Study of Serum Malondialdehyde Level in Opioid and Methamphetamine Dependent Patients

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Abstract- Opioid compound and methamphetamine are commonly used in drug abuse; these can disrupt the normal function of cellular and molecular systems, leading to several events such as oxidative stress, aging, apoptosis, and necrosis. Malondialdehyde (MDA) is the most important biomarker for evaluation of oxidative stress and determination of lipid peroxidation. In this study, 42 drug abusers and 22 healthy persons participated as case and control groups, respectively. MDA in volunteer sera was determined by high-performance liquid chromatography (HPLC) with fluorescence detection after pre-column derivatization using thiobarbituric acid. The analysis was performed on a ODS column by spectrofluorometer detection, operated at excitation of 515 nm and emission of 535 nm. A mixture of phosphate buffer (0.05 M, pH 6.8), containing potassium monobasic phosphate and methanol (60:40, v/v) at a flow rate of 1 ml/min, was used as the mobile phase. The retention time of MDA-TBA was 3.2 min. Our findings showed that the MDA level increased in the opioid and methamphetamine abusers when compared to the control group ($P<0.05$); however, no significant difference was observed between the opioid and methamphetamine groups. A state of oxidative stress during biological processes leads to lipid peroxidation, DNA damage, biomolecule dysfunctions, and many other diseases. Since it is impossible to eradicate the drug addiction, we should reduce the side effects of drug abuse, such as oxidative stress, by intake of proper nutrition and antioxidants.

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Introduction

Recently, drug abuse and addiction lead to serious public health problems and many physiological and pathological consequences (1,2). Drug abuse alters the normal brain function and disrupts the memory, behavior, neural cell life, and may even induce neuronal cell death (1). Methamphetamine, one of the most common drugs leading to drug abuse, can adversely affect the normal function of enzymes, cytokines, and neurotransmitters (3,4). Opioid compounds can alter the function of hormones, enzymes, and disrupt the endocrine system (5). In a healthy condition, oxidative stress and free radicals generation are balanced in the cellular system. Oxidative stress refers to the excess concentration of reactive oxygen species (ROS) occurring due to the imbalance in the production and removal rate of ROS (6,7) and is caused due to drug abuse (2). Amphetamine and its derivate induce oxidative stress in the nervous system (8,9) and increase ROS in CNS in vivo and in vitro (2). Induced lipid and protein oxidation associated with amphetamine increase the malondialdehyde (MDA) concentration in the brain (10,11). The exposure of CNS to drugs like morphine and heroin may also cause oxidative stress. Opioids consist of morphine, which causes an elevation of lipid peroxidation a chain process that affects the unsaturated fatty acids in the tissues (12); ROS, that induces lipid peroxidation; responsible for damaging the DNA and cell membrane (6,13,14). MDA is an important biomarker, indicating the lipid peroxidation in biological samples (15), and an increase in MDA leads to

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pathologies in human and animal models (16). In this study, we determined MDA to be an oxidative stress biomarker, in opioid and methamphetamine-dependent patients.

Materials and Methods

This study was approved by the ethics committee of Kermanshah University of Medical Sciences, Kermanshah, Iran.

Serum collection

Patients who decided to abandon their drug addiction were referred to the Farabi hospital of Kermanshah; after overnight fasting, peripheral blood sample was collected using vacationer tubes (BD Biosciences®). Furthermore, serum was separated, aliquots were prepared and labeled, and were frozen at -70° C until laboratory measurements. The test group comprised 42 drug-addicted patients and the control group comprised 22 healthy volunteers. The exclusion criteria were: dependency on any substance except methamphetamine and opium; any other major psychiatric diagnosis; and intake of any medication that can affect the serum MDA levels, such as alcohol, benzodiazepines, and caffeine.

Calibration curve

Standard solution of tetraethoxypropane (TEP) was prepared in alcohol, and 0.312, 0.625, 1.25, 2.5, 5, and 10 µM/l concentrations were selected for drawing the calibration curve.

