Effect of Antioxidant and Lipid Profile on the Coronary Heart Disease

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Abstract

Despite the declining coronary heart disease (CHD) mortality rate, CHD remains a major cause of premature death. The present study connect the relationship between SOD (antioxidant enzyme), Lipid profile, Angina and Myocardial Infarction. To achieve this, collected 105 of patient with Angina and MI aged between (38-82) years, then measurement of superoxide dismutase (SOD) activity in sera of patients with Angina and MI. The results indicated moderated significant decreases of SOD levels ($p<0.05$) in patient when compared with the control group. The degree inhibition of SOD enzyme with various concentration of cyanide, Hydrogen peroxide and diethyl dithiocarbamate, the results recorded that decrease more than 50% of SOD activity at 30 mM of cyanide ion, is measured, and achieve 50% of inhibition at 40 mM of both H_2O_2 and DDC, this indicated that SOD enzyme more sensitive to the cyanide ion, measurement of lipid profile in sera of patients with Angina and MI. It was indicated highly significant increases of cholesterol and LDL-c levels ($p<0.001$), and moderated significant increases of triglycerides ($p<0.05$) whereas no significant decrease in HDL-c levels when compared with the control group.

Keywords: Antioxidant, Superoxide dismutase, Lipid Profile, Heart Disease.

Abbreviation: A: Absorbance, CHD: Coronary heart disease, DDC: Diethyl Dithiocarbamate, HDL: High density lipoprotein, LDL: Low density lipoprotein, N.S: Not significant,MI: myocardial infarction, ROS: Reactive oxygen species ,V_S : Volume sample, N.S: Not significant, SD: Standard deviation, SOD : Superoxide dismutase, CuZn-SOD: Copper Zinc-Superoxide Dismutase.

Introduction

Reactive oxygen species: While oxygen is an essential molecule for aerobic metabolism, it also has adverse properties that induce cell damage and cell toxicity. Through redox reactions, oxygen and nitrogen atoms are candidates for the production of free radical species during electron transfer in living systems. ROS play an essential physiological role in maintaining cardiac and vascular integrity and a pathophysiological role in cardiovascular dysfunction associated with conditions such as hypertension, diabetes, atherosclerosis, and ischemic heart disease [1]. Clinical studies have demonstrated that essential hypertensive patients produce excessive amounts of ROS and have decreased antioxidant capacity. Under normal circumstances, cells are able to defend themselves against ROS damage(Fig.(1))with:

- enzymes: such as superoxide dismutases, lactoperoxidases, catalases, glutathione peroxidases and peroxiredoxins.
- Small molecule antioxidants such as vitamin C, vitamin E, uric acid, and glutathione also play important roles as cellular antioxidants.
- In similar manner, polyphenol antioxidants assist in preventing ROS damage by scavenging free radicals [2].

Antioxidant

Antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Therefore it can protect our cells against the effects of free radicals. Free radicals are molecules produced when our body breaks down food, or by environmental exposures like tobacco smoke and radiation. Free radicals can damage cells, and may play a role in heart disease, cancer and other diseases. Antioxidants can be complex molecules such as the SOD, catalases and peroxiredoxins, or simpler ones such as uric acid and glutathione. When Denham Harman proposed in the late 1950s that aging was a result of progressive changes caused by cumulative free radical damage, it immediately raised the possibility that antioxidant molecules might slow down

the aging process and prolong lifespan[3]. There are two main types of antioxidants:

1. Exogenous antioxidants lik: vitamins A,C and E.
2. Endogenous antioxidants like: Glutathione (GSH), Alpha Lipoic Acid (ALA), SOD, Catalase and CoQ10.

Superoxide dismutase:- SOD are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide(As such, they are an important antioxidant defense in nearly all cells exposed to oxygen)[4]. Among the common natural sources of SOD are cabbage, Brussels sprouts, wheat grass, barley grass and broccoli[5].The SOD-catalyseddismutation of superoxide may be written with the following half-reactions :

- $M^{(n+1)+}$-SOD + O_2^- → M^{n+}-SOD + O_2
- M^{n+}-SOD+O_2^-+$2H^+$→ $M^{(n+1)+}$-SOD + H_2O_2.

where M = Cu (n=1) ; Mn (n=2) ; Fe (n=2) ; Ni (n=2).

