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Estimation of Xanthine oxidase activity, related with Nitric oxide (NO) and lipid profile in serum of patients with atherosclerotic diseases

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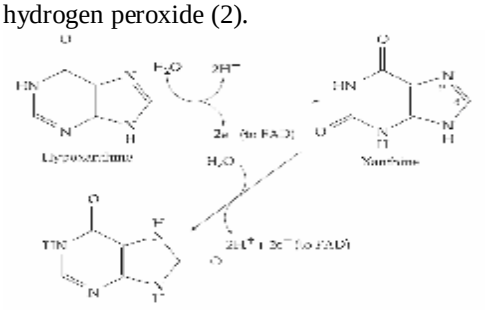
Abstract: This research outlines the study of xanthine oxidase activity (XO) , nitric oxide (NO)) and lipid profile with an atherosclerotic disease(angina and cerebral thrombus) . The study covers 46 atherosclerotic disease patients (26 males , 20 females) and 29 healthy subjects (control group) .The two groups contain males and females whose ages ranged between (38-65) years .The parameters ; Xanthine oxidase activity (XO) , Nitric oxide (NO) and lipid profile were investigated .
The results of these subjects studied (patient and control group) have significant increase in the activity of Xantine oxidase and concentration of Nitric oxide, triglyceride, cholesterol, low density lipoprotein and very low density lipoprotein in the serum of atherosclerotic diseases (angina and cerebral thrombus) compared to control groups. The increase amounts are to 360%, 240%, 2.3%, 23.5%, 54.8% and 2.3% respectively as compared to control groups.
The result indicates a significant decrease in the concentration of high density lipoprotein (HDL). The decrease amounts to 27% as compared to control groups. On the other hand, the results of these subjects studied show no significant different parameters in sex .

Keywords: , Xanthine oxidase , , Nitric oxide (NO) , lipid profile ,serum , atherosclerotic diseases

Introduction:

Xanthine oxidase contains two Fe2S2 clusters and bound FAD. The enzymes can also oxidize xanthine further by a repetition of the same type of oxidation process at positions 8 and 9 to form uric acid (1). In the dehydrogenase NAD+ is the electron acceptor that oxidizes the bound FADH2. Xanthine dehydrogenase, in the absence of thiol compounds, is converted spontaneously into xanthine oxidase, probably as a result of a conformational change and formation of a disulfide bridge within the protein. Evidently in the oxidase form the NAD+ binding site has moved away from the FAD, permitting oxidation of FADH2 by O2 with formation of hydrogen peroxide (2).



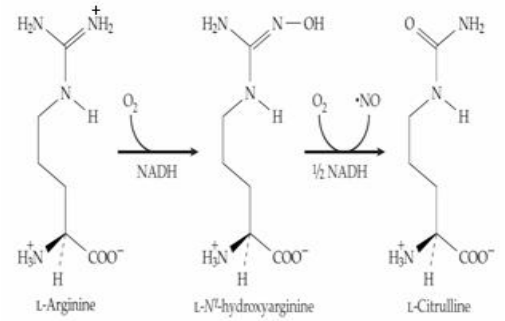
Nitric oxide (NO) is a reactive free radical whose formula is often written as ·NO to recognize its characteristic. However, .NO is not only a toxic and sometimes dangerous metabolite but also an important hormone which functions in the circulatory system, the immune system, and the brain (3).Nitric oxide reacts rapidly with O2 to form nitrite .

