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Investigation The Immunological And Genetic Role Of Interleukin 17 For Some Diabetes Patients In Diyala Governorate,

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ABSTRACT

Diabetes mellitus is chronic metabolic disorder, It is characterized by deficiency or fail in keeping normal level of glucose. This study aimed Investigation the immunological and genetic role of interleukine 17 in some diabetes patients in Diyala governorate in Iraq. Detection levels of IL-17 by using ELISA technique, Detection polymorphism of cytokine gen IL-17 by using PCR-RFLR technique. The Results of the study showed that the number female patients were higher than male patients without significance different between groups of study for the gender while the results showed that significance different in average age of patients and control group. The result of our study show high significance different in level of IL-17 in diabetes patients compared to control, Also, as well level of the IL-17 was increased with the increased age and increased in female compared to male in study groups. Genetic polymorphism of for IL-17 gene was inspected at the position (rs2275913), which thre genotypes, (GG, and AG) T1DM and T2DM patients and controls while third genotype AA (13.33%) show in T1DM patients only. AG genotype showed increased in T1DM (40.00%) and T2DM (66.66%) patients compared to controls while GG (80%) showed increased in controls with significant difference between genotypes in study groups T1DM (46.66%) and T2DM (33.33%) patients . Also showed positive correlation between genetic patterns of *IL-17* with IL-17 level.

Keywords: Diabetes mellitus, interleukine-17, *IL-17*, Genotyping, polymorphism, PCR-RFLR

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INTRODUCTION

According to ADA, 2016 there are mainly two types of diabetes mellitus Type 1 diabetes Mellitus (T1DM) it is causes approximately for 5-10% of all types of diabetes. It is a chronic genetic autoimmune disease. caused by T-cell mediated immune that causes pancreatic β-cell destruction and that leading to defects or stop insulin secretion and insulin dependence occurs (Cernea and Herold, 2010 ; William *et al.,* 2002).

Type 2 diabetes Mellitus (T2DM) is a multifactorial, chronic disease and occurs due to a mixed of hazard factors are environmental, and genetic factors. Type 2 diabetes is the most common form of diabetes, responsible for (90%-95%) of people of all types of diabetes (Miyawaki *et al.,* 2016). This the type occur and develops when the body fails to use the insulin due to insulin resistance (IR) with graual loss to ability beta cells to make enough insulin (Karuna**,** *et. al.,* 2013).

To Cytokines large role in pathogenesis of diabetes Mellitus, one of the cytokines is IL-17 that described as inflammatory cytokine. The biological role of IL-17 in T1DM that by destroying pancreatic beta cells at the onset of diabetes by stimulating increased immune response to beta cells, and also through an increase in the effectiveness of phagocyte cells Macrophages and effective T cells and other cells to secretion of inflammatory cytokines such as IL-1B, TNFA and IFN-E, which work on the other immune cells for the death of beta cells (Jin and Dong, 2013). Either the role of IL-17 in event of T2DM as proinflammatory cytokine where the pancreatic β-cell like other cells have strong defense mechanisms that contribute to the defense against infection (mostly virus) where leading to local inflammatory and cause insulin resistance defect in pancreatic β-cell. Where show result of studies to IL-17 shares in inflammatory process of T2DM, where IL-17 have crucial role in causes this the type of Diabetes, that found this the studies elevation level of IL-17 in T2DM patients (Randow *et al*., 2013 ; Chen *et al*., 2016).

IL-17 is a protein encoded by *IL-17* which consists of 1874 pairs of base (Yao *et al*.,1995), Located on the short arm for chromosome number 6 at site 12 consists three exons and the two introns (Chen *et al*., 2006). Although the IL-17gene, especially on-site (rs2275913), is proving to be many diseases, the effect is to be a factor in this report, which is signed in the logic of Promote IL-17 to produce that is IL-17 not clear good and the effect of this site of the gen on the IL-17 is still under discussion **(**Quan *et al.*, 2012). The existence of a correlation between IL-17gene (*IL-17*) and the increase in the incidence of diabetes has been raised through the replication of gene patterns that have proved to be linked to these genetic patterns in diabetes compared to the healthy*,* Therefor this study aimed to Investigation the immunological and genetic role of interleukine 17 for some diabetes patients in diyala governorate.

