

Article

**Estimation and Evaluation of The Antioxidant Enzymes
Catalase, Glutathione-S-Transferees And lipid Patterns in Patients With
Type 2 Diabetes in Basra Governorate – Iraq**

Baneen Abas KHalf and Sahera Ghareeb Sayyah

Department of chemistry – College of Education for pure Sciences –University of Basrah –
Basrah, Iraq

ABSTRACT

Type 2 diabetes occurs primarily as a result of two interconnected problems. Cells in the muscles, fat, and liver become resistant to insulin. Because the cells do not interact normally with insulin, they do not absorb a sufficient amount of sugar, and thus the pancreas becomes unable to secrete enough insulin to control blood sugar levels. Since antioxidants have a role in treating diabetes by defending beta cells against oxidative stress, the current research was conducted to determine the levels of enzymatic antioxidants (Glutathione - S- Transferees (GST), Catalase (CAT)) and lipid profiles (cholesterol, lipids). Triglycerides and lipoproteins (HDL, VLDL, LDL) in the blood serum of patients with type 2 diabetes compared to healthy people in Basra Governorate - Iraq, according to age. The number of patients in the study was (51) disease cases, including (19 males - 32 females) and (40) In a healthy case (20 males - 20 females), it was found that there was a significant decrease at a significance level of $P < 0.05$ in the levels of (GST and CAT) in patients compared to healthy controls, and the decrease increases with age, while there was a significant increase at a significance level of ($P < 0.01$) in Levels of (TG, TC, VLDL, LDL) in patients compared to healthy people with age, while high-density lipoprotein (HDL). The current study showed no statistically significant differences in patients and with age.

Keywords: Type 2 diabetes, Fat patterns, Catalase, Glutathione-S-Transferees

Introduction

Type 2 diabetes, may occur in more than 90% of all diabetes cases in the world (1). Type 2 diabetes is characterized by an increase in the level of blood sugar (glucose), due to a lack of secretion of the hormone insulin from beta (β) cells in The pancreas, or due to weak insulin action and body cell resistance to it, or both(2)(3). In 2015, according to statistics, there were 5 million deaths worldwide due to diabetes, and

blood clots are considered one of the main causes of death, and two-thirds of type 2 diabetes patients die due to blood clots (4). Since antioxidants have an important role in treating Diabetes by beta cells against oxidative stress resulting from programmed cell death (5). It has been found that catalase and glutathione S-transferase are powerful antioxidants.

Catalase

is considered one of the most important antioxidant enzymes and is present in all living organisms. Its function is to catalyze the decomposition of hydrogen peroxide into water and oxygen (6). As in the following equation.



The decomposition of hydrogen peroxide in the presence of CAT reduces oxidative stress significantly and also has a role in fighting diseases, including (diabetes, cancer, and aging) (7)

As for Glutathione - S-Transferees

(GST) is considered one of the enzymatic antioxidants. It is found in the liver, Which is considered a multi-functional eukaryotic and prokaryotic enzyme. This enzyme is produced through the glutathione peptide, which consists of three amino acids: glycine, glutamic, and cysteine. It is a powerful antioxidant, as it It inhibits the formation of free radicals and protects cells from damage (8, 9)

It is considered a powerful antidote to heavy metal toxins and drugs, and GST helps in treating liver and blood diseases (10,11)

Triglyceride

It is one of the types of fats that the body uses as a source of energy needed for cells and metabolic processes. Triglycerides consist of three fat molecules or fatty acids, and a glycerol molecule (12). Triglycerides are made within the body by the liver, and are stored in fat cells and liver cells. When excess calories are consumed, the body also converts them into triglycerides to store and use when needed. Measuring the concentration of triglycerides in the blood is important for diagnosing and monitoring hyperlipidemia. The increase can be of genetic origin or secondary to other metabolic disorders such as: diabetes mellitus, hyperthyroidism, liver disease, acute and chronic pancreatitis, nephritis and obesity. High triglycerides are also a risk factor for

atherosclerosis. Corticoid and estrogen/progestin treatments can also worsen hyperlipidemia (13)

Total Cholesterol:

Cholesterol is one of the types of fats, a molecule consisting of a steroid nucleus, which is involved in the composition of the body's tissues and organs, and bile acids necessary for the absorption of nutrients from food. It is also involved in the composition of the body's hormones necessary for growth and reproduction.

