

ORIGINAL ARTICLE

METFORMIN MAY AMELIORATE INFLAMMATORY EVENTS OF IL-18 IN SOME INFLAMMATORY CONDITIONS

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Summary

Background: Interleukin-18 (IL-18) belongs to the cytokine family IL-1. IL-18 is synthesized as inactive precursors which need to be processed into an active interleukin by the Caspase-1 enzyme. The role of IL-18 is implicated in several auto-immune disorders, myocardial function, emphysema, metabolic syndromes, psoriasis, bowel inflammation, sepsis, and acute kidney injury. IL-18 exhibits pro-inflammatory properties, such as increased cell adhesion molecules, nitric oxide production, enhancement of T-cell and natural killer cell maturation, and increasing the production of chemokines. This study was designed from November 2020 to February 2021 at Al-Shomali hospital, Babylon governorate, Iraq. This study aimed to assess the levels of IL-18 in patients with PCOS, T2DM and CAD before treatment with metformin and after metformin medication, and to evaluate the roles of IL-18 in the development of this disease. **Materials and methods:** The study design is a case-control study and patients are selected by simple randomization after diagnosis by a specialist based on clinical diagnosis and laboratory findings. An enzyme-linked immunosorbent test (ELISA) was used to estimate the level of serum IL-18 before and after metformin administration. A total of 300 patients were involved in this study, divided according to their chronic illness as 60 women with PCOS, 60 patients Type 2 diabetes mellitus (T2DM), 60 patients with myocardial infarction (MI), and 60 patients with T2DM and MI. In addition, 30 healthy people as a control group. **Results:** Before treatment with metformin, the results were exhibited a significant difference ($P \leq 0.0001$) in the concentrations of IL-18 in PCOS, T2DM, and patients with CAD as compared with control. While, after metformin treatment, a significant decrease ($P \leq 0.01$, $P \leq 0.0001$ and $P \leq 0.001$) in IL-18 level in patients with PCOS and T2DM and CAD respectively as compared to before metformin treatment. **Conclusion:** Metformin administration reduces the inflammatory events of IL-18 in patients with T2DM and CAD and PCOS.

Key words: metformin; IL-18; diabetes; inflammation; cytokines

Introduction

The IL-18 gene in humans encodes the protein IL-18 (1), This gene produces a pro-inflammatory cytokine protein. IL-18 can be released by an array of cell types, comprising both hematopoietic and non-hematopoietic

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cells (2). It has been first discovered in 1989 as a factor that stimulated the production of interferon (IFN) in spleen cells of the mouse. IL-18 synthesis was first discovered in Kupffer cells, non-hematopoietic cells like epithelial cells of the intestine, endothelial cells and keratinocytes. IL-18 has the ability to influence both innate and adaptive immunity (3, 4).

Several reports have been indicated that pro-inflammatory cytokines such as interleukin-6, IL-18, TNF, and C-reactive protein are elevated in patients with type 2 diabetes mellitus (5, 6, 7). Intracellularly, Interleukin-18 may lead to the secretion of TNF-Alpha, IL-1, and chemokines which are pro-inflammatory cytokines by mesangial cells, which activate tubular epithelial cells and podocytes to release further pro-inflammatory cytokines and chemokines, encouraging interstitial immune cells infiltration, tubular injury, and excessive growth in the fibrous tissues (8). The mechanisms of IL-18 increment in T2DM are still unclear. However, the activation of NF-κB by hyperglycemia-induced by oxidative stress raises the circulating pro-inflammatory cytokine concentrations. High levels of IL-18 have been elucidated as an indicator of a high risk of mortality in patients with coronary artery disease. The major action of IL-18 in CAD is the reduction in plaque stability (9).

