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Study the role of interleukin 1 beta in diabetic Iraqi patients with and without insulin treatment.

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Abstract: Diabetes mellitus is a long-term endocrine/metabolic disease with various causes, mainly related to insulin deficiency and resistance. Type 2 diabetes mellitus (T2DM) constitutes about 90% of all diabetes cases. In T2DM, th\e body's response to insulin is reduced, known as insulin resistance. Initially, the body compensates for this by producing more insulin to keep blood sugar levels in check, but over time, insulin production decreases, leading to T2DM. On the other hand, T1DM is caused by the immune system attacking and destroying insulin-producing beta cells, requiring lifelong insulin therapy. **Study design**: In a case-control study conducted at the Diabetes and Endocrinology Specialized Center in Nasiriyah between December 2023 and January 2024, 60 patients with Diabetes Mellitus type 2 were divided into two groups. One group consisted of 30 patients treated with insulin injections, while the other group consisted of 30 patients treated with oral medication only. Additionally, 30 people without Diabetes Mellitus were included as a control group.

Aim of Study: Estimate IL beta and other biochemical parameters in Diabetes mellitus patients to study their role in the disease's pathogenicity. Keywords: IL-1beta, DM, FBS, HOMO IR

Introduction: Diabetes mellitus is a chronic endocrine/metabolic disease with various causes (mainly due to insulin deficiency and insulin resistance) and diverse clinical manifestations. Symptoms of significant hyperglycemia include increased urination, excessive thirst, and (1) weight loss, sometimes accompanied by excessive hunger and blurred vision. Impairment of growth and susceptibility to certain infections may also occur with prolonged hyperglycemia. Type 2 diabetes mellitus (T2DM) accounts for approximately 90% of all diabetes cases. In (2) T2DM, there is a diminished response to insulin, known as insulin resistance. During this state, insulin becomes ineffective and is initially compensated for by an increase in insulin production to maintain glucose balance. However, over time, insulin production decreases, and T1DM is caused by autoimmune-mediated β-cell (3) leading to the development of T2DM. (4) dysfunction and apoptosis, leading to the lifelong need for exogenous insulin therapy. 2 Gestational diabetes mellitus is a classification used to identify women who develop diabetes mellitus during pregnancy, rather than being a specific pathophysiologic condition. It includes women who develop Type 1 diabetes mellitus during pregnancy and those with (5). undiagnosed asymptomatic Type 2 diabetes mellitus that is discovered during pregnancy. Insulin resistance is likely the initial issue in Type 2 diabetes and begins many years before

symptoms appear or high blood glucose levels signal the diagnosis. Insulin resistance affects the body's peripheral cells (mainly muscle and fat cells) as well as the liver. It is influenced by Insulin, a peptide hormone produced by pancreatic (6) by genetic and environmental factors. The main (7) beta cells, regulate blood glucose levels by promoting cellular glucose uptake. effects of insulin on tissues are as follows:

1. Carbohydrate metabolism: It increases the rate of glucose transport across the cell membrane in adipose tissue and muscle. It also increases the rate of glycolysis in muscle and adipose tissue. Furthermore, insulin stimulates the rate of glycogen synthesis in several tissues, including adipose tissue, muscle, and liver. (8)

2. Insulin also decreases the rate of glycogen breakdown in muscle and liver. resistance in humans is primarily caused by obesity, inflammation or infections, and high blood sugar levels.

Obesity is often linked to increased levels of free fatty acids in the blood and higher lipid oxidation. This can lead to insulin resistance by inhibiting certain enzymes and redirecting glucose into a different metabolic pathway. Inflammation is marked by higher levels of cytokines like tumor necrosis factor α (TNF-α) or interleukin (IL), which can affect glucose transport in cells. High blood sugar levels can increase the flow of glucose into muscle cells. Both obese individuals and those with type 2 diabetes often have reduced responsiveness to insulin in terms of glucose transport. In summary, insulin resistance resulting from obesity, inflammation, infection, or hyperglycemia is characterized by increased glucose transport through certain pathways and reduced insulin-mediated glucose transport in muscles. (9) The interleukin-1 (IL-1) family of mediators is associated with acute and chronic inflammation and is essential in the host response to infection. (10) IL-1β is a regulator of the body's inflammatory response and is produced after infection, injury, and antigenic challenge. It plays a role in various diseases, including autoimmune diseases such as rheumatoid arthritis and type 1 diabetes, as well as in diseases associated with metabolic syndrome such as atherosclerosis and type 2 diabetes. (11) IL-1B acts as a key regulator of inflammation. It is mainly produced by blood monocytes, tissue macrophages, skin dendritic, cells, and brain microglia. (12) Monocytic cells, such as macrophages and NK cells, are potent producers of IL-1. Additionally, they express mRNA for IL-1 and TNF-α in the inflammatory cell. (13) IL-1beta inhibits beta cell function and induces cell death in isolated islets of Langerhans and the isolated perfused pancreatic gland. (14)

