Effect of Steroid Treatment on Tissue Damage Markers in Diabetic Patients

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Abstract

Steroid-induced hyperglycemia challenges primary care providers. Understanding and managing these effects are insufficient despite widespread glucocorticoid use. Our study aimed to investigate the impact of steroid treatment on tissue damage markers in diabetic patients. We determined the liver enzymes, blood biochemistry, and interleukins (IL-12, IL-17A, IL-6, and IL1B) before and after steroid treatment. The results revealed a significant increase in creatinine levels among the active subjects compared to the control group. The mean concentrations of ALT and ALP in the active subjects (on steroids) were 43 ± 3.1 and 112 ± 10.2 U/L, respectively, significantly higher than the active control (24 ± 5.1). Our study also found that IL-12, IL-17A, IL-6, and IL-10 were upregulated 5.2, 16.2, 2.13, and 5.11 times, respectively, compared to the control. The blood sugar levels (RBS) showed positive correlations with Neutrophils ($r = 0.81$, $P = 0.012$), IL-12 ($r = 0.68$, $P = 0.028$), and IL-6 ($r = 0.51$, $P = 0.03$). In conclusion, our research demonstrated the significance of inflammation and tissue damage in the expression patterns associated with type 2 diabetes and its effects.

Keywords: Steroid-induced hyperglycemia, Glucocorticoid, Tissue damage markers, Interleukins (IL-12, IL-17A, IL-6, IL-1B), Type 2 diabetes

Significance | This research showed how diabetes and steroids worsen effects, providing a better understanding of the pathophysiology processes.

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Introduction

In terms of the metabolic adverse effects associated with steroid use, particularly in the context of diabetes, this study showed the potential impact of steroids on diabetic subjects. Type 2 diabetes (T2D) is the most common form of diabetes, accounting for about 90% of cases. It is associated with both macrovascular (stroke, coronary artery disease, and peripheral arterial disease) and microvascular (diabetic nephropathy, neuropathy, and retinopathy) complications. However, hyperglycemia, a condition marked by a partial or total lack of insulin action and insulin resistance specific to certain pathways, is a critical concern in diabetes. Diabetes is a complex metabolic disorder characterized by the development of disease in the retina, renal glomerulus, and peripheral nerve, with accelerated atherosclerotic disease being a notable complication. Diabetic cardiomyopathy is a significant consequence of diabetes, contributing to morbidity and mortality. The global prevalence of diabetes is on the rise, with an estimated 463 million people affected, projected to reach 700 million by 2045 (Giacco & Brownlee, 2010). Interleukin (IL) family proteins might significantly modulate obesity and T2DM through the physiology and pathophysiology of insulin resistance. For instance, IL-6 works as a pro-inflammatory marker protein to produce inflammation and T2DM and insulin resistance pathophysiology. Chronic inflammation creates insulin resistance to developing type 2 diabetes (Rehman, 2017). This Il-6-related inflammation upregulates IL-1 to activate beta-cell death and altered insulin sensitivity (Fève & Bastard, 2009). Furthermore, T2D is also related to the onset and progression of Non-Alcoholic Fatty Liver Disease (NAFLD) (Simmons et al., 2012). However, this inflammatory-oriented insulin resistance affect the liver cells through glucocorticoids and glucose metabolism. As a result, liver might prevent the glucose homeostasis system. Thus, we can

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determine the basic condition of the liver through the analysis of γ-glutamyl-transferase (GGT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) as, Insulin resistance, increases the risk of NAFLD and thereby developing diabetes (Ballestri et al., 2016)

Glucocorticoids, widely used in medical practice, exhibit short-term and chronic benefits in treating liver diseases and diabetes. However, long-term use, particularly in the context of solid organ transplants, can lead to metabolic adverse effects, including diabetes, osteoporosis, and hypertension (Simmons, 2012). Prednisolone is a commonly used steroid in primary care, especially in respiratory disorders like asthma and chronic obstructive pulmonary disease (COPD). Additionally, it is prescribed for conditions such as temporal arteritis, polymyalgia rheumatica, rheumatoid arthritis, lupus, myasthenia gravis, sarcoidosis, autoimmune renal disease, and inflammatory bowel disease due to its anti-inflammatory and immunosuppressive properties (Roberts et al., 2018; Morris, 2018). Steroids exert their effects by binding to cytoplasmic glucocorticoid receptors, influencing gene transcription and subsequently impacting glucose metabolism. Using steroids has potential side effects, such as increasing insulin resistance in adipose and muscular tissue and decreasing peripheral glucose absorption.

