Association Between a 50bp Ins/Del Genetic Variation at Promoter of the Superoxide Dismutase-1 (*SOD1*) and the Risk of Dependency to Opium and Methamphetamine

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Abstract- Superoxide dismutase-1 (SOD1, OMIM: 147450, copper-zinc superoxide dismutase) is one of the major antioxidant enzymes, which plays an important role in clearance of reactive oxygen species. A common genetic polymorphism of 50 bp insertion/deletion (Ins/Del) in the promoter region of the *SOD1* has been reported. The purpose of the present study was to investigate the association between this polymorphism and the risk of opium (OD) and methamphetamine (MD) dependency. The present report was consisted of two case-control studies. The first study consisted of 143 OD subjects and 570 healthy controls. The second study consisted of 65 cases with MD and 635 controls. The controls were selected randomly from the healthy blood donors. Genotyping were carried out using PCR based method. Statistical analysis indicated that neither the Ins/Del (OR=1.06, 95% CI: 0.69-1.62, *P*=0.788) nor the Del/Del (OR=0.57, 95% CI: 0.13-2.55, *P*=0.464) genotypes were associated with the risk of OD. Although the frequency of the Ins/Del genotype was lower among methamphetamine-dependent persons compared to healthy control subjects, there was no significant association between the Ins/Del polymorphism and the risk of MD (OR=0.82, 95% CI: 0.44-1.53, *P*=0.547). The present findings demonstrated that the *SOD1* 50bp Ins/Del polymorphism is not associated with the risk of OD and MD.

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Keywords: Drug-dependent; Ins/Del; Polymorphism; *SOD1*

Introduction

Superoxide dismutase-1 (EC 1.15.1.1; SOD1, OMIM: 147450, copper-zinc superoxide dismutase) metabolizes highly reactive and more dangerous superoxide radicals into less reactive molecules (O_2 and H_2O_2), therefore provide protection against superoxide radicals toxicity (1). Subsequently, H_2O_2 is converted into H_2O by catalase or glutathione peroxidase. The imbalance between the production of reactive oxygen species (ROS) and antioxidant systems (including enzymatic defense system) referred to as oxidative stress. Oxidative stress is associated with several chronic diseases such as psychiatric diseases (2-4).

Numerous genetic variations in the human *SOD1* gene have been reported. Previous studies have shown the association of *SOD1* genetic polymorphisms with the risk of numerous oxidative stress diseases such as senile cataract, cancers, etc. (1,5-11). A common 50bp insertion/deletion (Ins/Del) genetic variation in the promoter region of the *SOD1* (1684 bp upstream of the ATG start codon) has been reported (12). The 50bp-deleted region contains a number of transcription factor binding sites, such as SP1. Studies have been shown that the Del allele significantly reduces the *SOD1* promoter activity (12-14).

Opiates may cause oxidative stress in drug-dependent persons (3,4). Morphine decreases the expression level of the mu-opioid receptor via ROS production in morphine-treated SH-SY5Y cells (15). Oxidative stress is highly important in the brain, because it may change the function of the N-type and/or L-Type Ca^{2+} channel (16). It should be mentioned that the *SOD1* is expressed in the various parts of the brain (17). The *SOD1* expression level in SH-SY5Y cells showed significant alterations after the cells were exposed to methadone and morphine (18-20). Very recently, we reported the association between the *SOD1* Ins/Del polymorphism and the risk of heroin dependence

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(14).

Although the association studies on polymorphism of several genes involved in antioxidant defense and dependency to opium and methamphetamine have been reported (21-28), there is no published study considering the association between the *SOD1* Ins/Del polymorphism and the risk of dependency to opium (OD) or methamphetamine (MD). Therefore, the present study was carried out.

Materials and Methods

Subjects

The present report consisted of two case-control studies (Figure 1).

