

Study the Profile of Some Antioxidant Markers in Diabetic Mellitus and Non-diabetic Patients with Cardiovascular Disease

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Abstract

Background: Cardiovascular diseases (CVDs) claim the lives of millions worldwide. **Objective:** Evaluate the levels of some antioxidant biomarkers in sera of patients with CVDs with and without diabetes mellitus type 2. **Materials and Methods:** Includes 100 samples in 68 patients (40 with CVDs and 28 CVDs with diabetic), and 32 healthy subjects. **Results:** Serum (Albumin, thiol, thiol/T.protein, amine, free amine/ T.protein, carbonyl, carbonyl/ T.protein, and Ischemia modified albumin (IMA)) showed a stronger response, a significant rise in carbonyl and carbonyl/TP, and a significant reduction in thiol and thiol/TP in the patients with CVDs with T2D compared to CVDs may be a good factor to differentiate them or predict more serious complications. **Conclusions:** Oxidation markers may be useful in monitoring CVDs with and without diabetes.

Keywords: Antioxidant markers, cardiovascular diseases, HAS, IMA, type 2 diabetes

INTRODUCTION

Fatness, diabetes, too little exercise, smoking, and other variable lifestyle factors account for about 50% of the risk of “Cardiovascular diseases” CVDs.^[1] Death is the most dreaded complication of CVDs and remains one of the important global factors of mortality due to the alarming prevalence of CVDs in the population. The common complications of CVDs divided to several types including: Angina, cardiac arrest, atrial fibrillation, heart failure, Pulmonary embolism, Peripheral artery disease, heart attack, and Strokes.^[2] One of the major contributors to the growth and development of heart disease has been recognized as oxidative stress, Reactive oxygen species (ROS) have been identified to enhance the proliferation of cardiac fibroblasts.^[3] Decreased human serum albumin (HAS) concentrations are typically linked to an elevated risk of CVDs mortality; since it is the highest common extracellular type of protein, it performs a wide range of activities, such as transportation, controlling colloidal osmolality, and moderating sera redox homeostasis. Human serum albumin level decrease in chronic and acute illnesses, which provides accurate ideas on the early diagnosis of illnesses.^[4] Human serum albumin is a monomeric macromolecule having a molecular mass

of about 66.5 kDa,^[5] HAS are a significant antioxidant source and are the biggest thiol group in the blood due to their high content. They have 35 cysteine residues that produce 17 disulfide bonds and just a single free cysteine at location 34. In normal persons, the Cys-34 is reduced by 70–80 percentage of HSA, which circulates as mono mercaptalbumin, while the Cys-34 is completely oxidized to sulfonic or sulfonic acid in 20–30% of HSA and linked to small molecule groups (cysteine, homocysteine, and glutathione) in the remaining five percentage of HSA moves as mercaptalbumin.^[6] Due to the limited half-life (20 days) for HAS molecular, it is a reliable redox status indicator of the level of limited time systemically oxidative stress.^[7] The far more common and widely employed indicator is “carbonyl stress” is protein carbonylation, which is believed to reflect carbonyl modification of amino residues. Carbonyl content concentration is perhaps the most typical and frequently used marker oxidation

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of proteins.^[8] IMA, which is changed in oxidative stress situations have been linked to endothelial L-arginine/nitric oxide pathway dysfunction and ischemia-reperfusion injury (affecting NO levels).^[9] As a result of a continuing ischemia event, IMA is HSA that is unable to connect with cobalt,^[10] it showed particularly effective in detecting acute myocardial ischemia. The purpose of this research was to determine the levels of many oxidative biomarkers in the serum of individuals with CVDs and CVDs with T2D, comprising whole protein content, HSA, globulin, A/G, free amino, carbonyl, thiol, and IMA compare with healthy people as control, in order to evaluate the possible change in the serum proteins of the studied patients and afterward, examining the association among biomarkers. in the patients' groups.

MATERIALS AND METHODS

From mid-January to the end of April 2022, the research performed in the Department of Chemistry at the University of Kirkuk, Iraq, in partnership with the Daquq general hospital.