MATERIALS AND METHODS

Experimental design and Blood sampling: This study was conducted in diyala governorate province for Iraqi Arab Patients with diabetes mellitus type I and type II and started from Octoper 1, 2017 to June 17 2018. A total (90) blood sample, (30) sample collected from patients with diabetes mellitus type I where and (30) sample with diabetes type II also (30) samples were collected as control group.

Cytokine Assays. Serum concentrations of IL-17 measured using commercially available enzyme-linked immunosorbent assay ELISA kit.

Data extraction: Blood collected in tube contain EDTA and DNA of patients and controls was separated from blood by Go Taq® Green Master Mix Kit. The DNA concentration and quality of the DNA were analyzed by optical density in a Nano drop.

Genotyping

Primers Preparation: New Primer is designed on a Plus Primer3 program to suit the study interested area and has been accoutering by Alpha DNA company these primers are deicing with distilled water deionized distilled water to prepare the storage solution (100 pmol /µl)

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	primers	Sequence of primers
IL-17 rs2275913	F primer	5'- CTTGGTAGCATGCAGGGTTG -3'
	R primer	5'- ATGCCCACGGTCCAGAAATA -3'

Where and athermal gradient was made to get the optimum temperature was (Show as in Results and Discussion). The PCR amplification was performed in a total volume of 25µl mixture containing: Green Master 12µl, DNA template 3µl, Forward primer (10pmol) 1µl, Reveres primer (10pmol) 1µl, and dd water 8µl.

Than Conducted electrophoresis to PCR products were on 1.5% agarose with ethidium bromide staining. Than digested products to hour at 37C with enzyme *AvaII* were shown to be digested into 3 genetic patterns (GG, AG and AA) of the *IL-17.*

RESULTS AND DISCUSSION

Distribution of studys groups according to age

The results of study showed that significant difference in the age average between in study groups as shown in figure (1).

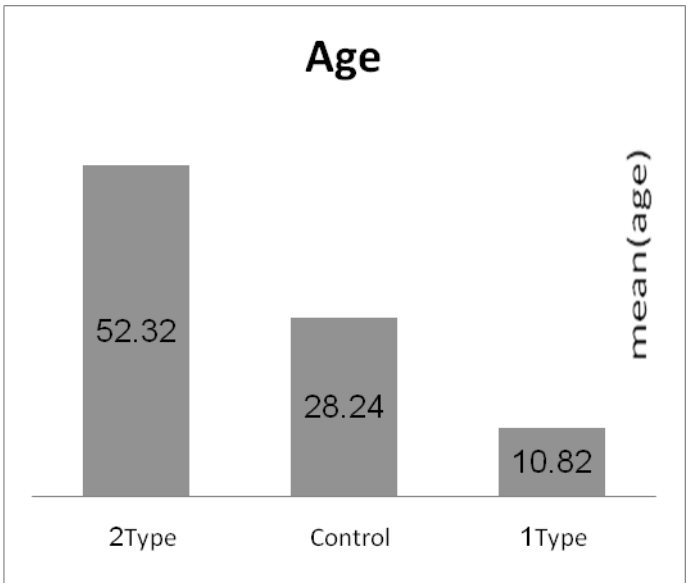

Figure 1: Distribution of studys groups according to age

And showed the results of the present study that the incidence of the first type of diabetes for ages (10-19) year was high, Due to the physical changes occurring in adulthood and the high level of genderual hormones as well as the need of peripheral devices to Insulin is also due to It may also be due to the impact of the environment such as healthy diet, living conditions and stress (Weets *et al.,* 2007). Either for Diabetes Type II, during age, physical activity, increase in fat mass and accumulation of fat in the visceral region and become more in this region, increasing insulin resistance and thus enhancing the risk of diabetes type II (Akram, 2013).