Metformin is the first-line in the treatment of T2DM by activating adenosine protein kinase (AMPK) in the hepatocytes. Also, metformin reduces the synthesis of glucose from non-carbohydrates sources (gluconeogenesis) (10, 11). Metformin decreases the overall glycogenolysis rate and reduces hepatic glucose-6-phosphatase activity and improves insulin-stimulated glucose uptake into skeletal muscle. Improve the sensitivity to insulin in muscle and adipose tissues by promoting the increase in the activity of insulin-mediated insulin receptor tyrosine kinase (12). In the cases of acute MI, exacerbation of heart disease, sepsis, or other conditions that might cause acute renal failure, metformin treatment is discontinued. Metformin has an anorexia effect in most people, which means it reduces caloric intake (this can be useful for PCOS). It inhibits gluconeogenesis (the creation of glucose in the liver) (13). Metformin inhibits growth hormone, adrenocorticotrophic hormone, follicle-stimulating hormone, and proopiomelanocortin expression in the pituitary gland, which helps to explain why it has an insulin-sensitizing effect on tissues like the liver, skeletal muscle, endothelium, adipose tissue, and the ovaries (14).

The purpose of the present study was to evaluate the level of IL-18 in patients with PCOS, T2DM and CAD before and after treatment with metformin, and to assess the impact of this cytokine in the pathogenesis of the disease.

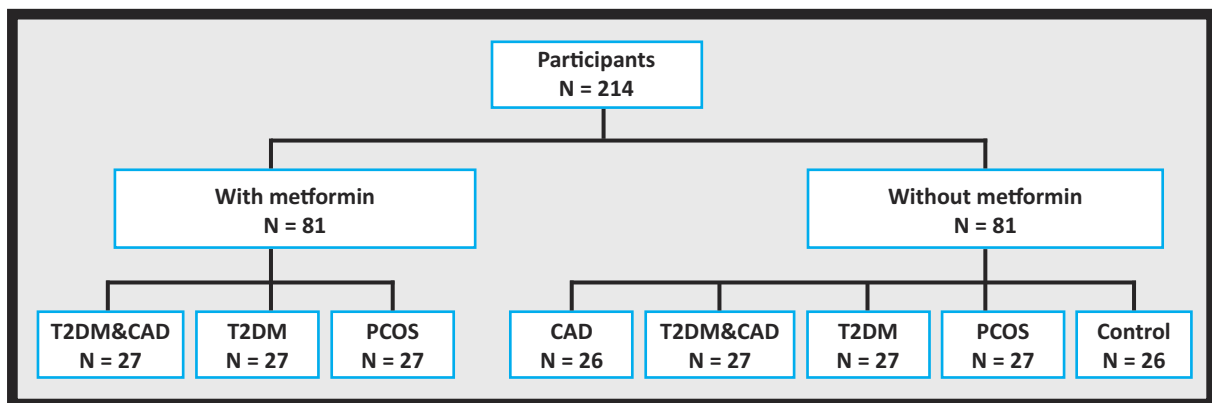


Figure 1. Diagram simplifying the studied groups enrolled in the present study.

Materials and methods

Subjects

The study was approved by the Medical Human Research Ethics Committee at the Faculty of Medicine, University of Al-Qadisiyah, Iraq (No. 1100 on 7th July 2020). The sample was distributed by the Simple randomization method and a total of 214 participants were enrolled from the Center for Diabetes and Shoumli

hospital in Babel Governorate (see Figure 1). A consent of agreement to participate in the study has been filled and signed by all participants. Two main groups were involved in this study, one without metformin administration and the other one including patients who have been using metformin for at least three months. Each main group was divided into subgroups listed below in Figure 1.

- Group I: Healthy individuals (26)
- Group II: PCOS women before metformin treatment (27)
- Group III: Patients with T2DM before metformin treatment (27)
- Group IV: Patients with CAD (26)
- Group V: Patients with T2DM and CAD before metformin treatment (27)

The second main group (totally known as after groups) also was further subdivided into

- Group VI: PCOS women after metformin treatment (27)
- Group VII: Patients with T2DM after metformin treatment (27)
- Group VIII: Patients with T2DM and CAD after metformin treatment (27)

The range of the duration is from 6 months into years. This duration was determined depending on previous studies which found effects of metformin on the levels of cytokines. The total duration of the study was 4 months starting from November 2020 to February 2021. Criteria of inclusion which were based in this study included: T2DM, CAD and PCOS, while the patients with any of the following conditions were excluded (Renal disease, liver disease, type I diabetes mellitus, thyroid functions disorders, COVID-19, and autoimmune disease). Diagnosis of participants is made by medical seniors based on clinical characteristics, history of patients, Electrocardiogram (ECG) and biochemical tests. These investigations were contained the fasting blood sugar or random blood sugar and troponin test. Ages, genders, smoking, family history, antihypertensive drugs or drugs for other diseases, were recorded.

