

Estimation of heat shock protein 90 concentration in patients with type 2 diabetes mellitus, nephropathy and diabetic foot.

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Summary

This study was conducted in the Department of Biology, College of Science, Al-Qadisiyah University, and Diabetes and Endocrinology Center in Al-Qadisiyah Governorate, during the period from July of 2022 to the February into 2023, 200 blood samples were collected from volunteer patients and visitors to the Diabetes Center of both sexes with different ages ranging between (45-70) and of both sexes in Diwaniyah Governorate. Fifty samples were used for each group, and the study design consisted of four groups divided between the first group (G1) the control group, the second group (G2) with type 2 diabetes, the third group (G3) with diabetes with diabetic nephropathy, and the fourth group (G4). (Type 2 diabetes with diabetic foot) In this study, blood sugar, HbA1c, creatinine, blood proteins of cholesterol, triglycerides, high and low density lipoprotein (HDL) and Low Density (LDL) were examined, as well as the concentration of heat shock protein 90 . The study results also showed a significant increase and a probability level ($P \leq 0.05$) in (HbA1C), total cholesterol and triglycerides for all groups Patients compared to the control group, and the study showed a significant increase and difference in the concentration of low-density lipoproteins ($P \leq 0.05$) and a significant decrease ($P \leq 0.05$) (Heavy Density Lipoprotein (HDL)) in the patients group compared to healthy subjects. Significant in the concentration of heat shock protein 90 in the injured groups and a significant difference with a probability level ($P \leq 0.05$), and the results of the study show that there is no significant increase and a probability level ($P \leq 0.05$) in terms of gender and age within the field of study. The results of a significant increase and difference between the age groups, as the highest infection rate was in the age (45-70).

Keywords: Hb1c,HDL,HSP90, LDL, Diabetes type2, nephropathy , diabetic foot.

1 - Introduction:

Type 2 diabetes mellitus (T2DM) is generally not dependent on drug therapy alone. Medical nutrition therapy is often hidden from patients for many reasons, such as the difficulty in determining the correct dietary pattern for themselves, or organizing their daily dietary patterns, or even not observing the recommended diet provided by specialists and doctors. Nutritional therapy management is one of the important efforts that It can be made by diabetics to prevent further complicating the disease. Establishing a diet with proper nutrition will help patients manage a healthy diet. The so-called Smart Plate Health to Eat system has been researched, a technical innovation that helps patients and users to know the type of food, weight, and nutrients present in some foods, all of which aim to prevent diabetes mellitus of the first and second types. (Salaki et al .,2023). Understanding the genetic background is important in determining the susceptibility to diabetic nephropathy, and tissue exposure to chronic hyperglycemia remains the most important factor(Gariani et al.,2012).

Diabetes mellitus

(DM) is essentially a chronic metabolic disorder characterized by hyperglycemia that may be caused by

defective and weak insulin secretion or insulin resistance. Thus, it leads to the development and occurrence of health complications that may threaten life in the end, most notably the small blood vessels, such as retinopathy, kidney and nerve impairment (Goyal, 2018). Type 2 diabetes (DM2) is a chronic disease characterized by varying degrees of insulin resistance and impaired insulin secretion. Besides, many evidences have shown that chronic inflammation, oxidative stress, and heat shock proteins are strongly implicated in DM2 and its complications, and different pharmacological and non-pharmacological treatment alternatives act on these processes/molecules to modulate them and alleviate disease. In addition, uncontrolled hyperglycemia is associated with many complications such as diabetic retinopathy, neuropathy, and liver, kidney, and heart complications. In other research, the involvement of different inflammatory and pro-oxidant pathways associated with DM2 has been discussed (Gabriela and Thiago, 2019).

The world and global healthcare centers are facing great challenges due to the significant increase in the prevalence of type 2 diabetes. Complications from diabetes are a major cause of death worldwide. Diabetic nephropathy (DN) is one of the most common micro vascular complications in diabetic patients, and is the leading cause of death in end-stage renal disease (Seyyed, 2022).

