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Study of serum levels of Melatonin, Paraoxonase, Oxidative stress in Iraqi patients with Acute Myocardial Infarction

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Abstract:

Background: *Acute myocardial infarction continues to be a major health concern. It contributes to morbidity and may end fatally. The aim of the study was to evaluate melatonin, paraoxonase levels and correlate these parameters to oxidative stress and lipid profile in AMI.*

Methodology: *A melatonin, paraoxonase (PON), malondialdehyde (MDA), Nitric oxide (NO), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were analyzed in 60 subjects, 30 AMI patients and 30 age/sex-matched controls.*

Results: *The current study reported that the patients with AMI had significantly lower level of melatonin, PON, SOD, TAC, NO and HDL and higher level of MDA, GPx, cholesterol, and TG levels than the healthy controls. The positive correlation was found between melatonin with paraoxonase, SOD, GPx, NO, TAS, HDL, and glucose, also there was positive correlation between paraoxonase with MDA, SOD, GPx, HDL.*

Conclusion: *This study indicates an imbalance between oxidant and antioxidant molecules in AMI patients, and suggests that the low serum Melatonin, and PON level may be a risk factor for AMI*

independent of other traditional cardiovascular risk factors, Melatonin may provide a novel therapeutic target.

Key words: Myocardial infarction, Melatonin, paraoxonase, Nitric oxide.

INTRODUCTION

Acute myocardial infarction (AMI) is defined as a part of acute coronary syndrome characterized by a typical clinical syndrome consisting of chest pain, dyspnea with rise and fall in troponin or creatine kinase–MB to values greater than 99% of a normal reference population with 7.2 million deaths and 12.2 % of total deaths, coronary heart disease (CHD) is a worldwide disease [(1)]. Melatonin (N-acetyl-5-methoxytryptamine), a tryptophan derivative secreted by the pineal gland, is a highly evolutionarily conserved molecule – present virtually in all organisms[(2)]. Evidence from the last ten years suggests that melatonin may influence the cardiovascular system in humans[(3)]. Furthermore, exogenous melatonin has induced several hemodynamic effects in healthy men and women[(4)].

Serum paraoxonase (PON1) EC 3.1.8.1 an arylesterasesynthesised in the liver. HDL-C associated enzyme which is responsible for the antioxidant properties of HDL. This enzyme plays an important role in preventing LDL–C oxidation, it is considered to protect against the development of coronary heart disease. Knowledge about the link between paraoxonase activity and atherosclerosis comes largely from the biological rather than epidemiological studies as there is evidence that peroxidation of LDL-C is an important factor for atherosclerosis [(5)]. About 25% of cell death in cardiomyocytes after reperfusion of acute myocardial infarction is caused by reperfusion injury.

Oxidative stress is the state of imbalance between the reactive oxygen species (ROS) and the ability of a biological system to detoxify readily the reactive intermediates. Development of oxidative stress because of free oxygen radical generation has been implicated in the pathogenesis of many diseases including Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction and even cancer[(6)]. Malondialdehyde (MDA), a carbonile group produced during lipid peroxidation, is used widely in determining oxidative stress[(7)]. Progression of atherosclerosis is correlated with oxidative stress and can be followed up by MDA measurements [(8)].

The key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), provide a defense system against oxidative stress by removing the OFR, thus protecting cells from oxidative damage [(9)]. However, the endogenous antioxidant activity is severely damaged after ischemia-reperfusion which makes the myocardium extremely vulnerable to OFR. Moreover, exogenous SOD and CAT cannot be delivered into living cells because of the poor permeability and selectivity of the cell membrane, which has limited its usage in protecting cells/tissues from oxidative stress damage [(10)]. Nitric oxide (NO), which is produced by the action of endothelial nitric oxide synthase (eNOS) in the vascular endothelium, is a potent antiatherosclerotic factor. Together, NO and the potent vasoconstrictor endothelin-1 (ET- 1) act as a pair of mutually constraining factors to play a key role in the regulation of vascular tone. An imbalance in either of these opposing factors can result in disease development[(11)].