About five ml of blood were collected from each participant through venipuncture. After that, the blood was left for 15 minutes at room temperature. The blood specimens were discrete by centrifuge at 11000 rpm for 5 minutes. Then, the serum was relocated into an Eppendorf tube (1.5 ml) labelled and kept at (-20) °C for subsequent analysis.

Determination of IL-18

Serum IL-18 level was estimated using Elabscience® kits as illustrated in figure 2.

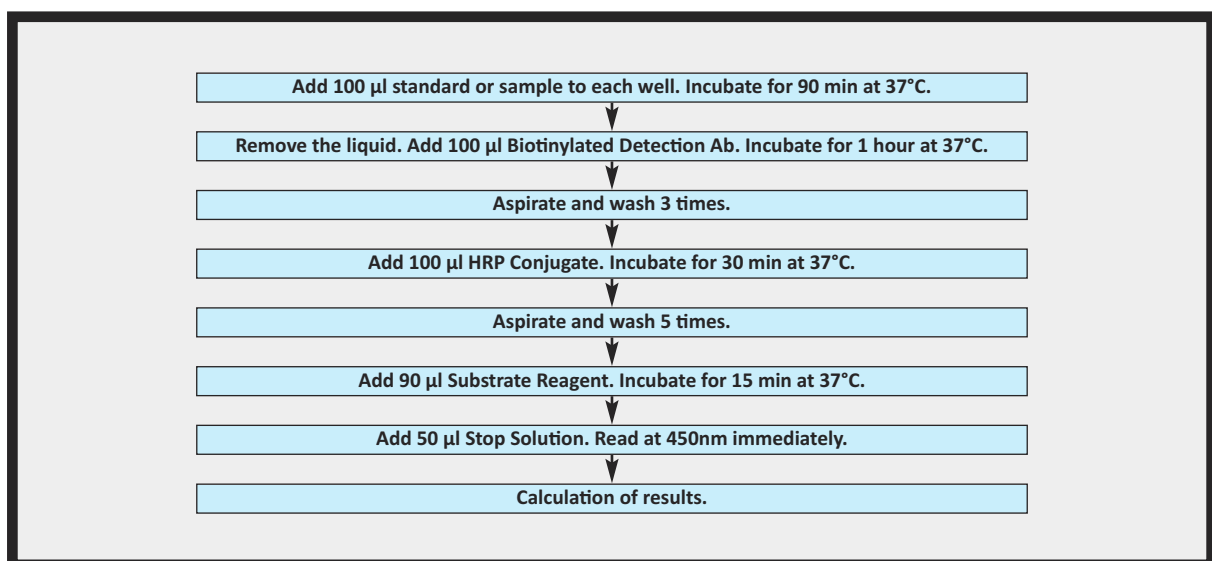


Figure 2. Method for detection of IL-18 by using ELISA. HRP, horseradish peroxidase.

Statistical analysis

Data were processed and presented using GraphPad Prism 9.2.0. and Microsoft Office Excel 2013. Numeric data were expressed as (Mean \pm SE). One-way ANOVA test and unpaired t-test were used for comparing values of the mean in the various groups when the values were within the normal distribution. Bivariate correlation was carried out by applying Pearson's correlation coefficient. The statistical significance was ascertained at $p < 0.05$.

Results

Comparison of demographic characteristics of the patient to that of the healthy control group

The Comparison of Demographic characteristics of patients with T2DM to that of the healthy control group is shown in Table 1. There was a highly significant difference in mean age between healthy individuals and T2DM without metformin 38.42 ± 4.90 years and 52.70 ± 4.86 years versus respectively ($P < 0.001$); the age was higher in the patients group than the control group.

