Status of Total Antioxidant Capacity and Malondialdehyde Level in Methamphetamine Addicts: A Cross Sectional Study

Shadnia S1, Gorgzadeh N2, Soltaninejad K3*, Abdollahi M4, Motevalian SA5

1 Toxicological Research Center, Excellent Center of Clinical Toxicology, Clinical Toxicology Department, Loghman Hakim Hospital Poison Center, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2 Faculty of Pharmacy, Islamic Azad University of Medical Sciences, Tehran, Iran
3 Department of Forensic Toxicology, Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran
4 Faculty of Pharmacy, and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran
5 Department of Epidemiology, School of Public Health, Iran University of Medical Sciences, Tehran, Iran

A R T I C L E   I N F O
Article Type: Original Article
Article History: Received: 26 Sep 2016
Revised: -
Accepted: 7 Oct 2016

Key Words:
Abuse
Antioxidant
Malondialdehyde
Methamphetamine
Oxidative Stress

A B S T R A C T

Background: Methamphetamine (MA) is an illegal amphetamine-like which stimulate the central nervous system. In recent years, MA has been widely abused worldwide. Previous studies have suggested that oxidative stress (OS) plays an important role in MA-induced toxicity. From this view, antioxidant therapy could be considered as a potential drug therapy in MA abusers. Therefore, the objective of this study was to evaluate OS status in MA abusers.

Methods: This was a cross-sectional study conducted on 21 MA abusers who referred to Iranian National Center for Addiction Studies and Congress 60 Humane Revivification Society, and 15 healthy males as a control group. The demographic data and peripheral blood sampling was obtained from both groups. The serum malondialdehyde (MDA) level as a marker of plasma lipid peroxidation and total antioxidant capacity (TAC) of plasma were analyzed.

Results: Significant decrease in plasma TAC in case group was observed (101.85±12.5 vs. 130.7±16.12 mmol/L). No significant increase in MDA serum level was detected in case group in comparison with control (27.35±2.6 vs. 26.67±2.22 µmol/L, respectively). Neither the serum MDA levels nor the plasma TAC were significantly correlated with the duration and amount of MA abuse.

Conclusion: It seems that, MA abuse is associated with prooxidant-antioxidant imbalance, which suggests evaluation the role of antioxidants administration for the prevention and treatment of MA-induced toxicity.

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Implication for health policy/practice/research/medical education: Total Antioxidant Capacity and Malondialdehyde Level in Methamphetamine Addicts

1. Introduction:
Methamphetamine (MA) is an amphetamine derivate and is categorized as a brain stimulant that has been abused due to its euphoric properties and potential in increasing energy (1). According to morbidity, mortality, and epidemiologic studies, MA abuse has been increased worldwide and could be considered as a major public health problem (2). Prolonged MA abuse can cause neurologic, psychiatric and several physical complications (3). Different pharmacologic agents have been investigated for the treatment of MA abuse (4); however no pharmacologic agent has been proven to have clinical efficacy (5). At present, cognitive-behavioral therapies including the Matrix Model are thought to be the most important methods for MA abuse treatment (6). As there is no specific pharmacotherapy protocol for the treatment of MA abuse, the evaluation of new drug treatment strategies in MA abuse is necessary.
Several mechanisms have been demonstrated to participate in MA mediated neurotoxicity, including apoptosis and necrosis (7), glutamatergic activity (8), dopamine quinine formation and protein modification (9). Previous cell culture experiments and animal studies have shown that free radical production increases in MA exposure (10-15). Also, the same findings were seen in few clinical studies (16-19).
Oxidative stress (OS) can be defined as an imbalance between the production of free radicals and the body’s antioxidant defense (20). OS can cause damages to biomolecules such as lipids, proteins and DNA (21) and is involved in the pathogenesis of many diseases (22). Lipid peroxidation refers to the oxidative degradation of lipids which forming malondialdehyde (MDA) that can be used as a biomarker to measure the level of OS in an organism (23).
Antioxidants are substances that play a major role in preventing the formation and in scavenging of free radicals. In early 1990s, a method for the measurement of total antioxidant capacity (TAC) was developed. The major advantage of this test is to measure the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of an individual compound (24).
According to different methods used to synthesize MA, and variation in its impurities between countries (25) as well as Iran (26); and with regard to findings that OS is playing an important role in MA toxicity, it may necessitate the evaluation of OS status in MA abusers in each country. The aim of this study is evaluation the OS status in Iranian MA addicts.