Material and method Ninety people, aged between 30 and 80, participated in this study. There were 60 diabetes mellitus patients divided into two groups: 30 patients were treated with insulin injections, and 30 patients were treated with oral tablets only. Additionally, there were 30 healthy controls. The patients were referred to the Nasiriyah Diabetes and Endocrinology Specialized Center between December 2023 and January 2024. The study focused on patients with type 2 diabetes mellitus. Data including clinical history, demographics (age, height, duration, and weight), chronic illnesses, and treatment plans were collected using a brief questionnaire. Sample Collection: Five milliliters were taken from each patient. It was divided into two parts. We put 3 ml in a test tube (10 ml Gel Tube), and 2 ml in an EDTA tube. The

specimen for the Gel tube was separated by centrifugation at 3000 rpm for 10 minutes to get the serum. FBS was done at the time of serum separation. - The three ml serum was immediately divided into two small tubes (Eppendorf tube 2.0) to do these tests later. - Three ml separated serum stored at –20 °C for the subsequent assay of IL 37 by ELISA, Insulin resistance, and C-peptide - FBS; Insulin resistance these analyses were measured by fully automated. Hemolysis samples were rejected. - Tow ml whole blood into EDTA tube use for HbA1c test. Ethical approval: Before participating in the study, all participants were asked to provide informed consent. They were informed of the study procedure and any associated risks to ensure they fully understood what was involved. The local ethics committee reviewed and approved the study protocol, subject data, and permission form. The approval was documented using the reference number 796, dated 27/11/2023.

Results 1. Demographic and clinical characteristics In this study, a total of 90 participants were involved, including 60 samples from patients with diabetes mellitus and 30 samples from healthy individuals as the control group. The sample size was determined using an equation based on the latest reported prevalence. An interview was conducted to gather the participants' history and demographic information. The patients were divided into subgroups based on age, gender, and disease duration. The clinical demographic characteristics and laboratory parameters of the study groups are summarized in Table 1. The mean age of the patients was 50.71 years with a standard deviation of 13.37, and the mean BMI was 29.64 with a standard deviation of 4.45. The age range of participants was as follows: more than 56 years old (50%), 41-56 years old (31.7%), and 25-40 years old (18.3%). The analysis of the data shows that approximately 36.7% of patients had a disease duration of more than 10 years, 6.7% had a duration of less than one year, and 31.7% of the patients had a duration of 1-5 years. Furthermore, 55% of the patients were obese, 33% were overweight, and 11.7% were of normal weight.

Table 1: Descriptive of the demographic characteristics of the study population (N=90)

Variables	Groups	Patient	Control
Age. groups (Years)	25-40 Years	11	8
	41-56 Years	19	20
	More than 56 Years	30	2
BMI.groups	Normal weight	7	8
	Overweight	20	12
	Obesity	33	10
Sex	Male	30	16
	Female	30	14
Type of treatment	Insulin	30	0
	Oral	30	0
	Control	/	30
Duration Disease	No	/	30
	Less than one Years	4	/

	1-5 Years	19	/
	6-10 Years	15	/
	More than 10 Years	22	/

2. Difference between the level of Immune marker (IL-1 beta) in the diabetes mellitus cases and control group.

In general, patients with diabetes mellitus showed higher levels of IL-1 beta compared to the healthy control group. The results indicated a significant difference in IL-1 beta levels among the groups, with the means and standard deviations presented in Table 3. The average level of IL-1 beta in patients was 248.92±106.87, which was significantly higher than that of the control group at 94.85±42.40 ($p \leq 0.001$).