Comparing the age between men and women in the patient with T2DM and control group which were revealed that no significant difference as shown in Table 1. The present study explained a significant increase ($P < 0.001$) in mean BMI between patients with T2DM and the control group. Also, there was a highly significant difference ($P < 0.001$) in the distribution of patients with T2DM and control subjects according to family history as shown in Table 1.

There was a highly significant difference ($P < 0.001$) in the mean age of patients with T2DM and CAD (63.19 ± 3.81 90) years without metformin treatment as compared with the control group (38.42 ± 4.90) as shown in Table 1. The ages of patients with T2DM and CAD without metformin treatment were higher than the control group. Performing of chi-square test revealed no significant difference ($P = 0.893$) in the distribution of patients with T2DM and control subjects according to gender. The present study explained a significant increase ($P < 0.001$) in mean BMI of patients with T2DM and CAD to that of the control group. Also, there was a highly significant difference ($P < 0.001$) in the distribution of patients with T2DM and CAD to that of control subjects according to family history.

There was a highly significant difference ($P < 0.001$) in mean age between healthy individuals (38.42 ± 4.90) years and PCOS without metformin, (30.41 ± 5.60) years; the ages of the PCOS group was lower than the control group. The present study revealed a highly significant increase ($P < 0.001$) in mean BMI between patients with PCOS and the control group. Also, there was a highly significant difference ($P < 0.001$) in the distribution of patients with PCOS to that of control subjects according to family history as shown in Table 1.

Table 1. Comparison of Demographic characteristics of patients with type 2 diabetes mellitus to that of the healthy control group.

Characteristic	Healthy individuals <i>n</i> = 26	Type 2 diabetes mellitus without metformin <i>n</i> = 27	<i>P</i>
Age (years)			
Mean \pm SD	38.42 \pm 4.90	52.70 \pm 4.86	< 0.001 I
Range	30 -45	44 -61	HS
Gender			
Male, <i>n</i> (%)	13 (50.0 %)	13 (48.1 %)	0.893 C
Female, <i>n</i> (%)	13 (50.0 %)	14 (51.9 %)	NS
BMI (kg/m²)			
Mean \pm SD	25.63 \pm 2.24	30.46 \pm 3.36	< 0.001 I
Range	21.5 -29	23.7 -35	HS
Family history			
Positive, <i>n</i> (%)	8 (30.7 %)	10 (37.0 %)	0.001 F
Negative, <i>n</i> (%)	26 (69.3%)	17 (63.0 %)	HS

(*n*: number of patients. SD: Standard deviations, NS: No significant. HS: highly significant. BMI: Body mass index. T2DM: Type 2 diabetes mellitus).

Table 2. Comparison of Demographic characteristics of patients with type 2 diabetes mellitus and coronary artery disease to that of the healthy control group.

Characteristic	Healthy individuals	CAD and T2DM without metformin	P
	n = 26	n = 27	
Age (years)			
Mean ± SD	38.42 ± 4.90	63.19 ± 3.81	< 0.001 I HS
Range	30 - 45	56 - 69	
Gender			
Male, n (%)	13 (50.0 %)	14 (51.9 %)	0.893 C NS
Female, n (%)	13 (50.0 %)	13 (48.1 %)	
BMI (kg/m²)			
Mean ± SD	25.63 ±2.24	31.45 ± 2.41	< 0.001 I HS
Range	21.5 -29	26.6 - 35	
Family history			
Positive, n (%)	8 (30.7 %)	7 (25.9 %)	0.010 F HS
Negative, n (%)	18 (69.3 %)	20 (74.1 %)	

(n: number of patients. SD: Standard deviations, NS: No significant. HS: highly significant. BMI: Body mass index. T2DM: Type 2 diabetes mellitus).

Table 3. Comparison of Demographic characteristics of patients with PCOS to that of the healthy control group.