Lipoproteins, measured during a total cholesterol test, are classified into three types

High-Density Lipoproteins (HDL) : Good cholesterol, which helps get rid of excess cholesterol and transports it to the liver to get rid of it. It also has an effect against heart diseases, and is considered an antioxidant(14)

Low-Density Lipoproteins (LDL): These help the deposition harmful cholesterol on the blood vessels. LDL is Considered a risk factor that contributes to atherosclerosis, vascular disease, and heart disease(15).

Very Low-Density Lipoproteins (VLDL): It is a type of harmful cholesterol, and it is made in the liver. This type enables (TG, Lipid, Cholesterol) to move within the aqueous solution of the blood

Research aims

Our current study aims to estimate the levels of the enzymatic antioxidants (Glutathione - S-) Transferees, Catalase) and lipid patterns in the blood serum of the study groups with age.

Materials and methods

Blood Sampling

Blood samples were combined for patients from Al-Fayha Military Hospital in Basra Governorate - Iraq. The study included (51) sick cases, including (19 males - 32 females) and (40) healthy cases (20 males - 20 females), and their information was recorded according to a questionnaire form that included (age, Sex , family history, duration of illness

Preparation of blood Samples

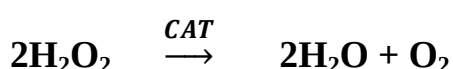
It was collected by drawing venous blood using a 5mL disposable syringe, and the blood was transferred into tubes containing the gel. After 10 minutes the blood clots,

the tubes were placed in a centrifuge at a speed of 3000 rpm for a period of time. (10-15) minutes to obtain the blood serum. The serum was divided into 8 small tubes (micro plastic tubes) and stored at a temperature of -4°C until laboratory tests were performed

Biochemical measurements

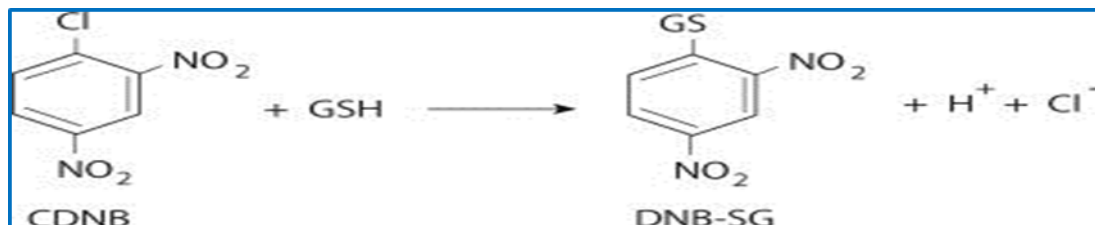
The concentration of lipid patterns in the blood serum of patients and healthy people was diagnosed using a kit equipped by the French company BIOLABO

As for the concentration of Catalase, it was determined by the decrease in absorbance due to the consumption of hydrogen peroxide according to the following equation



Absorbance is measured at 240nm.

diagnosing the concentration of Glutathione - S- Transferees in the blood serum of patients and healthy people, it is done by reacting it with the reaction (1-Chloro-2,4-dinitrobenzene (CDNB) and (GSH)) to estimate the enzyme activity according to the following equation



Absorbance is measured at 340nm.

Statistical Analysis

Statistical operations were performed in the statistical analysis program SPSS version (23) to compare the study groups using One-Way-Anova using the Least Significant Differences (LSD) method to determine the effect of significant coefficients and determine the presence of significant differences at a P value of <0.05 . Highly significant differences at $P < 0.01$, as well as the use of statistical analysis to extract the values of the mean and standard deviation for the characteristics of diabetic patients and healthy people

Results

The results of our current study in Table (1) showed a significant decrease at the significance level of ($P < 0.05$) in the average concentration of (CAT and GST) compared to healthy people. While there was a significant increase in the average concentration of (TG, TC, VLDL, and LDL) in the blood serum of patients compared to healthy people at a significance level of ($P < 0.01$), as for HDL, the study showed that there were no statistically significant differences compared to healthy people. Although there was a slight decrease in the concentration rate of patients

Table (1) represents enzymatic antioxidants (catalase, glutathione S-transferases) and lipid profiles in the blood serum of diabetic patients and healthy people

Parameters	(51) Patients SD $\pm$ mean	(40) Control SD $\pm$ mean	Probability
CAT K/m	0.33 $\pm$ 0.012	2.22 $\pm$ 0.13	P<0.05
GSST U/L	0.94 $\pm$ 0.23	2.51 $\pm$ 0.15	P<0.05
TG mg/dl	854.50 $\pm$ 84.32	516.7 $\pm$ 21.98	P<0.01
TC mg/dl	415.04 $\pm$ 15.54	360.03 $\pm$ 10.44	P<0.01
HDL mg/dl	191.95 $\pm$ 6.78	197.21 $\pm$ 10.07	P>0.05
LDL mg/dl	119.59 $\pm$ 12.34	70.51 $\pm$ 6.30	P<0.01
VLDL mg/dl	170.89 $\pm$ 16.86	103.47 $\pm$ 4.40	P<0.01

SD= Standard deviation

morale $P < 0.05$

High morale < 0.01

Table (2) Levels of enzymatic antioxidants (catelase, glutathione S-transferases, and lipid profiles in the blood serum of diabetic patients and healthy controls according to age factor

Parameters		Age(25-40)year	Age(41-56)year	Age(57-72)year
CAT K/ml	Patients	0.39±0.002	0.25± 0.003	0.0 90±0.005
	Control	3.20 ±0. 6	2.12 ±0. 8	2.1± 0.006
GST U/L	Patients	0.93±0.003	0.44±0.14	0.085±0.29
	Control	2.63±0.19	2.0± 0.97	1.12±0.00
TG mg/dl	Patients	247.68± 0.31	1007.81±133.98	716.30±64.10
	Control	21.60 ± 513.01	506.80±70.63	608.81±214.70
TCmg/dl	Patients	470.86±0.14	422.19±24.00	395.32±19.47
	Control	443.68 ±13.02	200.20±0.40	300.11±12.33
HDLmg/dl	Patients	275±0.00	175.55±11.70	218±17.75
	Control	183.71±7.70	225.92±9.36	200.75±35.75
LDLmg/dl	Patients	152.65 ±6.37	127.43 ±19.36	102.42 ±14.66
	Control	144.46± 17.95	93.32 ± 26.32	157.48± 53.98
VLDLmg/dl	Patients	49.53±0.06	201.55±26.79	143.25±12.82
	Control	102.60±4.32	102.13±14.24	121.76±42.94

Concentration Values Were expressed as mean ±SD** $P < 0.01$, * $P < 0.05$. NS: Non-significant.

Discussion

The results of the current study showed in a table a decrease in the average concentration of CAT compared to healthy people and with age, with statistically significant differences at ($P < 0.05$) Antioxidants in patients are low, and this is a sure

sign of oxidative stress. Some Studies have shown a decrease in the values of (CAT) in patients. (16)(17) Some studies have also shown that there is no change or difference between healthy people and patients (18)(

While the results of the current study in Table (1)(2) showed that there were statistically significant differences at the significance level of ($P < 0.05$) in the concentration rate of (GST), our current study is consistent with the study that shows a decrease in the activity of (GST) in red blood cells in. In Diabetics (19) due to oxidative stress, the status of antioxidants is weakened. It is clear that the rate of its concentration in females is higher than in males, and this is consistent with the study(20)

The results of our current study also showed a significant increase in LDL, VLDL, TC, and TG Levels in patients compared to healthy people and with increasing age at a significance level ($P < 0.01$). This is consistent with study (21). As for HDL, the study showed no significant differences with statistical significance. Compared to healthy people. Although there was a slight decrease in the concentration rate of patients.