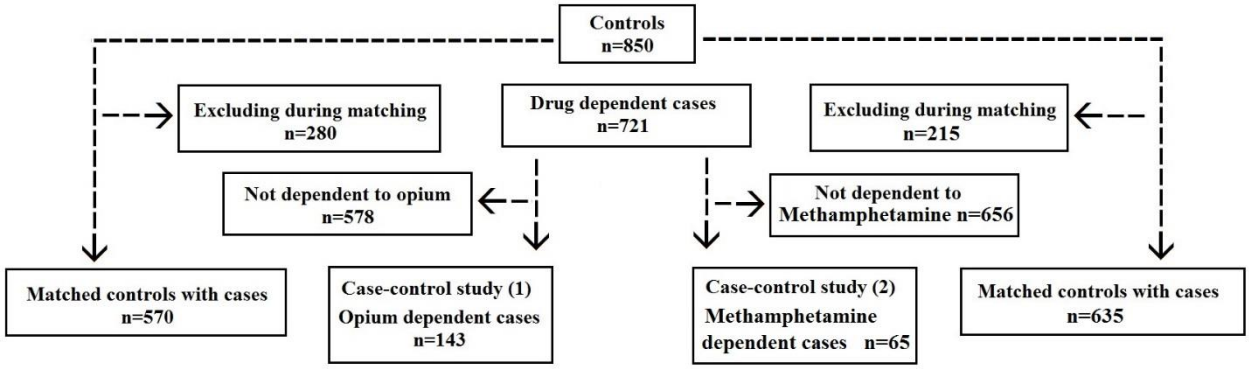

Figure 1. STARD flow diagram demonstrating the selection cases and controls

The first study consisted of 143 opium dependent subjects (12 females, 131 males) and 570 healthy controls (55 females, 515 males). The selection and characteristics of cases and control groups have been described in detail in our previous reports (23,24). The second study consisted of 65 patients (13 females, 52 males) with dependency to methamphetamine and 635 controls (110 females, 525 males) which were randomly selected from the healthy blood donors. A detailed description of these groups has been reported in our previous report (22). Considering that the Iranian population is very heterogeneous (29-32), we selected the participants from Persian (Caucasians) Muslims living in Shiraz (Fars province, south-west Iran). All patients were interviewed by a senior psychiatrist using the Structured Clinical Interview based on the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV) criteria for dependency to opium and methamphetamine. The patients were on methadone maintenance for treating drug dependency. Control individuals were blood donors, who declared that they did not have substance abuse.

The study protocol complied with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee on Biological Research of the Shiraz University (Iran). Informed consent was obtained from each volunteer before the study.

Genotyping

Genomic DNA was extracted from blood samples. All samples were genotyped for the *SOD1* Ins/Del polymorphism using the PCR method, as described previously (10).

Statistical analysis

The goodness-of-fit χ^2 test was used to verify whether the genotypic distributions were in accordance with the Hardy-Weinberg equilibrium. The associations between the genotypes and the risk of drug dependency were assessed by odds ratios (ORs) and its 95% confidence intervals (CIs). The Ins/Ins genotype was used as the reference group. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA; version 11.5). A probability of $P<0.05$ was considered statistically significant. All statistical tests were two-sided.

Results

Table 1 shows the genotypic prevalence of the studied polymorphism in the opium (OD) and methamphetamine-dependent (MD) patients and healthy control groups. The observed genotypic frequencies of the Ins/Del polymorphism in control groups were consistent with the expected frequencies based on the Hardy-Weinberg

equilibrium (For OD study: χ^2=0.92, df=1, *P*=0.335; For MD study: χ^2=0.01, df=1, P=0.973).