Diabetic foot ulcer is a preventable complication of diabetes that imposes a significant burden on society. It leads to amputation and increased disability if left untreated and the result has profound implications for the individual, society and the health system in general. (Abha et al., 2022).

Chronic kidney disease (CKD), a serious complication of type 2 diabetes (T2D) increases the co-morbid risk of cardiovascular disease (CVD) and end-stage renal disease. (Linda Fried et al., 2023). This study demonstrates that low muscle mass, as measured by CER, is associated with the prevalence of type 2 diabetes in the general population. The association is most pronounced in the lowest BMI category (18.5–25 kg/m²) and becomes less strong in the higher BMI categories in both men and women. Moreover, a loss of muscle mass over a five-year period was associated with an increased risk of type 2 diabetes in women of normal weight (Dionne et al., 2023). It has recently been proven that heat shock proteins (HSPs) play a role in the pathogenesis and development of some diseases such as cancer, as well as PCOS. HSPs are a class of proteins that act as molecular chaperones and maintain cellular proteome. Recently, research on the actions of these proteins that go beyond the actions of molecular chaperones has shed light on their pathogenic role in several diseases. Like in PCOS, different members of the HSP family show abnormal expression affecting ovarian cell proliferation and apoptosis rates as well as immune processes. Deregulation of HSP in the ovaries of PCOS patients results in an imbalance of proliferation/apoptosis that mechanically affects the development of the follicle stage, which leads to polycystic ovaries. In addition, HSPs may play a role in the pathogenesis of conditions associated with PCOS. It should be noted that modulating HSP activity may lead to new treatment strategies, and define areas for research on heat shock proteins in the future to have effective therapeutic approaches towards intractable diseases such as cancer. (Sara et al., 2023). Heat shock proteins (HSPs) are molecular chaperones involved in a variety of life activities. HSPs remodel unfolded proteins, which contributes to the maintenance of cellular homeostasis. Heat shock factor (HSF) is activated in response to various environmental stresses and binds to heat shock elements (HSEs), promoting HSP translation and thus production of high levels of HSPs to prevent organ damage. The researchers summarized, the role of molecular chaperones as anti-heat stress molecules and their involvement in immune responses and the modulation of apoptosis. Also, they speculated on the potential application of HSPs in cancer treatment, general medicine, and the treatment of heart disease (Shan Q. et al., 2020).

Heat shock protein 90 (Hsp90) is a well-conserved, cellular cofactor that aids in the maturation of several proteins involved in cellular signal transduction. Acting as a regulator of cellular signaling processes, it is vital for cellular protein maintenance and adaptation to environmental stressors. Current research shows that the function of Hsp90 in the organism goes beyond protein formation within cells. In metazoans, Hsp90, as an

environmental-responsive cofactor, is involved in tissue-specific stress signaling responses that coordinate and protect non-autonomous proteins of cells and organismal health. As such, Hsp90 has the potential to influence development and aging, influencing behavioral responses to facilitate tissue defense systems that ensure organism survival. In this paper, the organic roles of Hsp90 detected in multicellular organisms, from plants to invertebrates and mammals, were as curtailed (Patricija .,2023).

The 90 kDa heat shock proteins (Hsp90s) were initially identified as stress-responsive proteins and have been described to interact and participate in many homeostatic processes. In addition, extracellular Hsp90s may have the ability to bind to surface receptors and activate cellular functions related to the immune response (cytokine secretion, cell maturation, and antigen presentation), making them extremely important to be studied as immune modulators. In addition to , Hsp90s are proposed as novel adjuvants in the synthesis and design of novel vaccine formulations that require induction of a cellular immune response to prevent infectious diseases. Research, the adjuvant properties of Hsp90s when in a state alone, in complex or incorporated into a peptide have been summarized to add light to the knowledge of Hsp90s as vectors and aids in the design of vaccines against infectious diseases. Also, the mechanisms by which Hsp90s activate and modulate antigen-presenting cells have been studied.(Mariana .,2021).