$$4NO + O_2 + 2\,H_2O \longrightarrow 4NO_2^- + 4\,H^+$$

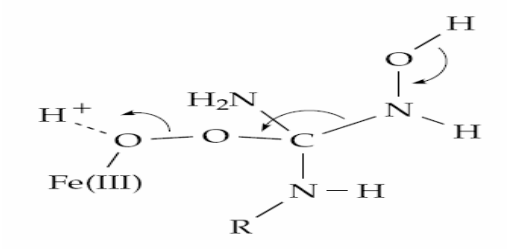
It also combines very rapidly with superoxide anion radical to form peroxynitrite. NO synthase is a very complex enzyme, employing five redox cofactors: NADPH, FAD, FMN, heme, and tetrahydrobiopterin. NO can also be formed from nitrite, derived from vasodilators such as glyceryl trinitrate during their metabolism. NO has a very short half-life (approximately 3–4 seconds) in tissues because it reacts with oxygen and superoxide. The product of the reaction with superoxide is peroxynitrite (ONOO-), which decomposes to form the highly reactive .OH radical. NO is inhibited by hemoglobin and other heme proteins, which bind it tightly. Chemical inhibitors of NO synthase are now

available that can markedly decrease formation of NO. Administration of such inhibitors to animals and humans leads to vasoconstriction and a marked elevation of blood pressure, indicating that NO is of major importance in the maintenance of blood pressure in vivo. Another important cardiovascular effect is that by increasing synthesis of cGMP, it acts as an inhibitor of platelet aggregation (4).

NO binds to the iron atoms in accessible heame groups such as those of hemoglobin (5) and of guanylate cylases (6) and in some Fe – S proteins such as aconitase. Some blood-sucking insects utilize salivary heame proteins called nitrophorins to carry NO into host tissues where it activates guanylate cyclase causing vasodilation. Nitrophorins also bind histamine and inhibit blood coagulation (7).Nitric oxide reacts with thiol groups of proteins and small molecules to give S-nitrosothiols. NO synthases are oxygenases, that carries out a two-step oxidation of L-arginine to L-citrulline with production of NO. In the first step, a normal monooxygenase reaction, L-Nγ-hydroxyarginine. In the second step NO is formed in a three-electron oxidation. The human body contains three types of nitric oxide synthase known as neuronal (nNOS or NOS1), inducible (iNOS or NOS2), and endothelial (eNOS or NOS3) (8) .



These enzymes have a broad distribution within various tissues, but NOS1 is especially active in neurons and NOS3 in endothelial cells. The inducible NOS2 originally discovered in macrophages is transcriptionally regulated. When these phagocytic cells are at rest the activity of NOS2 is very low, but it becomes highly active after Citrulline, and O-N–H, nitroxyl. This is one electron (e– + H+) more reduced than ·NO. Perhaps the adduct forms from Fe (III) – O – O·. On the other hand, there is evidence that NO synthases may produce nitroxyl or nitroxyl ion NO– as the initial product. NO and other products such as N2O and NO2– may arise rapidly in subsequent reactions. Nitrite is a major oxidation product of NO in tissues (2).



Materials and methods:

Table (1) Chemicals and their suppliers.

Subjects and methods:

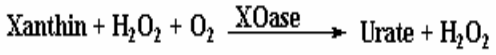
This study was conducted during the period from July 2008 until the end of November 2008. Ages of the subject ranged from (38 – 65) years. This study include two groups:

Group one: atherosclerotic vascular patients (angina, cerebral thrombus) plaques on artery wall causing reduced or cut off blood flow.

Group two: control (non- atherosclerotic disease). Twenty nine healthy subjects were included in this study as control group.

Estimation of xnathine oxidase (XO) activity in blood serum:

Xnathine oxidase (XO) activity was determined by the method of (Ackermann). This method depends on the enzymatic oxidation of xnthine which is followed spectrophotometrically by measuring uric acid formation (293 nm)(10).



Reagents:

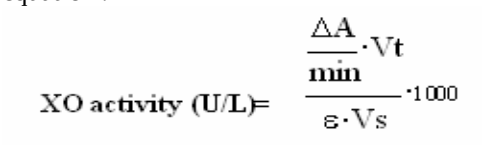
1-Sodium phosphate buffer (0.1M,pH 8.2)

2-Substrate solution (xnathine solution 1* 10-4 M) (15.2 mg) of xnathine was dissolved in 10 ml of NaOH solution (0.2 mg) by shaking and then (0.5ml) of this solution was diluted to (50ml) with phosphate buffer.