Furthermore, high hepatic insulin resistance causes high glucose production through gluconeogenesis and glycogenolysis in the liver. Steroids reduce insulin synthesis in pancreatic beta-cells but might cause hyperglycemia and diabetes (Tamez-Perez et al., 2015, Hala et al. 2024). Therefore, there is a complex interplay between the process of metabolic disease and the use of steroids, which we need to understand essentially. Since diabetes is a pandemic, hence the determination of the mechanism of action of steroids in causing diabetes through impaired glucose metabolism and inflammation. This will help us create a strategy to improve patient outcomes and guide therapeutic approaches. This study aims to determine the level of different interleukin expression and blood glucose marker analysis to understand the role of steroids in diabetes patients, which will shed light on the complex interplay between inflammation, steroid use, and diabetes.

Materials and Methods
Study design
50 diabetes patients were enrolled from Al-Yarmouk Teaching Hospital in January 2023 and November 2023. The patients have received Metformin (500mg/day) and Prednisone (5mg/day for 2 weeks) as standard treatment (Sun, 2019). We have selected the diabetes patients who have had steroid treatment for 6 months. We considered 20 healthy controls (n=20) with diabetes and 20 diabetes patients without steroid treatment. We have not considered the patients who had a history of respiratory

insufficiency, autoimmune, endocrine and metabolic, blood, nervous system, liver, or kidney diseases, as well as those with a history of cancer.

Patients who had undergone antibiotic treatment within three months before the study or were under 18 were also ineligible. All participants provided written consent, and their data, including any symptoms noted during sample collection, were documented. Blood samples (5cc) were collected before the initiation of treatment, and the separated sera were stored at -70°C in the hospital laboratory for subsequent analysis. The study received approval from the Scientific Ethical Committee at the AlFarahidi University, Iraq, adhering to Helsinki Declaration guidelines. Reporting in this article aligns with CONSORT standards.

Blood Biochemical analyses
This study employed the Dacie and Lewis approach for various hematological and biochemical analyses. The blood was collected in a standard capillary tube, filling it to approximately 75% of its capacity. Subsequently, the sealed capillary tube underwent centrifugation at 12000 rpm for 5 minutes using a Hawksley microhematocrit centrifuge (Hawksley, London) to prevent blood loss. The packed cell volume (PCV) was measured and represented as a percentage, utilizing a capillary tube and a micro hematocrit reader. The protocol of Dacie and Lewis was followed for the red blood cell (RBC) count. A dilution (1:200) of the blood was made using red blood cell diluting fluid. After a 2-minute incubation, RBCs were counted using a hemocytometer observed under a light microscope (Lawrence & Mayo) with x40 objectives. The total RBC count was expressed in mm3, with reference ranges for males (4.8-5.5 million/mm3) and females (4.5-5 million/mm3). White blood cell (WBC) count, lymphocyte count, hemoglobin, and platelet count were analyzed using the Cobas c 111® clinical chemistry automated system, with normal reference ranges provided. The processing of samples followed the manufacturer's instructions (Oluwatobi, 2022). Creatinine levels were determined using the p-nitrophenylglyoxal (PNPG) method, following modifications to the protocol by Aminlari and Vaseghi. Serum was mixed with a reagent mixture, and absorbance at 480 nm was recorded after a 10-minute incubation at 37°C using a UV 1800 recording spectrophotometer. Alanine aminotransferase (ALT) activity was determined using the alanine aminotransferase kit (Calchem). Serum was incubated with a reagent mixture, and absorbance at 340 nm was recorded for 5 minutes at 30-second intervals. ALT activity was quantified based on the enzyme's ability to catalyze the synthesis of 1 μmol/L of NAD per minute under specific assay conditions at 25°C. One unit of ALT activity was defined as the enzyme required to form 1 μmol/L of NAD per minute under these assay conditions.

Cytokines measurement

IL-1β levels in serum samples were determined using a Cytokine kit from HiMedia, Maharashtra, India, following the manufacturer's instructions. The measurements were conducted on the BD FACS Calibur Flow Cytometer by BD Biosciences, San Jose, CA, USA. Subsequent data analysis was carried out using BD FCAP ArrayTM Software from BD, US.