Patients

Sixty-eight cases, depending on their age of 24–70 years, admitted to the Daquq general hospital were dedicated to the investigation. Cases were identified and categorized (40 samples as CVDs and 28 as CVDs with T2D) with of cardiac enzyme tests, X-rays, and results of the Electrocardiography when a patient is admitted. Thirty-two healthy people who had undergone a diagnostic test but showed no signs of cardiac disease were chosen as the control group. Specimens of serum were collected (5–6ml) after 12 hours of fasting in gel tubes using a disposable syringe, and blood was taken by vein puncture without using the tourniquet. After 15 minutes at room temperature, centrifuged the gel tubes for (15) minutes at (2800 x g). The serum was collected and stored at deep freeze until utilizing it in experiments.

Exclusion criteria

The collected samples are free from decomposition and turbidity to avoid any interference with the results, and patients do not suffer from other diseases outside the scope of the research topic.

Methods

Bromocresol green (BCG) is used to assess HSA.^[11] The concept is that HSA is bound with bromocresol color to create a chrysolite measured at 630nm, with the amount of accessible HSA proportional to the rise in color. Lowry proposed a method in which bovine serum Albumin was employed as a control protein to calculate the total amounts of serum protein.^[12] The method proposed by Zaia, Barreto, and others was used to determine the free amino group spectrophotometrically.^[13] Elman^[14]

described a method for measuring total serum thiol concentration, the interaction between the thiol group and Ellman's reagent (DTNB) results in the formation of a colored complex peak point at 412nm. Calculated by utilizing the formula $A = \epsilon \cdot C \cdot l$, where: $\epsilon = 13,600 \text{ M}^{-1}\text{cm}^{-1}$.

The proteins' carbonyl concentration was determined using the Levine, Garland procedure,^[15] utilizing the formula $A = \epsilon \cdot C \cdot l$, where: $\epsilon = 22,000 \text{ M}^{-1}\text{cm}^{-1}$. IMA level was determined in serum by Albumin Cobalt Binding Assay; Myocardial ischemia induces alterations in HSA, as seen by reduced exogenous cobalt (II) binding. According to the assay, there is an opposite correlation between the amount of albumin-bound cobalt and the level of color generation.^[16]

Statistical analysis

Graph Pad Prism V 9.0 (Graph Pad Software, San Diego, CA, USA) was utilized to do the statistical analysis, and the outcomes were presented as (mean $\pm$ SD). The independent t-test was used to compare the mean and standard deviation, and the correlation between the parameters and $P \leq 0.05$ was used to assess statistical significance.

Ethical approval

The study was conducted by the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol information and consent form were reviewed and approved by a local ethics committee according to document number 3/7/6141 (including the number and the date on (23/11/2021) to get this approval.

RESULTS

The concentrations of T.protein, HSA, Globulin, Albumin/Globulin ratio A/G, Thiol, Free amine, Carbonyl, and IMA in the sera of all studied groups are shown in [Table 1].

The findings showed that there were no significant differences ($P \geq 0.05$) for TP, globulin, and AGR levels in patients' groups vs the control, HSA was significantly decreased ($P \leq 0.05$) in patients with CVDs and CVDs with T2D compared with control, still their was a no discernible difference ($P \geq 0.05$) among the two patient types. Both patients with CVDs and CVDs with T2D had a significant decrease for Thiol when vs the control, as well as a significant reduction ($P \leq 0.05$) in CVDs with T2D patients when compared to CVD patients. A significant rise ($P \leq 0.05$) in the concentrations of free amine, free amine/total protein, and IMA in patients' groups compared to the control and a non-significant increase in it of cases with CVDs with T2D compared to CVDs. Finally, in both patient groups, there was a significant

Table 1: Measurements of chemical parameters, that be expressed with (mean $\pm$ SD) for all groups under study

