Relationship Between Interleukin -33(IL-33) and C- Reactive Protein in Iraqi Women Patients with Celiac Disease.

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Abstract

Interleukin -33 is a new member of the IL-1 superfamily of cytokines that is expressed mainly by stromal cells.Its expression is upregulated following pro-inflammatory stimulation. Aim of the present study was to assess the serum IL-33 level and its relationship with inflammatory biomarker CRP in Iraqi females patients with celiac disease. Thirty five patients with celiac disease (CD) and thirty healthy individuals as control group were enrolled in this study,their age ranged (20-35) year.Anti-Gliadin IgA ,IgG and Anti-Tissue IgA ,IgG were estimated in all subjects as diagnostic parameters .ESR and CRP were assayed as inflammatory biomarkers. IL-33 was determined in patients and control groups.

The results of the present study revealed a highly significant increase in anti-gliadin (IgA , IgG) , anti-tissue (IgA, IgG) ,ESR,and CRP levels in CD patients compared to control. While no significant increase in IL-33 level in CD patients compared to control was found.

In conclusion, IL-33 could be considered as a biomarker for Celiac Diseases, and it is correlated positively with inflammatory biomarker"CRP" in Iraqi women patients with CD.

Key words: IL-33 , Celiac disease , C- Reactive Protein, autoimmune inflammatory diseases.

Introduction

Interleukin-33 (IL-33) is the 11th member of IL-1 cytokine family which includes IL-1 and IL-18. Unlike IL-1β and IL-18, IL-33 is suggested to function as an alarmin that is released upon endothelial or epithelial cell damage and may not enhance acquired immune responses through activation of inflammasome. ST2, a IL-33 receptor component, is preferentially expressed by T-helper type (Th) 2 cells, mast cells, eosinophils and basophils, compared to Th1 cells, Th17 cells and neutrophils[1].Identification of an IL-1 family member that we have named IL-33 (IL-1F11)[2]. The human gene is located on chromosome 9p24.1, while its mouse counterpart can be found on the syntenic chromosome 19qC1 region. The IL-33 cDNA sequences encode 270 and 266 amino acid polypeptides for human and mouse, respectively, corresponding to full-length proteins with calculated masses of 30 and 29.9 kDa. IL-33 is expressed as a prodomain containing polypeptide. Incubation of *in vitro* translated IL-33 with caspase-1 led to the production of mature IL-33 with a mass of 18 kDa as determined by SDS-PAGE. Human and mouse IL-33 are 55% identical at the amino acid level. The IL-1 family member is most closely related to IL-33 is IL-18 [3]. Using sequence and secondary structure alignment, Girard and colleagues demonstrated that IL-33consists of a homeodomain like helix turn helix motif (HTH) on the aminoterminal side [4].

Interleukin-33 (IL-33) (NF-HEV) is a chromatin-associated nuclear cytokine from the IL-1 family, which has been linked to important diseases, including asthma, rheumatoid arthritis, ulcerative colitis, and cardiovascular diseases[5]. IL-33 signals through the ST2 receptor and drives cytokine production in type 2 innate lymphoid cells (ILCs) (natural helper cells, nuocytes), T-helper (Th)2 lymphocytes, mast cells, basophils, eosinophils, invariant natural killer T (iNKT), and natural killer (NK) cells.Emma and others recently reported that, unlikeIL-1β and IL-18, full-length IL-33 is biologically active independently of caspase-1 cleavage and that processing by caspases results in IL-33 inactivation [6]. Interleukin -33 is a new member of the IL-1 superfamily of cytokines that is expressed by mainly stromal cells, such as epithelial and endothelial cells, and its expression is up regulated following pro-inflammatory stimulation. IL-33 can function both as a traditional cytokine and as a nuclear factor regulating gene transcription. It is thought to function as an ‘alarmin’ released following cell necrosis for alerting the immune system to tissue damage or stress.It mediates its biological effects via interaction with the receptors ST2 (IL-1RL1) and IL-1 receptor accessory protein (IL-1RAcP), both of which are widely expressed, particularly by innate immune cells and T helper 2 (Th2) cells[7,8].