Characteristic	Healthy individuals	PCOs without metformin	P
	n = 26	n = 27	
Age (years)			
Mean ± SD	38.42 ±4.90	30.41 ±5.60	< 0.001 I HS
Range	30 -45	30 -41	
Gender			
Male, n (%)	13 (50.0 %)	0 (0.0 %)	< 0.001 C HS
Female, n (%)	13 (50.0 %)	27 (100.0 %)	
BMI (kg/m²)			
Mean ± SD	25.63 ±2.24	29.38 ±3.79	< 0.001 I HS
Range	21.5 -29	22.8 -34.7	
Family history			
Positive, n (%)	8 (30.7 %)	7 (25.9 %)	0.010 F HS
Negative, n (%)	18 (69.3 %)	20 (74.1 %)	

(n: number of patients. SD: Standard deviations, NS: No significant. HS: highly significant. BMI: Body mass index. T2DM: Type 2 diabetes mellitus).

The effect of the duration of metformin use on demographic and some cytokine events

Pearson correlation analyses have revealed the relationships between demographic and some cytokines releases in the study groups. There was no significant correlation ($P>0.05$) between the duration of metformin treatment in patients with T2DM and the age. While there was a significant positive correlation ($P<0.05$) between the duration of metformin treatment in patients with T2DM and gender as explained in Table 4. There was no significant correlation ($P>0.05$) between the duration of treatment with metformin in patients with T2DM and IL-18 level as shown in Table 4.

As shown in Table 5, there was no significant correlation ($P>0.05$) between the duration of metformin treatment in patients with T2DM and CAD to that of age, gender, and IL-18. Furthermore, there was no significant correlation ($P>0.05$) between the duration of metformin treatment in patients with PCOS to that of age, gender, and IL-18 as represented in Table 6.

Table 4. Correlations of the duration of metformin treatment of patients with T2DM to demographic and IGF-1, IL-6 and IL-18.

Characteristic	Duration of metformin	
	R	P
Age (years)	0.158	0.433
Gender	0.415	0.032*
IL-18 (pg/ml)	-0.147	0.463

Table 5. Correlations of the duration of metformin treatment of patients with type 2 diabetes mellitus and coronary artery disease to demographic and biochemical characteristics of patients.

Characteristic	Duration of metformin	
	R	P
Age (years)	0.085	0.673
Female	-0.207	0.301
IL-18 (pg/ml)	0.092	0.650

Table 6. Correlations of the duration of metformin treatment of patients with PCOS to demographic and biochemical characteristics of patients.

Characteristic	Duration of metformin	
	R	P
Age (years)	-0.034	0.865
IL-18 (pg/ml)	-0.288	0.146

Estimation of IL-18 concentrations before treatment with metformin

In order to evaluate the effect of metformin, the level of IL-18 was estimated before the administration of the drug for PCOS, T2DM, and CAD patients. The results showed a significant increase ($P \leq 0.0001$) of IL-18 in the serum of all patients as compared to the control group as shown in Figure 3.

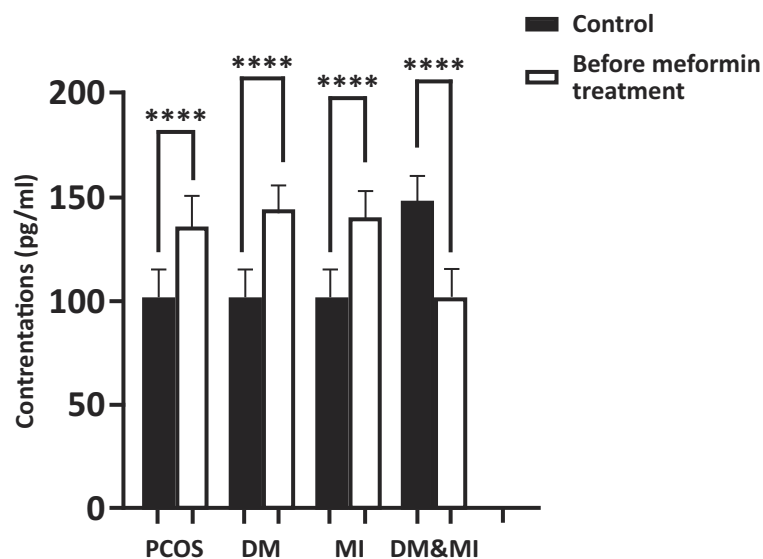


Figure 3. Concentrations of IL-18 in pg /ml in patients with T2DM, T2DM & CAD and PCOs before metformin treatment. Patients show a significant elevated ($P \leq 0.0001$) in the concentrations of IL-18 as compared with control.