The aim of this study was to evaluate melatonin, paraoxonase levelsin AMI individuals and to attempt to establish the relationship between melatonin, paraoxonase with oxidative stress and lipid profile.

SUBJECTS AND METHODS

A total of 30 patients (aged 40-60 years) with AMI who were admitted to Tikrit Teaching Hospital / Tikrit / Iraq were enrolled to this cross-sectional, case-controlled study. The diagnosis of AMI was established according to the presence of two of the following criteria: i-prolonged chest pain compatible with AMI, ii-typical electrocardiogram changes, iii-raising of cardiac enzymes. A second control group was comprised of 30 sex- and age-matched subjects with similar geographic and socioeconomic background without AMI. Exclusion criteria were valvular heart disease, surgery, trauma within the prior month, cardiomyopathy, liver disease, renal failure, malignant diseases, other inflammatory disease (such as septicemia and pneumonia) and oral anticoagulant therapy.

From each patient and control, five (5) ml venous blood samples were aspirated from a suitable vein. Samples were collected between (8-9 A.M.) after 12 hours fast. Blood samples were divided into two parts, three ml transferred to a plain tube for (lipid Profile and troponin .The remaining of blood was transferred to another sterile plain tubes for storage. The non-heparinized blood in the plain tubes were left to clot and then centrifuged at 4000 rpm for 5 minutes to separate the serum and dispensed into tightly closed tubes in 1.0 ml aliquot, and stored at -20 C° until assayed. Serum melatonin was measured by using the commercial enzyme-linked immunosorbent assay (ELISA) kits from United States Biological-Company.

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust[12]. Glutathione peroxidase was measured by using commercially available kits according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China)[13]. Superoxide dismutase activity in erythrocyte was determined by using a modified photochemical nitro-blue tetrazolium (NBT) method

utilizing sodium cyanide as peroxidase inhibiter [14]. Serum NO was assayed by the method of Najwa K. Cortas and Nabil W. Wakid by camium reduction method and color complex produced was measured at540nm[15]. Serum total antioxidant capacity was determined using Randox total antioxidant status Kit (Randox).

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS-21 (Statistical Packages for Social Sciences- version 21). All results are presented as mean ± SD. Student's t-test was used for the analysis of data. Values were considered to be significant at $P < 0.05$.

RESULTS

The study group included 30 patients of acute myocardial infarction with a mean age of 57.46 ± 10.95. The control group consisted of 30 healthy individuals with a mean age of 51. ± 10.47.

The results showed highly significant decrease in the levels of melatonin ,PON, SOD ,TAC, and NO in MI group versus the control group (5.4020±0.19422 vs 10.4857±0.24614 pg/ml; P<0.0001), (29.6±1.20465vs53±1.35504 KU/L; P<0.0001), (5.01±.29736 vs 6.24±.32004 U/mL; P< 0.007), (0.8233±.03920vs1.16±.05741 mmol/L; P< 0.0001), (37.8±1.15264 vs 51.32±.93204umol/L; P< 0.0001) respectively, while there was highly significant increase in the levels of GPx32.09±1.00487 in MI patients when compared to the controls12±.65379U/mL; P< 0.0001.

Total cholesterol, triglyceride and glucose levels were higher in the patients compared to the controls (5.7483±0.14793 vs 4.0483± 0.12095 mmol/l; P < 0.0001) (2.3172±.11091 vs1.4150

±.07196 mmol/l; P < 0.0001) ,(5.7225±0.15404 vs4.7452±0.10583 mmol/l; P< 0.0001) respectively. High-density lipoprotein, and creatinine levels were significantly lower in the cases than the controls (1.0853±0.02270 vs 1.6133±0.03577 mmol/l; p< 0.0001), (1.0432±.09976 vs0.8737±.01821 mg/dl; P < 0.0001) respectively.

The positive correlation was found between melatonin with paraoxonase, SOD, GPx, NO, TAS, HDL, and glucose.(r=703, 0.5, 0.892, 563, 864, 0.909, 0.630) respectively. The results showed that there was positive correlation between paraoxonase with MDA, SOD, GPx, HDL (r= 0.608, 0.763, 0.5, 0.646) respectively.