Distribution of studys groups according to Gender

The study showed that the proportion of females with diabetes and both type is more than that of males, without significant difference as shown in table (1). This can be due to the hormonal effect in the case of diabetes for women that is more than men and the feminine genderual hormonal differences are due to

endocrinopathy impairment, menstrual cycle menstrual, pregnancy, menopause and contraceptives Hormonal Contraceptive, as we are aware, there is a correlation between the functions of immune cells and of the receptor and the receptors of genderual hormones through the influence of receptors on these cells (Watson And Gametchu, 2001).

Table 1: Distribution of studys groups according to Gender

Gender of study groups			
study groups		**Male**	**Female**
Type 1	Count	11	19
	%	12.22	21.11
Type 2	Count	13	17
	%	14.44	18.88
Control	Count	16	14
	%	17.77 %	15.55
Total	Count	40	50
	%	.4444	.5555
p value		0.871^{NS}	
NS= No statistically significant difference P>0.05			

Estimation of IL-17A in the of study Groups

The results of the our study showed an increases IL-17 level in patients with diabetes for both types compared with the control group, as shown in Table (2) with high significance difference.

Table 2: level of IL-17A in the of study Groups

study groups	**Count**	**IL-17 pg/ml Mean± SD**
Type 1	N= 30	139.00±15.77
Type 2	N= 30	101.18±7.56
Control	N= 30	64.16±1.87
P-value		0.000**
**= high statistical difference P≤0.01		

Most studies support the large role played by IL-17 in breaking down beta cells that produce insulin for people with T1DM. In T1DM, $CD4^+$ T lymphatic cells are differentiated into TH17 cells in the pancreas tissue containing cells β Cells that are destroying beta cells mediated by the secretion of IL-17 as well as $CD4^+$ T cells can also differentiate into Th1 and Th2. (Grieco *et al.,* 2014), As confirmed that IL-17 contributes to the cause

of diabetes type I through two mechanisms that IL-17 exacerbates the programmed death of the beta cell as well as he is aware of the increase that the crown of chemokines, such as CXCL1, CXCL8 and consequently aggravated of the island and the of the beta cells, and also by IL-17 and Apotosis of the beta cells by linke of IL-17 to other anti-inflammatory cytokines, where the increase in the expression of cells TH17 in patients with T1DM acts as advanced indicators of autoimmune response to crash β cells destroying where the high production of IL-17 notes when the autoimmune response is advanced against the beta cells in diabetic patients type I (Hartwall *et al.*, 2015).

Either of the role of IL-17 in the events of the Diabetes type II as a cytokine was also demonstrated by the fact that the results of the studies indicate that IL-17 is involved in the cause of the T2DM epidemic, which has a crucial role in causing the type, where these studies have found a high level of IL-17 In T2DM but the IL-17 role in the event of this injury is not detected (Chen *et al.,* 2016). Where studies have confirmed that the leading Proinflammatory cytokine play a critical role in insulin resistance and event the T2DM (Carvalho MHC *et al*., 2006). Cytokine are considered to be toxic to cells and representing function roles is that they may cause the the cytostatic of the island cells in the pancreas, such as discouraging the insulin industry and secretion, or stimulating the production of nitric oxide (NOS) thereby These are the cytokines urging acute inflammation process (Cieślak *et al.,* 2015). Nitric oxide (NO) who causes the toxicity of the island cells in the pancreas of the mouse so IL-17 can participate in the subject inflammation and lead to the destruction of a beta cell in the pancreas in synergy with the Other cytokines leading inflammation (Milikovic *et al*., 2005).

The Relationship between the IL-17 with the age

The study showed that there is a positive correlation between the IL-17 level and progresses of the age more of 30 year where the highest level of IL-17 is greater in the age group more than 60 year without statistical significance as shown in Table (3).