γ-glutamyl transferase analysis

Gamma-glutamyl transferase (γ-GT) levels in human serum were quantitatively determined. This cellular enzyme is predominantly present in various tissues such as the kidney, pancreas, liver, and prostate. The measurement of γ-GT activity is a diagnostic and treatment tool for hepatobiliary disorders, including cirrhosis, biliary blockage, and liver tumors. The catalytic action of γ-GT involves transferring the γ-glutamyl group from the acceptor glycylglycine to γ-glutamyl-p nitroanilide. The photometrically determined rate of 2-nitro-5-aminobenzoic acid synthesis is directly proportional to the catalytically active γ-GT in the sample. To perform the assay, a Glutamyl reagent was prepared by combining the substrate Glycylglycine 100 mmol/L (L- γ - glutamyl-3-carboxy-4-nitroanilide) with 15ml of Buffer (Tris 100 mmol/L; pH 8.25). Briefly, 100 µl of the serum sample was mixed with 1ml of the glutamyl reagent and allowed to stand for 1 minute at room temperature. Absorbance readings were recorded at 405nm for 3 minutes at 1-minute intervals. The difference in absorbance and the average absorbance differences per minute were calculated using the formula (ΔA/min) x 1190, and the results were expressed in U/L.

ALP assay

Alkaline phosphatase was determined using the method outlined by Kanta (2021) with slight adjustments. In each well of a 96-well plate, approximately 0.1 ml of serum and 0.1 ml of AMP buffer (2 mg/mL of para-nitrophenyl phosphate; pH 10.5) were added. After thorough mixing, the contents were incubated at 37°C for 15 minutes and then treated with 0.006 ml of 6 N HCl. Adding 0.104 ml of 1N NaOH halted the alkaline phosphatase (ALP) activity. In this process, paranitrophenyl phosphate (PNPP) in the AMP buffer is transformed by alkaline phosphatase in the sample into yellow paranitrophenol (PNP) at pH 10.5, and its absorbance was measured at 410 nm.

RNA extraction

Following the manufacturer's instructions, we extracted the total RNA from each sample using the TransZol Up Plus RNA Kit Reagent (TransGen, No. ER501-01). In simple terms, 1 ml of blood was spun at 12,000 rpm for 1 minute, and the resulting pellet was mixed with 1000 µl of TransZol Up, incubated at 2-8°C overnight. We added 200 µl of chloroform, let it sit for 3 minutes, and centrifuged the mixture at 10,000 rpm for 15 minutes at 2-

8°C. The RNA in the colorless upper layer was transferred to a new tube, and cold ethanol was added. After a quick spin, the precipitate was placed in a spin column, spun again, and washed with ethanol. The purified RNA was air-dried, washed with RNase-free water, and finally recovered after a final spin. The obtained RNA was stored in a freezer at -20 °C for future use.

qPCR

We performed real-time PCR after reverse transcription-PCR using the cDNA products. Following the manufacturer's instructions, we used the SYBR Green PCR Kit (HiMedia, India). The two-stage cycling method involved denaturation at 95°C and combined annealing/extension based on primer Tm value. Primers were designed using Primer 3 software and obtained from Sigma Aldrich, India. The reaction mixture included SYBR Green PCR Master Mix (10 µl), primers (2 µl), template DNA or cDNA (2 µl), and RNase-free water (6 µl). Real-time PCR was conducted with 40 cycles at 94°C for 45 seconds, initial denaturation at 95°C for 5 minutes, and annealing at 55°C for 50 seconds. We compared the expression of each gene to that of β-actin, focusing on IL-6, IL-12, IL-17, and IL1β genes. The primer and probe sequences are listed in Table 2. Using the 2-ΔΔCT approach, real-time quantitative PCR tests allow the assessment of relative changes in gene expression.

Statistical analysis

We used SPSS version (faculty version) to analyze the collected data. One-way analysis of Variance (ANOVA) and the Tukey test were performed to find significant differences among the six groups (n = 5). We presented the mean and standard deviation (SD) results, considering statistical significance as $P < 0.05$. Spearman correlation coefficients were used for the cross-sectional analysis to examine the association between liver enzymes and anthropometric and metabolic factors.

