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Article

Evaluation of Interleukin-35 in Patients with Type 2 Diabetes Mellitus and Its Association with Oxidative Stress and Metabolic Parameters

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Abstract: The research samples were gathered from January to March of 2025. Ninety blood samples from both ill and healthy people were used in the investigation. These were split into two groups: the first group included sixty serum blood samples from male and female patients with type 2 diabetes who were between the ages of 35 and 65. Thirty serum blood samples from seemingly healthy men and women between the ages of 35 and 55 made up the second group. Specialist doctors made clinical diagnoses for every instance of diabetes. The results showed a significant decrease in the levels of both interleukin-35 and superoxide dismutase (SOD) in the type 2 diabetes group (1.954 ± 0.196 and 3.959 ± 0.203, respectively) compared to the control group at a probability level of 0.0001. Conversely, the results showed a significantly elevated level of the oxidative stress index, malondialdehyde (MDA), in diabetic patients (4.781 ± 1.396) and C-reactive protein (CRP) (7.072 ± 0.598) compared to the control group, with a high level of statistical significance (P ≤ 0.0001). Additionally, glycated hemoglobin (HbA1c) levels were significantly higher in diabetic patients compared to healthy individuals.

The discriminating ability of the investigated biomarkers in distinguishing between diabetes patients and healthy persons was also assessed using receptor operating characteristic (ROC) curve analysis. The findings demonstrated that every variable under investigation had the best discriminatory and diagnostic powers.

Keywords: Interleukin-35, oxidative stress, SOD, CRP

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1. Introduction

According to latest statistics on non-communicable disease risk factors (2022), type 2 diabetes affects 828 million people, with almost 95% of those individuals having the condition [1]. According to statistics, the prevalence of diabetes will continue to climb and reach 10.8% in the United States alone by 2050; however, this figure is probably greater because estimates of the present prevalence of diabetes differ [1, 2, 3]. One of the risk factors caused by the disease is high blood sugar levels resulting from impaired insulin secretion or insulin resistance, therefore it is classified as a multisystem disease [4, 5]. Hyperglycemia is a major cause of multiple disorders, as it stimulates glucose replacement pathways and their cytotoxic byproducts [6]. Oxidative stress, lipid peroxidation, and immune dysfunction also contribute to the increased disorders and morbidity caused by the disease [7]. Oxidative and inflammatory factors, including inflammatory and non-inflammatory cytokines, also contribute to disease progression via cellular alarm molecules (DAMPs) [8]. Long-term inflammation can be extremely harmful and cause several kidney issues, even tho the body may benefit from it in the short term [9]. Interleukin-35 (IL-35), belonging to the IL-12 cytokine family, is an anti-inflammatory molecule produced primarily by regulatory T cells (Tregs) and B cells [10]. It reduces the production of inflammatory cytokines and inhibits the activation of effector T cells. Dysregulation of IL-35 production has been found in metabolic diseases, including obesity and type 2 diabetes, suggesting its role in the development of inflammatory symptoms [11]. Some reports indicate that low IL-35 is a marker of insulin resistance and increased inflammation, while others describe elevated IL-35 in response to metabolic inflammation as an opposing regulatory response [12].

Interleukin-35 (IL-35) promotes the activity and differentiation of regulatory cells, such as regulatory T cells (Tregs) and regulatory B cells (Bregs), while inhibiting pro-inflammatory immune cell types like type 1 helper T cells (Th1) and type 17 helper T cells (Th17) [13, 14]. Additionally, it can activate iTr35, a specific subset of regulatory T cells that reduces immunological inflammation [15].

The increased energy release across cells due to elevated blood glucose levels leads to a higher rate of electron transporters (NADH and FADH2) flowing into the mitochondria. Consequently, complex III's activity is inhibited and electrons return to coenzyme Q when the potential gradient across the mitochondrial membrane reaches a critical threshold. Coenzyme Q then transfers electrons to molecular oxygen, hence boosting the formation of superoxide anion SOD ($.O_2^-$) [16]. Elevated blood glucose plays a major role in all of the classic pathways of type 2 diabetes complications, including increased flux in the polyol and hexosamine pathways, increased formation of advanced glycation metabolites, and activation of protein kinase C-PKC [17].