Heat shock protein HSP90 plays a major role in stimulating cell proteins, as HSP90 had a role in stimulating different biological processes, especially coordinating regulatory mechanisms in order to control the activity of these processes, and HSF1 which is the heat shock factor had a role in regulating HSP90, HSF1 integrates biological signals to regulate HSP90 levels, especially during times of stress, and not only regulates heat shock protein 90 chaperones, but also releases HSP90 from pancreatic beta cells when exposed to inflammatory conditions (Pinuinti et al , 2019).

3 -Materials and Methods3-

1 Experiment design

This study was conducted in the College of Science, Al-Qadisiyah University, in cooperation with the Diabetes and Endocrinology Center at Al-Diwaniyah Teaching Hospital, in the during the period between June 2022 to February 2023. Smoking and kidney disease patients were excluded. (200) blood samples were collected from both sexes, individuals whose ages ranged between (45-70) years, divided into four groups, the first representing the control group (G1),(50) without diabetes, the second group representing(G2), (50) patients with type 2 diabetes, and the The third (G3),(50) patients with type 2 diabetes with the presence of diabetic foot ulcers, and the fourth group(G4), (50) patients with type 2 diabetes with diabetic nephropathy. Venous blood (6 ml) was collected and collected according to the samples and groups mentioned, to measure blood indicators of cumulative sugar and blood proteins. Heat shock proteins concentration.

3-3 Collection of samples

(3ML) was withdrawn into tube free of anticoagulant materials (Gel tube) and left at room 3(Sample Collection

temperature for 20 minutes for clotting and then placed in a centrifuge at (300 rpm),for ten minutes, then the serum was taken the separated and transferred Eppendorf tubes ,then these samples were kept in continuous freezing below (- 20C)Until use The control group 50 healthy(control) both sexes. The samples were collected from different places i AL- diwnya city, without Diabetic and disorder in kidneys.

Analytical Methods and Procedures

Determination of Fasting Blood Glucose ,Determination of Glycated Hemoglobin (HbA1C (,Determination of Creatinineand Analytical Lipid profile, Total cholesterol (CHOL), Triglyceride (TG), HDL-Cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL) This was done using kit the factory of a German company and Determine the concentration of HSP-90(Heat Shock Protein 90) by kit kit the factory of a France company .

Statistical analysis:

Computerized SPSS (Statistical Packages for the Social Sciences) (V.20) program were used for the analysis of the results of the present study. The data were expressed as mean \pm standard deviation (mean \pm SD). Least significantdifferent test (LSD) was used to test the difference between means (groups) $P \leq 0.05$ was considered significant.The software was used for all statistical analyses (Spss Institue, Inc,USA).(Simon.,2001).

4 Results & Discussion.

Results and discussion of blood sugar levels, Changes in some chemical parameters in patients with diabetes mellitus(of intoxicated hemoglobin and creatinine), The results in Table (3-1) and Figures (3-2) and (3-3) showed a significant increase in the percentage of intoxicated hemoglobin and creatinine in G2 and G3 compared with G1, and for patients with kidney disease, a clear significant increase in G3 compared to G2 increase in creatinine (Diabetic nephropathy). The results showed a non-significant decrease in levels in G2 compared to G1 for patients with (creatinine) nephropathy.

Results of the current study that there is a study of the pathogenesis of type 2 diabetes if results with a study, (Al -Dahr and Jiffri .,2010) and induces hemoglobin production, induces hemoglobin production, induces hemoglobin production, and induces production of red blood molecules (Bunn et al., 1978; Majkowska and Homa 2010), and it was later realized that HbA1C reflects glycemia. , over a period of (2-3) months and was used as an early criterion for the diagnosis of diabetes mellitus (Davidson et al., 2000). It agrees with (Adriana , et al., 2023) Diabetes mellitus (DKD) is an important cause of end-stage renal disease in diabetic patients and includes no intrusion that appears mainly on the glomerular filtration unit, as for the basement membrane thickening, mesangial cell proliferation, and endothelial modification, and podocyte cell injury. These morphological abnormalities result in a persistent increase in urinary albumin to creatinine and a decrease in the estimated glomerular filtration rate and an understanding of the cell mechanisms and intracellular pathways of molecular interactions that play a role in the onset and progression of diabetes. agree with (Heri .,2022).

