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EVALUATION OF SERUM INTERLEUKINS 1 AND 10 IN TYPE 2 DIABETES MELLITUS SUBJECTS IN BENIN CITY

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ABSTRACT

Diabetes mellitus is a metabolic and endocrinological disease that results in hyperglycemia as a result of either insulin absence or non-responsiveness of insulin receptors. Interleukins have been implicated in the pathogenesis of diabetes mellitus**.** This study sought to evaluate serum interleukin-1 and 10 in diabetes mellitus. A total of three hundred subjects comprising of one hundred and eighty diabetes mellitus and one hundred and twenty apparently healthy subjects were recruited. Of the one hundred and eighty diabetes mellitus is made of 41.67% male and 58.33% females. Venous blood samples were collected into fluoride oxalate and plain containers and allowed to clot and the serum harvested. Glucose, IL-1 and IL-10 were estimated using standard methods. Statistical package for social sciences (SPSS) Chicago, USA, version 21 was used for data analysis. Our results show that IL-1 was significantly higher while IL-10 was observed to be significantly lower in diabetes mellitus when compared with apparently healthy subjects. It was observed that interleukin -1 and interleukin -10 were positively associated when correlated. Conclusively, interleukins are important in the pathogenesis of diabetes mellitus and should be evaluated for proper understanding of the pathogenesis of diabetes mellitus.

Keywords: Interleukin-1, Interleukin -10, Diabetes mellitus, Cytokine, Inflammation

INTRODUCTION

Diabetes mellitus (DM) has been considered metabolic disorder of multiple etiologies with chronic hyperglycemia and impaired carbohydrates, lipids, as well as proteins metabolism as a result of either complete or partial insufficiency of insulin secretion and/or insulin action (Lebovitz, 2000). Despite numerous persons suffering from this disease and its socioeconomic burden, the pathogenesis of type 2 diabetes mellitus has been associated with cytokines (Hamed *et al*., 2021). Insulin resistance has been observed to be an important component of type 2 diabetes mellitus - which is characterized by impaired response to insulin and in insulin – sensitive tissues, and β-cell failure – which is characterized by β-cell dysfunction and reduced β-cell mass (Zhao *et al*., 2014). It has been suggested that interleukins play a role in the pathogenesis of type 2 diabetes mellitus (Rehman et al., 2017)

Interleukins are regulatory proteins with ability to accelerate or inhibit inflammatory processes, as well as other tissue responses (Peiro *et al*., 2017). They play a great role in the immune system and rare deficiencies of a number of them have been described, which is found in autoimmune diseases. Majority of them are synthesized by T helper cells of the lymphocytes, monocytes, macrophages and endothelial cells. They promote the development and differentiation of T and B lymphocytes and haemotopoietic cells. Interleukin -1 family comprises of eleven (11) members with similar biological effects (March *et al*., 1987).These are IL-1α, IL-1β, IL-1Ra, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-36Ra, IL-37 and IL-38. Among these interleukins, IL-1α, IL-1β, IL-38, IL-33 and IL-36 are receptor-agonistic while IL-1Ra, IL-36Ra and IL-38 are receptor – antagonistic (Kaneko *et al*., 2019). Interleukin 1 has two forms which are IL-1α and IL-1β isolated from two distinct cDNAs, but is differentiated in their biological functions (Dinarello 1977). IL-1β is a 269 amino acid precursor protein which is processed by capase-1 (also known as IL-1β converting enzyme) activated in inflammasomes to the C-terminal 153 amino acid as mature IL-1β (Lachman *et al*., 1977). Earlier author have observed the association of IL-1α and IL-1β gene polymorphisms with central obesity and metabolic syndrome in a population with coronary heart disease (Carter *et al*., 2008). Failure of the β – cell has been implicated in the development of type 2 diabetes mellitus as a result of insulin resistance as β-cell function progressively deteriorates with an increasing duration of diabetes mellitus probably due to apoptosis (Donath *et al.*, 2011).