Sample preparation

Sample derivatization was carried out in 2 ml plastic centrifuge tubes, followed by the addition of 50 µl serum of each sample or TEP standard, 50 µl of 2,6-di-tert-butyl-4-methylphenol (BHT) solution, 400 µl H_3PO_4 (1M) solution, and 100 µl 2-thiobarbituric (TBA) solution. Sample tubes were capped tightly, mixed by vortex, and then heated for 1 h at 100° C in a dry bath incubator. After derivatization, the samples were kept in a water bath and were refrigerated at 4° C for 5 min; moreover, 250 µl of n-butanol was added to each vial for extraction of the MDA-TBA complex. All tubes were vortex mixed for 5 min and were then centrifuged for 3 min at 14,000 rpm to separate the two phases; 20 µl of supernatant was then injected.

Chromatographic apparatus and conditions

The chromatographic system consisted of an Agilent Technology® Series 1200 gradient quat pump (G1311A), an FLD detector (G1321), and a degasser (G1322A). Pump flow-rate was 1.0 ml/min with the mobile phase comprising ratio of methanol:buffer (60:40 v/v). The buffer used was 50 mM potassium monobasic phosphate (anhydrous) with an adjusted pH of 6.8. The fluorescence detector was set at an excitation wavelength of 515 nm and an emission wavelength of 553 nm. The column used was a Macherey-Nagel® of 5 µm with an ODS of 150×4.6 mm and a 5 µm Universal Phenomenex C18 guard®, placed in a column oven G1316 set to 37° C.

Results

The calibration curve is shown in Figure 1.

Chromatogram peaks of three samples are shown in Figure 2. The retention time was approximately 3.2 minute.

The data exhibited in Table 1 shows a significant increase in the serum levels of MDA in the drug abuse groups as compared to that of the control group (mean of opioid group=6.1173, mean of methamphetamine group=5.1367, and mean of control group=2.2819; P<0.000). The difference in the serum MDA levels between the study groups was statistically significant.

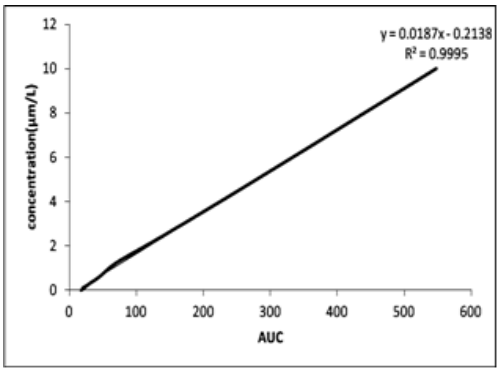

Figure 1. Calibration curve of malondialdehyde shows a good linearity at concentration from 0.312 (up) to 10 µM/l versus Area under Curve (AUC)