2. Materials and Methods:
Study Design and Patients: This was a cross-sectional study on MA abusers who were referred to Iranian National Center for Addiction Studies and Congress 60 Human Revivification Society for the treatment of chronic MA abuse from October 2012 to March 2013. The individuals whose conditions met the following inclusion criteria were considered: a) age between 18 to 60 years old b) to be diagnosed as MA addicts according to DSM-IV criteria c) not being abstinent at the time of sampling d) positive urine test results for amphetamines at the time of admission. The exclusion criteria were as follows: a) fulfilling the DSM-IV criteria for the diagnosis of polysubstance abuse and alcohol use disorder b) positive medical history for cardiac, pulmonary, hematologic, hepatic and renal diseases, diabetes mellitus, malignancies and recent infection c) extreme fatness or thinness d) regular medication intake specially including psychotropic drugs, statins, angiotensin converting enzyme inhibitors, angiotensin receptor antagonists, multivitamins, antioxidants and nitrates. All the individuals were informed that their legal status would not be influenced if they participate in the study.

Corresponding author: Soltaninejad K, Pharm D., PhD. Associate Professor, Department of Forensic Toxicology, Legal Medicine Research Center, Legal Medicine Organization, Behesht street, Khayyam Ave., Postal Code: 1114795113, Tehran, Iran
Tel/Fax Number: (+98) 21 55613731
E-mail: kamsoltaninejad@gmail.com
and for any of the patients the informed consent was obtained. Ethical Committee of Islamic Azad University of Medical Sciences approved this study. Healthy voluntary subjects with no known physical or psychiatric disorders which were identified by clinical interview were selected as a control group. They also did not meet the diagnostic criteria for previous drug or alcohol abuse or addiction. As approximately all participants in our study were cigarette smokers, the control group was either selected among this population to eliminate the effect of tobacco on OS parameters.

A questionnaire was filled to gather demographic data, history of MA abuse and cigarette smoking.

Blood Sampling and Bioanalysis: Venous blood samples were obtained from patients in both groups for the evaluation of OS status. The blood specimens were centrifuged at 3000 rpm for 10 minutes and plasma samples were frozen at -80°C until assay. Plasma lipid peroxidation was determined by measuring thiobarbituric acid reactive substances. In summary, 250 µL of trichloroacetic acid (TCA) (20 grams of TCA in 10 mL of sodium sulfate, 2 M) was added to 500 µL of plasma. The mixture was centrifuged at 1000 g for 10 minutes and the precipitate was reacted with 1 mL of 0.67% w/v thiobarbituric acid. The samples were heated at 100°C in a water bath for 30 minutes. After cooling, the samples were extracted with n-butanol and centrifuged at 3500 RPM. After centrifugation, the absorption of malondialdehyde-thiobarbituric acid chromogen was measured at 532 nm. 1,1,3,3-tetramethoxypropane was used as MDA standard (27). Thiobarbituric acid reactivity was calculated as micromole MDA per liter (µmol/L).

Plasma TAC was measured by using Ferric Reducing Ability of Plasma (FRAP) assay. At low pH, ferric tripyridyl-s-tirazine (Fe^3+–TPTZ) reduced to ferrous (Fe^2+) which has blue color and it can be monitored by measuring the change in absorption at 593 nm. 1500 µL of freshly prepared FRAP reagent (25 mM of acetate buffer 300 mM with a pH of 3.6 was added to 2.5 mM FeCl₃, 20 mM and 2.5 mM of TPTZ 10 mM in 40 mM HCl) was added into 50 µL of plasma. After 15 minutes incubation at 37°C in a water bath, absorbance was measured by ELISA microplate reader (Synergy, BioTek, Instruments Inc, Germany) at 593 nm at 0 minute after vortexing. Thereafter, samples were placed at 37°C in water bath and again absorption was measured after 4 minutes. FRAP values were determined by calculation the change in absorbance of sample from 0 to 4 minutes comparing with change in absorbance of trolox standard from 0 to 4 minutes (28).

Statistical Analysis: All data were analyzed with SPSS software version 19. The normal distribution of quantitative variables was tested by Kolmogorov-Smirnov test. We used the independent t-test to examine the difference of numeric variables between the MA and control group. Also Mann-Whitney U-test was used to explore the difference between groups for non-parametric variables. Pearson's correlation was used to estimate the correlation between numerical variables. P values of 0.05 or less were considered as a significant level of difference.