Diabetes mellitus has been classified as inflammatory condition due to over-secretion of pro-inflammatory cytokines. Interleukins -1β (IL-1β) have been identified as a major player in auto –inflammatory diseases, which act as key promoter of systemic and tissue inflammation in diabetes mellitus (Dinarello *et al.*, 2010). On the other hand, interleukin 10 (IL-10) is anti-inflammatory cytokines and plays a major role in regulating immune response and limiting inflammation (Chang *et al*., 2013). IL-10 also have inhibitory function against the action of inflammatory cytokines such as IL-12 (Yaghini *et al*., 2011). Previous authors (Giulietti *et al*., 2007) observed that diabetes mellitus is an immune dependent disease which affect the pattern of cytokine expression. Therefore, this study aims to examine the serum level of IL-1 and IL-10 in diabetes mellitus subjects.

MATERIALS AND METHODS

Study area

This study was carried out among patients attending the Diabetic Clinic of University of Benin Teaching Hospital, Benin-City. Benin City from March to August 2020.

Sample Size Determination

The sample size was calculated as 100 due to prevalence of diabetes mellitus of 8% as reported by Arugu and Maduka

(2017) in southern Nigeria using the formula proposed by Araoye and colleagues (2003).

$$N = \frac{2Z^2pq}{d^2}$$

Where:
N = Minimum sample size
Z = Standard normal deviation corresponding to 955 confidence interval =1.96
P = proportion of diabetes from a previous study
q = complimentary probability = (1-p)
d= degree of precision =0.05

$$N = \frac{2\ (1.96)^2 \text{ X } 0.08 \text{ X } 0.92}{(0.05)^2}$$

$$N = \frac{2(3.8416) \text{ X } 0.08 \text{ X } 0.92}{0.0025}$$

$$N = \frac{7.6832 \text{ X } 0.08 \text{ X } 0.92}{0.0025}$$

$$N = \frac{0.56548}{0.0025} = 226.$$

With 10% attrition of 22.6, therefore minimum sample size will be 249.

Study population
A total of three hundred (300) respondents were recruited for this study which comprises of one hundred and eighty (180) diabetic subjects attending the diabetic clinic and one hundred and twenty (120) sex and age matched apparently healthy subjects were used as control.

Ethical clearance and Informed consent
Ethical clearance was obtained from the ethical committee of University of Benin Teaching Hospital, Benin City while informed consent was taken from the subjects after properly explaining the procedure and protocol of the study to them.

Inclusion and exclusion criteria
Inclusion criteria includes both male and female who had been confirmed type 2 diabetic patients without any other underline ailment, non- alcoholics, non- smokers and not pregnant women who visited the diabetic clinic of University of Benin Teaching Hospital, Benin City while exclusion criteria are alcoholics, Smokers and Pregnant women and those that are not diabetes.

Sample Collection
After an overnight fast and using aseptic precautions, 6ml of venous blood was collected from the medial cubital vein using vacutainer and needle from each of the subjects and controls into vacutainer plain container and fluoride oxalate containers. The blood in plain containers was allowed to clot, retract and spun at 3000rpm for 10 minutes to harvest the serum. Using a sterile transfer pipette 1.8ml of serum was collected and aseptically dispensed into 2ml plain container and label appropriately. All samples were kept frozen at -20^{o}C until ready for analysis. The blood sample in the fluoride oxalate containers was used for the analysis of blood glucose immediately to confirm diabetes status of subjects.

Biochemical and immunological analysis
Interleukin 1 (IL-1) and Interleukin 10 (IL-10) were analysed using enzyme linked immunosorbent assay (ELISA) that employs Sandwich-ELISA method while fasting blood glucose was analysed using Glucose Oxidase Peroxidase method developed by Trinder (1969) using Randox reagent. Manufacturer's instructions were strictly followed in all procedures with control samples added to ensure quality control.

Statistical analysis
Data generated from analysis were analyzed statistically using Statistical Package for Social Sciences (SPSS) IBM, Chicago, version 21.0. The difference in mean were evaluated using student "t" test and significant difference is at <0.05 while Pearson correlation was at ≤0.001.

RESULTS
The results of this study shows the sample distribution of the respondents with Diabetes mellitus being one hundred and eighty (60%) while control is one hundred and twenty (40%) as shown in figure 1.