Study Objectives

This study aims to:

1. Evaluate serum interleukin-35 (IL-35) levels in patients with type 2 diabetes and compare them to levels in the control group.
2. Evaluate oxidative stress markers, specifically malondialdehyde (MDA) and superoxide dismutase (SOD), in diabetic patients compared to healthy individuals.
3. Determine the levels of C-reactive protein (CRP) and look into how it affects the inflammatory process linked to type 2 diabetes.
4. To assess the diagnostic efficacy of IL-35, MDA, and SOD in distinguishing between type 2 diabetes patients and healthy persons, as well as to ascertain the ideal threshold value, sensitivity, and specificity, do receptor operating characteristic (ROC) curve analysis.

2. Materials and Methods

2.1 Study Population and Design

This research employs a case-control study design. The study sample was collected between October 2024 and January 2025 at Al-Sharqat General Hospital. The sample consisted of 90 individuals divided into two groups: the first group comprised 60 individuals diagnosed with type 2 diabetes mellitus (T2DM) at the hospital's specialized clinics, based on the American Diabetes Association (ADA) criteria. The second group consisted of 30 individuals who were considered apparently healthy and served as the control group. The participants' ages ranged from 35 to 65 years, and both groups were matched for age and gender. Individuals with a history of diabetes, cardiovascular disease, kidney disease, tumors, or chronic inflammatory diseases were excluded from participation.

2.2 Eligibility and Exclusion Criteria

The study sample consisted solely of patients with type 2 diabetes. To ensure accurate and reliable results, overlapping conditions such as type 1 diabetes and gestational diabetes were excluded, as were patients with a known history of chronic liver or kidney disease, autoimmune disorders, or acute infections. Smokers and individuals who had taken anti-inflammatory drugs or antioxidant supplements in the three months prior to the study were also excluded.

2.3 Serum Collection and Separation

The sample collection procedure involved the following sequential steps: First, all participants were asked to fast overnight (8–12 hours) before 5 mL of blood was drawn from a vein. Second, the blood samples were allowed to coagulate spontaneously under normal laboratory conditions. Third, the samples were centrifuged at 3000 rpm for 10 minutes to isolate the serum. Finally, the serum was fractionated and isolated in a freezer at -32°C to be ready for subsequent biochemical measurements.

2-4 Quantitative Estimation of Biochemical Markers

The practical aspect involved measuring serum levels of IL-35 and C-reactive protein using an ELISA assay according to the manufacturer's instructions. The thiobarbituric acid reactants (TBARS) method was also used to determine the concentration of malondialdehyde (MDA) and SOD as indicators of lipid peroxidation. Glycated hemoglobin (HbA1c) was separately estimated using a standard immunoassay.

2.5 Statistical Analysis

The data obtained were subjected to statistical analysis using the Statistical Package for the Social Sciences (SPSS, Version XX; IBM Corp., USA). Variables were expressed as mean ± standard deviation (SD). To compare the two study groups and identify differences between them, an independent samples t-test was applied. The nature of the connections between interleukin-35 (IL-35) levels and other biochemical indicators was also evaluated using Pearson's correlation coefficient. Additionally, the diagnostic effectiveness of IL-35, MDA, and SOD in differentiating between people with type 2 diabetes and healthy individuals was assessed using receptor operating characteristic curve (ROC) analysis, which included calculating the cut-off value, estimating the area under the curve (AUC), and determining the sensitivity and specificity levels. As a measure of statistical significance, the probability value ($p < 0.01$) was used.

3.Results and Discussion

Table 1. displays the patients' levels of IL-35 and other biochemical indicators in comparison to the control group.