Celiac disease (CD)is an autoimmune inflammatory disease of the small intestine precipitated by the ingestion of gluten, a component of wheat protein, in genetically susceptible persons. Classically, the disease manifests with diarrhea, weight loss and anemia. There are very few reports of osteomalacia as the presenting symptom, and even fewer of osteomalacia as the only symptom of celiac disease at presentation[9]. Celiac disease is an immune-mediated enteropathy characterized by intolerance to gluten. Celiac disease is usually characterized by various gastrointestinal (GI) symptoms (e.g. diarrhea, malabsorption and weight loss) associated with consumption of grains containing gluten (wheat, barley and rye). Although some CD patients may have primarily GI symptoms, CD may be detected due to associated extraintestinal disorders, even without GI symptoms, or due to screening for CD based on a positive family history[10]. Celiac disease is an autoimmune enteropathy triggered by the ingestion of gluten. Gluten sensitive individuals (GS) cannot tolerate gluten and may develop gastrointestinal symptoms similar to those in CD,but the overall clinical picture is generally less severe and is not accompanied by the concurrence of tissue trans glutaminase autoantibodies or autoimmune comorbidities[11].

Until a few years ago, celiac disease (CD) was thought to be a rare food intolerance that was confined to childhood and characterized by severe malabsorption and flat intestinal mucosa. Currently, CD is regarded as an autoimmune disorder that is common in the general

population (affecting 1 in 100 individuals), with possible onset at any age and with many possible presentations[12]. The identification of CD is challenging because it can begin not only with diarrhea and weight loss but also with a typical gastrointestinal (constipation and recurrent abdominal pain) and extra-intestinal symptoms (anemia, raised transaminases, osteoporosis, recurrent miscarriages, aphthous stomatitis and associated autoimmune disorders), or it could be completely symptomless[13]. Over the last 20 years, the diagnostic accuracy of serology for CD has progressively increased with the development of highly reliable tests, such as the detection of IgA tissue transglutaminase and antiendomysial and IgG antideamidated gliadin peptide antibodies [14].

As far as to our knowledge,there is no data in the litreture concerning the serum level of IL-33 in patients with celiac disease.Thus the present study aimed to shed a light on the role and level of IL-33 and its relationship with CRP in such patients,which could exploure at least in part the contribution of this interleukin to the pathogenesis of the disease.

Materials and Methods

Patients and Control

Serum samples were obtained from (35) Iraqis women patients with celiac disease (CD), and (30) healthy individuals as a control group, age range (20-35) year, which enrolled in this study. The patients attended the Specialized Center for Endocrinology and Diabetes,Kindy Hospital,Baghdad, during the period from October to January, 2012.Serum was frozen until used for analysis.

Measurement of Anti-Gliadin IgA and IgG

Anti-Gliadin IgA and IgG are an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgA and IgG class autoantibodies against Gliadin in human serum or plasma.The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of celiac disease and dermatitis herpetiformis[15].

Measurement of Anti-Tissue IgA and IgG

Anti-Tissue-Transglutaminase IgA and IgG are an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgA and IgG class autoantibodies to tissue transglutaminase(tTG) in human serum or plasma.The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of celiac disease and dermatitis herpetiformis [16].

Measurement of Serum IL-33

Serum IL-33 levels were measured using specific enzyme-linked immunosorbent assay (ELISA) . kit (Ray Bio Human IL-33 for *in vitro* quantitative measurement of human IL-33 in serum) ,according to the manufactures protocol.[17]

Inflammatory Biomarkers Assessment

ESR and CRP were measured for patients and to the control group as inflammatory biomarkers[17].

Statistical Analysis

The data was expressed as mean ± SEM (Standar Error of Mean). The comparison between patients group and control group were analyzed by using student t-test . Pearson 's correlation

coefficient was used to examine between IL-33 and study parameters in patients group. P-value of < 0.001 and < 0.05 were considered highly significant and significant respectively.

Results and Discussion

Table (1) shows levels (means ± SEM) of Anti- Tissue and Anti- Gliadin in serum of control and women patients with celiac disease groups.

In the current study,the results in table (1) revealed highly significant ($P < 0.001$) increase in Anti-Tissue (IgA , IgG) and Anti- Gliadin (IgA ,IgG) levels in serum of women patients with CD when compared with control. These results are in agreement with reported results showing that CD is associated with an increase in Anti-Tissue and Anti-Gliadin[18].

The results in table (2) shows the levels of serum IL-33, CRP,and ESR in both of control and women patients with CD.There was no significant ($P > 0.05$) increase in serum IL-33 level in women patients with CD when compared to control group .There were a highly significant ($P< 0.001$) increase in serum CRP and ESR levels in women patients with CD compared with healthy control.These results are in agreement with those obtained by[19].