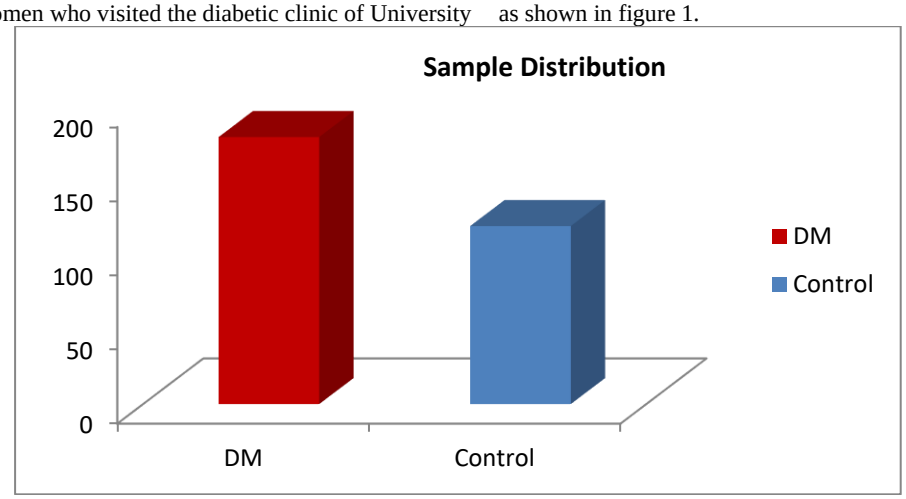

Figure 1: Sample distribution of respondents in the study

Figure 2 shows the sex distribution of diabetes mellitus subjects in the study with male having seventy five (41.67%) and female one hundred and five (58.33%).

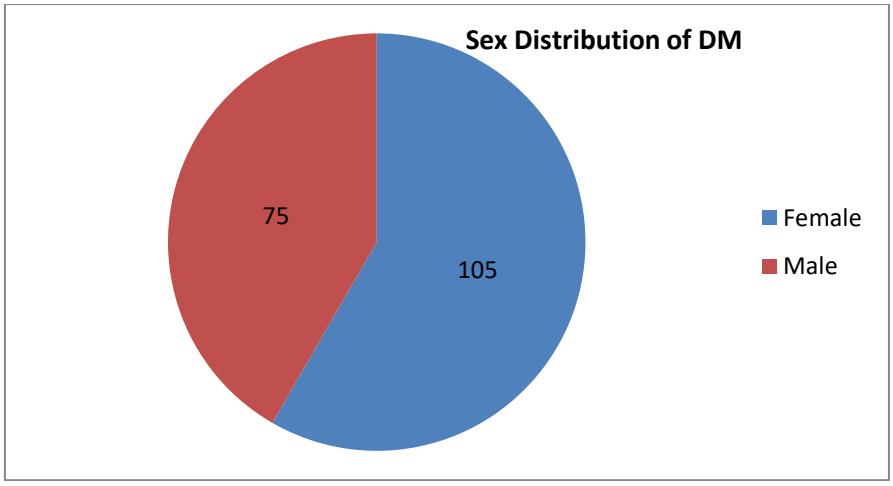

Figure 2: Sex distribution of Diabetes mellitus subjects in the study

Table 1 shows anthropometric variables of respondents. There was no significant difference observed in the age of both diabetes subjects and apparently healthy subjects when the means were compared. Also, there was no significant difference observed when the height of both diabetes mellitus subjects and apparently healthy subjects were compared. There was significantly higher weight and body mass index in diabetic subjects than apparently healthy subjects when compared.

Table 1: Anthropometric variables of the respondents

Parameter	Diabetics (n=180)	Control (n-120)	t value	Significant
Age (Years)	48.70±0.75	50.20±0.96	-1.243	0.217ŧ
Height (m)	1.62±0.01	1.60±0.01	1.320	0.190ŧ
Weight (Kg)	82.17±0.95	64.05±0.97	12.879	0.000*
BMI (Kg/m^2)	31.83±0.56	25.26±0.55	8.035	0.000*

Table 2 shows the mean ± SEM of fasting blood sugar, interleukin 1 and 10 of diabetes mellitus and apparently healthy subjects. Diabetes mellitus have a significantly higher (P<0.05) of fasting blood sugar when compared with apparently healthy subjects. In the same vein, diabetes mellitus have a significantly higher (P<0.05) interleukin 10 but significantly lower (P<0.05) when compared with apparently healthy subjects.