Table 3: The Relationship between the IL-17 with the age

Age Groups	Study groups	IL-17 Mean ± SE
1-7	Patients	14.34±108.0
	Control	56.30±4.75
8-13	Patients	23.181±42.69
	Control	3.835±59.00
14 -20	Patients	174.50±43.75
	Control	62.00±5.04
30-45	Patients	9.99±87.60
	Control	3.73±65.60
46_61	Patients	12.58±101.05
	Control	4.33±70.55
62-77	Patients	15.07±115.00
	Control	4.52±70.90
P-value	0.385^{NS}	

NS= No statistically difference P>0.05

The IL-17 concentration increase with age progresses as it indicated that IL-17 was involved in the inflammatory process and as a causative agent for diseases and complications of aging (Li *et al.,* 2017). As recent studies have been able to bolstering the Th17 cell with age, it has made it clear that these cells may contribute to significant changes in the immune function (Van Bruggen and Ouyang, 2014).

Relationship between IL-17 and Gender for study groups

When comparing IL-17 level with gender for patients with diabetes (type I and II) and control group results showed that the level of IL-17 in females was higher than that of males without significant difference as shown in the Table (4).

Table 4: Relationship between IL-17 and Gender for study group

Study groups	Gender	IL-17 pg/ml Mean± SD
Type1	Male	133.90±15.55
	Female	147.00±33.17
Type 2	Male	98.17±9.99
	Female	106.14±11.75
Control	Male	±2.9460.75
	Female	66.71±2.37
P-value	0.714^{NS}	
NS= No statistically difference P>0.05		

The lack of a statistically significant difference in the IL-17 Level with diabetes in both gendere may be due to the gender-based immune defence mechanisms, since males and females show the same immune cells to the immune response that occur in the patient's body, which may be somewhat similar regardless of the As the interaction within the patient's body leads to the activation of immune cells responsible for immunologic response in the serum of patients with diabetes, and we infer that gender is not affected by the cytokine level of this in the body (Voskuhl, 2011).

Genetic study

Determination of the IL-17A gene by PCR-RFLP
Select the Optimization temperature for the Primer

Different temperatures were used within the range (50-71)C. Therefore, the best temperature for the Primer was (61.8C) that selected according to shape and size the band as show table below and figure (2).

Well	1	2	3	4	5	6	7	8	9	10	11	12
TM	51	51.3	52.5	54.2	56.5	59.1	61.8	64.5	67.0	69.1	70	71.5

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Where 12 thermal degrees were used by the Gradient PCR system and used the temperatures is 61.8.

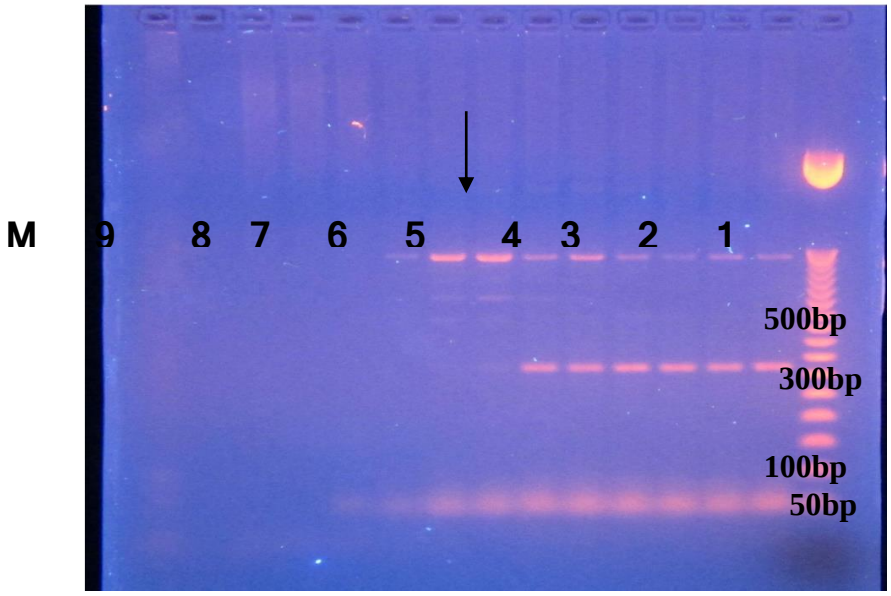

Figure 2: Select the Optimization temperature for the Primer, gel concentration was 1.5%, 70 volts/cm for 45-60 minutes. M = volumetric directory (50 bP).