In this reaction the oxidation state of the metal cation oscillates between n and n+1.

Lipids:- constitute a broad group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins, monoglycerides, diglycerides , triglycerides, phospholipids, and others. The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules [6].

Experiment and Methods

Assaying for Superoxide Dismutase Activity: Activity of SOD has been determined in to indirect methods; riboflavin/NBT method[7].

Reagent: 1-Phosphate buffer (131.19 mM) pH 7.8 containing EDTA 2Na (263 µM). 2- L-Methionine(300 mg/10mL) 3- NBT-2HCl(14.1 mg/10 mL) 4) Riboflavin solution (116.9 µM) 5- Potassium cyanide solution (0.292 mM). Working mixture solution:-

Component	*Volume (mL)*
Phosphate buffer (pH 7.8)	11.875
L-Methionine	1.5
NBT-2HCl	1

Procedure:1- A liquots of 0.5 mL of working mixture solution were delivered into small glass tubes, and then 0.4 mL of deionized water was added, followed by 0.07 mL of potassium cyanide solution. 2-A volume of 0.05 mL serum was mixed well in a test tube. A blank tube, in which serum was replaced by 0.05 mL of buffer solution, was run in parallel. 3-To each tube (10 µl) of riboflavin solution was added, mixed immediately. Then the absorbance of all tubes were measured at ג = 560 nm. 4- All tubes were illuminated at 25ºC in an aluminum foil lined box containing two 20-W fluorescent lumps for 7 min. 5-At the end of illumination time, the tubes were removed and the absorbance was measured immediately atג = 560 nm.

Calculation:- Maximum inhibition was calculated from the inhibition curve of each group, and SOD activity was calculated as the following:-

SOD activity (inhibition %)={[(AB_2-AB_1)–(AS_2–AS_1)]/(AB_2–AB_1)}x100

Where: AS_1= absorbance of sample tube before illumination, AS_2= absorbance of sample tube after illumination, AB_1=absorbance of blank tube before illumination, AB_2=absorbance of blank tube after illumination.

$$\text{SOD activity} = \frac{\text{Sample inhibition \%}}{\text{Max. inhibition \%}} \times \frac{2 \times 1000}{V_s}$$

Inhibition by Cyanide:

Different concentrations of Cyanide (5,10, 20, 30, 50, mM) was added in the reaction mixture in the presence of 50 µL of serum. The reaction mixture was incubated for 50 min. at 37ºC as recommended by Douglas R., Larry and Spitz W. The riboflavin was added to each tube to initiate the reaction in the presence of lightas shown in the assaying of SOD activity then calculate the SOD activity.

Inhibition by H_2O_2:

Different concentrations of H_2O_2 (10, 20, 30, 50, 100 mM) was added in the reaction mixture in the presence of 50 µL of serum and complete the procedure as shown in the assaying of SOD.

Inhibition by Diethyldithiocarbimate:

Different concentrations of DDC (10, 20, 30,40,50 mM) was added in the reaction mixture in the presence of 50 µL of serum and complete the procedure as shown in assaying SOD activity .

Determination of Lipid profile:

1. Cholesterol, triglyceride and HDL determination according to Cholesterol Kit [8], Triglyceride Kit [8] and HDL Kit[9] from BioSystems (Spain)**.**
2. LDL determination indirectly by using the Friedwald Equation:

$LDL–C_{(in\ mg/dL)} = TC – HDL–C – [TG / 5]$

Results and Discussion

It is of particular interest to explore the possible associations among antioxidant enzyme (SOD), the lipid profile and the risk of Cardiovascular disease in HD patients. In this study the results obtained for controls and patients are shown in Table (1) : serum lipid profile and SOD levels indicated highly significant increases of cholesterol and LDL-c levels ($p<0.001$), and moderated significant increases of triglycerides and decreases of SOD levels ($p<0.05$) whereas no significant decrease in HDL-c levels when compared with the control group.

Table (1)
Serum lipid profile and SOD levels in control and patient.