3. Results:
This study was performed on 21 MA addicts (19 male, 2 female) as a case group and 15 male voluntary subjects as a control group. The results showed that there was no significant difference with regard to age between case and control groups (33.8 ± 9.8 vs. 38.2 ± 8.8 years old, respectively, p=0.179) (Table 1). We observed significant differences between case and control groups with regard to duration of cigarette smoking (10.7 ± 8.7 vs. 18.9 ± 10.6 years, respectively, p=0.016) and the number of cigarette per year (5562 ± 2039 vs. 7422 ±1754, respectively, p=0.007). In all cases the route of exposure to MA was smoking. The mean daily amount of MA abused was 1.02 ± 0.52 grams. The mean duration of MA abuse was 4.4 ± 2.9 years (Table 2).

MDA serum levels were higher in the MA group compared to control group, however the difference was not significant (P=0.424).
FRAP as an indicator of total antioxidant capacity of plasma, was significantly decreased in MA abusers compared to control group (p<0.001) (Table 3). The results showed that there is no significant correlation between MDA serum level with amount (r=-0.085, p=0.713) and duration (r=0.036, p=0.876) of MA abuse. Also, we observed no significant correlation between plasma TAC with amount (r=0.25, p=0.26) and duration (r=0.1, p=0.64) of MA abuse.

4. Discussion:
Amphetamine-type stimulants which include MA, are the fastest rising drug of abuse worldwide (29, 30). Previous in vivo (9,11) and in vitro (12-15) studies have suggested that OS plays a key role in MA-induced toxicity. OS is associated with the emergence of a variety of diseases (22). The mechanisms, by which MA involves in formation of free radicals, include dopamine auto-oxidation (31), striatal glutamate release (32) and inhibition of mitochondrial function that increases mitochondrial-mediated reactive species generation (33).
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A variety of methods are available for the measurement of OS markers (34). In the present study, OS status in MA abusers was compared with that of normal controls based on plasma TAC and serum MDA level. In a previous study, plasma TAC was found to be lower in blood samples of MA dependent patients than in those of healthy controls (16). These results are in concordance with the results of our study which show that MA abusers have significantly lower levels of plasma TAC. As free radicals are produced, plasma TAC induction occurs to scavenge them. Malnutrition in chronic abusers of MA results in depletion of plasma TAC in comparison with healthy subjects (35).

Our results indicated that MA addiction is associated with non-significant increase in MDA serum levels. Despite our finding, a previous study showed that MA dependent patients have significantly elevated MDA serum levels (17). Another study on post mortem brain samples from chronic MA abusers showed significant elevation in MDA level (19). Different nutritional diet, MA dose and impurities could be considered for this discrepant finding. Also positive medical history for diseases such as anemia was not considered as an exclusion criterion in Suriyaprom et al. study (17). In Fitzmaurice et al. study, the known or suspected cause of death in 11 out of 16 chronic abusers was related to acute MA exposure, which was likely due to the abuse of a more toxic and higher dose than our subjects (19).

The previous study showed that there is no positive association between total MA level in the brain and aldehydes concentrations (19), which is the same as the results of our study, which showed no significant correlation between MDA serum level and plasma TAC with the amount and duration of MA abuse. A tolerance effect due to chronic MA abuse might be the explanation for this finding.

5. Conclusion:
MA addiction is associated with a prooxidant-antioxidant imbalance, which suggests evaluating the role of antioxidants administration for the prevention and treatment of MA-induced toxicity and morbidity.

| Table 2: Distribution of patients according to amount and duration of methamphetamine abuse |
|-----------------|------------------|
| Parameter       | Percent (%)      |
| Amount of methamphetamine abuse | Percent (%) |
| <1 gram/day    | 28.6             |
| 1-2 gram/day   | 66.6             |
| >2 gram/day    | 4.8              |
| Duration of methamphetamine abuse | Percent (%) |
| <1 Year        | 4.8              |
| 1-3 Year       | 42.8             |
| 4-6 Year       | 33.3             |
| 7-9 Year       | 14.3             |
| >10 Year       | 4.8              |

| Table 3: Level of Malondialdehyde and Total Antioxidant Capacity of Plasma in case and control groups |
|-----------------|-----------------|-----------------|-----------------|
| Parameter       | Case group (n=21) | Control group (n=19) | P value     |
| Malondialdehyde (μmoL/L) | 27.35±2.60 | 26.67±2.22 | 0.424 |
| Total Antioxidant Capacity of Plasma (mmoL/L) | 101.85±12.50 | 130.70±16.12 | <0.001 |
6. Acknowledgement:
We wish to give our thanks to Mr. Hossein Dezhakam, the founder and mastermind of Congress 60 Humane Revivification Society for his sophisticated help in patients' selection

6. References: