The Effect of Metformin Therapy on Serum IL-33 and ST-2 Receptor Levels in Patients With Type 2 Diabetes Mellitus

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Diabetes Mellitus, Interleukin-33, ST-2 Receptor

ABSTRACT
Introduction: Patients with type 2 had elevated levels of inflammatory biomarkers, including interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF-α). Significant elevations in serum levels of TNF-α and C-reactive protein were seen in individuals with type 2 diabetes mellitus (T2DM). The IL-1 superfamily (IL-1β, IL-18, and IL-33) plays a crucial role in the development of diabetes mellitus by modifying immunological and inflammatory immune responses. Objectives: Little is known about the role of metformin in type 2 diabetes mellitus with respect to serum levels of IL-33 and ST-2 receptors. Therefore, in the present study, we sought toexplore such role. Patients and Methods: This single arm study included 45 type 2 diabetes patients with an age range of 30 to less than 60 years. Patients were given metformin (500 mg orally; for two months then, serum level of interleukin- 33 (IL-33) and ST-2 receptors were evaluated. Results: Treatment resulted in significant reduction of serum the suppression of tumorigenicity suppressor 2 (ST2) from 0.71 ±0.157 µg/mlto 0.59 ±0.11 µg/ml ($p<0.001$), across with significant increase in serum IL-33 from 0.60 ±0.18 pg/ml to 0.75 ±0.13 pg/ml ($p < 0.001$). Conclusion: Treatment of type 2 diabetes mellitus using metformin revealed a crucial role for IL-33 and ST-2 receptors in glycemic control since increment in IL-33 and reduction in ST-2 receptors were associated with good glycemic control suggesting an inflammatory role in disease pathogenesis and response to treatment.

1. Introduction

Elevated concentrations of inflammatory biomarkers, including interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF-α), were identified in individuals diagnosed with type 2mellitus (1,2). Significant elevations in serum levels of TNF-α and C-reactive protein were seen in individuals with type 2 diabetes mellitus (T2DM) (3). The interleukin-1 (IL-1) superfamily, consisting of IL-1β, IL-18, and IL-33, plays a crucial role in the development of diabetes mellitus by modifying immunological and inflammatory responses (4). Furthermore, IL-1 regulates the concentration of blood glucose, pulse pressure, and basal metabolic rate. The IL-1 family comprises cytokines capable of both pro- and anti-inflammatory effects (5).

The cytokine interleukin 33 (IL-33) is a newly identified member of the IL-1 superfamily. It specifically interacts with two isoforms of tumorigenicity suppressor 2 (ST2); soluble ST2 (sST2) and transmembrane ST2L (6). Induction of Th2-mediated response is attributed to IL-33 (7). Reports indicate that IL-33 decreases the expression of resisting (8). A previous study has already documented the involvement of IL-33 in the development of type-1 diabetes (9, 10). A recent study proposed that IL-33 and sST2 exhibit diverse connection patterns with distinct metabolic indicators (11). Recently, Singh et al (12), examined30 patients diagnosed with type 2 diabetes mellitus (DM) and 30 control participants. The results revealed that blood IL-33 levels were notably lower and ST2 receptors levels were considerably higher in type-2 diabetic persons compared to healthy controls. They additionally found the significant correlations between the concentration of IL-33 in the blood and fasting plasma glucose as well as postprandial plasma glucose levels. This indicates that there are changes in the serum levels of IL-33 and sST2 in persons with type-2 diabetes.

Objectives

Little is known about the role of metformin in type 2 diabetes mellitus with respect to serum levels of IL-33 and ST-2 receptors. Therefore, in the present study, we sought to explore such role.

Patients and Methods

The present single arm study included 45 patients with an age range of 30 to less than 60 years. This kind of design enables the researcher to examine the relationship between independent and dependent

variables and to gain a deeper understanding of the elements influencing a given system those patients were diagnosed with type 2 diabetes mellitus by a specialist physician in the diabetes consultation clinic in diabetes center at AL-Diwaniyah teaching hospital, AL-Diwaniyah province, Iraq by a simple random method. Simple random sampling is a probability-based strategy that allows researchers to randomly and unbiasedly pick participants for their study. It is considered advantageous for studying populations that are homogeneous and evenly selected. In this form of selection, all participants are given an equal chance to participate in the study, with the selection process being solely dependent on chance. The minimum sample size in this study was (60) patients, according to the calculation of the minimal sample size software (surveymonkey.net, 2023; Select Statistical Services, 2023), based on a confidence level of 80% and a margin of error of 5%.Inclusion criteria of patients include fulfillment of type 2 diabetes mellitus criteria; since, the age of patients should be between 35 to 65 years. Exclusion criteria was pregnant women and co-morbidities such as cardiovascular disease, essential hypertension, liver disease and kidney disease.