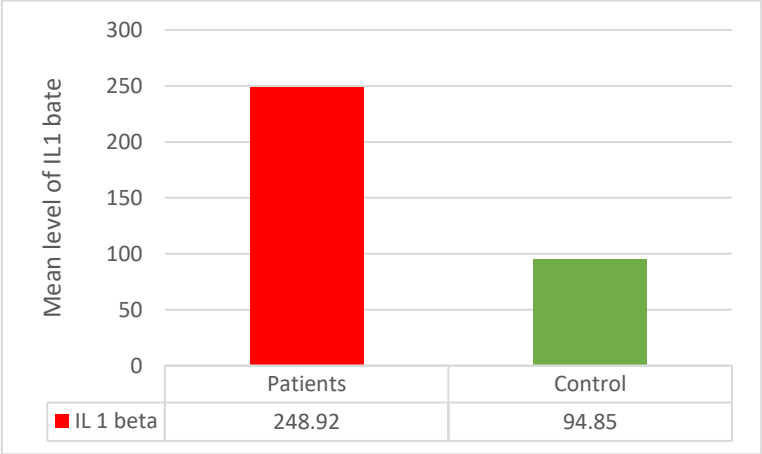


Figure 1: Results of the analysis of basic diabetes mellitus for patients with control groups (T-test was S= significant at $p \leq 0.05$, NS= Non- significant).

3. Difference between the level of Diagnostic biomarker of diabetes (FBS and HOMO IR) in the diabetes mellitus cases and control group

In general, patients with diabetes mellitus showed significantly higher levels of FBS and HOMO IR compared to the healthy control groups. The results indicated that there were increasing and highly statistically significant differences in the levels of FBS and HOMO IR among the groups. The means and standard deviations are presented in Table 3.3. The mean levels of FBS and HOMO IR in patients were 213.02±76.97 and 7.26±6.23 respectively, which were significantly higher than those for the control group, which were 88.10±10.94and 1.71±0.20 respectively ($p \leq 0.001$)

Table 2: Results of the analysis of basic diabetes mellitus characteristics for disease with control groups.

SGP-130	Patients Mean±SD N=60	Control Mean±SD N=30	P value
FBS	213.02±76.97	88.10±10.94	<0.001[S]
HOMO IR	7.26±6.23	1.71±0.20	<0.001[S]
T-test was *: significant at p ≤ 0.05 SD: standard deviation; S: significant; NS= Non-significant.			

The distribution of serum levels of FBS and HOMO IR in patients compared to the healthy control group is presented in Table 2.

4. Difference between the level of Immune marker for diabetes mellitus Patients (Insulin and Oral) compared to the control group. Generally, patients with diabetes mellitus showed an increasing level of IL-1 beta compared to three groups: G1, G2, and the healthy control group. The results indicated a significant difference in IL-1 beta levels among the groups. The means and standard deviations are presented in Figure 3. The mean level of IL-1 beta in G1 was 184.84 ± 87.95 and 94.85 ± 42.40, respectively. Using post hoc (LSD) analysis, the mean level was significantly higher in G1 than in the G2 and Control groups ($p \leq 0.001$), as indicated in Figure 2.

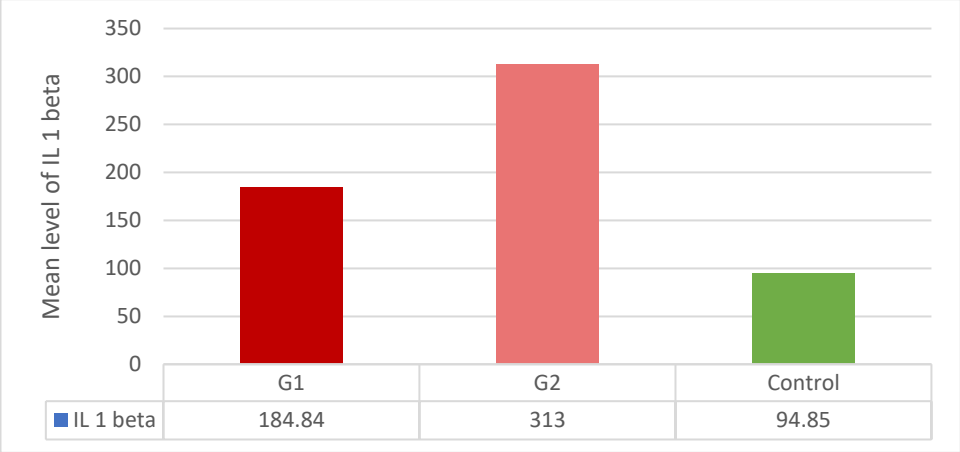


Figure 2: The difference in IL 1 beta for diabetes mellitus disease (G1 and G2) and control

5. Difference between the level of Diagnostic biomarkers of diabetes for diabetes mellitus Patients (G1 and G2) compared to the control group. The study found that patients with diabetes mellitus showed higher levels of FBS, HbA1c, IRI, and HOMO IR in Group G1 compared to Group G2 and the healthy control group. Meanwhile, the level of C-Peptide was higher in the healthy control group compared to groups G1 and G2. The results indicated significant differences in FBS and HOMO IR among the groups. The mean levels of FBS and HOMO IR (232.63±88.37) and (9.33±7.13) for Group G1, were significantly higher than for

Group G2 and the control group ($p \leq 0.001$). However, the level of C-Peptide did not show a statistically significant difference ($p \geq 0.05$).