Procedure:

(0.1ml) of serum was added to (3ml) of substrate solution, mixed well, then the increase in absorbance with time was recorded against water blank at 293nm.

The activity of XO was then estimated from equation .



induction by cytokines or by the ipopolysaccharides of bacterial cell membranes(9).

Where:

ΔA= change of absorbance

Vt = total volume

Vs = sample volume

ε = molar extinction coefficient

Estimation of peroxynitrite (NO3-) in blood serum:

Serum peroxynitrite level was measured by modified method of (vanuffelen). The principle of this determination is the radical peroxynitrite (ONOO-) mediate nitration of phenol resulting in formation of nitrophenol which is detected spectrophotometrically at (412 nm) (11).

Reagents:

1. 1-Phosphate buffer (50 Mm, pH 7.4) was prepared by diluting (62.5) ml of (0.2M) phosphate buffer to (100) ml with distilled water.
2. 2-pheno(l 5M) was prepared by dilation in distilled water.
3. 3-NaOH solution (0.1 M) was prepared via dissolving (0.04 gm) of NaOH in (10 ml) distilled water.

Procedure:

A mixture of (150 µL) serum and (150 µL) of (5ml) phenol was combined with (50 Mm) sodium phosphate buffer (PH 7.4) to give a final volume of (2ml) . The resulting solution was incubated for 2 hours at 37 C°, then (15 µL) of (0.1 M) NaOH is added. The absorbance was measured immediately at 412 nm.

The concentration of peroxynitrite was calculated after measuring the amount of nitrophenol produced which reflects the concentration of peroxynitrite according to the equation .

$$\text{Nitrophenol } (\mu \text{ mol/L}) = \frac{At}{\varepsilon \cdot L} \cdot 10^6$$

At= test absorbance

ε= extinction coefficient M-1.cm-1

L= light path (1cm)

Estimation of lipid profile in blood serum:

Enzymatic reagent for cholesterol, TG and HDL measurements were obtained from bioerieux SA au capital France. HDL were isolated from cholesterol measurement by phosphotungestic acid magnesium procedure.

LDL was calculated by the friedwald formula (12).

LDL = Tch – (HDL + TG / 5)

Results:

Determination of xanthin oxidase activity was estimated for (46) patients in table (2) showed the distribution of patients according to the atheroseclerotic disease, in addition to (29) normal used as control group.XO activity in seurm of atherosclerotic disease increase was about 360% (43.75 ±7.2 U/L ,9.5 ± 3.4 U/L) and the results of these parameters studied have significant increase in the concentration of Nitroxide,triacylglycerol,cholesterol, low density lipoprotein and vary low density lipoprotein in the serum of atherosclerotic disease patients as compared to control groups. The increase amounts are 240% (0.33± 0.93 µ mol / L , 0.097 ± 0.058 µ mol / L) , 2.3% (193.3 ± 19.14 mg/Dl , 188.9 ± 15.75 mg / Dl), 23.5%(218 ± 21.3 mg/Dl,176.5 ± 17.29 mg/Dl) 54.8% (143.13 ± 29.38 mg/Dl,92.5 ± 15.74 mg/Dl) and 2.3%(38.66 ± 7.2 mg/Dl,37.78 ± 4.6 mg/Dl)respectively as compared with control groups. The result indicated a significant decrease in the concentration of high density lipoprotein (HDL).The decrease amounts to 27% (33.6 ± 7.82 mg/Dl , 46.17 ± 10.43 mg/Dl) as compared to control groups.On the other hand, the results of these subjects studied show no significant different parameters in sex .

