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Original Article

Serum Level of Antioxidant Enzymes in Opium-Addicted Patients With Coronary Artery Disease

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ABSTRACT

Background: Opiates cause coronary artery disease (CAD), which is one of the most common complicated cardiovascular diseases and is responsible for morbidity and mortality rates. The mechanism of the association between opiates and CAD is not well known. Therefore, the aim of this study was to evaluate antioxidant enzymes serum levels in patients with CAD and opium addiction.

Methods: This case-control study was performed on 188 cases (40 in the CAD group, 39 in the CAD and opium addiction group, and 60 in the control group). Superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured by enzymatic methods and compared between the groups.

Results: The serum level of SOD was 744.55 ± 506.16 U/L, which was lowest among the patients who had CAD with opium consumption (465.46 ± 67.8 U/L) and highest in the control group (1304.46 ± 545.69 U/L) ($P < 0.001$). Furthermore, the serum level of GPx was 1076.92 ± 778.28 U/L, which was lowest among the patients who had CAD with opium consumption (769.79 ± 506.77 U/L) and highest in the control group (1661.41 ± 615.11 U/L) ($P < 0.001$).

Conclusions: The serum levels of SOD and GPx were significantly lower in our CAD cases with opium addiction. Opiates cause oxidative stress. Pharmacological and psychiatric approaches can reduce the toxicological effects of opiates. ***(Iranian Heart Journal 2019; 20(4): 31-37)***

KEYWORDS: Coronary artery disease, Opiates, Superoxide dismutase, Glutathione peroxidase

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Coronary artery disease (CAD) is one of the most common complicated cardiovascular diseases with high morbidity and mortality rates (accounting for 11.2% of all deaths). In the majority of patients, CAD is caused by atherosclerosis, vascular cavity stenosis, and/or occlusion. [1-3] The main risk factors of CAD are diabetes mellitus, dyslipidemia, smoking, hypertension, immobility, and obesity. The other less major

risk factors are aging, male gender, a positive family history of CAD, and menopause. [4,5] However, the role of many other risk factors in the development of CAD needs to be investigated. Recently, it has been reported that cellular antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) have a central role against the formation of atherosclerosis. A reduction in the amount of these enzymes increases the risk of CAD; nonetheless, little is known about their relevance to human disease. [6]

On the other hand, some studies have indicated that opium consumption is one of the risk factors of CAD. [7-9] Mohammadi et al [7] showed that opium consumption exacerbated atherosclerosis plaque formation by hypercholesterolemia. Sadat et al [10] in 2012 reported that increased levels of interleukin 1 (IL-1) in opioid consumers played an important role in atherosclerosis. It has also been reported that oxidative stress increases in opium consumers. Karajibani et al [11] in 2016 demonstrated that opium was capable of provoking oxidative stress, exerted negative effects on antioxidant enzymes and the lipid profile, and led to antioxidant vitamin deficiency. To the best of our knowledge, there are only a few studies on the comparison of the serum levels of cellular antioxidant enzymes such as SOD and GPx in CAD cases with and without opium addiction. We, accordingly, sought to assess the difference in the serum levels of SOD and GPx in CAD cases with and without opium consumption by comparison with a control group.

METHODS

Study Design

This cross-sectional study was conducted in the Cardiology Department of Kerman Shafa Hospital, from January 2017 to March 2018. The patients who referred to the cardiology department with a diagnosis of CAD and signed a consent form to participate in the study were included. The exclusion criteria consisted of left ventricular dysfunction (left ventricular ejection fraction < 50%), coronary interventions, a history of acute coronary syndrome in the preceding 3 months, myocardial infarction in the preceding 6 months, surgery within the preceding 3 months, cancer and infections within the preceding month, chronic inflammatory diseases, high C-reactive protein levels, high white blood cell counts, liver dysfunction, brain damage in the preceding 3 months, cardiac events after angiography, uncontrolled diabetes, a body mass index > 30 and < 15, pulmonary edema, tachyarrhythmias, heart valve diseases, pacemaker device implantation, hyperthyroidism, intracranial hemorrhage, all ischemic events within the preceding 3 months, acute trauma, pregnancy, and dissatisfaction to continue participation in study. Also excluded were patients with uncompleted data.

Participants

The study flowchart is depicted in Figure 1. Eighty-five patients with CAD (43 cases without opium addiction and 42 cases with opium addiction) diagnosed by cardiologists based on clinical and paraclinical findings who met the inclusion and exclusion criteria were included. Additionally, 40 subjects without CAD were included as the control group.