Biomarkers	G1 Mean±SD N=30	G2 Mean±SD N=30	Control Mean±SD N=30	P value
FBS	232.63±88.37	193.40±58.70	88.10±10.94	**<0.001[S]**
HOMO IR	9.33±7.13	5.18±4.39	1.71±0.20	**<0.001[S]**
ANOVA -test was *: significant at $p \leq 0.05$, N: number of cases; SD: standard deviation; S: significant; NS= Non-significant., G1= Insulin, G2= Oral				

6. Difference between the level of biomarkers for diabetes mellitus Patients compared to G1 and G2. The study revealed that patients with diabetes mellitus had higher levels of IL-1 beta and IL37 compared to the two other groups, G1 and G2. There were significant differences in the levels of IL-1 beta and IL-37 among the groups, and the means and standard deviations are presented in Figure 3. The average level of IL-1 beta in G1 was 184.84±87.95, which was significantly lower than in G2 ($p \leq 0.001$), as indicated in Figure 3.

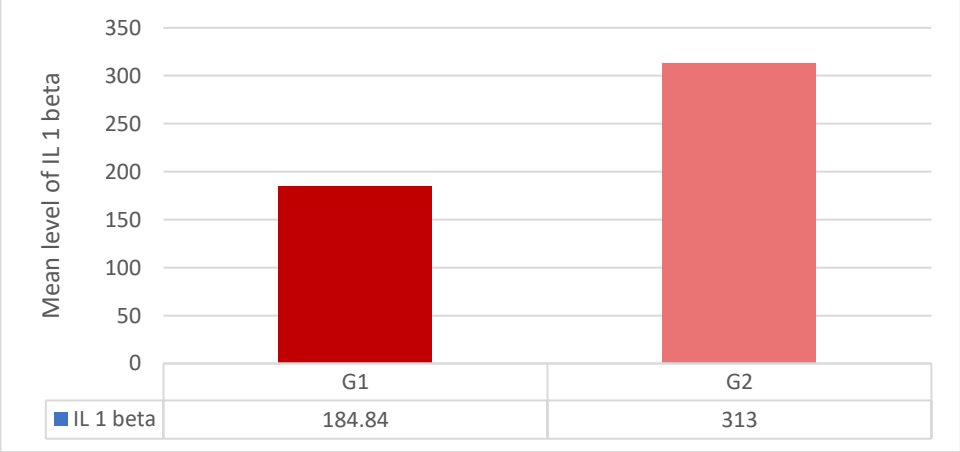


Figure 3: Results of the analysis of basic diabetes mellitus for patients with control groups (T-test was S= significant at $p \leq 0.05$, NS= Non-significant, G1= Insulin, G2= Oral).

Generally, Patients with diabetes mellitus in Group G1 showed higher levels of FBS and HOMO IR compared to Group G2. However, The results revealed a significant difference in FBS and HOMO IR levels between the groups. The mean levels and standard deviations are presented in Table 4. The mean levels of FBS and HOMO IR in Group G1 were 213.02±76.97 and 7.26±6.23, respectively, which were significantly higher than those in Group G2 ($p \leq 0.001$). as shown in Table 4. Table 4 The difference in biomarkers for diabetes mellitus disease compared to G1 and G2.

Table 4 The difference in biomarkers for diabetes mellitus disease compared G1 and G2

SGP-130	G1 Mean±SD N=30	G2 Mean±SD N=30	P value
FBS	213.02±76.97	88.10±10.94	**<0.001[S]**
HOMO IR	7.26±6.23	1.71±0.20	**<0.001[S]**
T-test was *: significant at p ≤ 0.05, SD: standard deviation; S: significant; NS= Non- significant. G1= Insulin, G2= Oral			

7. Examination of the mean differences in the IL 1 beta and IL -37 levels according to the Sex groups Figure (4) illustrates the mean level of IL 1 beta and IL -37 in the Patients and control groups according to Sex. Results showed that the levels of IL 1 beta and IL -37 were increased markedly in the patients group in both males and females compared to the control, p values were <0.001.