Groups / Parameters	Mean ± SD		P-value
	Control	Patients	
IL-35	3.465±0.214	1.954±0.196	<0.0001**
MDA	2.214±1.325	4.781±1.396	<0.0001**
SOD	7.103±0.244	3.959±0.203	<0.0001**
CRP	2.050±0.169	7.072±0.598	<0.0001**
HbA1c	4.863±1.702	9.244±1.032	<0.0001**

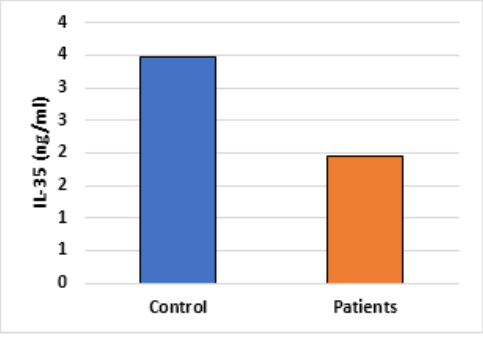

Figure 1. Shows the IL-35 level in patients Compared to the control group

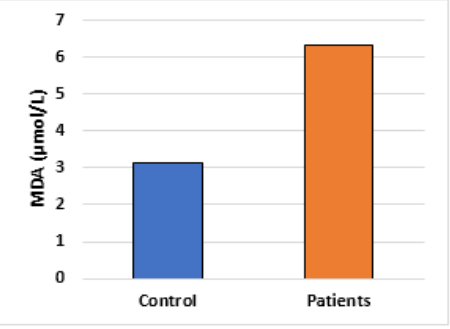

Figure 2. Shows the level of MDA in patients Compared to the control group

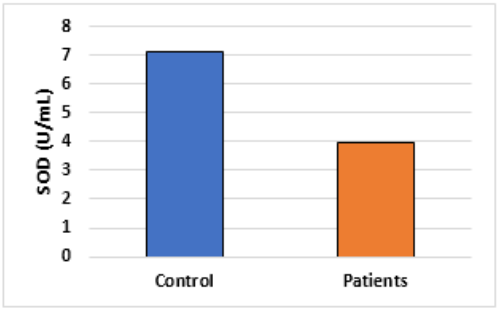

Figure 3. Shows the level of SOD in patients Compared to the control group

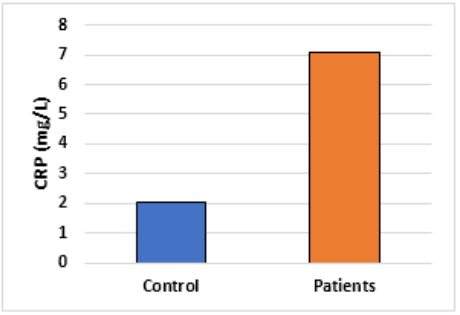

Figure 4. Shows the level of CRP in patients Compared to the control group

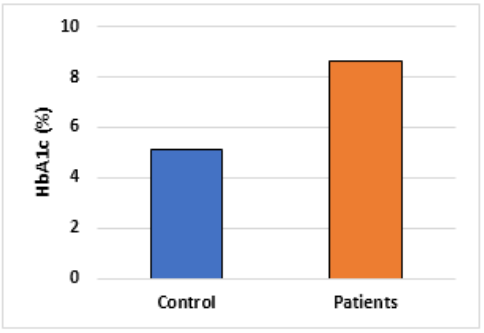

Figure 5. Shows the level of HbA1c in patients Compared to the control group

Table 2. Shows the correlation between IL-35 and all Parameters in the patient group

		IL-35	MDA	SOD	CRP	HbA1c
IL-35	**r**	1				
	p					
	N	60				
MDA	**r**	0.154	1			
	p	0.241				
	N	60	60			
SOD	**r**	0.990**	0.150	1		
	p	0.000	0.251			
	N	59	60	60		
CRP	**r**	0.980**	0.175	0.987**	1	
	p	0.000	0.180	0.000		
	N	60	60	60	60	
HbA1c	**r**	-0.549**	-0.190	-0.553**	-0.569**	1
	p	0.000	0.147	0.000	0.000	
	N	60	60	60	60	60

****. Correlation is significant at the 0.01 level (2-tailed).**
***. Correlation is significant at the 0.05 level (2-tailed).**
r. Pearson correlation
p. Sig. (2-tailed)
N. number of samples

Table 3. Shows the ROC analysis of IL-35 and biochemical variables of patients and healthy individuals

