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Salivary biomarkers of oxidative stress in methamphetamine users: a case-control study

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Abstract

Background: Methamphetamine abuse, a potent and highly addictive stimulant, continues to be a major concern globally due to its potential harmful effects on the human body. Research suggests that methamphetamine use leads to an increase in free radicals and oxidative stress in the body, resulting in various adverse consequences.

Objectives: This study aimed to compare the levels of oxidative stress biomarkers in the saliva of methamphetamine users versus healthy individuals.

Methods: A case-control study was conducted involving 36 methamphetamine users and 27 healthy controls recruited from Farshchian Hospital in Hamadan, Iran. Written, informed consent was obtained from all participants. Saliva samples were collected and analyzed for catalase activity, total thiol molecules, and total antioxidant capacity.

Results: There was no significant difference in the demographic characteristics between the case and control groups. However, the mean total antioxidant capacity in methamphetamine users (0.10±0.01 µmol/ml) was significantly lower than in healthy individuals (0.64±0.12 µmol/ml) ($P < 0.001$). Catalase activity and thiol groups in saliva showed no significant differences between the two groups.

Conclusion: Our findings suggest that long-term methamphetamine use triggers oxidative stress and elevates oxidants in the bodies of users.

Keywords: Methamphetamine, Oxidative stress, Saliva.

Introduction

Today, there is a growing concern about the pattern of drug abuse shifting from traditional drugs to industrial drugs and the use of chemical substances, stimulants, and narcotics on a global scale. Methamphetamine is a highly effective central nervous system (CNS) stimulant that is primarily used as a recreational drug but is also sometimes prescribed as a second-line treatment for attention deficit hyperactivity disorder and obesity. Methamphetamine was first synthesized in 1893 and consists of two enantiomers: levo-methamphetamine and dextro-methamphetamine.[1,2]

Methamphetamine is the second most widely used drug after cannabis globally.[3] Methamphetamine is commonly referred to as "Shisheh," "Gach," "Lachaki," "Ice," or "Crystal" in Iran. Recently, there has been an alarming increase in methamphetamine use in Iran, accompanied by a significant lack of understanding regarding the colloquial terms associated with this drug among healthcare professionals, policymakers, clinicians, researchers, and members of the general public.[4] Methamphetamine users often report experiencing

stimulating properties, hallucinations, feelings of pleasure, and happiness after using methamphetamine.[5,6] Methamphetamine can be used in a variety of ways, including smoking, snorting, injection, and swallowing.[7]

Depending on the method of use, this substance has severe and diverse destructive effects on individuals.[8] Acute methamphetamine use leads to several effects on the sympathetic nervous system, including hypertension, hyperthermia, elevated heart rate, vasoconstriction, tachycardia, and dilated pupils, while chronic methamphetamine use and abuse result in severe psychosis.[9]

Methamphetamine abuse, which can damage dopamine and serotonin nerve terminals, is associated with deficits in neuropsychological testing. It has been estimated that 40% of methamphetamine users exhibit abnormalities on neuropsychiatric tests. According to the available evidence, the primary effect of methamphetamine is to elevate the extracellular levels of monoamine neurotransmitters in synaptic clefts.[10]

The exact mechanism of action of methamphetamine in increasing the concentration of these neurotransmitters is not yet fully understood, but it appears to involve several possible mechanisms, including:

- Rapidly releasing serotonin and dopamine neurotransmitters from presynaptic terminals.
- Preventing these neurotransmitters from being reabsorbed and accumulating in the synaptic cleft.

These neurotransmitters play a crucial role in cognitive functions and motor activity, which is why disruptions in their systems and receptors are linked to various cognitive and motor disorders.[11,12]

Previous studies have shown that methamphetamine enhances pro-oxidant processes and causes oxidative stress damage. Oxidative stress is caused by an increase in the production of free radicals and uncontrolled peroxidation, both of which can injure cells. Reactive Oxygen Species (ROS) refers to a group of reactive molecules and free radicals derived from molecular oxygen. These ROS cause irreversible damage to macromolecules such as DNA, proteins, lipids, and carbohydrates within the body. There are specialized systems within the body to counteract free radical damage, known as antioxidant defense systems. When the rate of free radical production exceeds the capacity of these defenses, oxidative stress ensues.[13,14] In a healthy individual, there exists a balance between the production of free radicals and the antioxidant defense system. However, exposure to external factors such as environmental pollutants, drugs, and toxic substances can upset this delicate equilibrium by increasing free radical production. This imbalance leads to oxidative stress, which has been implicated in the development of various diseases and disorders, including drug addiction, particularly with regards to methamphetamine use.[15]

Objectives

Due to the high prevalence of methamphetamine abuse and its oxidative stress effects, the objective of this study was to assess the level of oxidative stress in the saliva of methamphetamine users compared to healthy individuals.