And then conducted electrophoresis for all PCR reaction products were then the best product was selected depending on the size and shape of the band. The IL-17 gene was determined in diabetic samples and the control group by amplifying it to a size of 770 bp with the help of specialized primers after selecting an Optimization temperature and then using the PCR technique and showed the electrolysis of the PCR products on agarose gel 770 bp gene in all studied samples as shown in Figure (3).

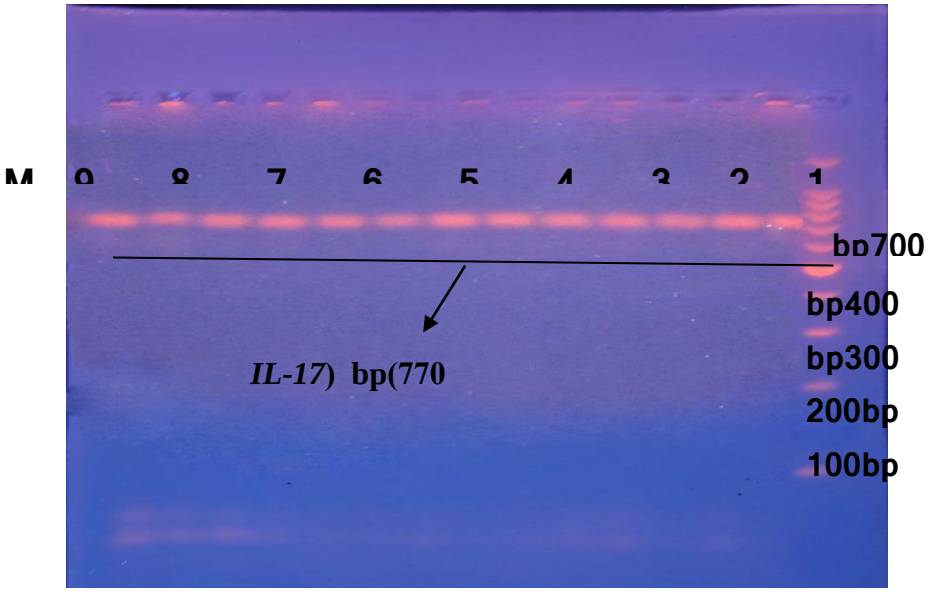

Figure 3: Electrophoresis on gel for the products of the PCR to a piece of Gene *IL-17* (770bp) Gel concentration was 1.5%, 70 volts/cm for 45-60 minutes. M = volumetric directory (100 bP).

Studies on this subject have indicated that there is a relationship between Single-nucleotide polymorphism (SNP) in the IL-17A gene with a variation in the IL-17 concentration, In particular, which strongly correlates bind to the single-nucleotide polymorphism (SNP) at site rs2275913 with diabetes. In light of this information, the analysis of single-nucleotide variants was performed at this site to highlight its relationship with diabetes, where this study is the first in Iraq.

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Genotyping of gene polymorphism of the gen *IL-17* in the site rs2275913by RFLP-PCR

Used Restriction fragment length polymorphism technique (RFLP-PCR) to conduct genotyping of the of polymorphism gene *IL-17* in site rs2275913 the product of PCR, which amounts to (770bp), was digested by a enzyme AvaII which cuts in the site rs2275913 and has been detection of digestion products by the electrophoresis on the agarose gel in the cutting region 5…CCGG...3, 3…GGCC…5.