Table 1. Association between the 50bp Ins/Del genetic polymorphism at the promoter region of the *SOD1* and the risks of opium and methamphetamine dependence

Genotypes		Controls N (%)	Cases N (%)	OR	95% CI	*P*
Opium dependency	**Ins/Ins**	424 (74.4)	106 (74.1)	1.0	--	--
	Ins/Del	132 (23.2)	35 (24.5)	1.06	0.69-1.62	0.788
	Del/ Del	14 (2.5)	2 (1.4)	0.57	0.13-2.55	0.464
Ins		980 (86.0)	247 (86.4)	1.0	-	-
Del		160 (14.0)	39 (13.6)	0.96	0.66-1.41	0.862
Methamphetamine dependency	**Ins/Ins**	467 (73.5)	51 (78.5)	1.0	--	--
	Ins/Del	155 (24.4)	14 (23.5)	0.82	0.44-1.53	0.547
	Del/ Del	13 (2.0)	0 (0.0)	--	--	--
Ins		1089 (85.7)	116 (89.2)	1.0	--	--
Del		181 (14.3)	14 (10.8)	0.72	0.40-1.29	0.277

Statistical analysis indicated that neither the Ins/Del nor the Del/Del genotypes were associated with the risk of OD (Table 1). There was no significant linear trend between the number of Del alleles and the risk of OD (χ^2=0.03, df=1, *P*=0.864). Although the frequency of the Ins/Del genotype was lower among methamphetamine-dependent persons compared to healthy control subjects, there was no significant association between the Ins/Del polymorphism and the risk of MD (OR=0.82, 95% CI: 0.44-1.53, *P*=0.547).

Discussion

It has been well established that oxidative stress is associated with opium dependency (3,4). It has been suggested that the SOD1 activity is potentially important in etiology of oxidative stress-related diseases. The Del allele was associated with reducing the promoter activity of the *SOD1* (12-14), and subsequently, it may alter the level of ROS detoxification. Therefore, we hypothesized that this polymorphism might be associated with the risk of dependency on opium and methamphetamine. However, we found no evidence for the association between the *SOD1* Ins/Del polymorphism and risk of OD and MD. Previously it has been reported that this polymorphism is not associated with the risk of heroin dependence (13), which is supported by the present study.

Our present study has some limitations. First, considering that several environmental factors are associated with the risk of dependency on drugs (33,34) and several factors are associated with expression level of the *SOD1* (35-37); therefore the interaction of the *SOD1* Ins/Del polymorphism and environmental factors on risk of dependency to opium and methamphetamine should be carried out simultaneously. Second, the estimated ORs in some comparisons revealed that the Del allele might be negatively associated with the risks of OD and/or MD. However, because of our small sample size, we failed to find statistically significant associations. It is important to gather data from several large studies before final conclusions regarding the association of the study polymorphism and risk of dependency on opium and methamphetamine can be drawn.

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References

1. Niwa J, Yamada S, Ishigaki S, Sone J, Takahashi M, Katsuno M, et al. Disulfide bond mediates aggregation, toxicity, and ubiquitylation of familial amyotrophic lateral sclerosis-linked mutant SOD1. J Biol Chem 2007;282:28087-95.
2. Smaga I, Niedzielska E, Gawlik M, Moniczewski A, Krzek J, Przegaliński E, et al. Oxidative stress as an etiological factor and a potential treatment target of psychiatric disorders. Part 2. Depression, anxiety, schizophrenia and autism. Pharmacol Rep 2015;67:569-80.
3. Ghazavi A, Mosayebi G, Solhi H, Rafiei M, Moazzeni SM. Serum markers of inflammation and oxidative stress in chronic opium (Taryak) smokers. Immunol Lett 2013;153:22-6.
4. Soykut B, Eken A, Erdem O, Akay C, Aydın A, Çetin MK, Dilbaz N. Oxidative stress enzyme status and frequency of micronuclei in heroin addicts in Turkey. Toxicol Mech Methods 2013;23:684-8.