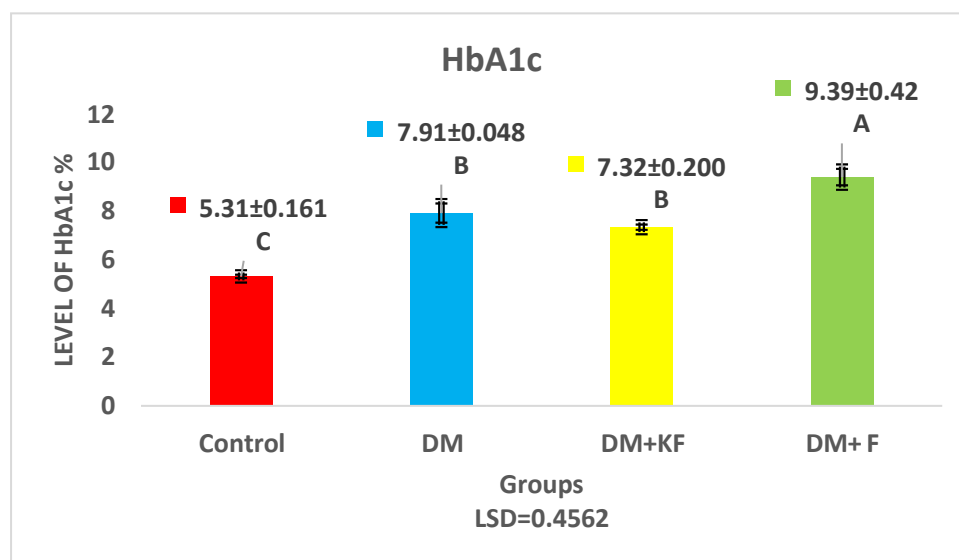
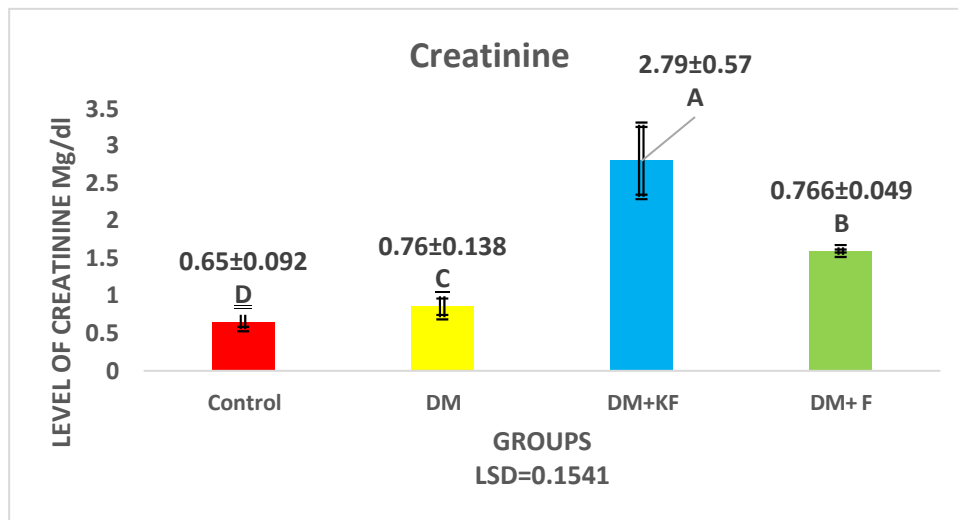