Parameters	Control (T.C=32) mean $\pm$ SD	CVDs (T.C=40) mean $\pm$ SD	CVDs with T2D (T.C=28) mean $\pm$ SD
TP gm/dl	7.115 $\pm$ 0.401	7.090 $\pm$ 0.486	6.941 $\pm$ 0.721
HSA gm/dl	4.474 $\pm$ 0.230	4.303 $\pm$ 0.326*	4.139 $\pm$ 0.363*
Globulin gm/dl	2.640 $\pm$ 0.451	2.787 $\pm$ 0.576	2.802 $\pm$ 0.733
AGR	1.754 $\pm$ 0.377	1.632 $\pm$ 0.488	1.753 $\pm$ 1.265
free amino mmole/L	3.913 $\pm$ 0.726	4.734 $\pm$ 0.735*	4.780 $\pm$ 0.765*
free amino/ TP mmole/gm	0.055 $\pm$ 0.011	0.067 $\pm$ 0.012*	0.070 $\pm$ 0.019*
Carbonyl nmole	9.226 $\pm$ 2.852	14.142 $\pm$ 1.564*	17.952 $\pm$ 1.581*†
Carbonyl /TP nmole	1.443 $\pm$ 0.448	2.356 $\pm$ 0.298*	3.251 $\pm$ 0.639*†
Thiol μ mole/L	402.494 $\pm$ 30.691	311.343 $\pm$ 24.288*	268.798 $\pm$ 17.204*†
Thiol/TP μ mole/gm	5.674 $\pm$ 0.533	4.415 $\pm$ 0.488*	3.913 $\pm$ 0.487*
IMA ABSU	0.684 $\pm$ 0.045	0.818 $\pm$ 0.101*	0.852 $\pm$ 0.098*

(T.C) total cases, (SD) standard deviation, (*) Significant differences ($P \leq 0.05$) between both patients with CVDs and patients CVDs with T2D vs control, (†) significant discrepancies ($P \leq 0.05$) between patients with CVDs and T2D in comparison to people with CVDs alone

Table 2: The correlation between the parameters that were assessed in CVD patients with and without T2D

Parameters		patients with CVDs		patients CVDs plus T2D	
		r	P value	r	P value
TP	Globulin	0.825	<0.001	0.875	<0.001
TP	A/G	-0.692	<0.001	-0.858	<0.001
TP	Carbonyl/TP	-0.479	0.002	-0.904	<0.001
TP	amino/TP	-0.474	0.002	-0.864	<0.001
TP	Thiol/TP	-0.713	<0.001	-0.844	<0.001
Globulin	A/G	-0.898	<0.001	-0.872	<0.001
Globulin	Carbonyl/TP	-0.426	0.006	-0.751	<0.001
Globulin	amino/TP	-0.340	0.032	-0.768	<0.001
Globulin	Thiol/TP	-0.572	<0.001	-0.767	<0.001
A/G	Carbonyl/TP	0.419	0.007	0.762	<0.001
A/G	amino/TP	0.378	0.016	0.784	<0.001
A/G	Thiol/TP	0.462	0.003	0.869	<0.001
Carbonyl	Carbonyl/TP	0.824	<0.001	0.714	<0.001
Carbonyl	Thiol	-0.924	<0.001	-0.941	<0.001
Carbonyl	Thiol/TP	-0.712	<0.001	-0.128	0.56
Carbonyl/TP	amino/TP	0.230	0.154	0.872	<0.001
Carbonyl/TP	Thiol	-0.755	<0.001	-0.719	<0.001
free amino	amino/TP	0.909	<0.001	0.822	<0.001
Thiol	Thiol/TP	0.767	<0.001	0.105	0.635

Significant variations ($P \leq 0.05$), (r) is Pearson correlation

increase ($P \leq 0.05$) for carbonyl, and carbonyl/TP when compared with control, also a significant rise ($P \leq 0.05$) in the patient CVDs with T2D when compared with CVDs patients.

[Table 2] summarized the significant Pearson correlation (r) between the examined variables in the groups of individuals with CVDs and those who also had T2D and CVDs

According to the findings, there was a favorable positive between [TP-globulin, A/G-CO/TP, A/G-amino/TP, A/G-thiol/TP, CO-CO/TP, CO-CO/TP, CO/TP-amino/TP, amino-amino/TP and thiol-thiol/TP] and there were negative correlation coefficient between [TP-A/G, TP-CO/TP, TP-amino/TP, TP-thiol/TP, globulin-A/G,

globulin-CO/TP, globulin-amino/TP, globulin-thiol/TP, CO-thiol, CO-thiol/TP and CO/TP – thiol], in both patients groups.