High triglycerides in diabetics are attributed to a defect in the functioning of insulin, and thus the activity of the enzyme lipo-protein lipase (LPL) decreases, whose role lies in converting triglycerides into fatty acids and cholesterol (22), which are absorbed by fat cells. Also, a defect in the action of insulin leads to Activating the hormone-sensitive lipase enzyme, which increases the work of fat decomposition, which in turn is transported to the liver, where it regenerates (TG). Thus, triglycerides Levels increases because they are an alternative source of energy (23). High cholesterol plays an essential role in raising the level of (VLDL, LDL). (24) LDL is considered a risk factor that contributes to atherosclerosis, vascular disease, and heart disease (25). High total cholesterol in patients and healthy people is explained by the decomposition of LDL lipoprotein or is due to the inefficiency of the receptors for the protein fragment (APO-B-100). For the lipoprotein LDL, the level of TC (26) increases. As for HDL, although there is a small difference in the values, no statistically significant differences appeared between patients and healthy men and between sick and healthy males and healthy males.

Conclusions

Since type 2 diabetes affects people between the ages of (40-70) years, this study was conducted to estimate and evaluate the levels of enzymatic antioxidants and lipid profiles for three different age groups to determine their effect on the development of

complications of type 2 diabetes with age. There was a significant increase in the levels of (TG, TC, LDL, and VLDL) in diabetic patients with age, while there was a significant decrease in the levels of (GST, CAT) with age. This indicates that oxidative stress increases in aging and plays a role. A major factor in the development of type 2 diabetes complications with age.

References

- [1] Abdulkader. A. Al-Shakou, N. A. N. Serum level of periostin in patients with Type 2 Diabetes Mellitus in Bsrh, Iraq. *Azerbaijan Medical Journal*. 2022, 62(6): 1745–1751.
- [2] Ozougwu J, Obimba KC, Belonwu CD, Unakalamba CB. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. *Journal of Physiology and Pathophysiology*. 2013; 4(4):46-57.
- [3] Jassim, S., & Abed, R. Relationship between A coping style and self- care activities of patients with type 2 diabetes mellitus (t2dm) at endocrinology and diabetes center in al-Basra city: a cross-sectional study. *Kufa Journal for Nursing Sciences*. 2021; 11(1): 1–8. <https://doi.org/10.36321/kjns.vi20211.452>.
- [4] Kadhim, S. N., Abdullah, A. S., & Sabah, A. I. Treatment modality, diabetic control, and blood homeostasis in type 2 diabetes mellitus patients in Basra. *Current Issues in Pharmacy and Medical Sciences*. 2021; 34(2): 70–73. <https://doi.org/10.2478/cipms-2021-0012>.
- [5] Rajendiran, D., Packirisamy, S., & Gunasekaran, K. A review on role of antioxidants in diabetes. *Asian Journal of Pharmaceutical and Clinical Research*. 2018; 11(2): 48. <https://doi.org/10.22159/ajpcr.2018.v11i2.23241>
- [6] Shi, Q., Xiong, X., Wen, Z., Qin, C., Li, R., Zhang, Z., ... & Wu, X. Cu/Zn Superoxide Dismutase and Catalase of Yangtze Sturgeon, *Acipenser dabryanus*: Molecular Cloning, Tissue Distribution and Response to Fasting and Refeeding. *Fishes*, 2022, 7.1: 35.

