70
	F	4.14		
	P.value	0.02 Sig		
	LSD	175.20		
TGF-β1	Mean	387.57	1082.09	72.39
	SD	830.49	1974.14	85.45
	F	4.91	1974.14	85.45
	P.value	0.01 Sig		
	LSD	631.64		

Transforming Growth Factor (TGF-β1)

GFD group had a significantly higher mean of TGF-β1 than CD patients, 1082.09 vs. 387.57, respectively (P. value = 0.01), GFD group also had a significantly higher mean of TGF-β1 than the healthy Group, 1082.09 vs. 72.39, respectively (P. value = 0.01), as shown in Table 2

Romero- Adriàn, 2015. It has been shown that the expression Transforming Growth Factor-β1 (TGF-β1) has been increased in lamina propria in children with intestinal villous atrophy, which is considered significant for CD

pathogenesis.

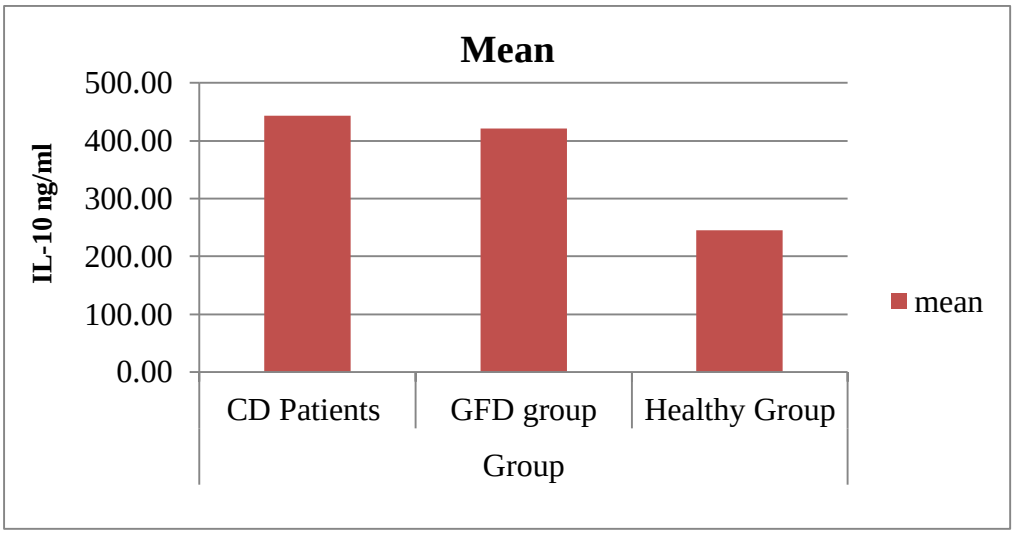

Figure 3: Graphical Comparison of the Mean IL-10 Level of CD Patients, GFD Group and Healthy Group

Gluten-free diet maintains the natural hormonal function of TGF-b in the intestinal epithelium of uncomplicated celiac disease. In fact, TGF-b1 released by TCRgd+ NKG2A+ intraepithelial lymphocytes in treated celiac patients reduces the expression of IFN-g, granzyme B and NKG2D by TCRab+ intraepithelial lymphocytes, thus reducing their pro-inflammatory and pro-apoptotic potential (Biancheri et al., 2014).

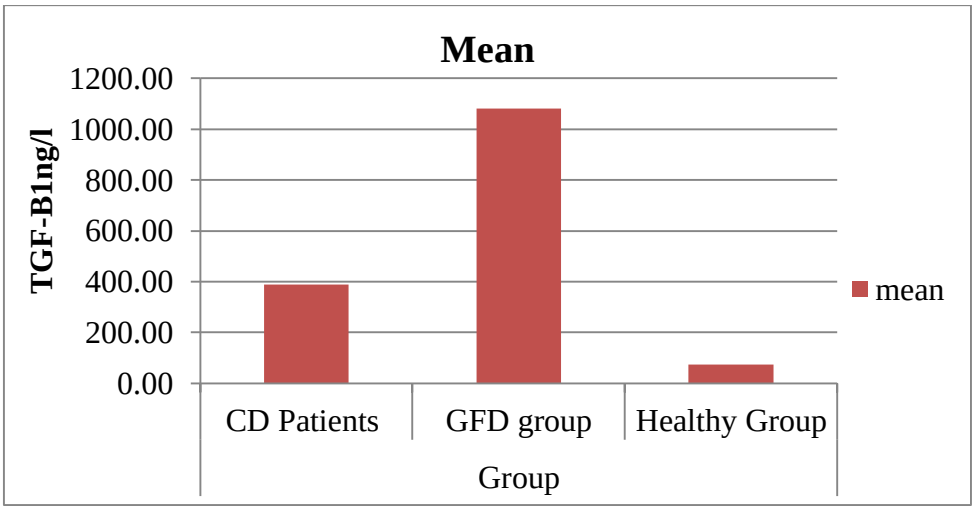

Figure 4: Graphical Comparison of the Mean TGF-β1 Level of CD Patients, GFD Group and Healthy Group

IV. Correlation Analysis Among IL-10 And TGF-B1

The results of correlation analysis showed that there is as significant correlation is a direct (positive) between IL-10 and TGF-β1 ($p<0.01$).

IL-10 and TGF-b play a non-redundant function in sustaining intestinal homeostasis (Biancheri et al., 2014). During TGF-b signalling, the IL-10 acts both upstream and downstream. For example, IL-10 can induce TGF-b

expression and secretion of lamina propria in T cells. In fact, it cooperates with TGF-b to facilitate the division of T-reg cells that generate more TGF-b and IL-10 cells. (Harrison and Powrie, 2013). Romero- Adriàn, 2016. Appointed that the proportion of TGF-β mRNA samples from Active CD patients was higher than from controls. The same thing happened to the IL-10. The amount of cytokine can be a significant marker of disease activity.

Ethical approval

All authors hereby declare that all actions have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

CONFLICT OF INTERESTS

The authors did not declare any conflict of interest.

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