Parameters	*Groups*	*Mean± SD*	*P-Value*
Cholesterol (mmole/L)	Control●	3.75 ± 1.17	0.001
	Patients♦	5.73 ± 0.53	
Triglycerides (mmole/L)	Control	1.76 ± 0.73	0.046
	Patients	2.26 ± 0.88	
HDL-c (mmole/L)	Control	1.14 ± 0.41	N.S
	Patients	1.00 ± 0.35	
LDL-c (mmole/L)	Control	3.54 ± 1.08	0.001
	Patients	5.76 ± 0.74	
SOD (U/mL)	Control	22.08 ± 3.06	0.03
	Patients	20.12 ± 6.19	

● ***Control=25,♦ Patients=105.***

Decreases of SOD may be due to excessive production and / or inadequate removal of ROS, especially superoxide anion which have been implicated in the pathogenesis of many cardiovascular diseases, including hypercholesterolemia, atherosclerosis, hypertension and may be due to progressive enzyme inactivation by its product H_2O_2. Free radical-scavenging enzymes such as SOD and CAT are the first line of cellular defense against oxidative injury, decomposing O_2• and H_2O_2 before interacting to form the more reactive hydroxyl radical (SOH). These enzymes protect the red cells against O_2• and H_2O_2-mediated lipid peroxidation[10].

The inhibition of SOD enzyme by **cyanide ion** was measured. Fig.(2). Cyanide ion is known as an inhibitor of SOD activity SOD was very sensitive to cyanide ion which was reported to inhibit CuZn-SOD completely by binding to the inner co-ordination sphere of the copper. It was observed that when the cyanide was present in the reaction mixture at concentration of (5,10, 20,30,50 mM), the color of the reaction mixture converted from colorless to a purple after incubation of the cyanide with the reaction mixture, for at least 30 minutes before the addition of the riboflavin and the presence of the lights which suppose to initiate the colored reaction. This change in the color that occurred, even though the exogenous initiator is absent, may be due to the reaction of the rest of the reaction mixture components with the serum during the preincubation period [12].

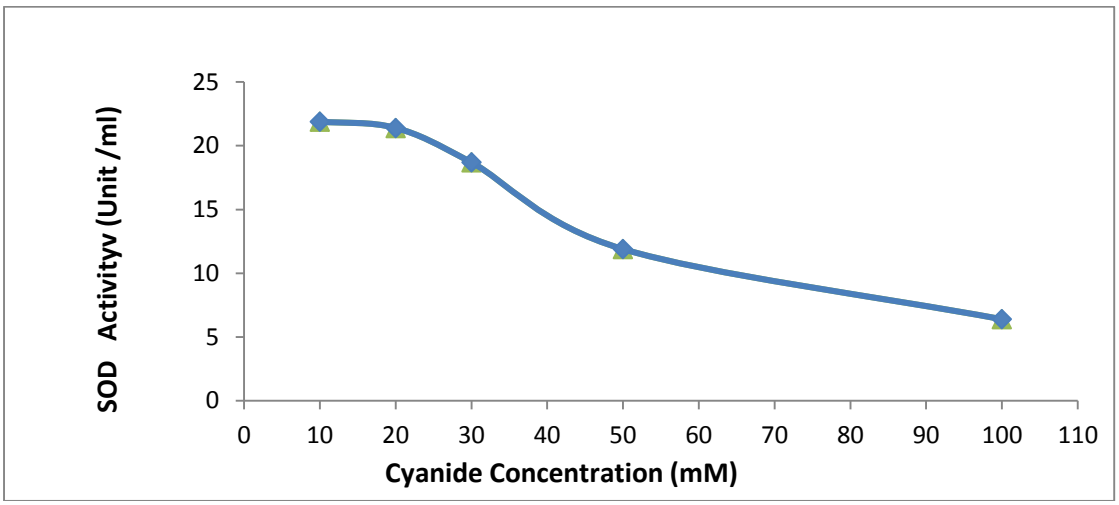

Fig.(2) Effect of cyanide ion on SOD activity.

