Xanthine Oxidase Activity as an Index of lipid Peroxidation in Sera of Patient with Mayocardial Infraction in Erbil City Population

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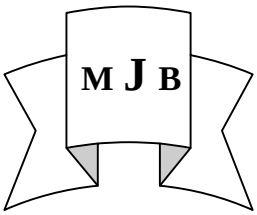

Abstract

Acute mayocardial infarction (MI) remains a leading cause of morbidity and mortality world wide. MI occurs when myocardial ischaemia exceeds a critical threshold and overwhelms mayocardial cellular repair mechanisms designed to normal operating functions and homostatus.

Activity of xanthine oxidase (XO), MDA and vit-C concentrations were determined in sera of 100 patients with MI. Their mean±SD age was (57.05±1.25) year. The same parameters were determined in sera of 100 apparently healthy matched subjects as a control group. The mean ±SE of XO, MDA, and vit-C in patients with MI was 0.031±0.016 unit/ mg protein, 1.28±0.053 µ mol/L, 0.520±0.137 mg/dl respectively while the mean ± SE of control group was 0.012±0.006 unit/ mg protein, 0.819±0.019 µ mol, and 0.648±0.302 mg/dl respectively. There were significant differences in the mean value of XO and MDA in patient with MI compared to control group (P<0.001). However the mean value of vit-C showed no significant differences in both groups.

فعالية الزانثين اوكسديز كدالة متابعة للاكسدة الشحمية الفوقية في مصول المرضى المصابين باحتشاء العضلة القلبية بين سكان مدينة أربيل

الخلاصة

يعتبر مرض احتشاء العضلة القلبية من الامراض القاتلة والواسعة الانتشار في العالم. يحدث هذا المرض متقدما لحالة ضيق تدفق الدم في شرايين القلب ومسببا عجزا في العمليات الطبيعية للانسجة الخلوية القلبية مع اضطراب في توازن عملها.

قدرت فعالية الانزيم وتراكيز كل من المالون دي الديهايد و فيتامين - س في امصال 100 شخص يعانون من مرض احتشاء العضلة القلبية وكان معدل اعمارهم 57.05±1.25 سنة. استخدمت امصال 100 متطوع يمثلون عينة السيطرة حيث قدرت فعالية كل من السابقة لهم.

كان معدل فعالية الانزيم والمالون دي الديهايد وفيتامين – س في امصال المرضى على التوالي :

0.031±0.016 unit/ mg protein, 1.28±0.053 µ mol/L, 0.520±0.137 mg/dl

بينما كان معدلها في المصول الطبيعية على التوالي

0.012±0.006 unit/ mg protein , 0.819±0.019 µ mol, 0.648±0.302 mg/dl

اوجدت الدراسة ان هناك علاقة معنوية ما بين فعالية الانزيم وتركيز المالون دي الديهايد في امصال المرضى والطبيعيين بينما لم توجد علاقة معنوية ما بين تركيز فيتامين – س في كلا المجموعتين.

Introduction

Oxidative stress occurs in aerobic organisms because of the generation of reactive oxygen species (ROS) during respiratory energy production. Increased oxidative stress impairs endothelial function in both human and animal models, partly by

reducing bioavailability of nitric oxide (NO) via its reaction with ROS [1].

Oxidative stress, lead to lipid degradation, forming such products as malondialdehyde (MAD)[2]. Malondialdehyde is one of low molecular weight end products of lipid peroxide decomposition . In monitoring lipid peroxidation [3], its plasma concentration is the most frequently used biomarker providing an indication of the overall lipid peroxidation level when produced caused by many diseases [4].

In addition to NAD(P)H– oxidase, as a source for oxidative stress, other specific enzymes like xanthine oxidase (XO) can induce ROS production [5]. Xanthine oxidase can lead to super oxide production during the purine degradation process, which involves metabolism of hypoxanthine and xanthine to uric acid [6]. Xanthine oxidase activity has been evaluated to contribute to endothelial dysfunction in animal and human [7, 8].

Since acute mayocardial infarction (AMI) may be related to thromboembolic process, tissue destruction, and secondary inflammatory process [9], it seemed reasonable to expect a raised XO activity in sera of patients with AMI that can cause elevation in MDA concentration of the patients.

It was also observed that the antioxidant vitamin C abolished the malondialdehyde-induced negative contractile response suggests that malondialdehyde may elicit its inhibitory effect associated with enhanced oxidative stress in the heart and has been shown to stimulate immune system been shown to blocks damaged pathways through the stimulation or maintenance of T cell proliferation in response to infection.