Totally, 118 individuals completed the study: 79 from the CAD group (40 cases without opium addiction and 39 cases with opium addiction) and 39 from the control group. The study received ethics approval from the Ethics Committee of Kerman University of Medical Sciences (Code IR.KMU.REC.1397.341), and all the participants gave written informed consent.

Coronary angiography was executed by standard Judkins technique. CAD was described as coronary artery stenosis > 50% in at least 1 coronary artery.

On the angiography day, blood samples were taken from the patients in fasting state. An EDTA anticoagulant blood sample was used to measure the biomarkers. After preparation, the plasma samples were immediately frozen in separate parts (aliquots) at −80 ° C until the time of measurement. GPx activity was measured over a fixed time via glutathione consumption. [12] The spontaneous or enzymatically catalyzed glutathione reaction with hydrogen peroxide was stopped after a fixed time by adding a strong acid, and the glutathione was later measured by polarography. SOD activity was determined through the following method: superoxide radicals produced by the xanthine oxidase reaction convert 1-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride quantitatively to a formazan dye (Ransod test kit, Randox). SOD inhibits dye formation and converts superoxide radicals into hydrogen peroxide and serves as a measure of SOD activity.

Data Analysis

The data were analyzed and reported only for the patients who completed the trial. The statistical analyses of the data were performed using SPSS, version 22 (SPSS Inc, Chicago, IL, USA). The χ^2 test was performed to compare the qualitative variables between the groups. The normal distribution of all the studied parameters was checked using the Kolmogorov–Smirnov test. The ANOVA test was used for the variables with normal distributions, and the Kruskal–Wallis test was performed for the variables without normal distributions. A 2-tailed *P* value < 0.05 was considered significant.