Results and Discussion

In this study, we determined the multifaceted impact of diabetes on hematological, immunological, and metabolic aspects, with steroid treatments exacerbating certain adverse effects. The findings showed a deeper understanding of the intricate relationships within diabetic pathophysiology. In this study, a total of 90 participants, 50 subjects were diabetic and were on steroids, 20 subjects were diabetic but with no steroid intervention, and 20 subjects were healthy individuals. The active case group with steroid intervention consisted of 34 (68%) males and 16 (32%) women, whereas the control group with no steroids consisted of 12 (60%) men and 8 (40%) women. On the other hand, healthy controls consisted of 14 (70%) men and 6 (30%) women. However, there was no statistically significant difference in the genders of the groups. The patients' mean ages in the active and control

Table 1. The List of primers used for the real time PCR study.

Gene	Primer sequence (5′–3′)	Base pairs	Tm	GC%	Product size (bp)
IL-12	AGCGGAGTGACTTTCCAAGA	21	65.6	55	97
	TTTTGGGGTTCATGATGGAT	21	59.4	55	
IL-17	ACCTTCATTGCCCAGGTTTCT	21	63	58	120
	TGTTTGGGGTCATCAGCCTCAA	21	63	56	
IL1β	CAAAGAACACAACTAAGAAG	20	66.5	55	181
	AGGTCTTTCAGCGTACTTGT	20	66	55	
IL-6	TCCAGAATGAGTATGAGG	18	62.6	58	236
	CATCCGAATAGCTCTCAG	18	62	54	
β-actin	CGCACCACTGGCATTGTCAT	21	65	58	227
	TCCAAGGCGACGTAGCAGAG	21	65	54	

Table 2. The blood biochemistry (haematological parameters) determined from the patients and controls (n=5).

Parameter	Control	Active	
		Steroids treatment	No treatment
PCV	41.2 ± 3.45	38.12 ± 3.11	40.18 ± 5.5
RBC	4.95 ± 2.11	4.51 ± 4.12	4.78 ± 3.12
Haemoglobin	14 ± 1.45	9.11 ± 1.15	11.23 ± 1.81
WBC	8300 ± 102.65	9840 ± 56.1	9120 ± 131.22
Lymphocyte	3150 ± 34.12	3850 ± 31.03	3500 ± 21.85
neutrophils	4100 ± 85.11	6250 ± 67.23	5600 ± 83.04
Platelets x 10^5	2.8 ± 1.1	4.1 ± 0.85	3.4 ± 0.71
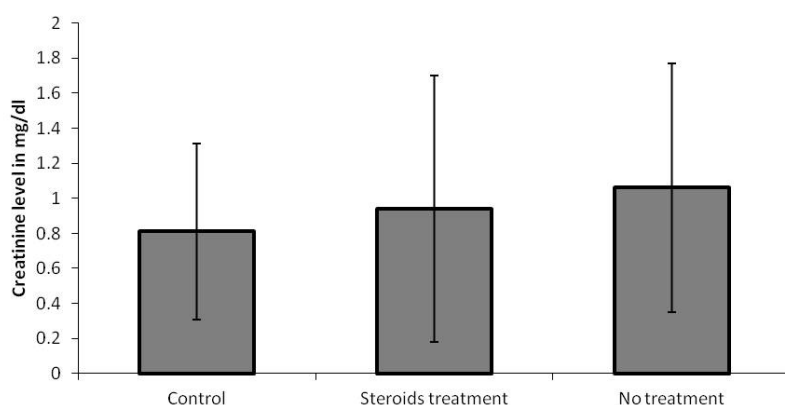
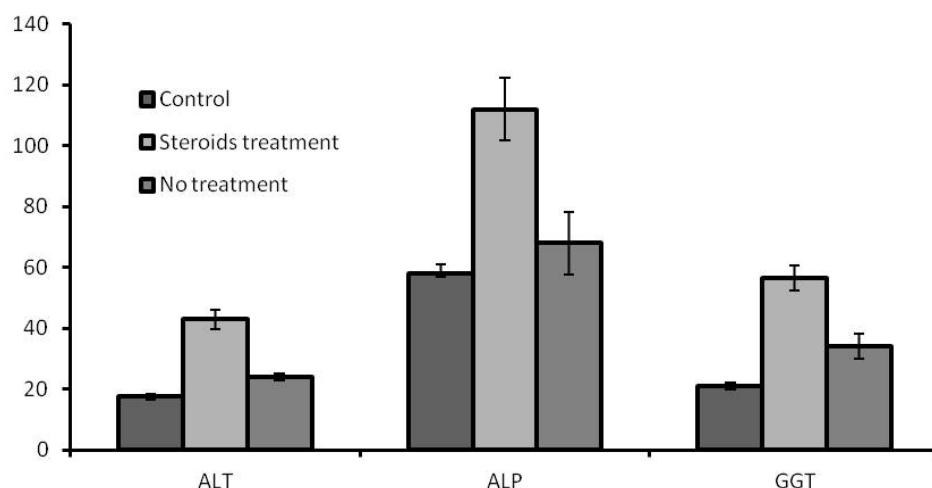Figure 1. The Creatinine levels of diabetic patients. N=5.