Parameters	Cut off	Sensitivity %	Specificity %	AUC	P-value
IL-35	≤2.3	100.0	100.0	1.000	<0.001
MDA	>4.27	88.3	86.7	0.934	<0.001
SOD	≤4.35	100.0	100.0	1.000	<0.001
CRP	>2.4	100.0	100.0	1.000	<0.001
HbA1c	>6.2	100.0	100.0	1.000	<0.001

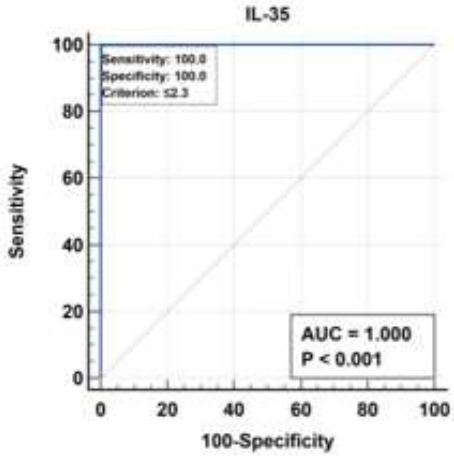

Figure 6. Represents the ROC curve for IL-35 In patients and healthy

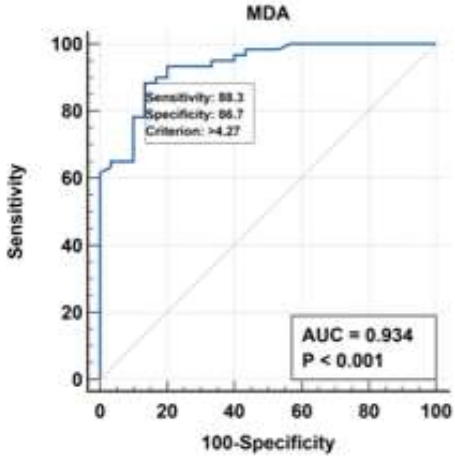

Figure 7. Represents the ROC curve for MDA In patients and healthy

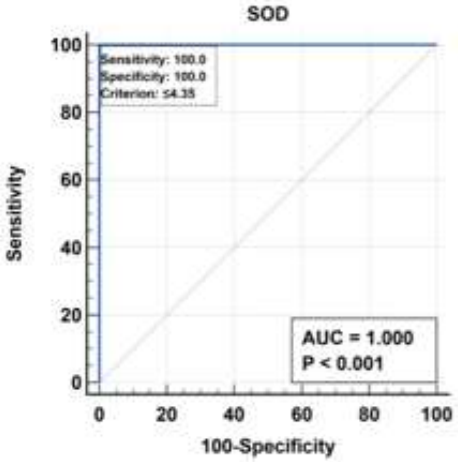

Figure 8. Represents the ROC curve for SOD In patients and healthy

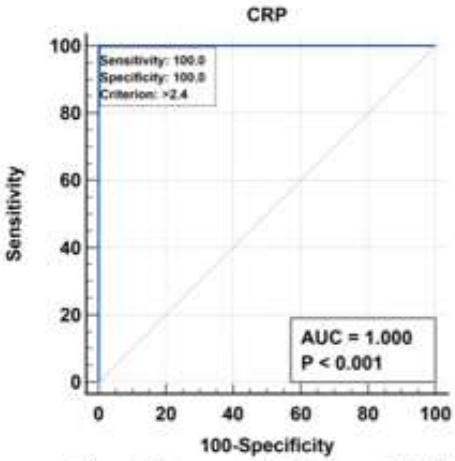

Figure 9. Represents the ROC curve for CRP In patients and healthy

We found that in our current study, the level of IL-35 was significantly lower ($p \leq 0.0001$) to 1.954±0.196 as compared to 3.465±0.214 in control group for patients with type 2 diabetes as observed with other studies [18]. The pathology behind all of this is the type 2 diabetes which stimulates and increases percentage of Th-2 cells over Th-1 cells. This would reduce the efficacy of factors inhibiting inflammation and cellular healing processes [18]. Another investigation conducted within a diabetic

periodontal population versus control group reported serum IL-35 concentrations too; however the outcomes were somewhat inconsistent with our findings, in that no correlation between the two conditions and interleukin levelsis clear [19]. This divergence is based on the measured fluids, which were blood serum and periodontal fluid. Low levels of IL-35 were detected in response to activated immune regulatory circuits mediating immunosuppression against metabolic stress and chronic inflammation. IL-35 is an immunosuppressant and an anti-inflammatory; therefore, it was considered that its secretion is a compensatory factor controlling the diabetes-associated inflammation [20]. These results were in agreement with another study showing that IL-35 levels in the gingival fluid decreased significantly in diabetic patients with generalized periodontitis [21]. A systematic review exploring the role of IL-35 in regards to endothelial dysfunction further also showed a global decrease of IL-35 [21] confirming this consistency. The results of our research also reported a significant elevation in the level of MDA(μmol/l 4.781±1.396) in the type 2 diabetes group Figure (2),Table No.(1)($P \leq 0.0001$) compared to its level in control humans(μmol/l 2.214±1.325). This finding agrees with what was obtained by [22], because the explanation of malondialdehyde concentration elevation could be attributed due to increase in oxidative stress processes and as a result that may occurs in type 2 diabetes patients, which produce free radicals pertaining to (lipid peroxidation) MDA is produced as an end product during lipid peroxides breakdown inducing proliferation of free radacl quenching state concentrations of malondialdehyde [23].

Previous acquired data show that the activity of SOD assayed by blood, urine and porcine retinal tissues are lower in diabetic patients than in random healthy controls. This finding supports previous literature reporting that type 2 diabetes is associated with impaired antioxidant protection systems and increased markers of oxidative stress, which are particularly evident during the development of complication to disease state [24-25] due to overproduction of active RONS molecules. In addition, a publication by Saffo et al. In their study, they found that although total antioxidant capacity was high, TBARS concentrations also increased in these diabetic patients【26】. In contrast, Kimura et al. [27] More specifically, there were greater levels of extracellular SOD (EC-SOD) in patient groups than found in control samples, and EC-SOD concentrations correlated with macrovascular and microvascular complications. In clinical practices, C-reactive protein (CRP) is the most commonly used nonspecific biomarker reflecting the degree of plasma cell inflammation [28]. Among the inflammatory markers most studied in the context of cardiovascular disease, CRP is probably the most well-studied and even a central element used for vascular risk prediction. CRP has been proposed as a mediator of the higher microvascular complication rates among this sample of patients with type 2 diabetes in a large prospective cohort study [29]. CRP induces overexpression of pro-oxidant, pro-inflammatory and pro-angiogenic mediators and activates CD32 regulation, as well as NF-kappa B cellular signaling pathway in the eye, which suggests that elevated CRP levels play a pathogenic role in diabetic retinopathy [30]. Analysis of the receiver operating characteristic (ROC) curve, as shown in Table 3, demonstrated that IL-35 exhibited high diagnostic performance in distinguishing between pathological conditions and healthy individuals.

4. Conclusion

Our study results indicated a significant decrease in serum interleukin-35 (IL-35) levels in patients with type 2 diabetes compared to healthy controls. This suggests that IL-35 may play a significant role in the progression and complications of the disease, particularly those related to inflammation and oxidative stress. Furthermore, the results showed a marked increase in malondialdehyde (MDA) and C-reactive protein (CRP) levels in the patient group compared to the control group. We also observed a

significant decrease in superoxide dismutase (SOD) activity, likely due to the increased oxidative stress and chronic inflammation associated with type 2 diabetes.

Moreover, receptor operating characteristic (ROC) analysis demonstrated that IL-35, MDA, SOD, and CRP variants possess significant diagnostic potential for identifying disease progression and complications, or for early disease detection. Therefore, they can be relied upon to assess the disease status of individuals with type 2 diabetes.

5.Recommendations:

1. Intensify research on these variables and determine their relationship to the disease and other illnesses.
2. Investigate the role of treatments and medications and their impact on the levels of the studied variables, which could be considered as future therapeutic agents for diabetes and other diseases.
3. Utilize variables that have demonstrated high diagnostic efficacy as biomarkers to aid in early disease detection, monitoring disease progression, and evaluating treatment response.

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