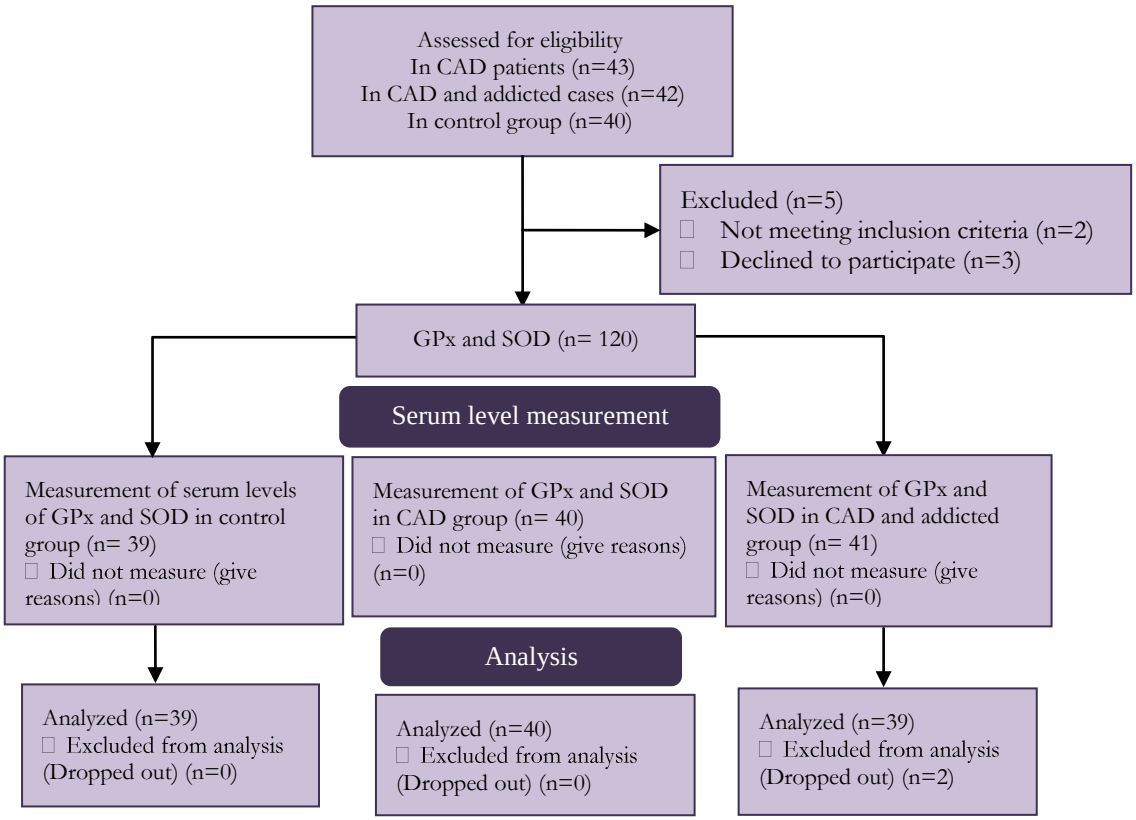

Figure 1. Study flowchart

RESULTS

A total number of 118 male and female CAD patients with and without opium addiction and a control group were included in the analysis. From this total, 66.9% of were diagnosed as CAD cases and 33.05% were diagnosed to be normal. The mean age at the time of visit for the total study population was 58.81 ± 10.93 years old (40–78 y). The overall body mass index was 27.35 ± 4.63, which was higher in the patients with CAD and opium addiction than in those without CAD (29.34 ± 4.14 vs 24.57 ± 3.25, respectively; $P < 0.001$). Lipid profiles (except for triglycerides) were highest in the CAD and opium addiction cases: the total cholesterol level of 202.076 ± 36.77 mg/dL and the low-density lipoprotein (LDL) level of 121.74 ± 34.19 mg/dL. However, the high-density lipoprotein (HDL) level was lowest in the CAD and opium addiction individuals (41.86 ± 7.21 mg/dL). On the other hand, the levels of total cholesterol (167.58 ± 25.36 mg/dL) and LDL (83.01 ± 22.86 mg/dL) were lowest and the level of HDL (48.35 ± 6.7 mg/dL) was highest in the control group ($P < 0.001$). Moreover, blood pressure (in both systolic and diastolic parameters) was higher in the CAD and opium addiction cases than in the other groups ($P < 0.001$).

The serum level of SOD was 744.55 ± 506.16 U/L, which was lowest among the patients who had CAD with opium consumption (465.46 ± 67.8 U/L) and highest in the control group (1304.46 ± 545.69 U/L) ($P < 0.001$) (Table 1), whereas it was not significantly different between the CAD cases with and without opium consumption based on the Mann–Whitney test ($P = 0.546$). Furthermore, the serum level of GPx was 1076.92 ± 778.28 U/L, which was lowest among the patients who had CAD with opium consumption (769.79 ± 506.77 U/L) and highest in the control group (1661.41 ± 615.11 U/L) ($P < 0.001$) (Table1), while it was not significantly different among the CAD cases with and without opium consumption based on the Mann–Whitney test ($P < 0.713$).

By performing linear logistic regression and eliminating the effects of confounding variables, we found that both SOD ($P < 0.001$) and GPx ($P = 0.004$) were lowest in the CAD cases with opium consumption, while the control group had the highest amount.

Table 1. Studied variables in all the study groups

Variable \ Group		CAD (n=40)	CAD and Opium Addiction (n=39)	Control (n=39)	*P* value
Age (y)		58.92 ±10.57	59.46 ±12.76	58.05 ±9.47	0.85
BMI (kg/m^2)		28.12 ±5	29.34 ±4.14	24.57 ±3.25	<0.001
Sex (male)		22 (55 %)	20 (51.3 %)	23 (59 %)	0.792
Lipid profile	triglycerides (mg/dL)	185.2 ±39.26	189 ±44.38	178.46 ±34.02	0.492
	cholesterol (mg/dL)	198.87 ±35.75	202.076 ±36.77	167.58 ±25.36	<0.001
	LDL (mg/dL)	118.73 ±34.11	121.74 ±34.19	83.01 ±22.86	<0.001
	HDL (mg/dL)	43.22 ±4.6	41.86 ±7.21	48.35 ±6.7	<0.001
Blood pressure	systolic (mm Hg)	147.85± 13.65	152.94± 15.87	135.46± 11.82	<0.001
	diastolic (mm Hg)	88.87± 8.04	90.38± 13.081	75± 14	<0.001
SOD (U/L)		470.75 ±75.57	465.46 ±67.8	1304.46 ±545.69	<0.001
GPx (U/L)		806.5 ±829.04	769.79 ±506.77	1661.41 ±615.11	<0.001

BMI, Body mass index; LDL, Low-density lipoprotein; HDL, High-density lipoprotein; SOD, Superoxide dismutase; GPx, Glutathione peroxidase

DISCUSSION

According to our results, SOD and GPx serum levels were significantly lowest in the CAD cases with opium consumption, while the control group had the highest amount. We also found that the body mass index, the lipid profile, and blood pressure were significantly higher in the CAD and opium-addicted individuals than in the other groups.