Table 1. Baseline biochemical parameters of MI and control groups

	Mean± Std. Error Mean		
Parameter	**Control group**	**MI group**	**p value**
Melatonin (pg/mL)	10.4857±.24614	5.4020±.19422	P<0.0001
Paraoxonase (KU/L)	53±1.35504	29.6±1.20465	P<0.0001
SOD(U/mL)	6.24±.32004	5.01±.29736	P< 0.007
GPx (U/mL)	12±.65379	32.09±1.00487	P< 0.0001
Nitric Oxide (umol/L)	51.32±.93204	37.8±1.15264	P< 0.0001
TAC(mmol/L)	1.16±.05741	0.8233±.03920	P< 0.0001
MDA(µmol)	3.71±0.6	9.34±1.8	p<0.001
Total cholesterol(mmol/l)	4.0483± 0.12095	5.7483±0.14793	P< 0.0001
TG (mmol/l)	1.4150 ±.07196	2.3172±.11091	P < 0.0001
HDL-C(mmol/l)	1.6133±0.03577	1.0853±0.02270	*p* < 0.0001
Glucose(mmol/l)	4.7452±0.10583	5.7225±0.15404	P< 0.0001
Creatinine (mg/dl)	0.8737±..01722	1.0432±0.01821	P< 0.0001

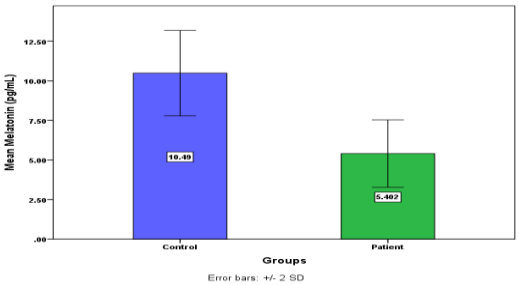

Figure (1):-The level of SerumMelatonin in MI group and the control group

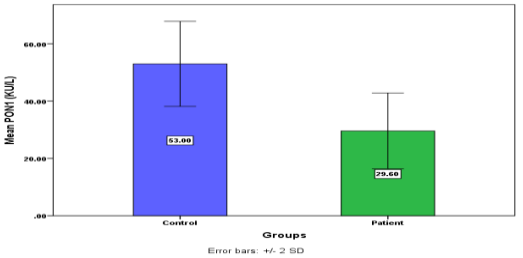

Figure (1):-The level of SerumPON in MI group and the control group

DISCUSSION

Despite the finding that circulating melatonin levels are reduced in patients with myocardial infarction [16], experimental myocardial infarction was shown to increase circulating melatonin levels, followed by enhancement of MI receptors expression[17]. The importance of these receptors in cardioprotection was further supported by the observation that luzindole, a melatonin receptor antagonist, was able to suppress the cardioprotection induced by melatonin [18]. These events may eventually affect the probability of the MPTP opening [19]. Petrosillo and coworkers [20] reported that melatonin protected hearts against reperfusion injury by inhibiting the opening of the MPTP probably via prevention of mitochondrial cardiolipin peroxidation.

The antioxidant actions of melatonin are well established [21-23]. Several reports indicated that melatonin protects the heart directly via its free radical scavenging actions and indirectly via its stimulatory effects on antioxidant capacity [24,25]. Indeed, melatonin was able to neutralize a number of toxic reactants including reactive oxygen species and free radicals induced by myocardial IRI [25,26]. For example, in an in vivo rat model of Myocardial Ischaemia and reperfusion injury, melatonin significantly increased GSH levels and reduced the MDA of the heart tissues after reperfusion [27]. Similar findings were observed in other in vivo and in vitro