Estimation of IL-18 concentrations after treatment with metformin

The level of IL-18 was evaluated for those who have been on metformin for at least three months. The concentrations of IL-18 have exhibited a significant decrease ($P \leq 0.0001$) PCOS women as compared to women before metformin usage, while the results for patients with T2DM and those who have both T2DM and CAD revealed a statistically significant reduction ($P < 0.0001$) in the serum concentration of IL-18 in comparison to those before metformin as showed in Figure 4.

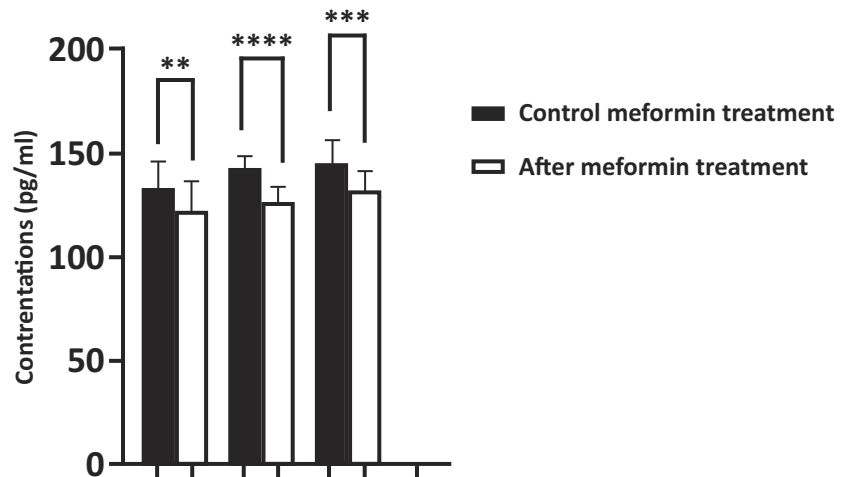


Figure 4. Concentrations of IL-18 in pg /ml in patients with T2DM and CAD, T2DM, and PCOs after metformin treatment. The figure shows significantly reduced ($P \leq 0.0001$) concentrations of IL-18 after treatment with metformin.

Discussion

T2DM is a chronic disease of the endocrine system that leads to multiple complications and an increased risk of early death. The incidence of T2DM has more than doubled globally and is on the rise in the United States (15). The study population comprised all men and women aged 30-65 years. Persons, less than 30 years were not included, due to the low prevalence of T2DM and CAD in this age group (16). The results of the current study have shown that there is an increase in the rate of T2DM and CAD with increasing age, these increases for the age group (50-65) years. A similar observation was reported in the studies which found rising the distribution of T2DM and CAD with rising age (17-19). The pathophysiology in which T2DM prevalence increase with ageing may be associated with many causes, but the most important causes of hyperglycemia are thought to be deficiency of insulin secretion developing with age and growing insulin resistance. Moreover, the sensitivity of pancreatic β cells for incretins decreases in the elderly. The distribution of adipose tissue in the elderly is changing (increased amount of visceral adipose tissue), and the amount of fat tissue grows in contrast to muscle mass, which decreases with age (20, 21). The prevalence of CAD is increased in aged adults as a complication of uncontrolled T2DM, oxidative stress, inflammation, apoptosis and Increased production of pro-inflammatory markers such as interleukin-6 (IL-6), tumour necrosis factor- α (TNF α), and CRP (C-reactive protein) (22, 23).

The results of the current study show that the range of age in the women with PCOS was (30-41). Several women have PCOS for years before diagnosis, resulting in a great variation between the age of onset and age of diagnosis (24). Many studies revealed the onset of clinical or diagnostic features such as hyperinsulinaemia in adolescents with hyperandrogenaemia and oligomenorrhoea depending on the presence of obesity and oxidative stress (25, 26).

The results of this study have shown that the majority of the patients were (62.1%) females and (37.9%) males with 134/82 female- to male- ratio. The greater part of participants were females due to the involvement of polycystic ovarian syndrome in this study which occurs in females only.