Discussion:

The results of this study showed that XO significantly has increased compared to the control group. The increase amounts was 360%.It is shown in table (2) & figure (1) and with Wooldridge and others who found that XO increased all lipoproteins in blood . XO an enzyme that is known to distribute widely in mammalian tissues (12). Among the tissue components, endothelial cells in micro vascular systems constitute the most abundant source of the enzyme as compared with those in larger vessels (13). Because the enzyme reaction that transfers electrons from hypoxanthine to uric acid is coupled with a reduction of molecular oxygen into superoxide anions, XO has been considered to play a crucial role in the pathogenesis of oxidant-induced micro vascular changes and tissue injury (12) .Also showed the results of this study that NO significantly increases compared to the control group. The increase amounts to 242%.It is shown in table (2) & figure (2).NO is an important protective agent produced by endothelium and may protect it against atherosclerosis accordingly, it may be suggested that oxidant stress and NO may have multiple effects on the initiation and progression of atherogenesis. Perroxy nitration (ONOO-) has a high affinity for sulfhydryl groups and thus inactivates several key sulfhydryl bearing enzymes. This effect of (ONOO-) is regulated by cellular content of

glutathione, since GSH is the major intracellular soluble sulfhydryl-containing compound. Factors that regulate the biosynthesis and decomposition of GSH may have important consequences. NO also has a significant effect on vascular smooth muscle tone and blood pressure. It is released by acetyl choline and other endothelium-dependent vasodilators .The vasodilator action of NO in vascular smooth muscle is mediated by its activation of soluble guanyly cyclase increase cGMP synthesis by guanyly cyclase results in smooth muscle relaxation .This observation confirms the high concentration of uric acid and XO activity in the present study. In addition, NO may act as antioxidant, blocking the oxidation of LDL and thus preventing the formation of foam cell in the vascular wall(14).

The results of this study showed that all lipid profile significantly increases Compared to the control group except HDL. It is shown in table (2) & figure (3). Lipid profile includes total cholesterol (T-ch), HDL cholesterol, LDL cholesterol, and triglycerides (TG). It is a group of tests that are often ordered together to determine risk of coronary heart disease (CHD) (15). There was a significant increase in serum levels of T-ch, TG, VLDL, and LDL, while HDL decreased significantly in the atherosclerotic disease as compared to the control group subjects. Overt proteinuria often accompanied by hypercholesterolemia and associated with increased serum lipoproteins. These lipid abnormalities are probably involved in the high incidence of macro vascular (16).

Recent studies have been postulated that insulin resistance stimulate glucose uptake and hyperinsulinemia, increases VLDL, TG concentration, decreased HDL concentration and small dense LDL particles and associated with metabolic abnormalities (17). TG is related to the presence of small dense LDL particles, now considered to be genetically influenced risk factor for cornary heart disease (CHD).Hyper triglyceride VLDL may affect endothelial cell and turn macrophages into foam cell. Hypertriglyceridemia with delayed clearance of chylomicrons remnants may also increase atherosclerosis (18).

There are several factors that may affect the metabolism of triglyceride- rich lipoproteins. These factors may decrease triglyceride catabolism and removal include decreased activity of lipolytic enzymes (lipoprotein lipase, hepatic triglyceride lipase, lecithin- cholesterol acyltransferase (LCAT).The potential contribution of elevated levels of TG to this increased risk for atherogenesis is unclear. Certainly, triglycerides do not contribute to a major extent to the composition of atherosclerotic plaques. However, high levels of TG are known to raise the concentration of other atherogenic lipoproteins that apparently do promote plaque formation. These include chylomicrons remnants (19). Results of this study demonstrate also that atherosclerotic disease patients have lower HDL levels than those found in the control group.

The epidemiological studies performed over a period of several decades indicated that HDL correlates negatively with the risk of development of CHD. The protective effect of HDL in the pathogenesis of atherosclerosis is due to its role in removal of cholesterol from peripheral tissues. Although cholesterol can be synthesized a number of tissues in the body, in the process of its normal metabolic turn over, it must be transported from peripheral tissues to the liver (20).It has been suggested that in such cases, transfer of cholesterol esters from HDL to VLDL, and triglycerides from VLDL to HDL, takes place, resulting in the appearance of smaller and denser HDL particles containing decreased amounts of cholesterol (21).