Methods

Study setting and participants

This match-case control study was conducted at Farshchian Hospital, Hamadan, Iran. Thirty-six methamphetamine users who were referred to the hospital were included in the case group. The control group consisted of 27 healthy individuals who were selected randomly and matched to the case group based on age, sex, and body mass index (BMI). The inclusion criteria for both groups were as follows:

- Methamphetamine use for more than 6 months
- No history of opioid or related substance abuse
- No antioxidant medication usage

Inclusion criteria for healthy controls were:

- No abuse of any drugs
- No smoking
- No history of systemic illness
- No long-term drug intake
- No previous history of malignancy
- No history of antioxidant medication usage

The exclusion criteria for both groups were:

- Any medical condition that could affect oxidative stress levels.

Study procedure and Collecting data

The demographic and anthropometric characteristics of participants, including age, sex, and BMI, were recorded. To minimize the potential impact of confounding variables (age, sex, and BMI), group matching was conducted between cases and controls. Once the data was collected, it was analyzed statistically between the two groups using appropriate methods.

Saliva samples

Unstimulated saliva was collected from subjects between 9:00 a.m. and 12:00 p.m. to mitigate any diurnal variations. Participants were instructed not to eat, drink, engage in oral hygiene activities, or chew for 60 minutes before the saliva collection process. Subjects were then seated on a dental chair and asked to spit into a graduated container every minute until 5 milliliters of saliva were accumulated. Throughout the saliva collection process, participants were advised not to speak or swallow. The salivary samples were then centrifuged at 3000 revolutions per minute for 5 minutes, and the supernatant was separated and frozen at -80 degrees Celsius until used for oxidative stress marker analysis.

Assay of total antioxidant capacity

The FRAP test was used to assess total antioxidant capacity (TAC). In this test, the amount of Fe^{3+} to Fe^{2+} reduction is measured. That is, the medium is exposed to Fe^{3+}, and the antioxidants present in the medium begin to produce Fe^{2+} as an antioxidant activity. The reagent containing TPTZ is dissolved in acetate buffer (pH 3.6) and $FeC1_3$. The complex between Fe^{2+} and TPTZ generates a blue color with absorption at 593 nm, which is determined based on a calibration curve obtained by measuring different concentrations of $FeC1_3$. [16]

Assay of Catalase activity

Catalase activity was estimated via spectroscopic measurements on saliva and expressed in units per milliliter. CAT activity was gauge in samples by assessing the absorbance decrease at 240 nm in a reaction medium containing 1682 10 nM H_2O_2 and 50 mM sodium phosphate buffer (pH 7.0) per minute. One unit of the enzyme consists of 1 mole H_2O_2 consumed per minute, with the specific activity being reported as units/milliliter saliva.[17]

Assay of Total thiol molecules

Salivary protein thiol concentration was determined using a spectrophotometric method employing dithionitrobenzene (DTNB)—Ellman's method.[18] Ellman's reagent, also known as 5,5'-dithiobis (2-nitrobenzoate, DTNB), is an asymmetric aryl disulfide that performs the thiol-disulfide exchange reaction in the presence of a free thiol.[19] In comparison with both disulfides, the TNB dianion has a rather intense absorbance at 412 nm. The absorbance of the TNB complex in the assay mixture at 412 nm was measured using established standard concentrations and absorbance values to calculate the salivary protein thiol concentration.[20]

Ethical considerations

The study was conducted in adherence with the Declaration of Helsinki. Institutional Review Board (IRB) approval was obtained prior to initiation of the study. To ensure that the study did not interfere with the diagnostic or treatment processes of patients, we took steps to avoid any potential conflicts of interest. In conducting the study, we clearly explained the objectives and methodology to volunteer participants, obtained their written and informed consent, and maintained confidentiality throughout the process.

Statistical analysis

The continuous variables were expressed as the mean±SD, and the categorical variables were presented as a percentage. Chi-square and independent t-test were used to compare data between the two groups. All statistical analyses were performed with SPSS (version 16.0, SPSS Inc, Chicago, IL, USA). A “P value” less than 0.05 was considered significant.

Results

In this case-control study, according to the inclusion and exclusion criteria, 36 methamphetamine users were included in the case group and 27 healthy individuals in the control group. Most individuals were male in both

groups, and there was no significant difference between the two groups. The mean age of methamphetamine users was 32.4 ± 9.8 years, which was similar to the mean age of 30.5 ± 11.2 years in the control group. Additionally, BMI was similar in both groups [Table 1]. The levels of TAC and thiols in methamphetamine users were lower than those in the control group, and this decrease was statistically significant for TAC. Catalase activity was also higher in methamphetamine users compared to the control group [Table 2].