Figure 2. The enzyme activities of ALT, ALP and GGT in the diabetic patients.
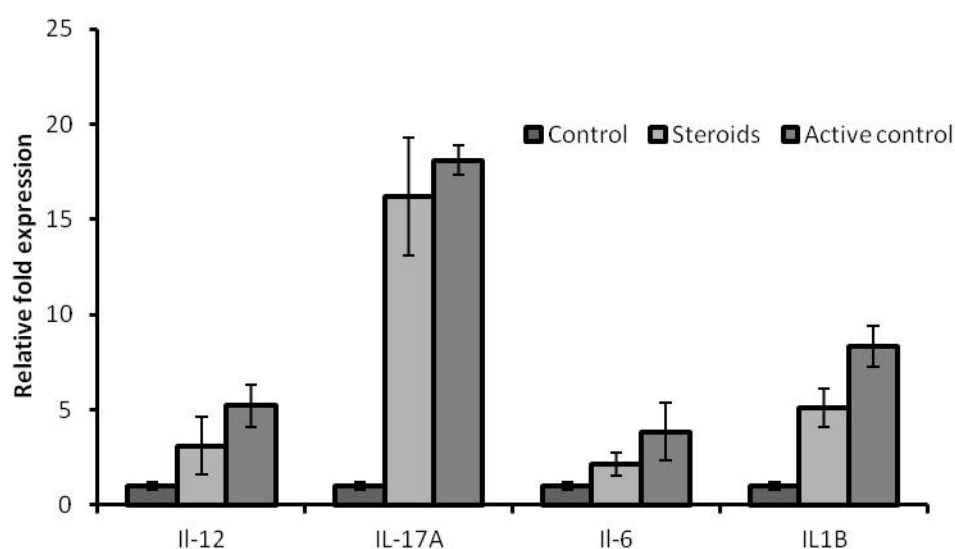

Figure 3. Relative fold expression of the cytokines (IL-12, 17A, IL-6 and IL-1B) of the patients (steroids and controls. Healthy control expression is considered 1 or 100%. Beta actin was used as a housekeeping gene (p<0.05).
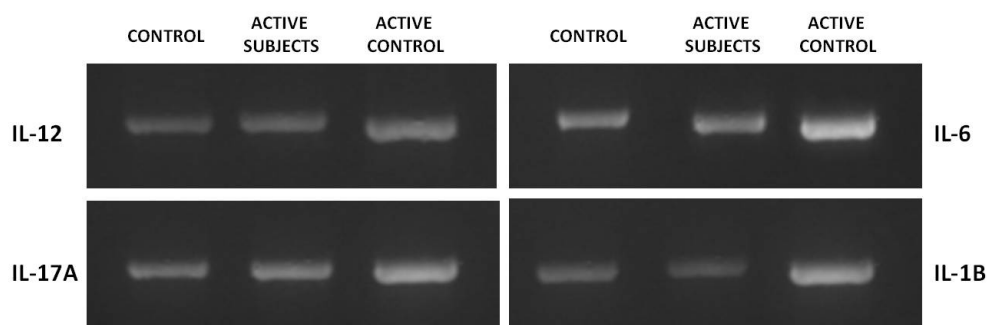

Figure 4. Gene expression analysis of the inflammation markers as seen on 1% agarose gel post amplification through RT-PCR.

groups were 32 (21-50 years) and 34 (22-50 years), respectively. The blood biochemistry results showed an adverse effect in Diabetic Patients.