5. El-Kheshen G, Moeini M, Saadat M. Susceptibility to ulcerative colitis and genetic polymorphisms of A251G SOD1 and C-262T CAT. J Med Biochem 2016;35:333-6.
6. Rajkumar S, Vasavada AR, Praveen MR, Ananthan R, Reddy GB, Tripathi H, Ganatra DA, Arora AI, Patel AR. Exploration of molecular factors impairing superoxide dismutase isoforms activity in human senile cataractous lenses. Invest Ophthalmol Vis Sci 2013;54:6224-33.
7. Kasznicki J, Sliwinska A, Kosmalski M, Merecz A, Majsterek I, Drzewoski J. Genetic polymorphisms (Pro197Leu of Gpx1, +35A/C of SOD1, -262C/T of CAT), the level of antioxidant proteins (GPx1, SOD1, CAT) and the risk of distal symmetric polyneuropathy in Polish patients with type 2 diabetes mellitus. Adv Med Sci 2016;61:123-9.
8. Spisak K, Klimkowicz-Mrowiec A, Pera J, Dziedzic T, Aleksandra G, Slowik A. rs2070424 of the SOD1 gene is associated with risk of Alzheimer's disease. Neurol Neurochir Pol 2014;48:342-5.
9. Jamhiri I, Saadat I, Omidvari S. Genetic polymorphisms of superoxide dismutase-1 A251G and catalase C-262T with the risk of colorectal cancer. Mol Biol Res Commun 2017;6:85-90.
10. Eskandari-Nasab E, Kharazi-Nejad E, Nakhaee A, Afzali M, Tabatabaei SP, Tirgar-Fakheri K, et al. 50-bp Ins/Del polymorphism of SOD1 is associated with increased risk of cardiovascular disease. Acta Med Iran 2014;52:591-5.
11. Kordestanian N, Saadat M. A 50-bp Ins/Del polymorphism at the promoter region of the superoxide dismutase-1 and bipolar disorder type 1. Nordic J Psychiatry 2017;71:750-3.
12. Broom WJ, Greenway M, Sadri-Vakili G, Russ C, Auwarter KE, Glajch KE, et al. 50bp deletion in the promoter for superoxide dismutase 1 (SOD1) reduces SOD1 expression in vitro and may correlate with increased age of onset of sporadic amyotrophic lateral sclerosis. Amyotroph Lateral Scler 2008;9:229-37.
13. Ingre C, Wuolikainen A, Marklund SL, Birve A, Press R, Andersen PM. A 50 bp deletion in the SOD1 promoter lowers enzyme expression but is not associated with ALS in Sweden. Amyotroph Lateral Scler Frontotemporal Degener 2016;22:1-6.
14. Saify K, Saadat M. Influence of a 50bp Ins/Del polymorphism at promoter of the superoxide dismutase-1 on gene expression and risk of heroin dependency. Environ Health Prev Med 2017;22:4.
15. Ma J, Yuan X, Qu H, Zhang J, Wang D, Sun X, et al. The role of reactive oxygen species in morphine addiction of SH-SY5Y cells. Life Sci 2015;124:128-35.
16. Hool LC. Evidence for the regulation of L-type Ca2+ channels in the heart by reactive oxygen species: mechanism for mediating pathology. Clin Exp Pharmacol Physiol 2008;35:229-34.
17. Pardo CA, Xu Z, Borchelt DR, Price DL, Sisodia SS, Cleveland DW. Superoxide dismutase is an abundant component in cell bodies, dendrites, and axons of motor neurons and in a subset of other neurons. Proc Nat Acad Sci U S A 1995;92:954-8.
18. Saify K, Saadat M. Expression patterns of antioxidant genes in human SH-SY5Y cells after treatment with methadone. Psychiatry Res 2015;230:116-9.
19. Saify K, Saadat I, Saadat M. Down-regulation of antioxidant genes in human SH-SY5Y cells after treatment with morphine. Life Sci 2016;144:26-9.
20. Saify K, Saadat M. Expression levels of antioxidant genes in human SH-SY5Y cells long term exposed to methadone. Turk J Biochem 2016;41:493-4.
21. Nakatome M, Miyaji A, Mochizuki K, Kishi Y, Isobe I, Matoba R. Association between the GST genetic polymorphisms and methamphetamine abusers in the Japanese population. Leg Med (Tokyo) 2009;11:468-70.
22. Khalighinasab MR, Saify K, Saadat M. Association between GSTM1 and GSTT1 genetic polymorphisms and susceptibility to methamphetamine dependence. Mol Biol Res Commun 2015:4:25-32.
23. Khalighinasab MR, Saify K, Saadat M. Association between null alleles of GSTM1 and GSTT1 and dependence to heroin and opium. Psychiatry Res. 2015;228:977-978.
24. Saify K, Khalighinasab MR, Saadat M. No association between GSTM1 and GSTT1 genetic polymorphisms and susceptibility to opium sap dependence Mol Biol Res Commun 2016;5:59-64.
25. Koizumi H, Hashimoto K, Kumakiri C, Shimizu E, Sekine Y, Ozaki N, et al. Association between the glutathione S-transferase M1 gene deletion and female methamphetamine abusers. Am J Med Genet B Neuropsychiatr Genet 2004;126B:43-5.
26. Saify K, Saadat I, Saadat M. Influence of A-21T and C-262T genetic polymorphisms at the promoter region of the catalase (CAT) on gene expression. Environ Health Prev Med 2016;21:382-6.
27. Boroumand F, Saadat M. Lack of association between two genetic polymorphisms of SOD2 (rs2758339 and rs5746136) and the risk of opium dependency. Pol Ann Med 2017;24:194-8.
28. Boroumand F, Mahmoudinasab, H, Saadat M. Association of the SOD2 (rs2758339 and rs5746136) polymorphisms with the risk of heroin dependency and the SOD2 expression levels. Gene 2018;649:27-31.
29. Rafiee L, Saadat I, Saadat M. Glutathione S-transferase genetic polymorphisms (GSTM1, GSTT1 and GSTO2) in