The study is dated back to September the 21st 2023 and extended to March 31st 2024. The age of patients were between 30 to 60 years. Patients were given metformin (500 mg orally; Glucomet, Furat Pharmaceutical Industries, Iraq) for two months. Serum level of IL-33 was evaluated using the Human Interleukin 33, by enzyme-linked immunosorbent assay (ELISA)kit (BT lab, China).Serum ST-2 serum level was evaluated using Human sST2 (soluble ST2) ELISA kit (Elabscience, USA).Insulin level was measured using a fasting patient sample for 8 hours. Hemoglobin A1c test was measured according to the specified time, i.e. after three months, taking into account the period before the intervention.

Statistical analysis

Statistical analysis was conducted using SPSS (version 16, IBM, USA). Quantitative variables were displayed using measures such as mean, standard deviation, minimum, and maximum values to find the different between two groups. The means of variables before and after therapy were compared using a paired T-test. A significance criterion of $p<0.05$ was established.

Results

Table 1 shows that the percentage of men was somewhat more than that of females, with 25 (55.6%) compared to 20 (44.4%). The average age of all 45 patients included in the study was 54.13±8.58 years, with a range of 35 to 65 years. Table 1 displays the comparison of mean body mass index and waist circumference before and after administration of therapy. The mean BMI decreased significantly from 30.41±4.45 kg/m^2 to 28.47±4.53 kg/m^2 ($p<0.001$). Moreover the mean waist circumference decreased significantly from 112.91±18.80 cm to 101.33 ±14.56 cm ($p<0.001$).

Table 1. Comparison of mean body mass index and waist circumference before and after treatment

Characteristic	Before n = 45	After n = 45	p. value
BMI (kg/m^2)			
-Mean ±SD	30.41 ±4.45	28.47 ±4.53	<0.001 Pa ***
-Range	21.10 -42.30	20.10 -41.40	
Waist circumference (cm)			
-Mean ±SD	112.91 ±18.80	101.33 ±14.56	<0.001 Pa ***
-Range	74 -154	70 -133	

n: number of cases; SD: standard deviation; Pa: paired *t*-test; ***: significant at $p \leq 0.001$; BMI: body mass index.

InTable 2 comparisonof mean glycemic control parameters before and after treatment is shown. Treatment resulted in significant reduction of plasma insulin from 13.07±7.07 µU/ml to 8.13±2.92 µU/ml ($p< 0.001$), along with across with significant reduction of fasting plasma glucose (FPG) from 163.09 ±52.84 mg/dl to 129.35±34.67 mg/dl ($p< 0.001$), along with significant reduction of postprandial glucose (PPG) from 213.27±71.78 mg/dl to 171.67 ±49.68 mg/dl ($p< 0.001$), and significant reduction of glycated hemoglobin A1 % (HbA1c %) from 7.48 ±0.76 % to 6.90 ±0.87 % ($p< 0.001$).

Table 2. Comparison of mean glycemic control parameters before and after treatment

Characteristic	**Before** ***n* = 45**	**After** ***n* = 45**	***p. value***
Plasma insulin (µU/ml)			
-Mean ± SD	13.07 ±7.07	8.13 ±2.92	< 0.001 Pa ***
-Range	5.6 -34.3	3.4 -14.5	
FPG (mg/dl)			
-Mean ± SD	163.09 ±52.84	129.35 ±34.67	< 0.001 Pa ***
-Range	95 -300	80 -210	
PPG (mg/dl)			
-Mean ± SD	213.27 ±71.78	171.67 ±49.68	< 0.001 Pa ***
-Range	111.3 -421	100 -310	
HbA1c %			
Mean ±SD	7.48 ±0.76	6.90 ±0.87	< 0.001 Pa ***
Range	6.4 -8.7	4.9 -8.4	

n: number of cases; **SD**: standard deviation; **Pa**: paired *t*-test;***: significant at $p \leq 0.001$; **FPG**: fasting plasma glucose; **PPG**: postprandial glucose; **HbA1c%**: glycated hemoglobin A1

Comparison of mean serum ST-2 and IL-33 before and after treatment is shown in Tables 1 to 3.Treatment resulted in significant reduction of serum the suppression of tumorigenicity 2 receptor (ST-2) from 0.71 ±0.157 µg/ml to 0.59 ±0.11 µg/ml ($p< 0.001$).Figure 1 show that Treatment with metformine resulted in significant reduction of serum the suppression of tumorigenicity 2 receptor (ST-2) from 0.71 ±0.157 (µg/ml) to 0.59 ±0.11 (µg/ml) ($p < 0.001$), 3.15. while ,Figure 2 show that metformine leads to significant increase in serum interleukin- 33 (IL-33) from 0.60 ±0.18 pg / ml into 0.75 ±0.13 pg / ml (p; < 0.001), 3.16.