Obesity is a complicated, prolonged medical condition correlated with a high rate of mortality (27). The results of this study have shown a highly significant difference in mean BMI between control and patient groups. Moreover, the majority of patients who participated in the study were overweight and obese. These findings agree with that of (28) who found that the risk of T2DM and CAD diagnosis was larger for persons in higher body mass index classes than for persons in lower body mass index classes, also they found that the chance of acquiring T2DM to persons who were obese or overweight was around (1.5–5) times higher than for persons with normal body mass index. The results of our study disagree with (Rossi *et al.*, 2011) which revealed that BMI is not significantly associated with the greater extent of T2DM and CAD (29). Also, the observations of our study disagree with the studies (30) (Fan *et al.*, 2016), which show that BMI may not be the optimal measurement for predicting the T2DM and CAD in the Iraqi population because it does not differentiate muscle from fat or various fat distributions, thus it may not be a very precise index of excess adipose tissue, while waist/hip ratio was the best indicator (31). This may explain why there are patients in our study with normal weight. In our study, PCOS women are overweight with a mean BMI (29.38 ± 3.79) kg/m². This consequence was agreed with the studies (32, 33) (Ollila *et al.*, 2017), (Artini *et al.*, 2020) which record that most women with PCOS are overweight.

Regarding family history, The results of our data show that 37% of patients with T2DM 25.9% of patients with T2DM&CAD and 25.9% of women with PCOS have a positive family history. Family history of type 2 DM seems to increase the risk of hypertension, dyslipidemia and atherogenesis leading to coronary heart disease (CHD) (Thejaswini *et al.*, 2012), (Adams & Lammon, 2007) (34, 35). Family history (FH) of coronary artery disease (CAD) has been established as an independent risk factor for CAD (36). The results of this study demonstrate that a positive family history of coronary artery disease is an important predictor of impaired endothelium-dependent coronary blood flow regulation in humans. The influence of a positive family history aggravates endothelial vasodilator dysfunction associated with hypercholesterolemia and increased age, suggesting important interacting effects between genetic and environmental risk factors (37).

The results of our study show there was highly significant difference ($p < 0.001$) in the distribution of patients with PCOS to that of control subjects according to family history. Family history of diabetes, notably an inherited metabolic disorder, also poses a significantly high risk for PCOS. Many studies The results of our study are contraindicated with other studies which observed that cases and controls did not differ significantly regarding the history of PCOS. This might be argued that as history of PCOS is strongly related to the incidence of PCOS, however, persons who don't need to have PCOS always have a history of PCOS. Although family history is an important risk factor, environmental triggers are also playing a role, e.g. diet, and exercise (38, 39).

IL-18 is one of the pro-inflammatory cytokines that is believed to be increased in women suffering from PCOS. The results of our study explain the above principle and these results matched with Yang *et al.*, 2011 (40) and Al-Musawy *et al.*, 2018 (41) which showed IL-18 level increase in PCOS, and correlated with insulin resistance, obesity and hyperandrogenism and also indicated that IL-18 can be synthesized by adipose tissues. This elevation is thought to be related to the increase in the activity of the caspase enzyme in visceral adipose tissues in females with PCOs (42).

T2DM is marked by chronic low-grade inflammation. Elevated concentrations of inflammatory cytokines cause insulin resistance in hepatocytes, adipocytes, and muscles by phosphorylating insulin receptor substrate (IRS) (43), inhibiting insulin receptor tyrosine kinase activity, ubiquitination and degrading both IRS1 and IRS2, and decreasing IRS1 mRNA transcription (44, 45). The exact mechanisms in which IL-18 promote pathological degradation in T2DM have not been quietly clarified. Various studies show an increase in IL-18 levels in subjects with T2DM (46, 47). The outcomes of our data are matched with these studies.