studies where it was shown that melatonin also suppressed superoxidase (˙O2) production, reduced myeloperoxidase (MPO) activity and increased SOD, reduced hydroxyl radical (OH˙), as well as total ROS generation)[28,29]. Interestingly, these antioxidant properties were also confirmed in rat models of coronary artery ligation-induced ischaemic heart failure [30], and isoproterenol induced MI [31]. Melatonin does not undergo an enzymatic pathway of reduction after oxidation, but binds irreversibly to free radicals, and these compounds are removed by the kidneys. Not only melatonin but also precursors of its synthesis and products of its metabolism (eg, tryptophan, serotonin, 6-sulphatoxymelatonin) are able to reduce oxidative reactions [29,32]. examined the role of MPO activity in melatonin-induced cardioprotection. They found that N(omega)-nitro-L-arginine-methyl ester(L-NAME) (a NOS inhibitor) treatment innonischemic animals increased blood pressure and lipidperoxidation and depressed glutathione (GSH) levels in themyocardial tissue when compared to the non-L-NAMEtreatedanimals.

Paraoxonase1 is tightly bound with HDL particles in serum. It may play an important role in cardioprotection, as it prevents LDL cholesterol oxidation, metabolizes oxidized LDL-C, and interferes the macrophage uptake of LDL particles [33]. In present study, we found decreased levels of PON in AMI as compared to controls. Decreased PON1 activities might be due to enhanced protection 26 from free radical damage in AMI during the ischaemic process [34]. Few studies reported that OFRs generation in AMI patients is substantiated by measuring PON1 activity[35]. The decrease in PON1 activity could be the result of lower HDL concentration in AMI, given that HDL is the main serum carrier of PON1. It has been demonstrated that not only homocysteine, but also homocysteine thiolactone may modulate properties and function of endothelial cells, which may be contribute to cardiovascular

diseases. Recent studies showed that PON1 playsimportant role in detoxification of harmful compound homocysteine thiolactone (HTL) synthesized in altered homocysteine metabolism. It has been reported that HTL served as substrate for PON1. Low activities of PON1 in present study could due to increased utilization of PON1 to detoxify HTL [36].

Nitric oxide has various physiological properties including vasodilatation, inhibition of platelet aggregation, neutrophil adhesion, scavenging superoxide (O^{-2}) radical and inhibition of xanthine oxidase activity[37]. The production of nitric oxide is decrease in response to oxidative stress; this may be one of the possible causes of complications associated with dyslipidemia[38]. It has been shown that L-arginine serves as a precursor for the synthesis of nitric oxide. Decline nitric oxide may be due to either deficiency of L-Arginine (Arg) or a deficiency of some cofactors required for nitric oxide production[39]. Melatonin incapacitates NO and prevents NO induced apoptosis and induction of inducible NO-synthase melatonin may potentiate the effects of NOS augmentation during beta-adrenergic stimulation[40]. Evidence exists for beta-adrenergic mechanisms in the control of NO generation in cardiomyocytes; for example, isoproterenol has been demonstrated to upregulate NOS expression [41]. And to activate eNOS via Giα, causing a rise in cyclic adenosine Monophosphate(cGMP) [42]. In a study by Genade *et al.* [43]. Forskolin caused a significant elevation in tissue cGMP, suggestive of prior NOSactivation. In addition, inhibition of NOS activation with L-NAME before the onset of regional ischemia caused a significant decline in infarct size, suggesting a role for NOin the associated tissue damage. It has been noted that neuronal NOS, and eNOS, attenuate the b1/b2 adrenergic-induced increase in inotropy and chronotropy, thereby protectingthe heart against excessive stimulation by catecholamines[42, 44].

Nitric oxide is a potent stimulus for the expression of SOD. SOD is an important antioxidant enzyme having an antitoxic effect against super oxide anion. The over-expression of SOD might be an adaptive response, and it results in increased dismutation of superoxide to hydrogen peroxide [45]. This study showed that SOD activity were significantly decreased in AMI patients. The results of this study are Similar to Kumar and Das [46]. while in agreement with those reported by Kayyum *et al.*[47]. SOD is an important enzymatic antioxidant which is positioned in the arterial wall where NO may be inactivated by $O2^{-}$.Hence, pathological states like atherosclerosis which results in NO depletion may be associated with a fall in extracellular SOD.