Table 2: Interleukins- 1 and10 of diabetes mellitus and apparently healthy subjects

PARAMETER	Diabetics (n=180)	Control (n-120)	t value	Significant
FBS (mmol/l)	15.37±0.79	2.60±0.06	13.205	0.000*
IL-1 (ng/ml)	1.76±0.02	0.06±0.00	27.269	0.000*
IL-10 (ng/ml)	1.32±0.04	4.12±0.16	-14.028	0.000*

Figure 3 shows a negative correlation between BMI and interleukin 1. This is also same for BMI and interleukin 10 as shown in figure 3. There was also, negative correlation observed between fasting blood sugar and interleukin 1 and 10 as shown in figures 4 and 5. However, there was a positive correlation between interleukin 1 and interleukin 10 when compared as shown in figure 6.

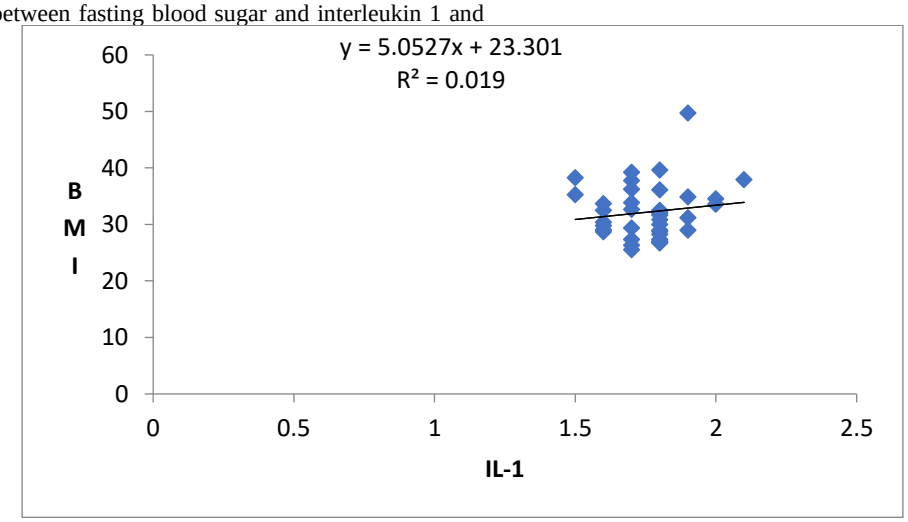

Figure 3: Positive correlation between body mass index and interleukin -1

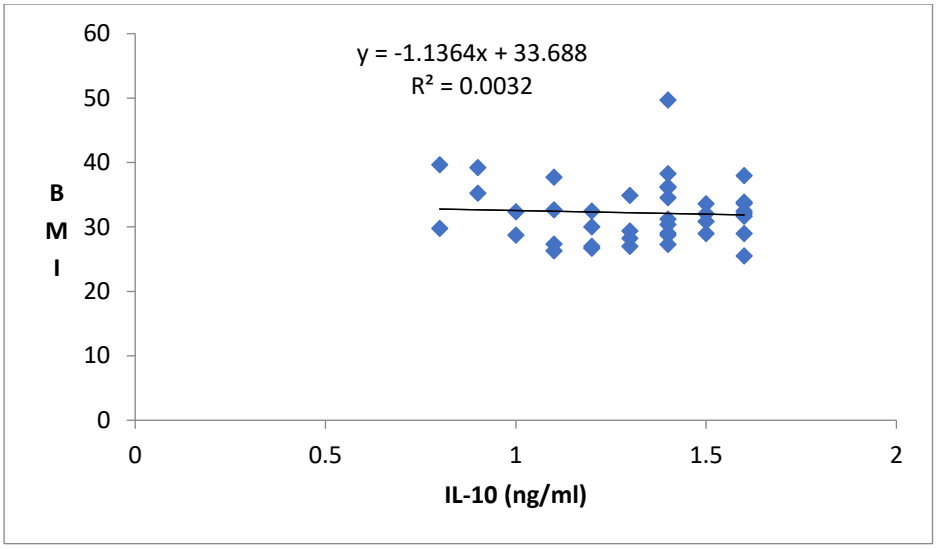

Figure 4: Negative correlation between body mass index and Interleukin -10

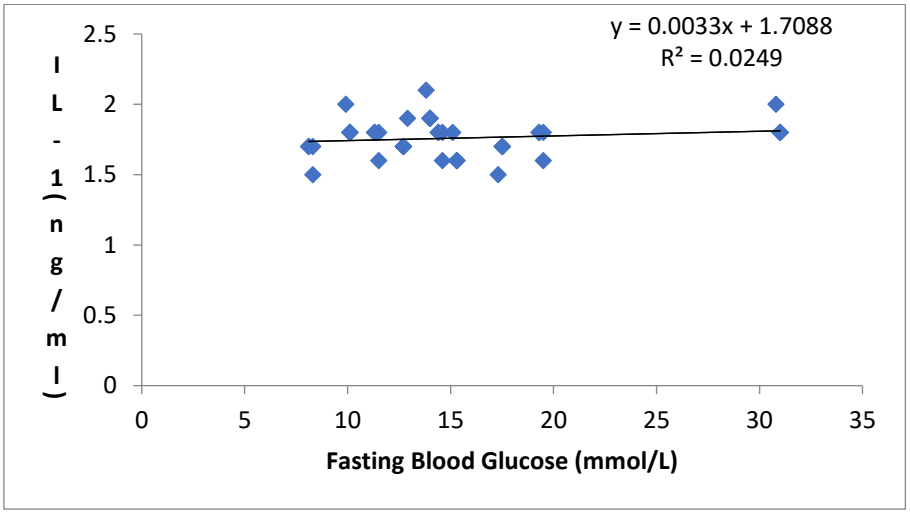

Figure 5: Positive correlation between fasting blood glucose and interleukin -1

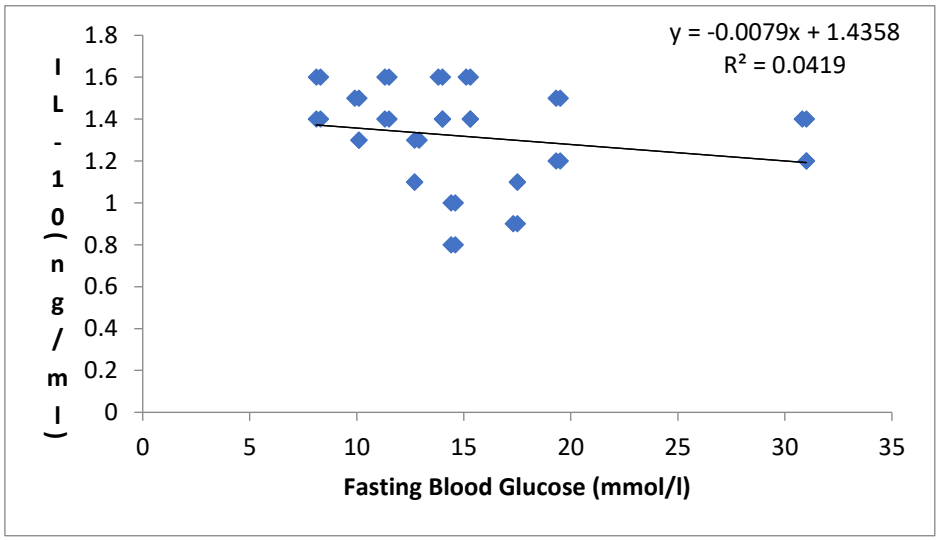

Figure 6: Negative correlation between fasting blood glucose and interleukin - 10