Figure (3-1): HbA1C concentration in Blood



figure(3-2) : Creatinine concentration in Blood

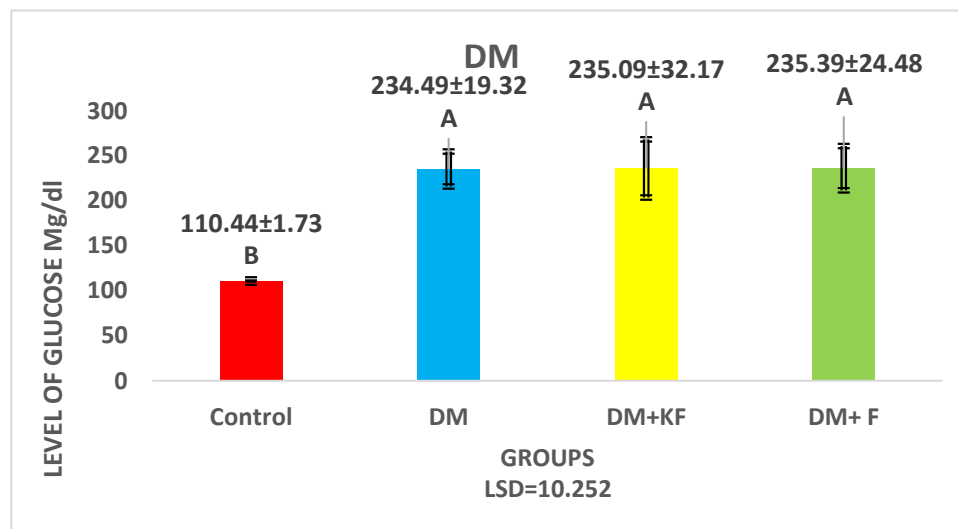


Figure (4-1): Glucose concentration in Blood

The study showed in the results a significant increase at the level of probability ($0.05 \geq P$) and as shown in Table (3-2) and figures (3-6), (3-7), (3-4), (3-5) fat levels (TC, TG, LDL-c) in all study groups increased in (TC, TG, LDL-c) levels in G2 and G3 compared to G1, while a significant decrease in HDL levels was observed in G2 and G3 compared to G1, and in G3 compared to G2, while a non-significant decrease in HDL level was found in G3 compared to G2

Also, the results of the current study, table (3-2), showed that there was a significant difference ($p \leq 0.05$) in the level of total cholesterol, as it increased in the group of patients with type 2 diabetes and those with nephropathy compared to the healthy group of the disease, and these results are consistent with the study (Faraj et al., 2004), the study of (Al-Dabbagh., 2005), the study of (Suryawanshi et al 2006), and the study of (Al-Dahr and Jiffri., 2010) (Hasban and his., 2012)

It is consistent with (Huijuan .,2023) type 2 diabetes mellitus (T2DM) There was a relationship between triglycerides to HDL cholesterol (TG/HDL-C) and T2DM, baseline TG/HDL-C ratio was positively associated with risk of developing diabetes, there was a positive relationship between baseline TG/HDL-C and the incidence of type 2 diabetes.