Table (3) shows the correlation between IL-33 and other studied parameters ,which revealed no significant ($P> 0.05$) positive correlation between IL-33 and CRP (Fig.1) ,Anti-gliadin(IgA) (Fig.2),while there was no significant ($P> 0.05$) negative correlation between IL-33 and ESR (Fig.3),Anti-tissue(IgA) (Fig. 4),(IgG) (Fig.5) ,and Anti-gliadin(IgG) (Fig.6).

This is the first study which found the elevation in IL-33 level in patients with Celiac disease, which is the only treatable autoimmune disease by restrickting diet without glutin.Interleukin-33 appears to be a crucial cytokine for Th2-mediated host defense and plays a central role in controlling immune responses in barrier tissues such as skin and intestine. It is able to activate cells of both the innate and adaptive immune system, and depending on the disease can either promote the resolution of inflammation or drive disease pathology.Interleukin -33 is a new member of the IL-1 superfamily of cytokines that is expressed by mainly stromal cells, such as epithelial and endothelial cells, and its expression is upregulated following pro-inflammatory stimulation. IL-33 can function both as a traditional cytokine and as a nuclear factor regulating gene transcription [20].

Interleukin-33 is thought to function as an 'alarmin' released following cell necrosis for alerting the immune system to tissue damage or stress[20].By reviewing the references,there is no data in the literature concerning the serum level of IL-33 in patients with celiac disease,but there are some studies which stated that Inflammatory bowel disease (IBD) is a group of chronic inflammatory conditions of the colon and small intestine, including ulcerative colitis (UC) and Crohn's disease, resulting from dysregulated immune responses. Serum IL-33 and sST2 levels were elevated in UC patients compared with controls[21]. Marina García ,etal in their paper review the role of the IL-33/ST2 system in innate immunity of the intestinal mucosa and its importance in inflammatory bowel diseases, especially ulcerative colitis[22].Patients with active CD and those on gluten-free diet GFD with positive antibodies had significantly higher levels of proinflammatory cytokines, such as interferon-, interleukin(IL)–1, tumor necrosis factor, IL-6 and IL-8, and also Th-2 cytokines such as IL-4 and IL-10, compared withnormal controls[23].Some researchers investigated that ,there was associations between genetic polymorphisms in *IL-33, IL1R1* and risk for inflammatory bowel disease[24].Recently results showed a significant increase in IL-33 level in serum of patients with hyperthyroidism, knowing that is a well established autoimmune diseases [25].

Celiac disease, also known as gluten-sensitive enteropathy and nontropicalsprue, is a prevalent autoimmune disorder that is triggered by the ingestion of wheat gluten and related proteins of rye and barley in genetically susceptible individuals[26]. The immune response in celiac disease involves the adaptive, as well as the innate, and is characterized by the presence

of anti-gluten and anti-transglutaminase 2 antibodies, lymphocytic infiltration in the epithelial membrane and the lamina propria, and expression of multiple cytokines and other signaling proteins. The disease leads to inflammation,(villous atrophy, and crypt hyperplasia in the small intestine[26].CRP is a major inflammatory cytokine that functions as a nonspecific defense mechanism in response to tissue injury or infection[27].

Conclusion

From this study, a conclusion could be drawn, that IL-33 could be considered as a biomarker for Celiac Diseases, and it is correlated positively with inflammatory biomarker CRP in Iraqi women patients with CD.

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Table (1):Diagnostic Parameters (Anti-tissue and Anti-gliadin) levels (mean ± SEM)for Control and Women Patients with Celiac Disease .

Parameters / Groups	Anti-tissue U/ml		Anti-gliadin U/ml	
	IgA	IgG	IgA	IgG
Control No.(30)	5.45±0.48	7.63±0.58	5.56±0.52	7.06±0.73
(CD) Patients No.(35)	153.46±76.84 *HS*	126.11±43.09 *HS*	102.08±26.96 *HS*	129.83±33.80 *HS*

HS highly significant($P < 0.001$)

Table (2):Serum IL-33 , CRP , and ESR levels (mean ± SEM) in Control and Patient with Celiac Disease (CD).