In their study, TAC levels were also significantly lower in AMI patients Fazendas *et al.*,[48]. Reported that TAC levels decreased in young survivors of acute Myocardial Infarction, Yegin *et al.*,[49]. Also showed reduction in the level of TAC. Our findings are in consistent with them, and a decreased TAC level might be associated with an enhanced protective mechanism to oxidative stress in AMI.

In AMI patients, we found significantly higher levels of GPx in patients group. GPX, a selenium containing enzyme, is an important antioxidant enzyme of erythrocytes [50,51]. GPX plays a significant role in the peroxyl scavenging mechanism, and in maintaining functional integration of the cell membranes[52].

In the present study, MDA levels have been increased significantly in serum of the patients with AMI as compared to the controls. Elevatedlevels of lipid peroxidation is thought to be a consequence ofoxidative stress, which occurs when the imbalance between oxidant and antioxidant. In ischemia, the conversion of ADP toATP is drastically reduced and ADP is converted to hypoxanthine and uric acid by xanthine oxidase upon reperfusion. During this process, enormous amounts of

superoxide radicals formed which can stimulate Haber-Weiss reaction for further generation of ROS, initiating lipid peroxidation [53]. This is similar to work of Dubois Rande *et al.*[54] and McMurray.[55]. who showed a decrease in antioxidant enzyme activities and increase in lipid peroxidation products (MDA, TBARS) in patients with unstable angina and chronic heart failure. Belch *et al*[56]. showed that progression of atherosclerosis is correlated with oxidative stress and can be followed up by MDA measurements. Results of the studies of Pezeshkian *et al.*[57]. Showed that, MDA levels increased significantly in heart diseases.

Fasting levels of triglycerides, LDL-C and total cholesterol in patients of AMI were significantly higher as compared to those in controls whereas the levels of serum HDL-cholesterol was significantly lower as compared to that in controls. In a prospective cardiovascular Munster study, elevated TG has been found to be significant and independent risk factor for major coronary events even after adjustment for LDL-C and HDL-C levels and other risk factors [58]. Assessing the lipid ratio in a normal individual as it is one of the atherogenic factors for development of myocardial infarction and other coronary complications. Lehto *et al.*[59]. Have demonstrated that there was a direct correlation between the incidence of acute myocardial infarction and plasma lipid abnormalities. Abdulla[58]. Has found that significant increase in lipid and lipoprotein, total cholesterol and LDL-C in the sera, which showed severity of clinical symptoms of endothelial symptoms. According to him, abnormal lipid profile along with other crucial factors in the cascade leading to ischemic damage in the cardium.

The physiological function of PON seems to be a degrade specific oxidized cholesteryl ester and oxidized phospholipids in lipoproteins and cell membranes[60]. The lower activity of PON1 can depress the ability of circulating HDL particle to protect

LDL from oxidation, to participate in reverse cholesterol transport pathway and to inhibit monocytes-endothelial cell interaction. All these appear to be important in the inflammatory response in artery that promotes atherogenesis(61). Andan Akey *et al.*(61). Have found that no significant correlation in between PON1 activity and other metabolic parameter like HDL-C, TG, Insulin in the CAD and metabolic syndrome .Amur Ayab *et al.*(62). Have established that sustained myocardial infarction did not show markedly decreased HDL-C concentration but PON1 activity and PON concentration were profoundly decreased Kumar *et al*(46). Have studied serum PON1 activity in normolipidimic patients with acute myocardial infarction. According to them, no correlation was observed between PON1 activity and HDL-C in acute myocardial infarction which suggested that decreased PON 1 activity could be oxidative stress in acute myocardial infarction (58).

CONCLUSION:

This study shows the low serum paraoxonase activity may be an independent risk factor for AMI furthermore it can be used as primitive marker of progression of atherosclerosis and AMI, Serum PON may play an important role in the instability of atherosclerotic plaque, Melatonin may provide a novel therapeutic target. Depression of antioxidant system in these patients confirms this conclusion.

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