The plasma lipid and lipoprotein concentrations were comparable between the control group where as the plasma TG, VLDL, T-ch concentration were significantly increased in atherosclerotic .The serum total cholesterol levels in atherosclerotic group is greater than that in control group. One of the diseases associated with increased concentrations of cholesterol is arteriosclerosis, or hardening of the arteries, among the most common of the disease of aging. With increasing age, human normally develop decreased capacity to metabolize fat and therefore cholesterol concentration in membranes increase (22).

The role of LDL and scavenger receptors is disturbed lipid metabolism of microalbuminuric is multi factorial. In the circulation of these patients, the unchanged LDL level is associated with low HDL level, making the LDL / HDL ratio high (23).In the present study, size of LDL index (expressed as TG to HDL ratio) has been evaluated to determine the risk factor for cardiovascular disease.

These relationships are difficult to interpret in terms of cause. One hypothesis is that the presence of TG- rich lipoproteins in the circulation leads to increase exchange of their TG for cholesterol esters in HDL as well as LDL by cholesterol ester transfer protein. These neutral lipid exchanges decrease the HDL concentration. In addition, TG enriched

lipoprotein may be removed by hepatic lipase, leading to small dense LDL particles (24). Table (3) a significant positive showd and negative correlation between all parameters in atherosclerotic patients and Table (4) a significant positive showd and negative correlation t-test between all parameters in atherosclerotic patients with control group.

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Table(1): The chemical s and their suppliers.

Chemicals	Suppliers
sodium phosphate	Gainlad chemical G.C.C.compa
substrate xnathine	Randox laboratory,Ltd.Englan
NaOH	Fluka, Switzerland
phenol	Fluka , Switzerland.
HDL Kit	bioerieux SA au capital France
TG Kit	bioerieux SA au capital France
T-ch Kit	bioerieux SA au capital France

Table(2): Concentration of all parameters study in patients and control group.

The	patients	control
XO (U / L)	43.75 ± 7.2	9.5 ± 3.4
NO (µ mol / L)	0.33 ± 0.93	0.097 ± 0.058
TG (mg/ Dl)	193.3 ± 19.14	188.9 ± 15.75
T-ch (mg/ Dl)	218 ± 21.3	176.5 ± 17.29
LDL (mg/ Dl)	143.13 ± 29.38	92.5 ± 15.74
VLDL (mg / Dl)	38.66 ± 7.2	37.78 ± 4.6
HDL (mg/ Dl)	33.6 ± 7.82	46.17 ± 10.43

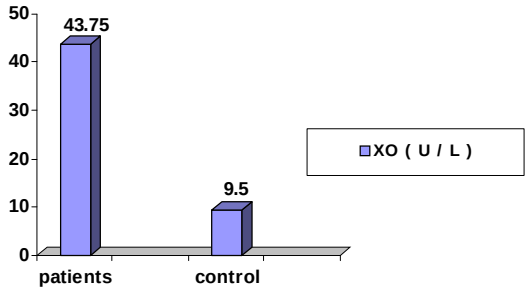

Figure (1) : Comparison of XO between two patient groups.

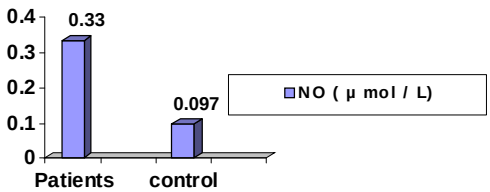

Figure (2) : Comparison of (NO) between two patient groups.

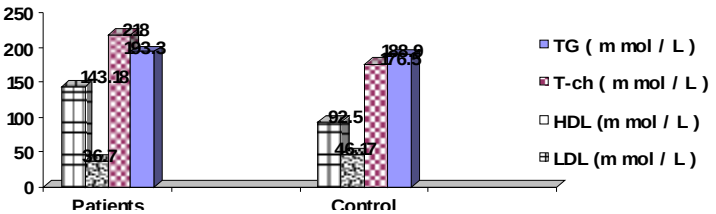

Figure (3) : Comparison of lipid profile between two patient groups.