Therefore, in order to evaluate this possibility, serum XO activity together with MDA and vit-C concentrations was quantified in a group of AMI patients and the data produced were compared with those of healthy subjects as a control group.

Materials and Methods

a.Materials

Chemicals: Standard ascorbic acid (Vit-C), EDTA, Methanol, and all other chemicals used were of analytical grade.

Instruments: UV-Visible Spectrophotometer, water bath, centrifuge, and all related apparatus were used.

Subjects: 100 objects with AMI, their mean±SD ages was (57.05±1.25) year. They were admitted to the coronary care units at Erbil Teaching Hospital. They were diagnosed as AMI by consultant and their diagnosis based on a history of prolonged ischemic chest pain, characteristic electrocardiogram (ECG) changes and elevated CK enzyme activity and serum troponin T within 12 hr after the onset of pain. Patients are carefully screened to exclude evidence of congestive heart failure, hepatic and renal failure, endocrinological disorders. In addition, 100 apparently healthy individuals of the same mean±SD were used as controls.

Collection of sera: Eight to ten mls of peripheral venous blood were drawn from both patients and healthy controls individuals. Blood was transferred into centrifuged tubes, waiting for 30 minutes for clotting and then centrifuged for 15 mns at 3000 rpm. Sera were aspirated and used for the determination of xanthine oxidase activity, MDA and vit-c concentrations at the same time of preparation.

b. Methods:

Xanthine oxidase activity: A xanthine oxidase activity was determined using assay kit provided by Worthington Biochemical Corporation - Uk). XO catalyzes the hydroxylation

of hypoxanthine to xanthine and then further catalyzes the oxidation of xanthine to uric acid. The rate of urate formation is determined by measuring increased absorbance at 290nm. A unit of activity is conversion of one micromole of urate per minute at 25ºC.

Calculations:

$$\text{Units/min} = \frac{\Delta A/\text{min} \times 1000 \times 3\text{ml} \times \text{dilution}}{1.22 \times 10^4 \times 0.1\text{ml}}$$

Where:

$1.22 \times 10^4\ cm^{-1}$ is the molar absorbency of uric acid

ΔA is the difference in absorbance.

MDA concentration determination : Concentration of MDA was determined colorimetrically by Burtis and Ashwood [10]. The principles was based on the spectrophotometer measurement of the color intensity occurred during the reaction of thiobarbuteric acid with the formed MDA. The measured intensity was at 532nm after complexation and lefting the mixture to stand for 20 min at room temperature.

Colorimetric determination of Vit-C concentration

Principle: Vit-C in plasma is oxidized by Cu^{2+} to form dehydroascorbic acid, which reacts with acidic 2,4-dinitrophenylhydrazine to form a red bis-hydrazone, which is measured at absorbance 520 nm (11-Titez etal, 1986). 0.5ml of serum react with 2.0ml meta-phosphoric acid (freshly prepared). The reactants is mixed vigorously then centrifuged at 900 X g for 10 mn. 0.4ml of DTCA reagent is added to the filtrate and the mixture incubated in water bath at 37ºC for 3hr. The mixture then chilled for 10 mn in an ice bath. While mixture is mixing slowly an addition of 2.0ml of cold H_2SO_4 (12 mol/L) is made. A standard curve is prepared up to a concentration of 2.omg/dL of Vit-C.

Results

Serum XO activity (fig-1), was seen to be significantly elevated ($P<0.001$) in patient with mean±SE (0.031±0.016 unit/gm protein) compared with that of normal individuals (0.012±0.006 unit/g protein), (table-1). Table (2), reflects higher MDA concentration in serum of MI patient (1.280±0.053 µmol/L), compared to normal individuals (0.819±0.019 µmol) . Also. The concentration of Vit-C in serum of MI patient was seen to be lower (0.520±0.137µmol/L) than that in normal individuals (0.684±0.302 µmol/L). The obtained data were found to be significant as $P < 0.001$ (fig-2,3).

Discussion

It has been shown that one of the sources of super oxide anion during oxidative stress is XO, which can lead to super oxide production during the purine degradation process, that involve metabolism of hypoxanthine and xanthine to uric acid. Circulatting XO has been suggested to be specifically involved in the mechanism of peripheral endothelial dysfunction and it could play a crucial role in the generation of ROS in the body [12]. The authors observed that MI patients were characterized by increased activity of the XO system. Therefore, it could be established that XO can be regarded to be cardiovascular risk factor for human [13]. In healthy individuals, XO is present in appreciable amounts and it was

released into circulation during disturbances of the body metabolism. Therefore, XO activity improve the vascular function and oxidative stress in patients with MI disease. This was confirmed by the observations that an elevation in XO activity and MDA concentrations was seen during this study.