Inhibition of SOD enzyme by **H_2O_2** was measured caused inhibition to CuZn-SOD and this has been attributed to the "peroxidase activity" according to this equations[13]:

$$SOD\text{-}Cu^{2+} + H_2O_2 \leftrightarrow SOD\text{-}Cu^{1+} + O_2^{\bullet -} + 2H^+$$

$$SOD\text{-}Cu^{1+} + H_2O_2 \rightarrow SOD\text{-}Cu^{2+}\text{-}{}^{\bullet}OH + OH^-$$

His-61 Oxo-His61

$$SOD\text{-}Cu^{2+}\text{-}{}^{\bullet}OH \rightarrow Cu^{2+} + \text{inactive SOD}$$

This peroxidase activity results in the generation of a highly potent, "site-specific" hydroxyl radical-like species that inhibition the enzyme by oxidative attack on a histidine residue (His-61) at the active site [13]. It achieve 50% of inhibition at 40 mM and that mean thecyanide is more sensitive to inhibit the CuZn-SOD in our study. Fig.(3).

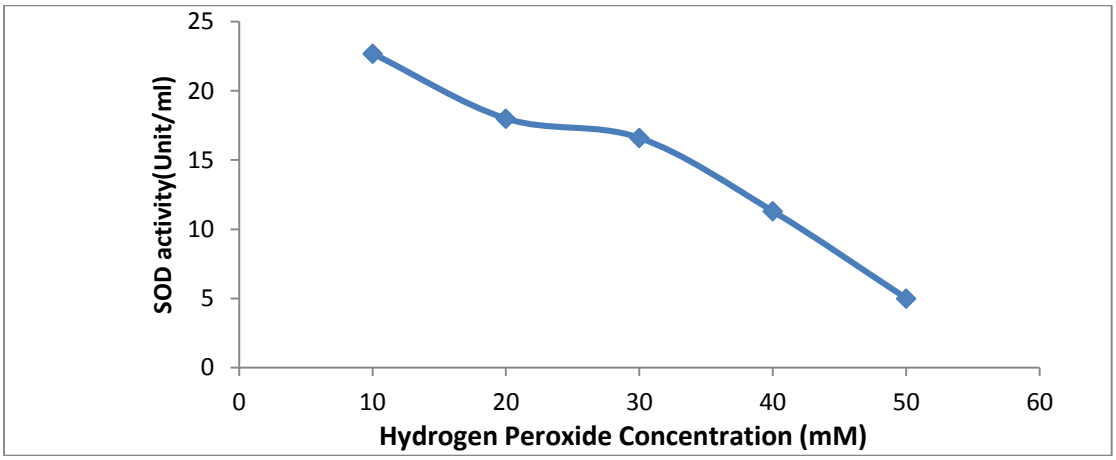

Fig.(3) Effect of H_2O_2 on SOD activity.

Inhibition of SOD enzyme by DDC was measured causing inhibition to the Cu-Zn SOD both in *vitro* and in *vivo*. It acts by liganding and removing the copper ions from the enzyme thus inhibiting the Cu-Zn SOD activity by more than 50% in the 40mM [14] as it had seen in Fig.(4).

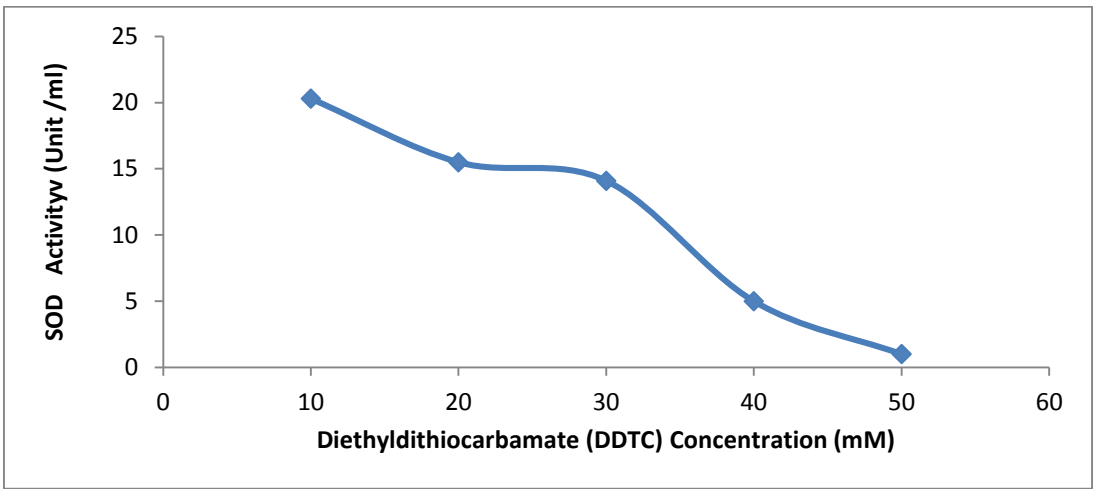

Fig.(4) Effect of DDC on SOD activity.