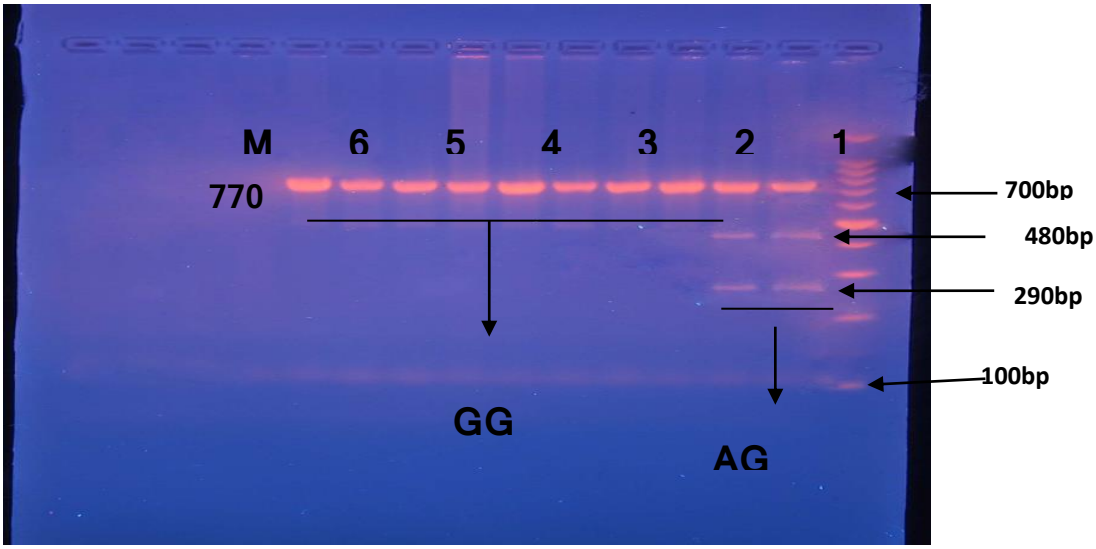

Figure 4: Electrophoresis on gel for the products of the PCR to a piece of Gene *IL-17* (770bp) in a Control sample. Gel concentration was 1.5%, 70 volts/cm for 45-60 minutes. M = volumetric directory (100 bP).

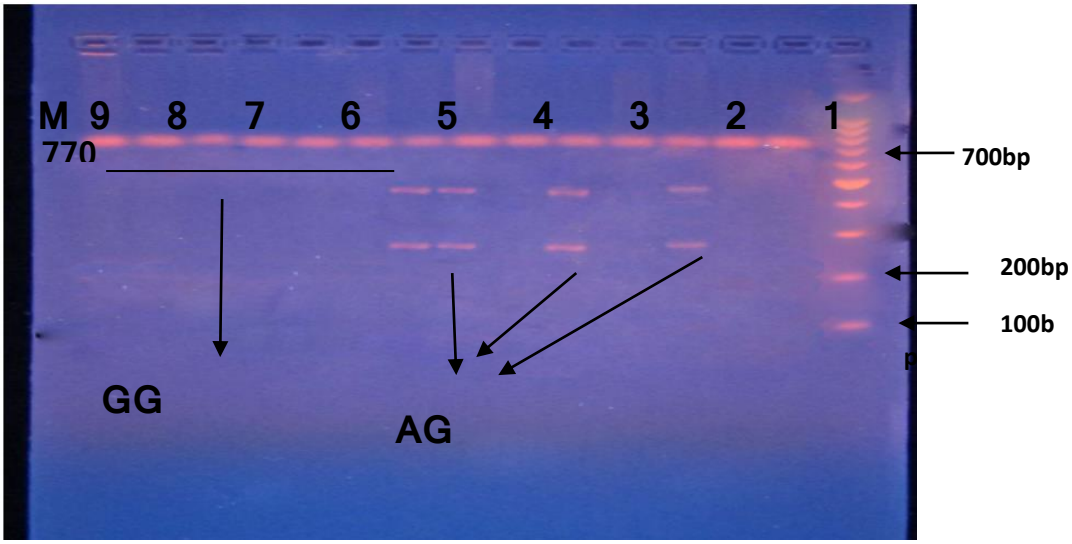

Figure 5: Electrophoresis on gel for the products of the PCR polymerase reaction to a piece of Gene *IL-17* (770bp) in a T2DM sample. Gel concentration was 1.5%, 70 volts/cm for 45-60 minutes. M = volumetric directory (100 bP).