Blood biochemistry results provided valuable insight into the relationship between diabetes and hematological abnormalities for better patient outcomes management. Clinical adverse effects were observed in RBC count, PCV, and Haemoglobin during the study period. We observed a significant decline in packed cell volume (PCV), hemoglobin, and red blood cell counts (p<0.05), though they were within the normal range. PCV declined to 38.12 ± 3.11 in treatments compared to active control (40.18 ± 5.5). PCV was found to be 41.2 ± 3.45 in healthy controls in the normal range (35.5 - 48.6). RBC count declined to 4.51 ± 4.12 in treatments compared to active control (4.78 ± 3.12). The RBC count was 4.95 ± 2.11 in healthy controls, which is in the normal range (4.5 - 5.5). Hb declined to 9.11 ± 1.15 in treatments compared to active control (11.23 ± 1.81). Hb was found to be 14 ± 1.45 in healthy controls in the normal range (12 to 18 g/dl). Random blood sugar levels of more than 120 mg/dl were considered diabetic and all the subjects in the study were screened for RBS. The mean RBS of the active subjects and active controls were 205 ± 7.32 and 135 ± 3.12 mg/dl, respectively. on the other hand, healthy subjects showed a mean of 86 ± 5.4 mg/dl.

Our studies are on those reports where a sharp decline was seen in people with diabetes (Ravid, 1998), (Anderson, 2007). This decline was also seen in people with diabetes who were on steroids receiving intervention for chronic infections (Cavusoglu, 2009). Administering steroids reduced the levels of Hb and PCV in diabetic individuals (Schijvens, 2019), (Czock, 2005). The finding above could be caused by oxidative damage and an inflammatory response from a chronic hyperglycaemic state (Kirschke, 2014), (Vaya, 2015).

WBC count is a biomarker for tissue damage, inflammation, and other degenerative disorders. According to studies, the progression of coronary artery disease is closely connected with a rise in WBC count. Additionally, it raises CVD incidence, fatality rates, and frequency (Kannel et al., 1992). Chronic DM problems have a close correlation with an increase in WBC count. This study discovered that DM was substantially linked with WBC, Hb, polymorphs, and MCH from the normal range (P <0.05). We found a significant clinical trend in the WBC (WBC count, neutrophils, Lymphocytes) and platelet parameters. Steroids showed a significant increase in the WBC and platelet parameters compared to the control. The WBC count was 9840 ± 56.1 and 9120 ± 131.22 for treated and active control, respectively (p<0.05). Though a significant rise was seen, it was within the normal range. Healthy individuals showed 8300 ± 102.65. The lymphocyte count was 3850 ± 31.03 and 3500 ± 21.85 for treated and active control, respectively (p<0.05). Though a significant rise was seen, it was

within the normal range. Healthy individuals showed 3150 ± 34.12. The same trend was also seen with neutrophils. The neutrophil count was 6250 ± 67.23 and 5600 ± 83.04 for treated and active control, respectively (p<0.05). Though a significant rise was seen, it was within the normal range. Healthy individuals showed 4100 ± 85.11. Although the white blood cell count, neutrophils, and lymphocytes tended to rise over time, these trends were not statistically significant and did not exceed the reference period in any individuals. No additional significant changes were found in the CBC or chemistry results.

WBC, Hb, Haematocrit, and MCH levels were abnormal. The study found that uncontrolled diabetes was associated with increased leukocyte counts. Chronic inflammation, which this factor can indicate, may be linked to the pathophysiology and development of certain diabetes-related problems when combined with other markers (Mehak, 2021).

The patients showed high polymorphs in this study. According to Arruda-Olson et al., (2009), an increase in polymorphs is connected to the formation of thrombi and ischemia damage. WBC subtypes can exhibit a variety of inflammatory responses or acute infectious illnesses. According to prior studies, persistent cardiac arrest in patients with diabetes is associated with high levels of polymorphs and relatively low levels of lymphocytes in the blood (Rudiger et al., 2009).

Platelets increased to 4.1 ± 0.85 in treatments compared to active control (3.4 ± 0.71). Platelets were found to be 2.8 ± 1.1 in healthy controls in the normal range (1.5 - 4.5 x 10^5). A major predictor of worsened microvascular and macrovascular problems in diabetes patients is an increase in average platelet volume (Hekimsoy, 2004). Even before vascular disease manifests itself, platelet functional abnormality starts to emerge at the time diabetes first develops.