Table-1. Comparison of demographic and anthropometric data in methamphetamine users (Case) and healthy individuals (Control)

		Case (n=36)	Control (n=27)	P value
Gender	Male	29 (80.5 %)	23 (85.2 %)	0.63
	Female	7 (19.5 %)	4 (14.8 %)	
Age (years)	Mean	30.5±11.2	32.4±9.8	0.48
	Min-Max	11-60	18-65	
BMI	Mean	26.5±3.2	25.4±3.8	0.21
	Min-Max	23-29	21-28	

Table-2. Comparison of oxidative stress biomarkers in methamphetamine users (Case) and healthy individuals (Control)

	Case (n=36)	Control (n=27)	P value
Total antioxidant capacity (mmol Ml^{-1})	0.1±0.01	0.64±0.12	0.0001
Total thiol molecules (mmol mL^{-1})	0.027±0.054	0.032±0.066	0.74
Catalase activity (UmL^{-1})	6.927±5.557	1.036±1.481	0.0001

Discussion

Oxidative stress has been shown to play an important role in the toxic effects of methamphetamine. Current studies have found that oxidative stress increases in methamphetamine users compared to healthy individuals. Specifically, the levels of total antioxidant capacity and thiol groups in methamphetamine users are lower than those in healthy individuals. Additionally, catalase activity in methamphetamine users is significantly higher than in the control group. Yamamoto and Zhu previously explored the effects of methamphetamine on free radical formation and oxidative stress in rats, finding that the substance promotes pro-oxidant processes and offers evidence for oxidative damage induced by the drug.[21]

Another study by Walker et al. investigated the link between stimulant dependence and oxidative stress. Catalase (CAT) enzyme activity and total antioxidant capacity were measured in peripheral blood samples from 48 methamphetamine-dependent participants and 30 normal controls. The results showed that methamphetamine-dependent patients had significantly lower antioxidant capacity compared to controls, indicating that they may be at a higher risk of oxidative damage to the brain and other organs.[22]

Mitochondria are a significant source of reactive oxygen species (ROS) and mitochondrial dysfunction has been linked to several neurodegenerative disorders. Additionally, amphetamines inhibit mitochondrial function, but the exact mechanisms behind this inhibition are unclear. A study titled "Enhanced oxidative stress and aberrant mitochondrial biogenesis in human neuroblastoma SH-SY5Y cells during methamphetamine-induced apoptosis" found that elevated oxidative stress and production of irregular mitochondria play a crucial role in the neurotoxic effects of methamphetamine.[23]

The results of the current study demonstrated the effect of methamphetamine on the induction of oxidative stress in saliva. Similar to a previous study, it was found that methamphetamine altered the activity and expression of antioxidant enzymes in mice, leading to oxidative stress.[24]

Methamphetamine appears to be capable of producing hydrogen peroxide via monoamine oxidase mediators and radical reactions, resulting in the formation of reactive oxygen species (ROS) via the mitochondrial electron transport chain and changes in mitochondrial membrane potential, which leads to oxidative stress and damage.[25]

Given the harmful effects of methamphetamine and its ability to induce oxidative stress, using antioxidants or similar compounds may be beneficial in minimizing these damages.[26]

Therefore, methamphetamine users should consume more natural antioxidants like vegetables and fruits. The enhancement effect of natural products on methamphetamine-induced neuronal apoptosis and their potential molecular mechanisms in regulating dopamine release, oxidative stress, mitochondrial-dependent apoptotic pathway, endoplasmic reticulum stress-mediated apoptotic pathway, and neuroinflammation has been demonstrated. This may underscore the potential value of natural products in moderating methamphetamine-induced oxidative stress and offer valuable insights for future research and development of novel and effective pharmacotherapies in this area.[27]

Conclusions

The current findings demonstrate that oxidative stress increases in methamphetamine users. The total antioxidant capacity, thiol groups, and catalase activity decreased while comparing methamphetamine users to healthy individuals.

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Competing interests

The authors declared no conflict of interest.

Abbreviations

Central nervous system: CNS; Reactive Oxygen Species: ROS; Body Mass Index: BMI; Total antioxidant capacity: TAC; Fluorescence recovery after photobleaching: FRAP; 2,4,6-tris(2-pyridyl)-s-triazine: TPTZ; Dithionitrobenzene: DTNB.

Authors' contributions

All authors pass the four criteria for authorship contribution based on the International Committee of Medical Journal Editors (ICMJE) recommendations. All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

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Role of the funding source

None.

Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The study was in accordance with the latest revision of the Helsinki Declaration and approved by the ethical committee of Hamadan University of Medical Sciences.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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