Table (3)Correlations between all parameters patient .

umber	XO	NO	TG	T-ch	HDL	LDL	VLDL
XO	1	0.913618	0.70279	-0.2587	0.319094	0.758057	0.701302
NO	0.913618	1	0.765303	-0.36113	0.238644	0.844577	0.763639
TG	0.70279	0.765303	1	-0.26184	0.074026	0.731022	0.999977
T-ch	-0.2587	-0.36113	-0.26184	1	-0.05297	-0.21433	-0.26285
HDL	0.319094	0.238644	0.074026	-0.05297	1	0.14598	0.07413
LDL	0.758057	0.844577	0.731022	-0.21433	0.14598	1	0.729174
VLDL	0.701302	0.763639	0.999977	-0.26285	0.07413	0.729174	1

Marked correlations are significant at p < 0.05000, N=46

Table (4)Correlations t-test between patients with control parameters.

parameter	XO	NO	T-ch	TG	HDL	LDL	VLDL
XO	4.97969**	1.05934	1.8301	1.1386	6.89514**	8.20058**	1.0597
NO	6.33525**	1.909	8.1042**	1.5315	2.5324*	2.79835*	1.18496
T-ch	8.94806**	1.02098	0.000491	5.78067**	1.95396	9.2349**	1.01367
TG	4.29318**	6.19409**	0.148374	0.000304	2.20662*	6.66042**	2.9706*
HDL	7.60937**	5.98255**	3.26253*	2.44252*	5.5234**	9.01366**	0.271828
LDL	8.35802**	2.49503*	3.98796**	0.000424	4.41905**	1.57241	1.27988
VLDL	6.829**	7.20164**	9.1016**	1.59524	1.54854	1.83348	0.14945

*** significant difference (p<0.05)**
**** highly significant difference (p<0.001)**

تقدير فعالية أنزيم الزانثين اوكسديز وعلاقته مع اوكسيد النايتروز والمحتوى الدهني في مصل الدم لمرضى تصلب الشرايين

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الخلاصة:

تضمن البحث دراسة فعالية أنزيم الزانثين اوكسديز (XO) وأوكسيد النتريك (NO) وأنواع من دهون الدم للمرضى المصابين بأمراض تصلب الشرايين (بالذبحة الصدرية والجلطة الدماغية). غَطّتْ الدراسة ست وأربعين عينة من المرضى المصابين بأمراض تصلب الشرايين (بالذبحة الصدرية و الجلطة الدماغية) وكانو 26من ذكور و20 من الإناث و 29 شخصاً من الأصحاء (مجموعة قياسية). تراوحت أعمارهم بين(38-65) سنة من كلا الجنسين . أشارت النتائج بأنَّ هناك زيادة معنوية في فعالية أنزيم الزانثين اوكسديز (XO) ، وكذلك في تركيز اوكسيد النتريك (NO) والكليسيريدات الثلاثية والكوليستيرول والبروتين ألدهني واطئ الكثافة والبروتين الدهني واطئ الكثافه جدا في مصل الدم للمرضى المصابين بأمراض تصلب الشرايين (الذبحة الصدرية والجلطة الدماغية) وبمقدار: 360% و 240% و2,3 % و 23,5% و 54,8% و2,3% على التوالي بالمقارنة مع المجموعة القياسية . بينما أظهرت النتائج انخفاضاً معنوياً بتركيز البروتين العالي الكثافة، وكانت نسبة النقصان 27% ، مقارنة مع ا لمجموعة القياسية.من جانب اخر بينت الدراسة عدم وجود أي علاقة معنوية للمتغيرات ألمقاسه لمجموعة المرضى المصابين بأمراض تصلب الشرايين (الجلطة الدماغية والذبحة الصدرية) نسبة لاختلاف الجنس.