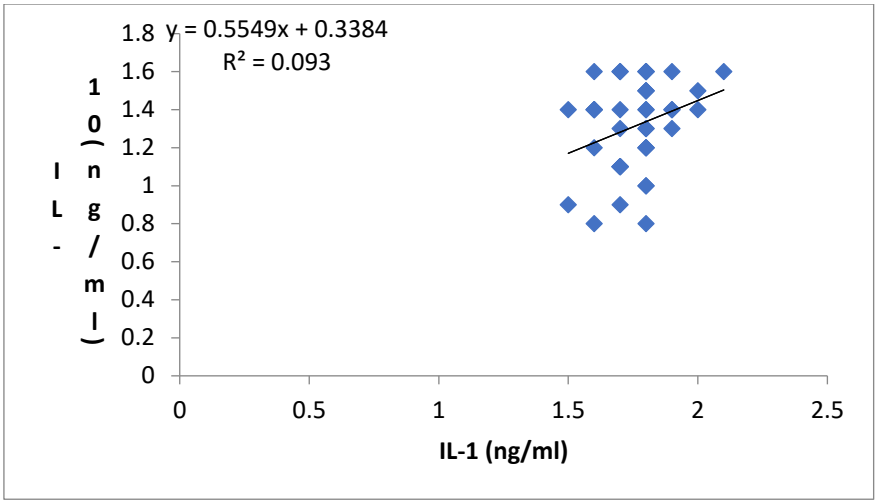

Figure 7: Positive correlation between Interleukin -1 and Interleukin- 10

DISCUSSION

Diabetes mellitus is considered as an inflammatory disease and has affected the pattern of cytokines in the system (Xiao et al., 2014). In this study, interleukin 1(Pro-inflammatory cytokine) and interleukin 10 (anti-inflammatory cytokine) were evaluated. There was no significant difference ($p>0.05$) observed in the age of both diabetic and control subjects when compared. This is due to the fact that the respondents were in the same age range. There was significantly higher ($p<0.05$) weight in diabetes mellitus than apparently healthy subjects. This implies that diabetic subjects are weightier than apparently healthy subjects, which is a risk factor for diabetes mellitus. It was observed that diabetes mellitus had a significantly higher ($P<0.05$) body mass index than apparently healthy subjects. The mean body mass index of diabetes mellitus in this study was observed to be 31.83±0.56 which falls under the classification of obese subjects, and obesity is one of the risk factors for diabetes mellitus.

There was a significantly higher ($p<0.05$) fasting blood sugar levels in diabetes mellitus when compared with apparently healthy subjects. Blood sugar has been observed as a gold standard indicator of diabetes mellitus (Kianpour et al., 2021). This is in tandem with earlier work by Adu (2022) when he observed hyperglyceamia in diabetes mellitus subjects. Interleukin-1 was observed to be significantly higher in diabetes mellitus subjects when compared with apparently healthy subjects. This confirms that diabetes being an inflammatory disease had more of pro-inflammatory cytokines than apparently healthy subjects. Giulietti and colleagues (2007) in their study observed that in type 2 diabetes mellitus, monocytes of peripheral blood produce more inflammatory cytokines than those from apparently healthy subjects. Dinarello (2009) observed that interleukin-1β is associated with the destruction of insulin producing beta (β) cells of the pancreas. Donath and Mandrup-Poulsen (2008) in their study concluded that administration of neutralizing monoclonal antibodies to IL-1β improves glycemic control and beta cell function in type 2 diabetes mellitus. Likewise, there was positive correlation between interleukin-1 with both fasting blood sugar and body mass index. This implies that interleukin -1 increases as blood sugar and body mass index increases as earlier observed by Hamed and colleagues (2021).

There was a significantly lower ($P<0.05$) interleukin-10 in diabetes mellitus when compared with apparently healthy subjects. Interleukin -10 being an anti-inflammatory cytokine is smaller in circulation of diabetes which is an inflammatory disease than in apparently healthy subjects. This is in tandem with previous authors (Yaghini *et al.*, 2011) which did similar work on this area. They opined that the key role of IL-10 is to work as inhibitory cytokine against action of inflammatory cytokine (Yaghini *et al.*, 2011). However, there was negative correlation between interleukin-10 with both body mass index and fasting blood sugar. This implies that interleukin-10 decreases as body mass index and blood sugar increases. Bosutti and colleagues (2008) opined in their study that low level of circulating IL -10 has been associated with obesity while Heeschen and colleagues (2003) linked low IL-10 with cardiovascular disease. This is in tandem with previous observation by previous authors (Van Excel *et al*., 2002) that did similar work on this area and concluded that low levels of serum interleukin-10 can be considered as a risk factor of type 2 diabetes mellitus.

This study also observed a positive association between interleukin -1 and interleukin -10 as shown in figure 6. This implies that both interleukins (1& 10) are directly proportional to each other. This is in agreement with earlier observation by previous authors (Chang *et al*., 2013) who did similar work on interleukins.

In conclusion, it has been observed that cytokines takes part in the pathogenesis of diabetes mellitus with an increased level of IL-1 as a result of β- cell failure in type 2 diabetes mellitus subjects. Also, IL-10 was observed to be lowered in diabetes mellitus subjects. It is therefore recommended that these cytokines should be incorporated in the test menu of type 2 diabetes mellitus subjects in the course of their management for a better understanding of the pathogenesis of the disease.

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Conflict of interest: None

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