The Creatinine Levels showed Tissue Damage in Diabetic Patients.

Understanding the dynamics of creatinine levels in diabetic patients contributes to a comprehensive approach to diabetes management, allowing healthcare professionals to address potential complications promptly. We found a significant rise in the creatinine level among the active subjects compared to the control. The creatinine levels were found to be 0.94 ± 0.76 mg/dl and 1.06 ± 0.71 mg/dl, respectively, for the active subjects and control. On the other hand, the control value was found to be 0.81 ± 0.5 mg/dl. An increase in creatinine levels suggests chronic tissue damage, especially kidneys. Our findings align with those reported by Roy, 2011, where increased creatinine levels were associated with tissue damage. Anderson, 2007. also found a similar trend, wherein increased creatinine was observed in people with diabetes, though within the normal range. Ani et al., 2009 and Cavusoglu, 2009, observed lowered creatinine levels in diabetic

individuals who are on steroids.

Liver Enzyme Levels increased in Diabetic Patients on Steroid Treatment

The results showed the potential impact of steroid treatment on liver enzyme levels in diabetic patients and emphasized the need for close monitoring. Understanding the correlations between sugar levels and cytokines provides valuable insights into the complex interplay between diabetes, steroid treatments, and liver health. The study revealed elevated levels of liver enzymes, including ALT, ALP, and GGT, in diabetic individuals undergoing steroid treatment. Active subjects on steroids showed significantly higher ALT (43 ± 3.1) and ALP (112 ± 10.2) levels compared to the active control (ALT: 24 ± 5.1, ALP: 68 ± 4.12) and healthy controls (ALT: 17.5 ± 0.9, ALP: 58 ± 1.1). Although within the normal range, the rise in values was notably sharp ($p<0.05$), suggesting potential liver damage. The study aligns with previous research linking liver enzyme elevation to corticosteroid use and liver damage in diabetics. The GGT levels were 56.5 ± 10.2 mg/dl and 34 ± 4.1 U/L, respectively for the active subjects and control. On the other hand, in the healthy controls, the value was 21 ± 1.1 U/L. An increase in the GGT levels suggests chronic tissue damage, especially in the liver.

Corticosteroids, a class of hormones closely resembling the natural and synthetic hormones in the human body, are widely used in medicine due to their strong anti-inflammatory and immunosuppressive effects. Liver damage associated with corticosteroid medication varies, with some instances attributed to the treatment itself and others presumed to be directly linked to it (Chitturi, 2013). Our findings align with Mandal, 2018, who observed increased liver enzyme levels in individuals undergoing steroid treatment. Vozarova et al. 2002, investigated the association between elevated hepatic enzymes (ALT, AST, or GGT) and potential changes in liver tissue damage, reinforcing our study's findings of heightened liver enzyme levels in diabetic individuals. Hua et al., 2021 also reported similar outcomes, noting elevated levels of liver enzymes (ALP and ALT) in Hispanic adults.

Gamma-glutamyl transferase (γ-GT) is an enzyme in various body tissues, including the kidney, pancreas, liver, and prostate. High oxidative stress from reduced insulin action and ß-cell malfunction may increase serum GGT activity. This suggests that GGT levels could indicate various mechanisms related to the development of diabetes. Over the past four decades, numerous epidemiological studies have consistently demonstrated heightened GGT levels in diabetic patients, as highlighted by research findings from Haghighi (2011). Similarly, Gan et al. (2020) and Afarideh et al. (2019) reported increased GGT levels in individuals with chronic diabetes and those undergoing steroid treatments.

We observed a significant relation between blood sugar levels and neutrophils and cytokine members' expression. In the active subjects, neutrophil levels increased with rising RBS levels. Additionally, Il-12 and Il-6 exhibited similar correlation trends among the subjects. The positive correlation between RBS value and Neutrophils was significant ($r = 0.81$, $P = 0.012$). Similarly, we found a comparable positive correlation between RBS value and IL-12 ($r = 0.68$, $P = 0.028$) and IL-6 ($r = 0.51$, $P = 0.03$).