DISCUSSION

Proteins are important targets for oxidation reactions described as a mismatch in how free radicals are formed and the antioxidant reaction within cells, because they are abundant in tissues, extracellular cells, and physiological fluids, as well as their quick reaction rates with oxidants, furthermore, oxidative stress can cause. Lipids and carbohydrates decompose into extremely reactive compounds that attack proteins in a variety of functional places. As a result, a wide range of proteins that modify

non-reversible modifications contribute factors that speed up the development of many diseases.^[17] Because of the chemical stability of some protein oxidation products and produced in high quantities, they are attractive candidates as oxidative damage indicators. The current investigation demonstrates strong observational links between HSA and the likelihood of CVDs and CVDs with T2D when compared with control. In Comparison to the control, HSA concentrations in both groups decreased significantly ($P \leq 0.05$) Andreas Ronit *et al*^[18] they found similar results, they also discovered a drop in HSA levels in individuals with CVDs. Several theories have been offered to clarify the mechanisms that may help to explain the link between a drop in HSA and an increased risk of CVDs, the most obvious mechanism is inflammation. The liver produces HSA, When there is widespread inflammation, several acute-phase proteins instead of HSA, which is a negative acute-phase reactant, are produced.^[19] Another explanation is that decreased HSA indicates increased vascular permeability in inflammatory conditions, whereas C-reactive protein (CRP) does not detect, as fluid redistribution to the intercellular can swiftly and greatly change HSA levels.^[18] Furthermore, in the case of patients who have diabetes in addition to CVDs, other mechanisms affect the decrease in HSA level. First, diabetes can cause a reduction in liver albumin production.^[20] Second, processes involved in the formation of glycated albumin (GA) may result in a reduction in HSA. GA functions as an intermediate for “advanced glycation end products” (AGE) which result in inflammation and have an aberrant capacity to bind various ligands.^[21] Furthermore, there is proof that GA causes an immunological reaction that causes HSA levels to drop much lower, and increased oxidative stress has been related to insulin resistance and diabetes development.^[22] Third, as previously state, inflammation has a role in the etiology of diabetes.

When comparing the patient groups with control the results of free amino concentrations showed a statistically significant rise ($P \leq 0.05$) in the cases groups. These results are supported by Li, Tie *et al*^[23] they found that in CVDs, the concentration of free amino increased. Furthermore, the findings of this study contradicted those of Trifunovic-Macedoljan J *et al*,^[21] they showed a reduction in the amount of free amine in Diabetes Mellitus patients. This study's findings people with higher free thiol levels exhibited better glycemic control than those with lower amounts, and patients with CVDs also had low levels compared with control. The findings showed a significant decline ($P \leq 0.05$) in the free thiol amounts in the sera of CVDs with T2D compared to CVDs patients, there was also a significant decrease ($P \leq 0.05$) in thiol amounts in patients with CVDs compared with control. These findings were consistent with those of Emmelien E.M. *et al*^[24] and Amaal E Abdulla *et al*^[25] they discovered that individuals with T2D and CVDs had significantly lower thiol