The malondialdehyde-induced cardiac depression may involve enhanced oxidative stress and activation of MAP kinase indicated by pharmacological and immunoblotting studies. In addition, malondialdehyde shortened duration of shortening and had little effect on resting intracellular Ca^{2+} levels and intracellular Ca^{2+} clearing rate. These results may suggest a convincing link between the elevated lipid peroxidation end product malondialdehyde (fig-2) and impaired ventricular contractile function under pathological conditions such as heart failure. Oxidation of membrane lipid is a process generated by ROS and is responsible for the membrane dysfunction under various disease states [14]. The ROS radicals may initiate a rapid self-propagating chain reaction by attacking the polyunsaturated fatty acids in the membrane. Polyunsaturated fatty acids display the highest sensitivity among cellular macromolecules to ROS-induced damage [15]. Lower degree of fatty acid unsaturation of cell membrane is believed to be protective against lipid oxidation-derived damage. Membrane damage due to ROS-induced lipid peroxidation has been considered the predominant mechanism for cellular membrane dysfunction and subsequently, alteration of cellular function [15, 16]. These damages including DNA but primarily phospholipid, a cellular membrane component, which are converted to MDA by lipid peroxidation. MDA can react with the free amino group of proteins, phospholipids, and nucleic acids leading to structural modification which induced dysfunction of the immune system [17]. Level increases of MDA may be explained to be due to the enhancement of serum lipid peroxidation removal by aldehyde dehydrogenase enzyme in mitochondria cell. Aldehyde dehydrogenase has a function to destroy toxic aldehyde and protects tissue from aldehyde accumulation. In addition, serum MDA can be moderated by enhancement of the degradation of excretion. These findings were demonstrated that patients with AMI were prone to accumulation of potentially harmful oxidative stress, which may resulted in an obvious elevation in MDA concentration, and as those found by Sahin etal [18], Peerapatdit etal [19], and Lykkesfeldt etal [20]. The fact that peak cardiac contraction, duration and velocity of contraction and relaxation were altered by malondialdehyde indicated that lipid peroxidation may affect different cardiac contractile or regulatory components[19]. Although the mechanism(s) of action underlying reduced myocyte contraction in response to malondialdehyde is not fully clear at this time, several speculations may be made. First, the observation that the antioxidant vitamin C abolished the malondialdehyde-induced negative contractile response suggests that malondialdehyde may elicit its inhibitory effect associated with enhanced oxidative stress in the heart. Oxidative stress and damage are known to impair cardiac contractile function [22, 23]. Vit-C has been shown to stimulate immune system by enhancing T-cell proliferation in response to infection. These cells are capable of lysing infected targets by producing large quantities of cytokines

and by helping B cells to synthesize immunoglobulins to control inflammatory reactions. Further, it has been shown that ascorbic acid blocks pathways that lead to apoptosis of T-cells and thus stimulate or maintain T cell proliferation to attack the infection. Therefore, the author expected a decrease in Vit-C concentration as an anti oxidant factor that can consumed during the proceeding of the case studied

In addition, Lipid peroxidation and oxidative modification of low density lipoproteins (LDL) are implicated in development of atherosclerosis. It has been shown that, mammalian cells therefore have evolved effective antioxidant defense systems to cope with the toxic ROS [24, 25] generated in the course of aerobic ATP generation. The health of cells in tissues is influenced by the balance of antioxidants and ROS. Although Vitamin C is a well known antioxidant whose precise role in protecting cells from oxidative challenge is uncertain [17, 20], it is known to prevent the oxidation of LDL primarily by scavenging the free radicals and other reactive oxygen species in the aqueous milieu. In addition, in vitro studies have shown that physiological concentrations of ascorbic acid strongly inhibit LDL oxidation by vascular endothelial cells.

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Table 1 activity of xanthine oxidase in sera of control and MI patients Unit/mg protein.