- [7] Sinha, N., Patra, S. K., Sarkar, T. S., & Ghosh, S. Secretome analysis identified extracellular superoxide dismutase and catalase of *Macrophomina phaseolina*. *Archives of Microbiology*, 2022, 204.1: 1-11.
- [8] Josephy PD (2010). "Genetic variations in human glutathione transferase enzymes: significance for pharmacology and toxicology". *Hum Genomics Proteomics*. 2010:
- [9] Uppugunduri, C. R. S., Muthukumaran, J., Robin, S., Santos-Silva, T., & Ansari, M.. In silico and in vitro investigations on the protein–protein interactions of glutathione S-transferases with mitogen-activated protein kinase 8 and apoptosis signal-regulating kinase 1. *Journal of Biomolecular Structure and Dynamics*, 2022, 40.3: 1430-1440-
- [10] Oakley A (2011). "Glutathione transferases: a structural perspective". *Drug Metab. Rev.* 43 (2): 138–51. doi:10.3109/03602532.2011.558093. PMID 21428697 .
- [11] Hayes JD, Flanagan JU, Jowsey IR (2005). "Glutathione transferases". *Annu. Rev. Pharmacol. Toxicol.* 45: 51–88. doi:10.1146/annurev.pharmtox.45.120403.095857. PMID 15822171
- [12] Heartuk.org.uk. Triglycerides. Retrieved on the 17th of May, 2023
- [13] TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 809-857
- [14] Kosmas CE, Martinez I, Surlas A, Bouza KV, Campos FN, Torres V, Montan PD, Guzman E. High-density lipoprotein (HDL) functionality and its relevance to atherosclerotic cardiovascular disease. *Drugs in context*. 2018;7.
- [15] Chruściel P, Stemplewska P, Stemplewski A, Wattad M, BieleckaDąbrowa A, Maciejewski M, Penson P, Bartłomiejczyk MA, Banach M. Associations between the lipid profile and the development of hypertension in young individuals—the preliminary study. *Archives of Medical Science: AMS*. 2022;18(1):25.

- [16] Sundaram RK, Bhaskar A Vijayalingam S, Viswanathan M, Mohan R Shanmugasundaram KR. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications, *Cin Sc.*, 1996, 90, 255-260 .
- [17] Parthiban A Vijayalingam S. Shanmugasundaram KR, Mohan R. Oxidative stress and the development of diabetic complications antioxidants and lipid peroxidation in erythrocytes and cell membrane, *Cell Bol int*, 1995, 19, 987-993.
- [18] Song F, Ja W, Yao Y, Hu Y, Le L, Un J, Sun X, Liu L, Oxidative stress, antioxidant status and DNA damage in patients with Impaired glucose regulation and newly diagnosed Type 2 diabetes, *Clin Sci*, 2007, 112, 599-606.
- [19] Vairamon SJ, Babu M, Viswanathan V, Oxidative stress markers regulating the healing of foot ulcers in patients with type 2 diabetes, *Wounds*, 2009, 21, 273-279- 27.
- [20] Sushma Verma', Nibha Sagar', Pushpank Vats', KN Shukla', Mohammad Abbas', Monisha Banerjee" Molecular & Human Genetics Laboratory, Department of Zoology, University of Lucknow, Lucknow-226007, India 'Department of Medicine, Balrampur Hospital, Lucknow-226001, India
- [21] Robert, et. al. Introduction Biostatistics for the health sciences (1991).
- [22] Kestelein J.J., Ordovas J.M., Wittekoek M.F. and Pimstona S. Two common mutations in lipoprotein lipase: accumulative analysis of their influence on plasma lipid and lipoprotein in men and weman. *Clinical Genetics* 1999; 56: 297-305.
- [23] Hameed, I.K.; Rashid, N.F. and Abed, B. A. (2013). Homestasis model assessment- adiponectin ratio and adiponectin -resistin index as markers of insulin resistance in type 2 diabetes mellitus. *J.FacMed Baghdad*. ,55(2): 175-178.
- [24] Turk, Z.; Sesto, M.; Skodlar, J. and Ferencak, G. (2002). Soluble LDL immune complexes in type 2 diabetes and vascular disease. *Horm. Metab. Res.*, 34(1): 196 – 201

- [25] Chruściel P, Stemplewska P, Stemplewski A, Wattad M, BieleckaDąbrowa A, Maciejewski M, Penson P, Bartłomiejczyk MA, Banach M. Associations between the lipid profile and the development of hypertension in young individuals–the preliminary study. Archives of Medical Science: AMS. 2022;18(1):25.
- [26] Cumming M.H., Watts G.F. and Naoumova R.N. (1995). "Increase hepatic secretion of() VLDL apolipoprotein B-100 in NIDDM". Diabetologia., 38: 959-980