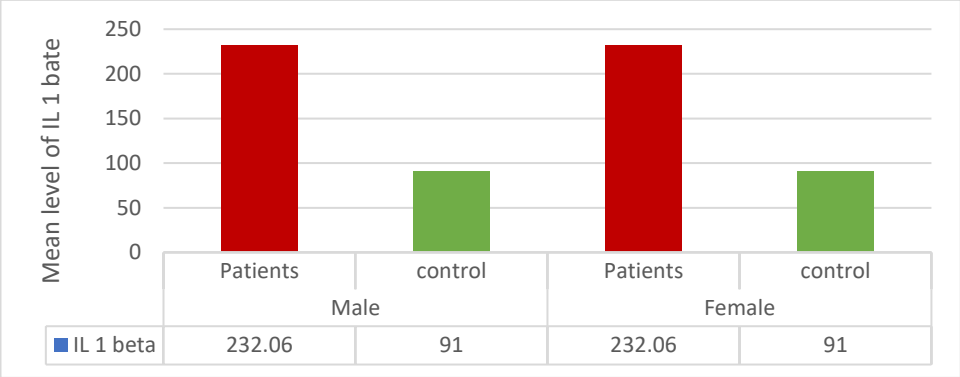


Figure 4: The effect of gender on the biochemical parameters according to the Patients and control groups

8. Examination of the mean differences in the IL 1 beta levels according to the BMI groups In Figure (5) a comparison of serum levels of IL 1 beta in different BMI groups was highly were performed. Both levels of IL 1 beta were increased within all the BMI ranges and were statistically significant (p= <0.001).

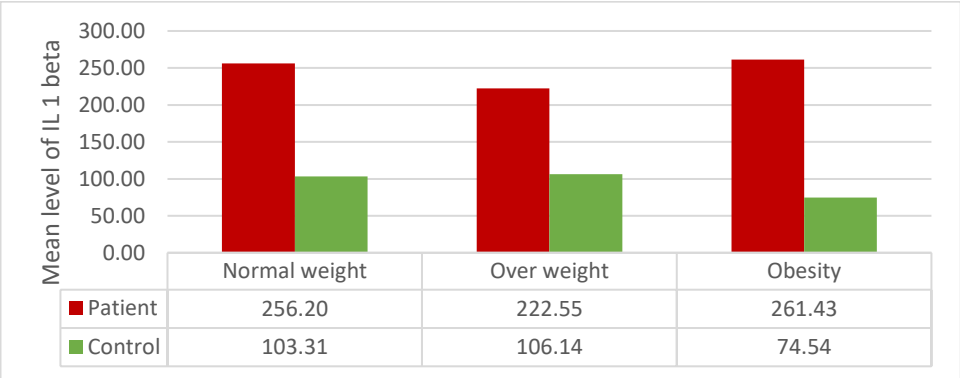


Figure 5: The effect of BMI groups on the IL-1 beta according to the patient and control groups (T-test was S= significant at $p \leq 0.05$, NS= Non-significant)

9. correlation

Considering the important role of the measured biomarkers, the Spearman rank test analysis of diabetes mellitus was used to analyze the response relationship between parameters. The correlation study shows many significant correlations among the measured parameters (figure 6).