				Control		MI		
No.	Parameters	Age	Size No.	Mean ± S.E.	Range	Mean ± S.E.	Range	P value
1	Serum Xanthine Oxidase (units/mg protein)	36-79	100	0.012 ± 0.006	0.004 -0.029	0.031 ± 0.016	0.004 - 0.066	P<0.001

Table 2 levels of MDA and vit. C in control and MI sera

				Control		MI	
No.	Parameters	ze No.	Age	Mean ± S.E.	Range	Mean ± S.E.	Range	P value
1	Serum MDA (µmol/L)	100	36-79	0.819 ± 0.019	0.588 - 1.36	1.280 ±0.053	0.8 - 1.67	P<0.001
2	Serum Vitamin C (mg/dl)	100	36-79	0.684 ± 0.302	0.058 - 1.31	0.520 ±0.137	0.18 - 0.86	P<0.001

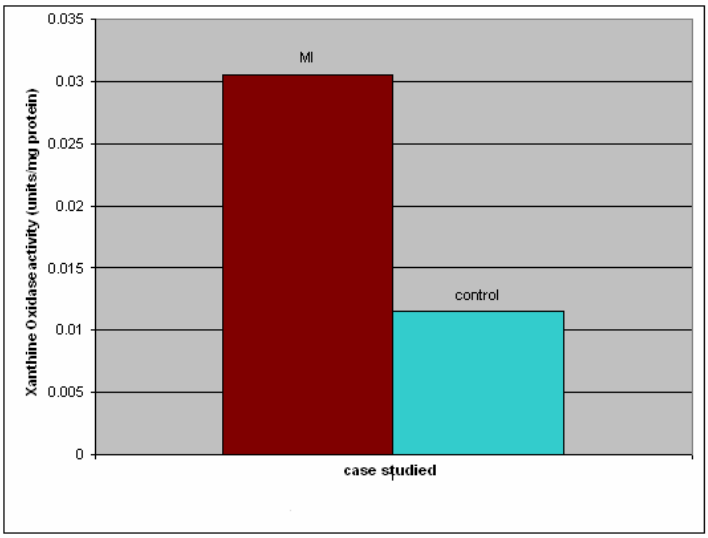

Figure 1 Serum xanthine oxidase activity in control and MI individuals.

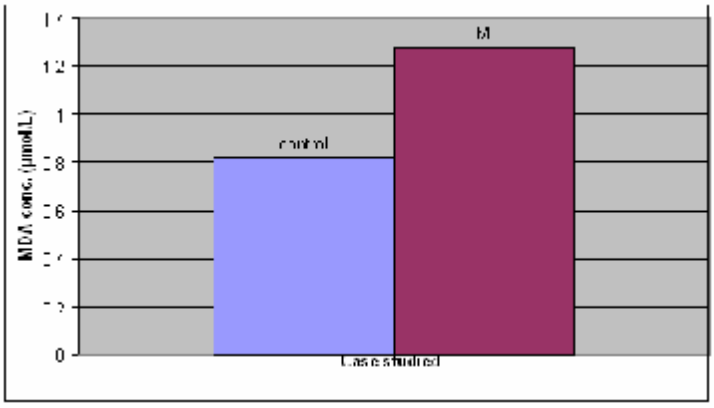

Figure 2 MDA concentrations in sera of control and MI individuals.

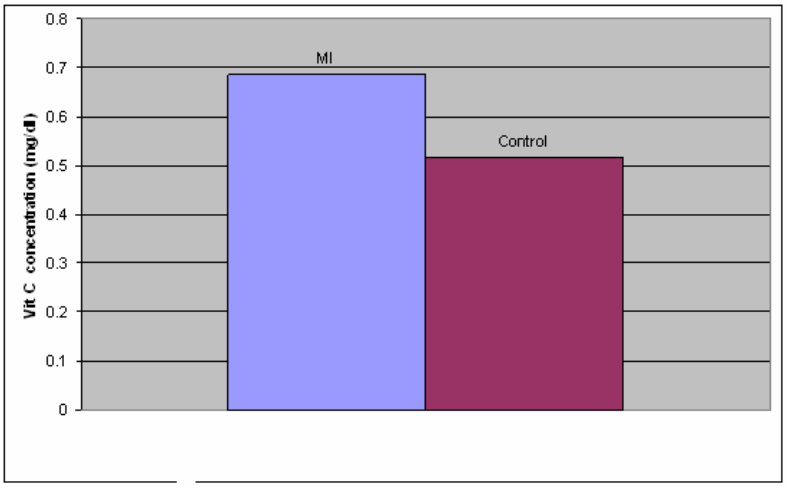

Figure 3 Vit. C concentrations in sera of control and MI individual.