Soykut et al [13] observed a significant decrease in copper zinc-SOD activity and increases in malondialdehyde levels and micronuclei frequencies in addicts, while they did not find significant differences in selenium-dependent GPx. However, we found a significant reduction in both GPx and SOD in the cases with opium addiction and CAD. This differences may be due to CAD in our cases. Karajibani et al [11] reported an increase in the production of reactive oxygen species (ROS) and free radicals and a reduction in the enzymatic and non-enzymatic antioxidants such as GPx, glutathione (GSH), and SOD; catalase activities; total antioxidant capacity; and the concentration of vitamins A, E, and C in opium addiction. They also reported that atherogenic indices such as the LDL/HDL ratio and malondialdehyde increased in opium addiction, which might contribute to the increased risk of cardiovascular disease. Zahmatkesh et al [14] demonstrated that opioids elevated the level of ROS and decreased the function of SOD, catalase, and GPx. They also reported that these changes increased the gene expression of target cells through ROS production and vitamin deficiency. GPx is an internal antioxidant that protects cells from the attack of oxidative stress. In addition, we should note that the maintenance of normal cellular actions depends on antioxidants. GPx can be reduced to organic peroxides. [15] Moradi-Sardareh et al [16] demonstrated that the plasma concentration of malondialdehyde markedly increased in their opium cases compared with their healthy hamsters, whereas the SOD, GSH, and catalase levels were markedly reduced in the opium cases. The authors concluded that oxidative stress was increased in the opium-treated animals.

Lamsal et al [17] showed that chronic opium administration in rats significantly reduced GSH depletion in the heart, while it increased glutathione reductase, oxidized glutathione, SOD, and GPx, resulting in cardiac oxidative stress. It appears that GSH-Px, SH-groups, and GSH impair antioxidative defenses. [18,19]

SOD is a first-line enzymatic antioxidant that causes the dismutation catalysis of the superoxide anion into H_2O_2, which is converted into H_2O by catalase and GPx in synergy with GSH. [15] SOD detoxifies $O\bullet_2$- but increases H_2O_2 levels. [17] Previous research has demonstrated that opium decreases GSH levels in hepatic mitochondria, induces the activity of the mitochondrial isoform of SOD and Mn-SOD, and decreases the activities of catalase and GPx. [19,20] On the other hand, treatment with antioxidants prevents opium-induced cardiac dysfunction. Previous investigations have also shown that ROS takes part in the progress of cardiomyopathy after opium consumption. [21,22]

A previous study reported a significant reduction in SOD in opium-treated animals as compared with the control group. [16]

Mohammadi et al [23] showed that opium provoked oxidative stress when administered to the animals in their study. Moreover, opium harmfully increased total cholesterol, LDL, triglycerides, very low-density cholesterol, the atherogenic index, and non-HDL-C in the animals. We found similar results, although we did not find significant differences in triglyceride levels.

CONCLUSIONS

Our results showed that SOD and GPx serum levels are significantly lower in CAD cases with opium addiction; therefore, opiates cause

oxidative stress. Pharmacological and psychiatric approaches can reduce the toxicological effects of opiates.

Conflict of Interest

The authors hereby declare that they have no conflicts of interest regarding the content of this article.

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REFERENCES

1. Dickens C, Cherrington A, McGowan L. Depression and health-related quality of life in people with coronary heart disease: A systematic review. Eur J Cardiovasc Nurs. 2012;11:265–275.

2. Zwadlo C, Meyer GP, Schieffer B, Westhoff-Bleck M. Anomalous intramural course of coronary arteries in congenital heart disease-three case reports and review of the literature. Congenit Heart Dis. 2012;7:139–144.

3. Mehra M, Somohano T, Choi M. Mandibular fibular graft reconstruction with CAD/CAM technology: A clinical report and literature review. J Prosthet Dent. 2016;115:123–128.

4. Khot UN, Khot MB, Bajzer CT, Sapp SK, Ohman EM, Brener SJ, et al. Prevalence of conventional risk factors in patients with coronary heart disease. JAMA. 2003;290(7):898–904.