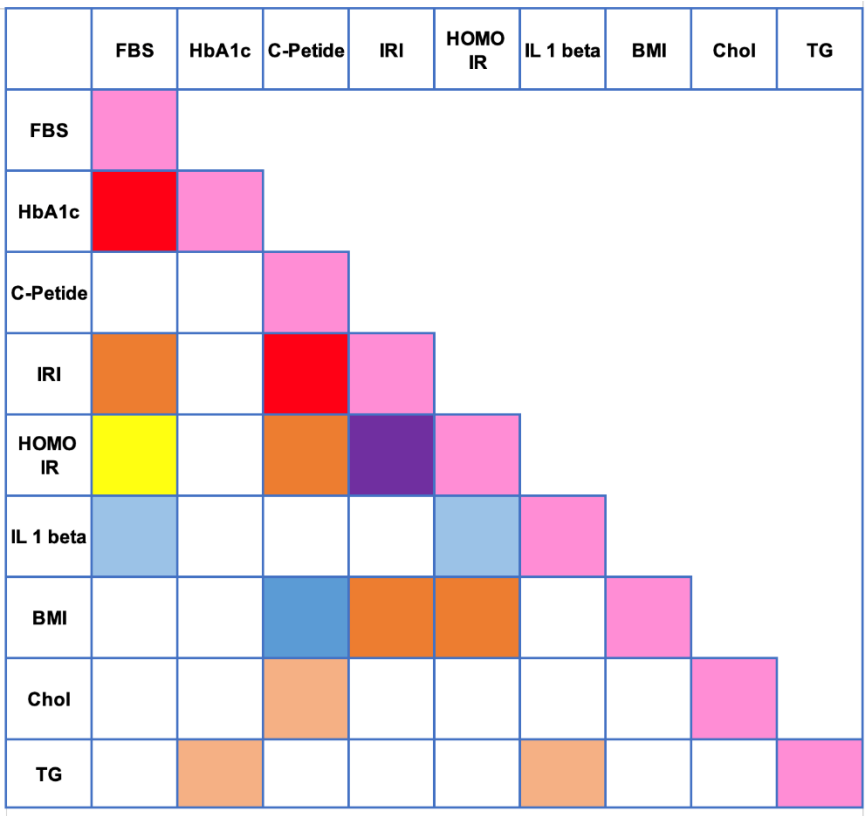
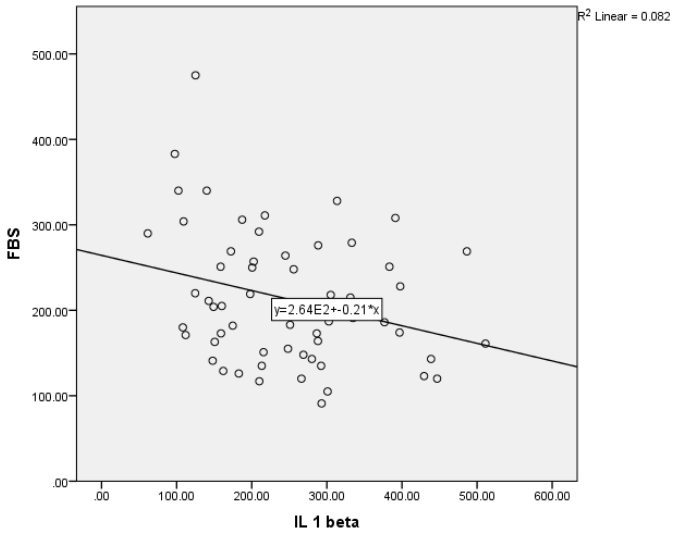
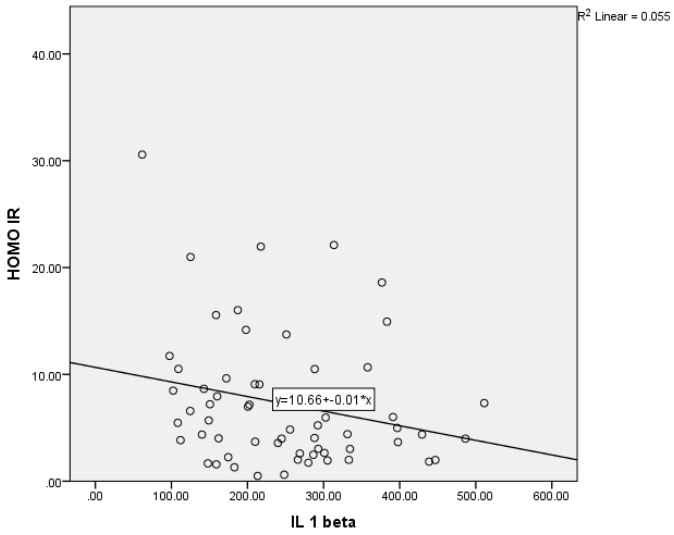


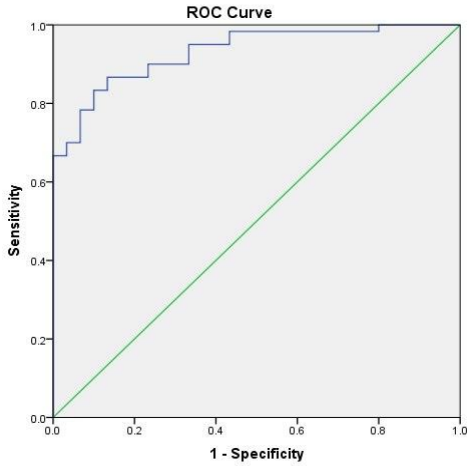
Figure 6 Heatmap of the Spearman rank test analysis of diabetes mellitus disease. white boxes indicate a lack of correlation ($p>0.05$) while colored boxes reported statistically significant direct and indirect correlations, respectively. The intensity of the colour indicates the following relation: Yalow (r= 0.7); Red (r=6), Purple (r=0.9) Orange (r=0.3); Light Orange (r=-0.3); Blue (r=-0.4); Light Blue (r=0.4)