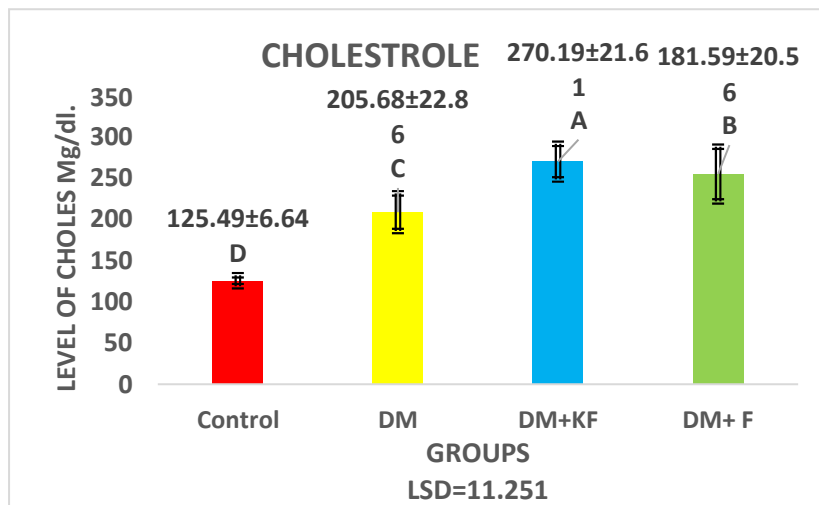


Figure (3-4):Total Cholesterol concentration in Serum

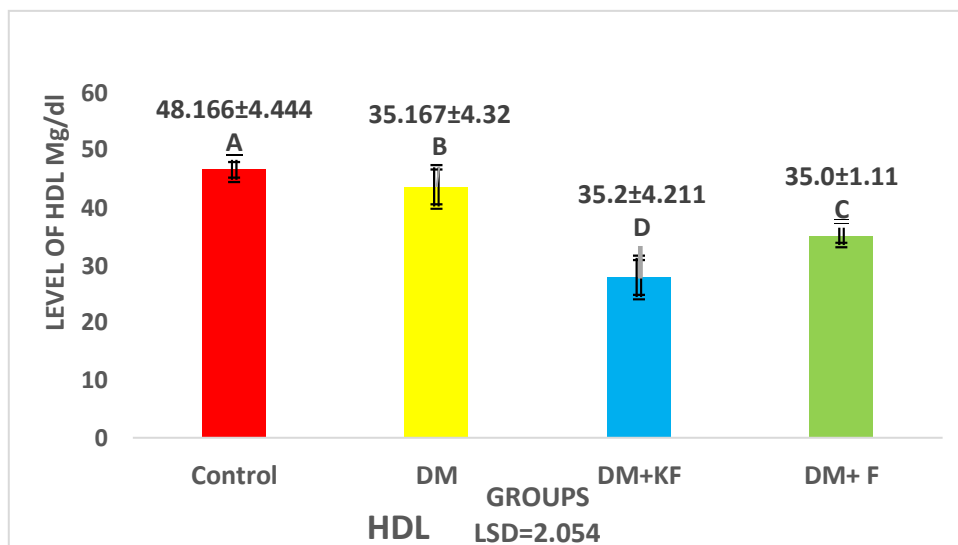


Figure (3-5): HDL-c concentration in Serum

Also, the lack of insulin secretion or the presence of insulin resistance and inefficiency in its work leads to the decomposition of fats and the release of fatty acids to obtain energy, and this leads to a decrease in the rate of consumption of very low density lipoprotein, which causes an increase in the level of triglycerides in the blood (2001, Robert). Disturbed lipid metabolism and subsequent dyslipidemia significantly contribute to plaque formation, with low-density lipoprotein (LDL-C) cholesterol being the main responsible factor, attributed to disturbances of other lipid components, namely triglycerides (TG) and lipoprotein cholesterol. High-density lipoprotein (HDL-c). Increased plasma TG and decreased levels of HDL-C have been associated with metabolic syndrome (MetS) and cardiovascular disease.(Constantine et al.,2023).