concentrations in patients compared with control. The findings of the thiol levels contradicted those of Adnan Khalaf, M, *et al*,^[7] who found a significant rise ($P \leq 0.05$) in thiol levels in diabetic patients compared to control. Diabetes results in metabolic pathway abnormalities, an increase in free radicals, and higher glucose levels due to the impaired antioxidant systems. Protein thiol groups are one of the antioxidant systems, when proteins are exposed to oxidative stress, the thiol groups decrease and functional problems occur.^[26] The significance of oxidative stress in CVDs has received more attention in recent decades, as it has been linked to endothelial dysfunction.^[27] Thiol is an important component of redox signaling networks that are dynamic and complicated in the extracellular environment, where they have significant antioxidant action because of their ability to remove circulated reactive species quickly.^[28] Homocysteine, glutathione, and other small molecular weight thiols, account for a lesser percentage of extracellular thiols incorporated in plasma proteins.^[29] The most redox-active thiol groups are found in blood proteins. Because of its abundance and ability to transport thiols, experimentally HSA is this pool's more significant component (Due to the presence of its lone free cysteine residue, Cys34).^[28] As a result of the preceding, serum levels of free thiol may be inversely connected to oxidative stress and the hazards it causes, such as CVDs and diabetes. Protein carbonyl production, which can result from direct free radical breakage of the side chains of amino acids is key evidence that proteins have oxidized.^[30] Protein oxidation was measured in this study by detecting protein carbonyl content. The outcomes showed a significantly rise ($P \leq 0.05$) for carbonyl content in the sera of CVDs with T2D compared to CVDs and control, also revealed a significantly ($P \leq 0.05$) higher carbonyl content in patients with CVDs compare with control. These findings were consistent with those of Abd I.K.^[31] and Sushmita Bora, *et al*^[32] they found that there was an increase in carbonyl levels in diabetes and CVDs patients respectively, however, this was in contrast to Adnan Khalaf, M. *et al*. findings.^[7] Platelets, which are circulating anucleate cells produced from megakaryocytes, play a critical role in the formation of CVDs. In prothrombotic cardiovascular events, ROS plays a significant part in platelet stress-induced signaling processes, platelet activation induces a pro-thrombotic phenotype and increases the risk of CVDs.^[33] IMA concentrations were found significantly rise ($P \leq 0.05$) in CVDs with T2D and CVDs compared to control in this study. This result is confirmed by UVPU Sowjanya *et al*^[34], and Amira Abdel Moneam Adly, *et al*^[35] they reported that the enhanced oxidative stress and free radical production resulted in wide vascular endothelial inflammation and the subsequent tissue hypoxia could be the causes of the strong correlation between higher serum IMA and HbA1c.^[9] For the generation of IMA, various mechanisms have been proposed. Acidosis, free iron, and copper ion release, hypoxia, rise free radical damaging, Na-Ca pump

disruptions that depend on membrane energy are all possible consequences of cardiac ischemia, in each of which result in damage to the amino terminus of HSA.^[36]

For both patient groups, there was a significant inverse relationship between (Globulin-A/G, Carbonyl-Thiol, TP-A/G, and TP-Thiol/TP) and a significant positive correlation between (Carbonyl-Carbonyl/TP, and TP-Globulin). Current study outcomes were consistent with earlier investigations Adnan Khalaf, M, *et al.*^[7] This explains how a drop in HSA, which serves as an antioxidant in endothelial function and causes a relative rise in globulin levels, can result in a decrease in A/G (inverse relation), while also achieving (Positive association) between (TP-Globulin). Since protein thiol groups are one of the antioxidant systems,^[37] any increase in carbonyl content may result in a drop in thiol levels since protein carbonyl synthesis is a sign of dependent oxidative stress on the type of ROS^[38] (inverse relation).

CONCLUSIONS

The findings of this investigation showed that, in contrast to controls, oxidative protein markers increased in CVDs with T2D, it an increase in the patients with CVDs with T2D compared to CVDs. The aforementioned data could support a link between these

oxidation proteins and the aforementioned illnesses. Serum (HSA, thiol, thiol/T.protein, amine, free amine/ T.protein, carbonyl, carbonyl/ T.protein, and IMA) showed a stronger response, a significant rise in carbonyl and carbonyl/TP, and a significant reduction in thiol and thiol/TP in the patients with CVDs with T2D compared to CVDs may be a good factor to differentiate them or predict more serious complications. Patients with CVDs with T2D higher than patients with CVDs had more high correlations between biochemical markers. It's possible that having T2D along with CVDs will enhance the likelihood that serious vascular problems. The correlation between (Carbonyl-Thiol/TP, and Thiol-Thiol/TP) is strong in Patients with CVDs but, is negligible in patients with CVDs with T2D this may serve as a good prognostic marker for patients with CVDs alone without T2D, whereas the strong correlation between (Globulin-amino/TP, and Carbonyl/TP-amino/TP) in patients with CVDs and T2D, and low correlation for same parameters in patients with CVDs may be referred to as significant indications of CVD patients with complication. Future research must confirm any possible associations between redox balance and the cardiovascular profile.

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Conflicts of interest

There are no conflicts of interest.

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