Table 3.Comparison of mean serum ST-2 and IL-33 before and after treatment

Characteristic	**Before** ***n* = 45**	**After** ***n* = 45**	***P. value***
Serum ST-2			
Mean ±SD	0.71 ±0.157	0.59 ±0.11	< 0.001 Pa ***
Range	0.45 -1.17	0.31 -0.78	
Serum IL-33			
Mean ±SD	0.60 ±0.18	0.75 ±0.13	< 0.001 Pa ***
Range	0.08 -0.87	0.44 -1.05	

n: number of cases; SD: standard deviation; Pa: paired *t*-test; ***: significant at $p \leq 0.001$; ST-2:The Suppression of Tumorigenicity 2 receptor; IL-33: Interleukin-33

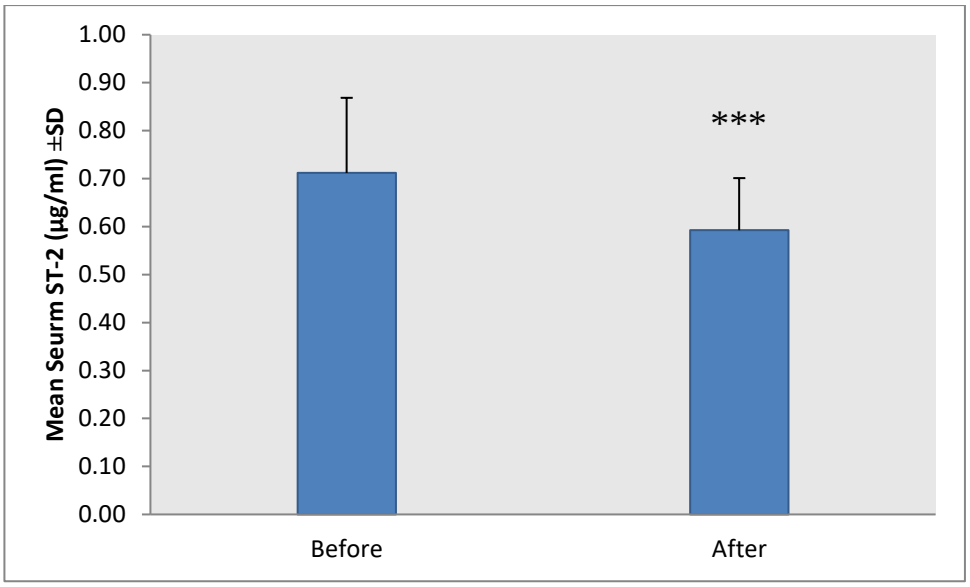

Figure 1.Bar chart showing comparison of mean serum ST-2 before and after treatment

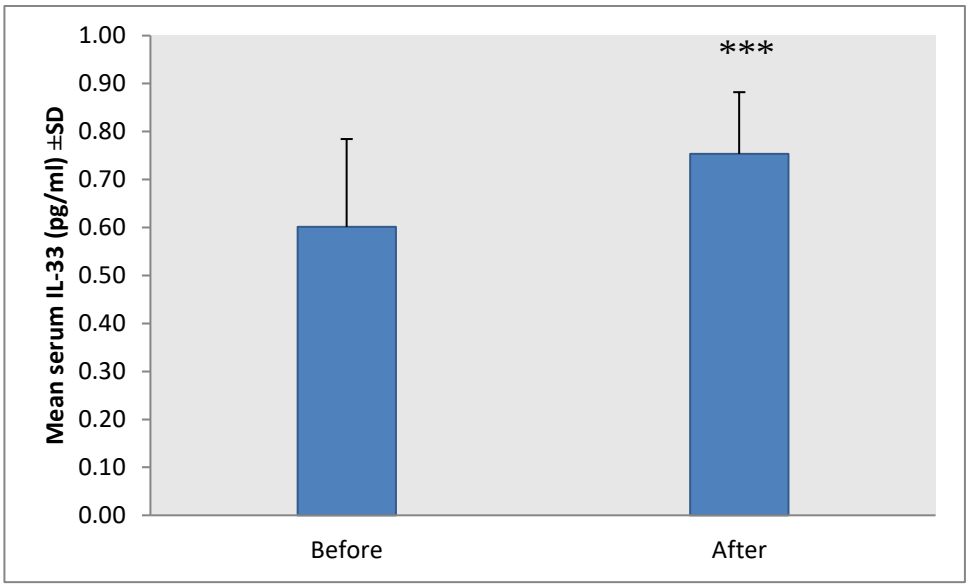

Figure 2.Bar chart showing comparison of mean serum IL-33 before and after treatment