10. Receiver Operating Characteristic Analysis. ROC curve and AUC analysis for the biomarkers of diabetes mellitus disease compared to the control group Results of the receiver operating curve (ROC) and area under curve (AUC) analysis for the CPeptide, IL 1 beta, and IL 37 as diagnostic parameters were done. IL 1 beta was shown a good performance for predicting diabetes mellitus disease compared to the control group, data are presented in Table (5). For IL 1 beta levels: (sensitivity = 88.5%, specificity 86.7 %) at a level = 140.09, the p-values of the AUC were <0.05 and highly statistically significant, as shown in table (4). The p-values of the AUC were <0.05 and statistically significant. Youden's J statistics of the IL1 beta. for parameters in Figure (7) confirm these results.

Variable	AUC	Sensitivity	Specificity	Youden index	Cut off	CI	P value
IL 1 beta	0.93	88.50%	86.70%	0.752	140.09	0.883-0.981	**<0.001[S]**



Discussion

Diabetes mellitus results from inadequate insulin action. This common metabolic disease is characterized by varying levels of chronic hyperglycemia and increased availability of free fatty acids. (15) Type 2 diabetes mellitus develops when the beta-cell function fails to compensate for insulin resistance. Beta-cell function deteriorates progressively with increasing duration of diabetes, partly due to beta-cell demise through apoptosis. (16) T2DM has been identified as an immune-mediated disease that leads to impaired insulin signaling and selective destruction of insulin-producing β-cells, in which cytokines play an essential role. Disturbance of the anti-inflammatory response could be a crucial component of chronic inflammation in T2DM. (17) In our research, patients with diabetes mellitus disease showed an increasing range of IL1-beta compared to the healthy control groups. This is what we found in a study by Safaa I Tayel (17) she found diabetic patients had significantly higher IL 1- β gene expression transcript than the control group. In general, data from patients with diabetes mellitus showed a higher range of levels for FBS and HOMA-IR when compared to the healthy control groups. The results indicated increasing and highly statistically significant differences in the levels of FBS and HOMA-IR. agreement with our research, as we found a substantial (18) among the groups, N. Yaghoobi suggests that (19) correlation between IL-1β expression and glycemia. and M Böni-Schnetzler variable blood glucose levels could contribute to the variable expression levels of IL-1β. J. Obesity, insulin resistance, and type 2 diabetes are linked to the chronic A. Ehses (20) activation of the innate immune system, which leads to an inflammatory condition in both pancreatic islets and insulin target tissues in animal models and humans with type 2 diabetes. In the case of pancreatic islets, individuals with type 2 diabetes have higher

levels of IL-1β and various chemokines compared to nondiabetic controls. Moreover, islets from individuals with type 2 diabetes are infiltrated with macrophages. When human islets are exposed to metabolic stress (elevated glucose and palmitate), they release increased levels of cytokines.) Research has shown (21 This information is based on research conducted by Theresa V. Rohm. that IL-1β, a substance produced by islet macrophages, is known to hinder insulin secretion, leading to glucose intolerance and the development of type 2 diabetes (T2D). Additionally, IL-1β has been found to promote insulin resistance. In adipose tissue, IL-1β disrupts insulin signaling in fat cells. Studies have demonstrated that patients with diabetes mellitus have higher levels of IL-1β compared to three other groups: those who receive insulin injections (G1), those who take oral anti-diabetic medication (G2), and healthy control groups. The results indicated a significant increase in the levels of IL1-beta in both male and female (22) patients compared to the control group. This is consistent with the findings of S. Mirza which showed no significant gender differences. Additionally, individuals with diabetes were found to be significantly older than those without diabetes. there were no significant gender differences but as expected individuals with diabetes were significantly older than those without diabetes.