5. Fruchart JC, Nierman MC, Stroes ES, Kastelein JJ, Duriez P. New risk factors for atherosclerosis and patient risk assessment. Circulation. 2004;109(23 Suppl 1):III15–9.

6. Blankenberg S, Rupprecht HJ, Bickel C, Torzewski M, Hafner G, Tiret L, Smieja M, Cambien F, Meyer J, Lackner KJ; AtheroGene Investigators. Glutathione peroxidase activity and cardiovascular events in patients wit h coronary artery disease. N Engl J Med. 2003 Oct 23;349(17):1605-13.

7. Mohammadi A, Darabi M, Nasry M, Saabet-Jahromi MJ, Malek-Pour-Afshar R, Sheibani H. Effect of opium addiction on lipid profile and atherosclerosis formation in hypercholesterolemic rabbits. Exp Toxicol Pathol. 2009;61(2):145–9.

8. Sadeghian S, Darvish S, Davoodi G, Salarifar M, Mahmoodian M, Fallah N, et al. The association of opium with coronary artery disease. Eur J Cardiovasc Prev Rehabil. 2007;14(5):715–7.

9. Marmor M, Penn A, Widmer K, Levin RI, Maslansky R. Coronary artery disease and opioid use. Am J Cardiol. 2004;93(10):1295–7.

10. Saadat H. Z, Ghanemnia M, Namazi M. H, Safi M, Vakili H, Dabbagh A, et al. Opium Addiction Increases Interleukin 1 Receptor Antagonist (IL-1Ra) in the Coronary Artery Disease Patients. Plos one. 2012;7(9)

11. Karajibani M, Montazerifar F, Khazaei Feizabad A. Study of Oxidants and Antioxidants in Addicts, Int J High Risk Behav Addict. 2017; 6(2):e3505. doi: 10.5812/ijhrba.35057.

12. Flohe L, Gunzler WA. Assays of glutathione peroxidase. Methods Enzymol 1984;105:114–21.

13. Soykut B1, 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 Nov;23(9):684-8.

14. Zahmatkesh M, Kadkhodaee M, Salarian A, Seifi B, Adeli S. Impact of opioids on oxidative status and related signaling pathways: An integrated view. JOM. 2017; 13(4): 1-12.

15. Cheng TY, Zhu Z, Masuda S, Morcos NC. Effects of multinutrient supplementation on antioxidant defense systems in healthy human

beings. J Nutrition Biochem. 2001; 12(7): 388-95

16. Moradi-Sardareh H, Mohammadi N, Oubari F, Nikbakht MR, Hosseini KR, Farnoosh G, et al. Biochemical factors, MDA levels and Antioxidant activity in Opium addicted Hamsters. In Res J Biol Sci . 2014; 3(2): 21-4

17. Lamsal M, Gautam N, Bhatta N, Toora BD, Bhattacharya SK, Baral N. Evaluation of lipid peroxidation product, nitrite and antioxidant levels in newly diagnosed and two months follow-up patients with pulmonary tuberculosis. Southeast Asian J Trop Med Public Health. 2007; 38(4): 695-703

18. Pacifici R, Fiaschi AI, Micheli L, Centini F, Giorgi G, Zuccaro P, et al. Immunosuppression and oxidative stress induced by acute and chronic exposure to cocaine in rat. Int Immunopharmacol. 2003; 3(4): 581-92

19. Devi BG, Chan AW. Impairment of mitochondrial respiration and electron transport chain enzymes during cocaine-induced hepatic injury. Life Sci. 1997; 60(11): 849-55

20. Devi BG, Chan AW. Cocaine-induced peroxidative stress in rat liver: antioxidant enzymes and mitochondria. J Pharmacol Exp Ther. 1996; 279(1): 359-66

21. Boess F, Ndikum-Moffor FM, Boelsterli UA, Roberts SM. Effects of cocaine and its oxidative metabolites on mitochondrial respiration and generation of reactive oxygen species. Biochem Pharmacol. 2000; 60(5): 615-23

22. Moritz F, Monteil C, Isabelle M, Bauer F, Renet S, Mulder P, et al. Role of reactive oxygen species in cocaine-induced cardiac dysfunction. Cardiovasc Res. 2003; 59(4): 834-43

23. Mohammadi A, Mirzaei F, Jamshidi M, Yari R, Pak S, Sorkhani A, et al. The in vivo biochemical and oxidative changes by ethanol and opium consumption in Syrian hamsters. Int J Biol. 2013; 5(4): 14