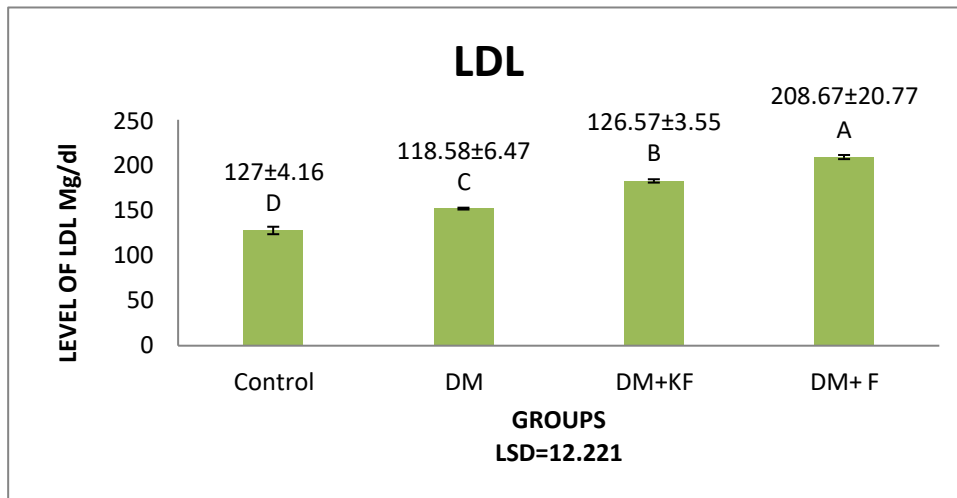


Figure (3-6): LDL-c concentration in Serum

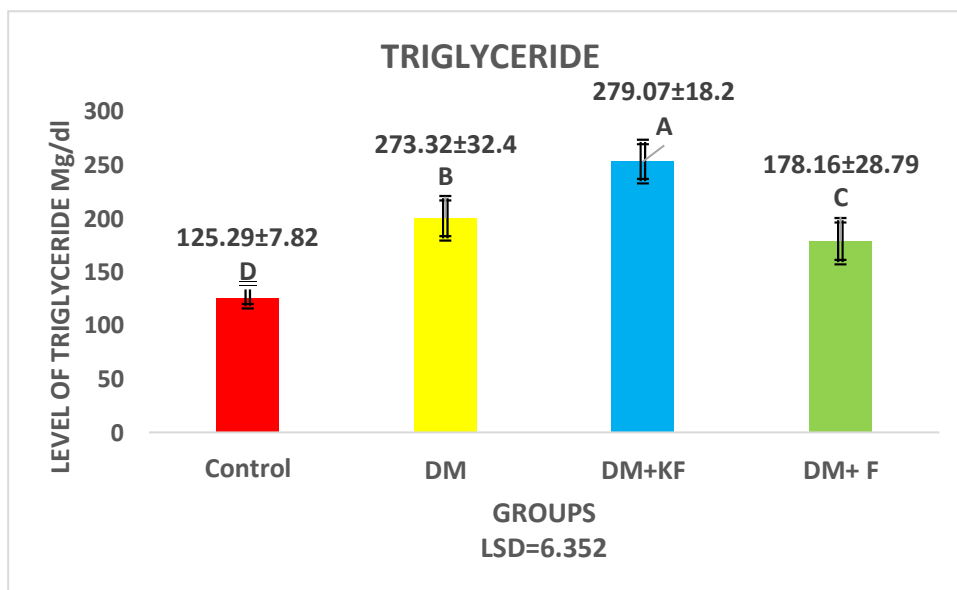


Figure (3-7): Triglyceride concentration in Serum

Results and discussion of HSP90 concentration

The results of the current study showed that there was a significant difference ($P \leq 0.05$) in the concentration of heat shock protein 90 between the patients group compared to the healthy group or the control group, as there was a significant increase in G3 and G2 compared to the G1 group, as well as a significant increase between the G3 group and the control group, G2 and the results showed a significant decrease between group G4 and group G3.