Measurement of lipid profile: The major plasma lipids of interest are total cholesterol and the triglycerides. When one or more of these major classes of plasma lipids is elevated, a condition referred to as hyperlipidemia exists. The major exceptions are individuals with excessive amounts of LDL whose plasma cholesterol is kept within normal limits by a concomitant decrease in HDL. Hyperlipidemia may be associated with the development of atherosclerosis and its complications, including angina and myocardial infarctions. Many theories have been discussed the change in lipid profiles after Angina and MI. One study has shown that acute myocardial infarction causes an observed decrease of plasma cholesterol levels early after it, therefore, there will be a parallel increase of LDL receptor activity and thus cholesterol catabolism increased[15]. Another aspect shows that cytokines act on the systemic targets, including the liver, to generate changes in the concentration of various heterogeneous plasma proteins including lipoproteins. At this early stage of MI, there is a significant decrease in the serum concentrations of apoprotein A-I and apoprotein B (lipoprotein B-100 which binds to LDL receptors and acts as an atherogenic protein and apoprotein A which resembles plasminogen and competes with the latter for binding to fibrinogen), reflecting the maximum decrease in the serum cholesterol level by this time[16].

Conclusion:- We reach to the relationship between Angina and MI & lipid profiles We have a more decrease in SOD activity in patient when comparing the results with control. In this study we find that Cyanide ion is more potent than H_2O_2 and DDC.

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الخلاصة

بالرغم منانخفاضالإصابة بأمراض القلب التاجية، لكنها لا تزال تشكلسببا رئيسيا للوفاة المبكرة. هذه الدراسة ربطت العلاقة بين(أنزيم مضاد للأكسدة)، الدهن الشخصي الذبحة الصدرية، واحتشاء عضلة القلب. ولتحقيق ذلك، جمعت١٠٥ من مرضى الذبحة الصدرية و احتشاء عضلة القلب الذين تتراوح أعمارهم بين (٣٨-٨٢) وبعد ذلك تم قياس نشاط أنزيم سوبرأوكساید ديسميوتاز في أمصال المرضى الذين يعانون من الذبحة الصدريه واحتشاء العضله القلبيه وأشارت النتائج الى انخفاض نشاط أنزيم سوبرأوكساید ديسميوتاز ($p<0.05$) في المرضى بالمقارنة مع الاصحاء. وبعد ذلك تم قياس تثبيط هذا الانزيم بمعاملته مع تراكيز مختلفه من بيروكسيد الهيدروجين، أيون السينايد وثنائي اثيل ثنائي كبريتات الكاربامیت، وتم الحصول على انخفاض اكثر من ٥٠ ٪ من نشاط أنزيم سوبرأوكساید ديسميوتاز عند اضافة ٣٠ ملي مولر من ايون السيانيد و انخفاض اكثر من ٥٠ ٪ من نشاط أنزيم سوبرأوكساید ديسميوتاز عند اضافة ٤٠ ملي مولر من بيروكسيد الهيدروجين وثنائي اثيل ثنائي كبريتات الكاربامیت وذلك يثبت ان أنزيم سوبر أوكساید ديسميوتاز أكثر حساسية لأيون السيانيد وكذلك تم قياس الدهون في مصل المرضى الذين يعانون من الذبحة الصدرية و احتشاء العضله القلبيه. واشيرت الدراسة الى زياده كبيرة جدا في الكوليسترول ومستويات الكولسترول المنخفض الكثافة ($p<0.05$)، وزيادات متوسطه في الدهون الثلاثية ($p<0.05$) في حين لا يوجد انخفاض ملحوظ في مستويات الكولسترول العالي الكثافة مقارنه مع الاصحاء.

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