reference

1. Reddy SSK, Tan M. Diabetes mellitus and its many complications. Diabetes Mellitus2020. p. 1-18.

2. American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33 Suppl 1(Suppl 1): S62-9.

3. Goyal R, Jialal I. Diabetes mellitus type 2. 2018.

4. Baynest HW. Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus. Journal of Diabetes & Metabolism. 2015;06.(05)
5. Sharma S. Diabetes mellitus: an overview. Journal of Advanced Scientific Research. 2018;9.3-10:(01)
6. Guthrie RA, Guthrie DW. Pathophysiology of Diabetes Mellitus. Critical Care Nursing Quarterly. 2004;27(2):113-25.
7. Puddu A, Sanguineti R, Montecucco F, Viviani GL. Evidence for the gut microbiota short-chain fatty acids as key pathophysiological molecules improving diabetes. Mediators of inflammation. 2014;2014.
8. Newsholme E, Dimitriadis G. Integration of biochemical and physiologic effects of insulin on glucose metabolism. Experimental and Clinical Endocrinology & Diabetes. 2001;109(Suppl 2):S122- S34.
9. Ebeling P, Koistinen HA, Koivisto VA. Insulin-independent glucose transport regulates insulin sensitivity. FEBS letters. 1998;436(3):301-3.
10. Netea MG, van de Veerdonk FL, van der Meer JW, Dinarello CA, Joosten LA. Inflammasome-independent regulation of IL-1-family cytokines. Annu Rev Immunol. 2015;33:49-77.
11. Maedler K, Dharmadhikari G, Schumann DM, Størling J. Interleukin-1 beta targeted therapy for type 2 diabetes. Expert opinion on biological therapy. 2009;9(9):1177-88.
12. Tulotta C, Ottewell P. The role of IL-1B in breast cancer bone metastasis. Endocrine-related cancer. 2018;25(7):R421-R34.
13. Mandrup-Poulsen T, Zumsteg U, Reimers J, Pociot F, Mørch L, Helqvist S, et al. Involvement of interleukin 1 and interleukin 1 antagonist in pancreatic β-cell destruction in insulin-dependent diabetes mellitus. Cytokine. 1993;5(3):185-91.
14. Reimers JI. Interleukin-1 beta-induced transient diabetes mellitus in rats. A model of the initial events in the pathogenesis of insulin-dependent diabetes mellitus? Danish Medical Bulletin. 1998;45(2):157-80.
15. Borilova Linhartova P, Poskerova H, Tomandlova M, Bartova J, Kankova K, Fassmann A, Izakovicova Holla L. Interleukin-1 gene variability and plasma levels in Czech patients with chronic periodontitis and diabetes mellitus. International journal of dentistry. 2019;2019.
16. Larsen CM, Faulenbach M, Vaag A, Vølund A, Ehses JA, Seifert B, et al. Interleukin-1–receptor antagonist in type 2 diabetes mellitus. New England Journal of Medicine. 20.26-1517:(15)356;07

17. Banerjee M, Saxena M. Interleukin-1 (IL-1) family of cytokines: role in type 2 diabetes. Clinica chimica acta. 2012;413(15-16):1163-70.
18. Yaghoobi N, Al-Waili N, Ghayour-Mobarhan M, Parizadeh S, Abasalti Z, Yaghoobi Z, et al. Natural honey and cardiovascular risk factors; effects on blood glucose, cholesterol, triacylglycerol, CRP, and body weight compared with sucrose. The Scientific World Journal. 2008;8:463-9.
19. Böni-Schnetzler M, Thorne J, Parnaud G, Marselli L, Ehses JA ,Kerr-Conte J, et al. Increased interleukin (IL)-1β messenger ribonucleic acid expression in β-cells of individuals with type 2 diabetes and regulation of IL-1β in human islets by glucose and autostimulation. The Journal of Clinical Endocrinology & Metabolism. 2008;93(10):4065-74.
20. Ehses JA, Lacraz G, Giroix M-H, Schmidlin F, Coulaud J, Kassis N, et al. IL-1 antagonism reduces hyperglycemia and tissue inflammation in the type 2 diabetic GK rat. Proceedings of the National Academy of Sciences. 2009;106.4003-13998:(33)
21. Rohm TV, Meier DT, Olefsky JM, Donath MY. Inflammation in obesity, diabetes, and related disorders. Immunity. 2022;55(1):31-55.
22. Mirza S, Hossain M, Mathews C, Martinez P, Pino P, Gay JL, et al. Type 2 diabetes is associated with elevated levels of TNF-alpha, IL-6, and adiponectin and low levels of leptin in a population of Mexican Americans: a cross-sectional study. Cytokine. 2012;57(1):136-42.