In this study, treatment using metformin resulted in significant increase in serum interleukin- 33 (IL-33) along with a significant reduction of serum the suppression of tumorigenicity 2 receptor (ST-2). In contrary to our findings, Zhang et al (13) have shown that metformin can cause reduction of serum IL-33 level in diabetic patients who have diabetic nephropathy. However, Asensio-Lopez et al (14) in their experimental study have shown that administration of metformin is associated with increased IL-33 level and reduced level of ST-2.

Chen et al (3) reported the detection of increased levels of inflammatory markers such as TNF-α and C-reactive protein in persons diagnosed with type 2 diabetes mellitus. Banerjee and Saxena (4) have proposed that the IL-1 superfamily, including IL-33, exerts a substantial influence on the development of diabetes mellitus via regulating immunological and inflammatory responses. Singh et al (12) recently observed a significant disparity in serum IL-33 levels and sST2 levels between individuals with type-2 diabetes and healthy controls. Also, The authors proposed that their study findings demonstrate an opposite effect of IL-33 and sST2 in diabetic patients, which aligns with previous studies carried out by Hoogerwerf et al (15) and Lin et al (16). An In-vivo study showed

that the delivery of rIL-33 to genetically obese diabetic (ob/ob) mice resulted in a reduction in FPG levels and obesity, as well as improvements in insulin tolerance (8). animal experiment study illustrated that administrating rIL-33 to (ob/ob) mice that are obese diabetics because of genetic manipulation, led to a decrease in FPG (fasting blood glucose) levels and adiposity, along with enhancements in insulin tolerance(190. Moreover, a recent study has clarified the protective effect of IL-33 on the mass β-cells, insulin content, and release of β-cells, emphasizing the beneficial function of IL-33 in protecting the insulin-producing cells of the islets (17). Our findings point indirectly into the to two important suggestions; first suggestion is that higher levels of IL-33 are associated with good glycemic control and that metformin effect in increasing serum IL-33 is providing beneficial health outcomes; second suggestion on the contrary is that higher serum ST-2 is associated with poor glycemic control, however the reducing effect subjected by metformin is going to help diabetic patients to get rid of the harmful effect of ST-2.

Interleukin-33 is a cytokine that belongs to the IL-1 family and is categorized as an alarmin. It is well recognized for its important role in initiating Th2 biological responses and regulating metabolism (18). The production of IL-33 is ascribed to mesenchymal cells situated in pancreatic islets, thereby promoting the augmentation of insulin secretion, therefore impacting the activity of islet β-cells (17). Dependent on the particular illness condition and experimental model used, the effect of IL-33 might appear either as a pro-inflammatory or anti-inflammatory response. Our findings pointing out indirectly in a fact that reduced levels of IL-33 may be a risk factor for the development of insulin resistance, despite the numerous positive benefits of IL-33 in cardiovascular illnesses, obesity, and diabetes (19, 20).

Persistent low-grade inflammation is detected inside the pancreatic islets of persons with diabetes. The stimulation of innate lymphoid cells residing in the islets by interleukin-33 (IL-33) generated by mesenchymal cells results in an increased ability of myeloid cells to synthesize retinoic acid, therefore promoting the production of insulin in islet β cells. Meanwhile, Shi et al (21) have shown a favorable correlation between normal or increased levels of IL-33 and good glycemic management. Interleukin-33 is a constituent of the IL-1 family and has been previously demonstrated by Schmitz et al (22) to engage in interactions with the mechanism of tumorigenicity 2 (ST2) receptor suppression. Griesenauer and Paczesny (23) shown that the ST2 receptor exists in two main isoforms; a transmembrane form (ST2L) and a soluble form (sST2). The investigations conducted by Chackerian et al (24) and Hayakawa et al (25) have specifically shown that the interaction between IL-33 and sST2 has the capacity to suppress cellular processes that are activated by IL-33 binding to ST2L. Therefore, based on the findings of our study, it can be deduced that the decrease in sST2 levels caused by metformin leads to beneficial results by promoting the release of more IL-33 molecules, thereby enhancing glycemic control.