three Iranian populations. Mol Biol Rep. 2010;37:155-8.
30. Saadat M. Distribution of ACE insertion/deletion (I/D) polymorphism in Iranian populations. Mol Biol Res Commun 2015;4:63-6.
31. Fallahzadeh-Abarghooei L, Zahedi T, Mirabedi F, Saadat M. Alleleic prevalence of intron 3 insertion/deletion genetic polymorphism of DNA double-strand break repair gene XRCC4 in four Iranian populations. Egypt J Med Hum Genet 2015;16:215-8.
32. Nasseri G, Zahedi T, Mousavi-Kazerooni F, Saadat M. Prevalence of null genotypes of glutathione S-transferase T1 (GSTT1) and M1 (GSTM1) in seven Iranian populations. Iran J Public Health 2015;44:1655-61.
33. Ajonijebu DC, Abboussi O, Russell VA, Mabandla MV, Daniels WM. Epigenetics: a link between addiction and social environment. Cell Mol Life Sci. 2017;74:2735-47.
34. Badiani A, Spagnolo PA. Role of environmental factors in cocaine addiction. Curr Pharm 2013;19:6996-7008.
35. Mahmoudinasab, H, Saadat M. Expressions of some antioxidant genes in SH-SY5Y cells treated with β-lapachone, morphine and electromagnetic field. Mol Biol Rep 2018;45:379-87.
36. Saw CL, Cintrón M, Wu TY, Guo Y, Huang Y, Jeong WS, et al. Pharmacodynamics of dietary phytochemical indoles I3C and DIM: Induction of Nrf2-mediated phase II drug metabolizing and antioxidant genes and synergism with isothiocyanates. Biopharm Drug Dispos 2011;32:289-300.
37. Mahmoudinasab H, Saadat M. Electromagnetic field could protect SH-SY5Y cells against cisplatin cytotoxicity, but not MCF-7 cells. DNA Cell Biol 2018;37:330-5.