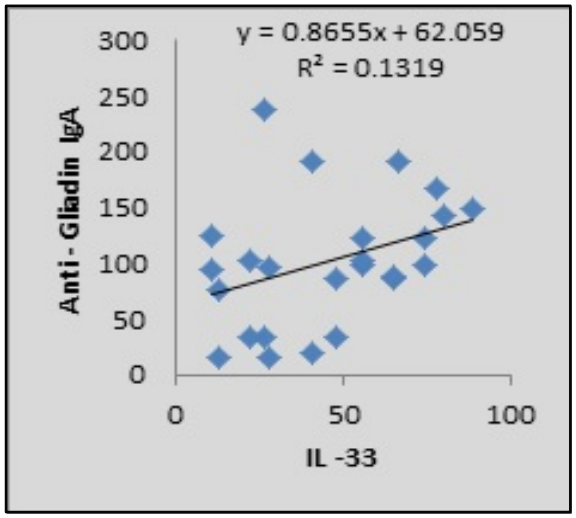
Groups / Parameters	Control No.(30)	(CD) Patients No.(35)
IL-33 (Pg/ml)	42.12 ± 22.85	151.41 ± 100.35 *NS*
CRP (mg/L)	3.3 ± 0.52	17.2 ± 1.16 *HS*
ESR (mm/h)	6.4 ± 0.91	30.1 ± 0.65 *HS*

HS highly significant($P < 0.001$), NS No significant($P \geq 0.05$)

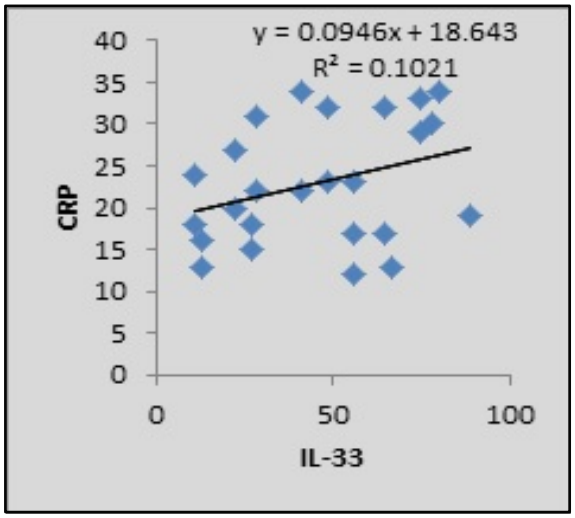
Table (3) :Correlation Between Interleukin-33 (IL-33) and the Study Parameters.

Pearson Correlation	CRP mg/L	ESR mm/L	Anti-tissue		Anti-gliadin	
			IgA	IgG	IgA	IgG
IL-33 Pg/ml	0.196 *NS*	- 0.185 *NS*	-0.149 *NS*	-0.070 *NS*	0.016 *NS*	-0.154 *NS*

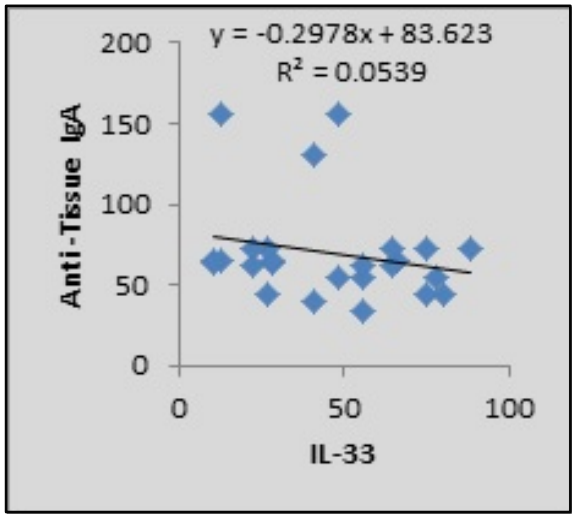
NS No significant($P \geq 0.05$)



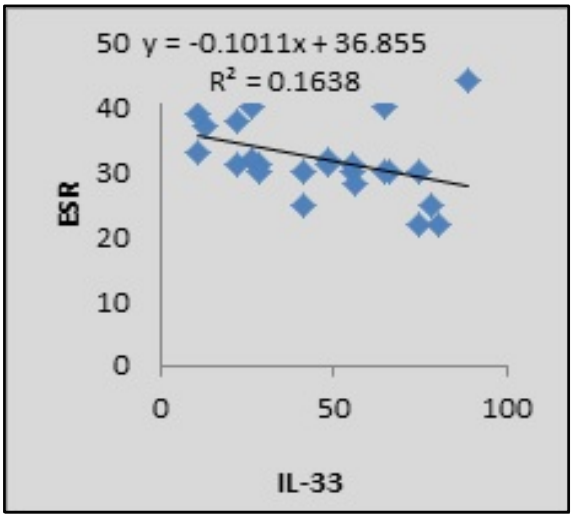
Figure(1):correlation between IL-33 and CRP