Attempts to find pertinent data within the network were fruitless in identifying a study that investigates the concentrations of serum IL-33 and ST-2 in diabetic patients receiving metformin therapy, save for the research conducted by Zhang et al (13). The present investigation provided evidence that the administration of metformin can result in a reduction in blood IL-33 levels in individuals diagnosed with diabetic nephropathy. Therefore, it is difficult to determine the fundamental underlying mechanism responsible for the observed rise in serum IL-33 levels and decline in serum ST-2 levels in persons diagnosed with type 2 diabetes mellitus. Therefore, the experimental results of Asensio-Lopez et al (14) imply that the transcription factor Yy1 may have a role in controlling the expression of serum ST2. Moreover, their study suggests that the suppression of Yy1 by metformin may lead to decreased levels of sST2. The ongoing inquiry has led to the following discoveries: Significant reductions in mean BMI and mean waist circumference were seen in our study following the treatment of metformin. Reiterating the results of the current study and randomized controlled clinical studies have shown that metformin is associated with weight loss and decrease in waist circumference in obese and overweight diabetic patients, as compared to the placebo (26).

The meta-analysis conducted by Hui et al (27) shown that metformin has the ability to significantly reduce weight in obese and overweight persons, even in the absence of diabetes mellitus. Clinical investigations have shown that metformin is effective in reducing weight gain and promoting weight loss in obese persons, whether or not they have type 2 diabetes mellitus (28, 29). Therefore, several extensive systematic reviews have provided insights into the administration of metformin for weight control and the treatment of obesity (30-32).

However, the exact processes responsible for the pharmacological effects of the medicine are not fully understood. Metformin may exert its anti-obesity effects via regulating the function of adipose tissue, as demonstrated in research conducted by Yuan et al (33) and Dludla et al (34). Through inhibition of white adipocyte differentiation, primarily by influencing fibroblast growth factor 21, a crucial metabolic regulator known to enhance lipolysis in white adipose tissue and prevent fat deposition, metformin was shown to reduce body weight and improve metabolic parameters in murine models of obesity (35). More rigorous studies have shown that metformin can reduce weight gain in obesity experimental models by increasing the metabolic activity of brown adipose tissue. This effect may be influenced by and not dependent on the specific activities of uncoupling protein 1, a molecular marker associated with the dissipation of energy as heat (36-39).

In addition reductions in plasma insulin, fasting plasma glucose, and postprandial glucose levels, as well as a substantial decrease in glycated hemoglobin A1 %, were seen in our study following treatment with metformin. The observed alterations can be ascribed to the pharmacological effects of metformin, which include the enhancement of peripheral insulin sensitivity by increasing glucose uptake and oxidation through the activation of adenosine monophosphate protein kinase enzyme (AMPK). Likewise, metformin inhibits the breakdown of hepatic glycogen and the production of glucose. Accordingly, metformin does not enhance the production of insulin from pancreatic β cells, hence preventing the development of low-blood sugar (40).

Conclusion

Treatment of type 2 diabetes mellitus using metformin revealed a crucial role for IL-33 and ST-2 receptors in glycemic control since increment in IL-33 and reduction in ST-2 receptors were associated with good glycemic control suggesting an inflammatory role in disease pathogenesis and response to treatment.

Limitations of the study

Short duration of study and lack of compliance and drop out of some patients were the main limitations of the present study leading to enrollment of relatively small sample size. We suggest more studies with large sample size with comparing the effect of metformin with other diabetic medications.

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Ethical considerations

The study was conducted in accordance with the principles of the Declaration of Helsinki. The Ethics Committee of AL-Qadisiyah University of Medical Sciences approved the research (Ethical code 14/30 in 4/1/2024). All participants provided written informed consent before any intervention. The authors have taken care to address ethical issues, including plagiarism, data fabrication, and double publication.

Conflict of interests

The authors of the current study want to declare no conflict of interest regarding the current research.

Study highlights

- In addition to its role in improving metabolic aspects of diabetes, metformin can help reducing and optimizing inflammatory responses in type 2 diabetes mellitus.
- Metformin can participate at least partially in limiting rate of development of diabetes complications related to augmented inflammatory responses.

Authors' contribution

Thara Hussein Abdullah: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation,Methodology,Project administration, Resources, Software, Visualization, Writing–original draft, Writing–review & editing.

Hussein Ali Saheb: Supervision, Validation.

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