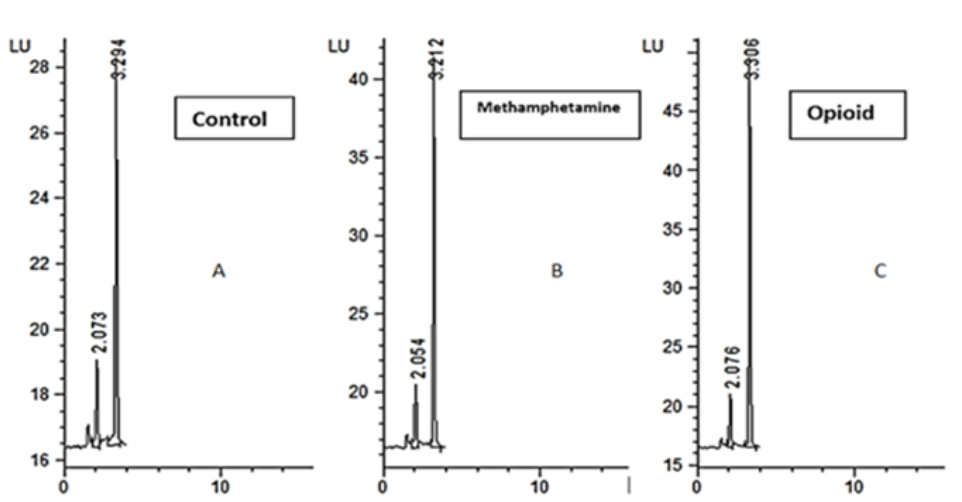

Figure 2. Typical chromatograms of (A), (B), and (C) serum samples obtained from control, methamphetamine, and opioid volunteer, containing 2.94, 8.58, and 12.3 µM/l of MDA, respectively

Table 1. Serum MDA levels were assessed in all groups including the control (healthy), opioid, and methamphetamine drug abuser groups. A comparison was made between each of the abuser groups and the control group. Depicted data are presented as mean±SEM. µM/l: micromole per liter

Drug		N	Mean (µM/L)	Std. deviation	Std. error mean	*P*
MDA	**Control**	22	2.2819	0.53640	0.11994	0.000
	Opioid	22	6.1173	6.22090	1.32630	
MDA	**Control**	22	2.2819	0.53640	0.11994	0.000
	Methamphetamine	20	5.1367	4.89301	1.06774	
MDA	**Opioid**	22	6.1173	6.22090	1.32630	0.459
	Methamphetamine	20	5.3533	4.75291	1.03717	

Discussion

Drug abuse due to methamphetamine and opioid, disrupts the normal physiological and biological pathways, and reduces the antioxidant content, increases ROS production, and leads to lipid peroxidation. Therefore, induction of oxidative stress in biological processes may lead to lipid peroxidation, which can alter the cell structure and lipid metabolism in the human biological system (1,11,12). MDA is a most important biomarker for determination of lipid peroxidation. Our findings correlate with those of Sebnem *et al.*, who investigated the toxic effects of opioids on liver and kidney in rats; thus, the serum MDA level was determined, and significant difference was observed between the case and control groups (17). Pervious study by Goran *et al.*, showed an increase in the MDA level in rats, which were treated by morphine; hence, this study correlated with our results (18).

A study by Kevin *et.al.*, showed that methamphetamine administration in rats for evaluation of left ventricular function increased the ROS level in serum by more than twice the standard value (19).

Moreover, a study by Bryan *et al.*, showed that treatment of rats with methamphetamine could change the oxidative stress parameters such as MDA and cause oxidative brain damage (20).

Our results revealed a significant difference in the serum MDA levels between the drug abusers and control groups. Mean value of MDA in the opioid group was higher than the methamphetamine group, but no significant difference was observed.

In opioid abuse patients, opium was traditionally used with Wafoor (traditional instrument), which produced smoke and induced hypoxia, exacerbating the oxidative stress and increasing MDA in greater amount than the methamphetamine abuse.

An increase in the oxidative stress may have adverse consequences. Oxidative modification of DNA, protein, and lipids play an important role in many diseases, cancers, and malignancies (13). Increase in ROS causes damage to the DNA and other biomolecules, and alters the normal function of tissues and cells, leading to human aging and diseases (7). Oxidative stress causes

cell damage and death, and progresses to pathologies such as cardiovascular disease, neuropathies, inflammatory diseases, acquired immune deficiency syndrome (AIDS), diabetes mellitus, renal disease, cancer (6), atherosclerosis, Parkinson's disease (21), Alzheimer's, schizophrenia, rheumatoid arthritis, thalassemia, hypertension, male and female infertility, muscular degeneration, asthma, heart failure, and many other diseases (7). Oxidative stress induced by drugs contributes in their cytotoxicity. In chronic drug abuse, antioxidant defense system is defeated; (2) eventually, an increase in the oxidative stress and insufficient antioxidant defense mechanism disrupts the physiological balance and leads to pathologies in drug abusers. Since it is impossible to eradicate the drug addiction, we should reduce the side effects of drug abuse, such as oxidative stress, by intake of proper nutrition and antioxidants.

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