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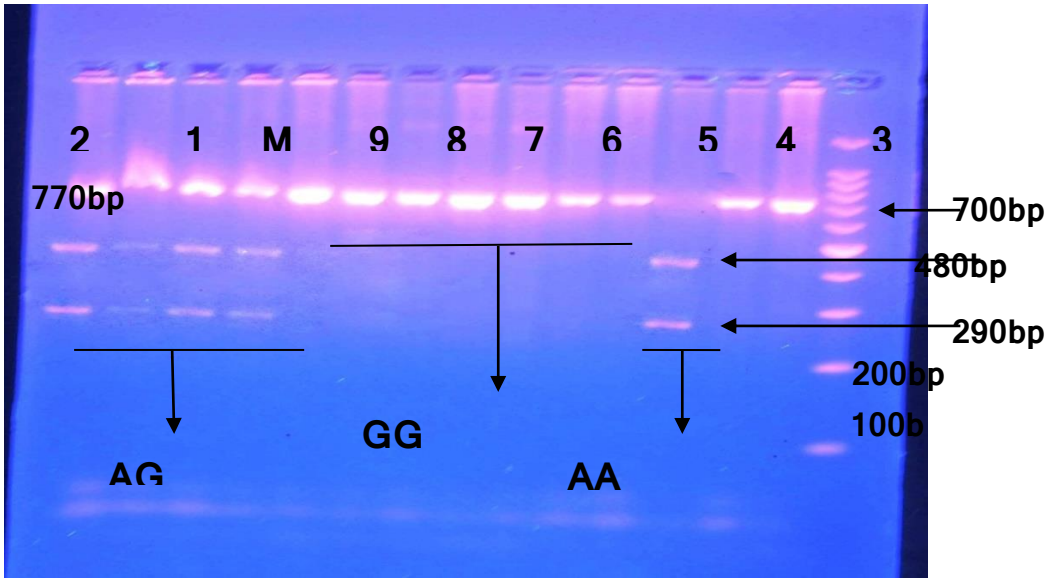

Figure 6: Electrophoresis on gel for the products of the PCR to a piece of Gene *IL-17* (770bp) in a T1DM sample. Gel concentration was 1.5%, 70 volts/cm for 45-60 minutes. M = volumetric directory (100 bP).

Distribution of genetic patterns of the gen IL-17 in the site rs2275913 in patients with diabetes

Gene *IL-17* at the site rs2275913 includes of each of the following genetic patterns (GG, AA and AG). The results indicated that there were clear differences with a statistically difference in the distribution of gene patterns in the sample of patients with diabetes and the sample of the group control and as we observe in the Table (5)

Table 5: Distribution of genetic patterns of the gen IL-17 in the site rs2275913

Study groups		GG	AA	AG	X^2	p value
Patients	Type1 **N=15**	9 (46.66%)	(13.33%) 2	4(40.00%)	**10.649***	**0. 036**
	Type 2 **N=15**	5(33.33%)	0(0%)	10 (66.66%)		
ontrol N=15		13(80%)	0(0%)	2(20%)		
=statistically difference P>0.05*						

We note that the AG genetic pattern was high among patients with diabetes (type I and II) while it was low (20%) in the control group, either the AA genotype was shown in the patients of T1DM only. Also we noted the genotype GG was high (80%) in the control group compared to the patient groups.