Significantly Altered Expression of Cytokines in Diabetic Patients showed Potential Links to Inflammation

Interestingly, the result showed a targeted intervention to manage inflammation and improve outcomes for diabetic patients. The study identified significant upregulation of interleukin (IL)-12, IL-17A, and IL-10 gene expressions in diabetic patients, shedding light on potential connections to inflammatory processes. IL-12 showed a 5.2-fold increase in active subjects and a 3.1-fold increase in active controls compared to healthy controls. Similarly, IL-17A exhibited upregulation by 16.2 and 18.11 times in active subjects and controls, while IL-10 was upregulated by 5.11 and 8.32 times, respectively. The research also highlighted a favorable link between serum IL-1β concentrations and glycaemic profiles in people with diabetes, emphasizing the potential role of IL-1β in diabetes. However, our study is in agreement with the findings from previous research on IL-1β concentrations in type 2 diabetes. Moreover, elevated IL-12 plasma concentrations in type 2 diabetes were associated with macrovascular problems and atherosclerotic plaque formation.

We have determined the upregulated expression of IL-17, indicating the high inflammation and the cause of diabetes. We have also observed serum IL-1β concentrations, glycaemic profile, fasting blood sugar (FBS), 2-hour postprandial (PP), and HbA1c level to understand the relation of the cause of diabetes and steroid consequences. We found that uncontrolled diabetes patients are significantly related to glycaemic and serum IL-1β concentrations compared to well-managed diabetes patients.

These findings align with a study by Eizadi et al. (2011), which investigated IL-1β concentrations in 30 male patients with type 2 diabetes and 36 BMI-matched healthy individuals, revealing considerably higher serum IL-1β concentrations in type 2 diabetics than in the control group. In contrast, no significant correlation was found between blood IL-1β concentrations and inflammatory markers or cytokines (IL-6, TNF-α, IL-1β, and IL-8) in a study involving 367 obese or overweight Mexican Americans conducted by Mirza et al. (2011). Our research results are consistent with Maedler et al.'s (2007) mouse experiments, demonstrating that exposure of islet cells to varying concentrations of IL-1β inhibits β-cell proliferation. Furthermore, exposure to 2 and 5 ng/ml IL-1β increased the rate of apoptosis by 2.3 and 3.6 times, respectively. The study shows that plasma concentrations of IL-12 are elevated

in individuals with type 2 diabetes (T2DM), potentially contributing to the acceleration of macrovascular issues and the formation of atherosclerotic plaques. Our findings align with Wegner, 2008 reported increased serum levels of IL-12 in type 2 diabetic patients treated with sulphonylureas. Mishra, 2011 supported similar conclusions, linking IL-12 to endothelial dysfunction and insulin resistance. The pro-inflammatory cytokine IL-17 has been investigated about the onset of diabetes. In our study, we found that patients with T2DM exhibited higher mean levels of IL-17 (normal level < 10 pg/ml) compared to non-diabetic controls (mean = 5.232 ± 2.867 pg/ml) [10]. These results are consistent with the research conducted by Takeuchi, M. et al. (2017), revealing elevated IL-17 levels in newly diagnosed diabetics compared to healthy controls. Notably, patients with acute problems in group B had a much higher mean value of IL-17 at 20.386 pg/ml. Our findings also align with Yousefidaredor et al. (2014), who found that IL-17 upregulates various inflammatory markers associated with the JAK 2 STAT 3 pathway and the angiotensin II type I receptor, playing a significant role in the development of T2DM and its consequences. These results suggest that IL-17 affects the inflammatory processes associated with type 2 diabetes and its consequences.

Conclusion

In conclusion, we determined the significance of a complete blood count test and screening for inflammatory markers or cytokines as valuable clinical examinations for the early diagnosis and prevention of microvascular and macrovascular problems in diabetes mellitus. The results could contribute to reducing morbidity and mortality of diabetes and might help develop preventive measure strategies in managing this complex diabetic condition, such as diabetic cardiomyopathy, obesity, hepatic steatosis, insulin resistance, dyslipidemia, and hyperglycemia.

Author contribution

M.S.A., A.A.S., Q.A.I., O.A.I. and M.H.A.B. conceptualized, performed the methodology, analsyzed the data and wrote the paper.

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None declared.

Competing financial interests

The authors have no conflict of interest.

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