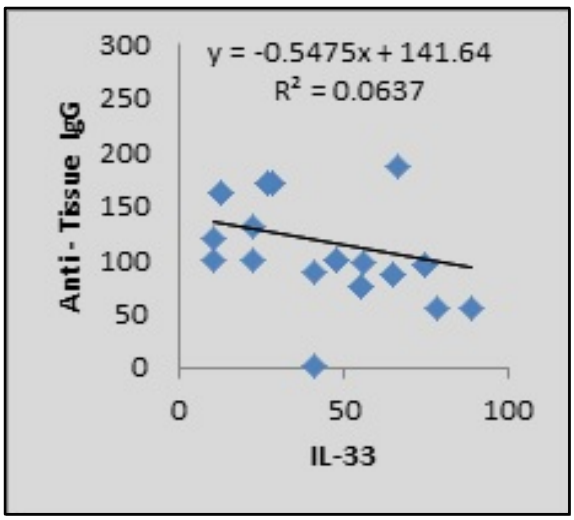
Figure(2):correlation between IL-33 and Anti-Gliadin IgA



Figure(4):Correlation between IL-33 and Anti-Tissue IgA



Figure(3):Correlation between IL-33 and ESR



Figure(6):correlation between IL-33 and Anti-Gliadin IgG

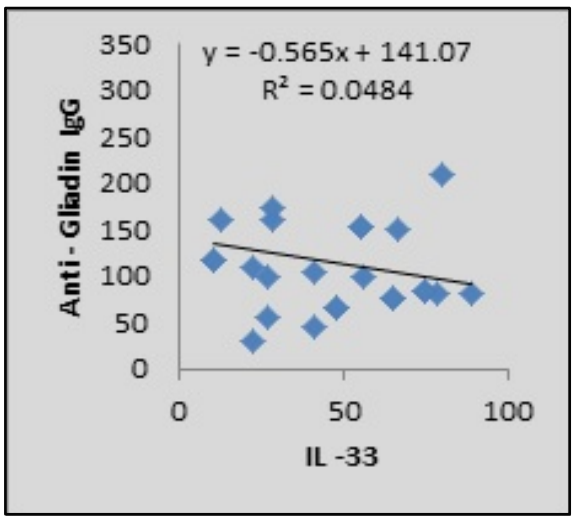


Figure (5):correlation between IL-33 and Anti- Tissue IgG

الطور الحاد (بروتين سي التفاعلي) لدى العلاقة بين الانترليوكين-33 وبروتين النساء العراقيات المصابات بمرض حساسية الحنطة

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استلم البحث في : 7 تشرين الأول 2013 ، قبل البحث في :26 كانون الأول 2013

الخلاصة

الهدف من هذه الدراسة هو تقييم مستوى IL-33 وعلاقته مع علامة الالتهاب البايلوجية"CRP " في مصل النساء العراقيات اللاتي يعانن من مرض الداء الزلاقي والمعروف بـــ (حساسية الحنطة).

تتعامل هذه الدراسة مع (35) مريضة تعاني من مرض حساسية الحنطة (CD) فضلا عن (30) شخصا من الاصحاء بوصفهم مجموعة تحكم في هذه الدراسة ،معدل اعمارهم مابين (20- 35) سنة. شخصوا من خلال قياس مستوى Anti-GliadinIgA ,IgG and Anti-Tissue IgA ,IgG . كما قيس مستوى IL-33 وعلامات الالتهاب البايولوجية (ESR,CRP) في امصال المرضى والمجموعة الضابطة.

وأظهرت نتائج الدراسة الحالية ان هناك زيادة معنوية كبيرة في مستوى Anti-Gliadin IgA ,IgG and Anti-Tissue IgA,IgG ومستوى ESR,CRP ، بينما توجد زيادة غير معنوية في مستوى IL-33 في امصال مرضى حساسية الحنطة مقارنة مع المجموعة الضابطة .

نستنتج من هذه الدراسة ان IL-33 هو علامة بايولوجية لمرض حساسية الحنطة ،ويرتبط بشكل ايجابي مع علامة الالتهاب البايولوجية CRP لدى النساء العراقيات المرضى بحساسية الحنطة (CD) .

الكلمات الافتتاحية : IL-33،مرض حساسية الحنطة ، امراض المناعة الذاتية ، CRP