Previous research on the role of IL-18 in predicting coronary events has shown mixed results. After controlling for established risk variables, IL-18 links to various metabolic syndrome components but not to subclinical atherosclerosis in the Dallas Heart Study. The authors speculated that the link between IL-18 and atherosclerosis is dependent on the coexistence of additional deleterious conditions including overweight and diabetes and that IL-18 may mediate some of these factors' proatherogenic effects. Inflammatory indicators such as CRP, IL-6, and IL-18 have been found to provide additional predictive information for cardiovascular mortality in people

with established coronary artery disease and metabolic syndrome (44, 45). IL-18 and its receptors were detectable in cardiomyocytes in patients with coronary artery disease and surrounded by inflammatory infiltrates. It appears that the ischemic insult with the inflammatory response of this state is the main stimulator of IL-18 expression since increased mRNA of IL-18 concentrations were found in the serum of patients with CAD. The conversion of IL-18 to its active form by caspase-1 was boosted in the myocardium suggesting pathophysiological mechanisms of IL-18 have been involved in CAD (48). In our study, we demonstrated that IL-18 is noticeably more measurable in the existence of high glucose levels, and since utmost patients with metabolic syndrome have deranged fasting glucose or diabetes, it's likely that IL-18's prognostic value in the present study is further affected by hyperglycemia. In line with our results (49) also suggest the strong relation between the concentration of IL-18 and T2DM patients suffering from CAD. These cytokines are increased in patients with T2DM and CAD through oxidative stress. IL-6 and pro-inflammatory cytokines can jeopardise insulin receptor signalling in adipose tissue and skeletal muscle, and hence deteriorating insulin-stimulated glucose uptake and encouraging insulin resistance conjoined with overweight and type 2 diabetes (50).

Metformin has been linked to several benefits in PCOs women, like retrieving ovulation, weight loss, lowering circulating testosterone levels, lowering the risk of miscarriage, and lowering the risk of gestational diabetes mellitus (GDM). (51) show a significant decrease in the levels of interleukin-18 and interleukin-6 in patients with polycystic ovary syndrome after treatment with metformin. Hussein, 2017 (52) has revealed that metformin inhibits the inflammatory response by suppression NF κ B via AMP-activated protein kinase (AMPK). In our study, the IL-18 level also showed a significant difference between before and after metformin groups. However, patients with T2DM showed a significant reduction in IL-18 levels after metformin administration. The same trend was observed for those suffering from both T2DM and CAD.

The mechanism by which metformin diminish the levels of IL-18 remains unclear but could be due to inhibition of the production and the induction of NF-kB by metformin. NF-Kb induces the transcription of the gene responsible for encoding IL-18. These findings are matched with another study conducted by Isoda et al., 2006 which show similar results and disagreed with the study Chen et al., 2008 which indicate that there are no associations between metformin and the concentrations of IL-18 (53, 54). Further investigations are required to confirm the mechanism that might be responsible for the inflammatory reaction modulated in the context of this study; since hyperlipidemia which commonly coexists with diabetes, might be responsible for the modulation of cytokine levels (55). Moreover, antidiabetic medication in current use e.g. glibenclamide (12) could have a role in inflammation and these need specific attention and research conduction.

Conclusions

The serum levels of IL-18 were significantly elevated in patients with PCOS, T2DM and coronary artery disease as compared with the control group. Patients with T2DM and CAD who have metformin medication exhibit a significant reduction in the concentrations of IL-18 as compared with patients without metformin treatment. Thus, metformin reduces the inflammatory events by lowering the concentrations of IL-18. The major limitation is the difficulty in sample collection due to the increased prevalence of COVID-19 during the period of sample collection.

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Adherence to Ethical Standards

The study was approved by the ethical committee at the University of Al-Qadisiya.

Conflict of Interest

The authors have no conflicts of interest regarding the publication of this article.

Abbreviation	Mean
AMPK	Adenosine monophosphate protein kinase
CAD	Coronary artery disease
CHD	Coronary heart disease
CRP	C-reactive protein
COVID-19	Coronavirus disease - 2019
DM	Diabetes mellitus
ECG	Electrocardiogram
ELIZA	Enzyme linked immunosorbent assay
HS	Highly significant
IL-18	Interlukin -18
IL-4	Interleukin - 4
IL-6	Interlukin - 6
IR	Insulin resistance
IRS-1	Insulin receptor substrate-1
Mean \pm SD	Mean \pm Standard deviation
NF- κ B	Nuclear factor kappa-B
NS	Non-significant
PCOS	Polycystic ovarian syndrome

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