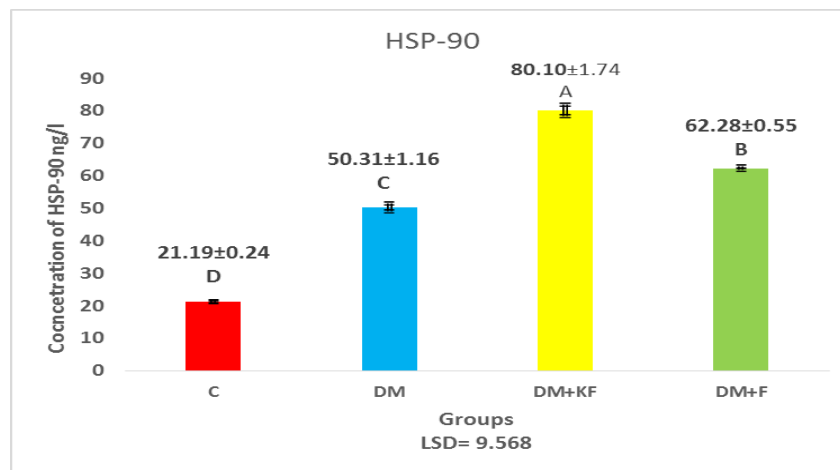


Figure (3-7): concentration of Hsp 90 in serum

This study is consistent with (Marzie and Saeed Shirali.,2016) heat shock proteins (HSPs) are a family of highly conserved proteins that are expressed by all cells exposed to environmental stress, and have numerous functions. in diabetics, but these accompaniments can help improve some of the complications of diabetes, such as oxidative stress and inflammation. Diabetes is a condition in which oxidative stress and inflammation are elevated. Heat shock proteins (HSPs) are a family of highly conserved proteins that are expressed by all cells exposed to environmental stress, and have diverse functions. In diabetic patients, heat shock proteins 90 can help improve some complications of diabetes, such as oxidative stress and inflammation. Research has shown an important role for HSPs in diabetes as well as its complications(Marzie and Saeed ., 2016). This study agrees with (Gabriela and Thiago ., 2019) showing that chronic inflammation, oxidative stress, and heat shock proteins 70 kDa (HSP70) are strongly implicated in DM2 and its complications, and various pharmacological and non-drug treatment alternatives act on these processes/molecules to modify them and reduce disease symptoms.

This study is consistent with(Wei Li, et al., 2012) Diabetic foot is another complication of diabetes. Extracellular heat shock protein 90 alpha (eHsp90 α). In contrast to the restricted cell type specificity of PDGF-BB, eHsp90 α is a common pro-factor for all skin cells, and eHsp90 α bypasses TGF β inhibition and hyperglycemia. Topical application of eHsp90 α rapidly resulted in acute and diabetic wound closure much more effectively than PDGF-BB. This makes Hsp90 α superior to conventional growth factors in wound. Healing. And this happens by knowing the role of heat shock proteins (HSPs), which are precisely preserved. They have major roles in the protection and integrity of cells from stress, and in the presence and spread of the obesity epidemic. Type 2 diabetes (T2DM). Importantly, through tumor necrosis factor-alpha (TNF α), endoplasmic reticulum and oxidative stress are all obesity-related and stimulate inflammatory kinases such as Cajun amino terminal kinase (JNK), inhibitor of NF- κ B kinase (IKK). and protein kinase C (PKC), whose activation inhibits insulin signaling. Mitochondrial dysfunction in skeletal muscle is important in the pathogenesis of T2DM either by reducing the ability to oxidize fatty acids, which causes the accumulation of harmful lipid species in peripheral tissues such as skeletal muscle and liver, or by altering the cytokinesis. redox state. Because HSPs function as molecular chaperones and exhibit important protective and protective functions in stressed cells, manipulation of HSP expression in metabolically relevant tissues represents a potentially important therapeutic approach for insulin resistance induced by the presence of obesity(Darren et al .,2014).

Conclusions and recommendations

There is a significant increase in diabetes levels and cumulative sugar(HAC1) in people with type 2 diabetes and people with diabetic nephropathy and diabetic foot. As well as a significant increase in creatinine levels, total cholesterol and triglycerides, and a decrease in low-density lipid levels(LDL).A significant increase in the

concentration of heat shock proteins 90 in all groups compared with the control group. significant increase in the levels of type 2 diabetes with an increase in body mass in all study groups. There is no significant difference in terms of gender and age between the study groups compared with the study groups.

Recommendations

- 1 - Increasing the sample size to include multiple regions and different races .
- 2 - Adoption of this study to genetic diagnose future complications for patients with type 2 diabetes.
- 3 - Adopting genetic diagnosis for the early detection of people with type 2 diabetes, taking into account the environmental aspect, balanced and healthy food, exercise, and the approved lifestyle.

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