We found that the AG and AA genotypes was associated with an increased risk to diabetes (type I and II), the study agree with a study carried out in (2016) by Linhartova, it was shown AA genotype increase in T1DM compared to the control group with statistically difference P<0.05. This A allele displayed a higher affinity for the nuclear factor of activated T-cells (NFAT), a critical transcription factor in IL-17 regulation (Espinoza *et al.*, 2011) .The studies showing that the gene IL-17 in diabetes mellitus-type I shows a toxic role for beta cells in the pancreas in the laboratory as this study showed that the activated T-cells and $CD4^+$ cells in the diabetic patients are urging on level elevate of transcription factor RORγT which stimulus of IL-17 (Honkanen *et al.,* 2010). Also another study linked that IL-17 response and motivation occurs through reliance on the transcription factor NF-KB (Awane *et al*., 1999). Where NF-KB regulates the expression of many genes

involved in immunity as well as in inflammatory responses. This transcription agent acts on the genes of the inflammatory cytokines and participates in cellular responses to stimuli such as stress, cytokines, free radicals such as the production nitric oxide (Perkins, 2007).

The relationship Genetic patterns of *IL-17* with IL-17 level

The present study showed a significant difference in level of IL-17 different values relative to the different genotypes of the *IL-17* in site rs2275913 in the diabetic patients and the control group as shown in Figure (2).

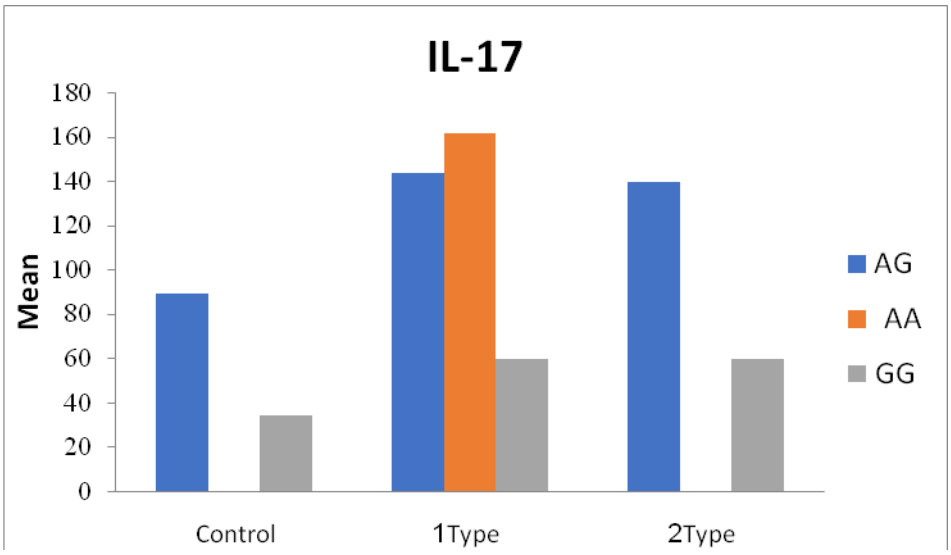

Figure 6: The relationship Genetic patterns of *IL-17* with IL-17 level

This finding present that the two genotypes (AG/AA) are associated with increased production of IL-17 on the contrary to the genotype GG genotype has a negative impact on the production of IL-17.

The proinflammatory cytokines including IL-17A, use the NF-κB pathway, which is a powerful catalyst for the expression of genes (Fujisawa *et al*., 2011). Where the effect IL-17 gene on protein level expression at the is through the NF-κB factor by complex method (Dragan *et al.,* 1998).

The area of promoter of genes cytokines for in humans is a combination of elements identified by transcription factors such as NF-κb, AP-1 and CREB and these latter factors mediate gene transcription caused by a variety of external signals including That's cytokines (Chandra *et al*., 1995).

CONCLUSIONS

During the current study, there was high significant increase in level of IL-17 in sera of patients diabetes (T1DM and T2DM) compared to control. The number female patients were higher than male patients and increased of IL-17 in female compared to male and no significance different between groups of study for the Gender. Significant significance difference between distribution genotype of gen *IL-17* in groups study, We found that the AG and AA genotypes was associated with an increased risk to diabetes (type I and II), Also showed positive correlation genetic patterns of *IL-17* with IL-17 level**.**

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