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# **Original Research Article**

# Assessment of Erythrocyte Superoxide Dismutase as Biomarker of Oxidative Stress in Sudanese: Male Smokers in Khartoum State

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**Abstract:** Oxidative stress plays an important role in the pathogenesis of some diseases. Smoking may enhance oxidative stress not only through the production of reactive oxygen radicals in smoke but also through weakening of the antioxidant defense systems. Study done To assess the erythrocyte superoxide dismutase activity in cigarette smokers. This is case control study. The study was conducted from February to May 2015, sixty samples from non smokers chosen randomly as control and sixty sample from smoker as test group. All samples were tested for superoxide dismutase activity using fortress diagnostic reagent by spectrophotometer analyzer, and results were analyzed using statistical of package social science. The study result showed that the level of superoxide dismutase activity was significantly decrease (P = 0.00) in smokers. Mean  $\pm$  SD cases versus controls  $(907\pm134.47$  versus  $1159\pm93.44)$ . There are a significant moderate negative correlation with the number of cigarettes per day (P = 0.00) also weak negative correlation with duration of smoking (P = 0.048). From this study, concluded that superoxide dismutase level was significantly decreased in smokers and correlated negatively with number of cigarettes and duration.

**Keywords:** Cigarette, Oxidative stress, erythrocyte superoxide dismutase, smokers.

### INTRODUCTION

Superoxide dismutase is a naturally occurring enzyme that protects the body against active oxygen free radicals by scavenging excess superoxide. Cigarette smoke contains abundant amount of oxidants and superoxide  $(O_2^-)$  anion from cigarette smoke may rich the vascular endothelium and can then react with nitric oxide (NO-) to form peroxynitrite anion, a highly reactive intermediate with strong cytotoxic potency. Thus the damaging free radicals in cigarette smoke may cause either direct arterial wall injury or protein peroxidation and activation of phagocyte platelet endothelial cell interaction [1].

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) or free radicals and antioxidant defense, which may induce tissue injury. Oxidative stress can be assessed by measurement of reaction products of oxidative damage, like lipid peroxidation, DNA oxidation and protein oxidation [2].

Cigarette smoking is a serious health problem and most important avoidable causes of death in world. The leading causes of death from smoking are cardiovascular diseases (1.69 million deaths), chronic obstructive pulmonary disease (0.97 million deaths) and

lung cancer (0.85 million deaths) [3].

In Sudan, it has been estimated that the prevalence of cigarette smoking in the adult is 24% in 2009, according to a WHO report, published in 2010. Cigarette consumption is one of the main risk factors for a number of chronic diseases, including cancer, lung diseases, and cardiovascular diseases. So, the SOD is considered as an antioxidant that enhance oxidative stress in smoking people [4].

This study is the first one done in Sudan, people must take care to the substance To assess erythrocyte superoxide dismutase activity in Sudanese male cigarette smokers induced to their body because oxidant kill our cells and cause diseases. This study aimed to compare erythrocyte superoxide dismutase activity in cigarette smokers and non smokers. ALSO correlate between erythrocyte superoxide dismutase activity and number of cigarette per day and duration of smoking per year.

### MATERIALS AND METHODS

Analytical case control study. The study was conducted in Khartoum states. The study was carried during the period from February to May 2015.Non hospitalized healthy Sudanese smoker individuals.

Cigarette smokers (more than 5 years) as test group and non smokers as control group, both test and control group were apparently healthy individual. Smokers less than 5 year and individuals with clinical history of any chronic disease had been excluded.

The study sample size was 120 (60 smoker as test group and 60 non smoker as control) were enrolled in this study. After informed consent and use local antiseptic for skin (70%) ethanol, 3ml of venous blood was collected from each volunteer in this study using disposable plastic syringe. The venous blood poured in EDTA container.

Objective of this study were explained to all participating in this study. Information were obtained from all participating in this study were kept as highly confidence, data and specimen result we're not be permitted.

Interview and questionnaire: interview with cigarette smoker and control were done to obtain the clinical data, and it was specifically designed to obtain information which help in either including or exclusion certain individual in or from study. Superoxide dismutase activity was measured using spectrophotometer analyzer and reagent from fortress diagnostics.

# **Principle**

Superoxide dismutase (SOD) role is to accelerate the dismutation of the toxic superoxide radical (02•), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen.

Fortress method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay .

Data was analyzed using SPSS (independent T-test and pearson correlation test). The precision and accuracy of method use in study was cheeked and was analyzed.

### **RESULTS**

Erythrocyte superoxide dismutase was measured in 60 smokers as test group and 60 non smokers as control group, during the period of February to May 2015.

Table 1, Comparison of superoxide dismutase activity in cigarette smokers and non smokers control group (mean  $\pm$  SD:  $907\pm134.47$  versus  $1159\pm93.44$ , P = 0.00).

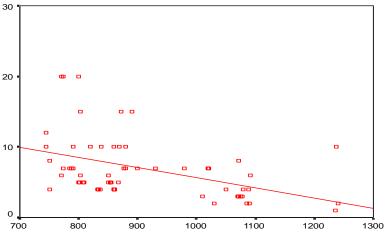
Figure 1, A scatter plot shows negative moderate correlation between the level of superoxide dismutase and number of cigarette per day (r = -.044, P = 0.00).

Figure 2, A scatter plot shows significant weak negative correlation between the level of superoxide dismutase and duration of smoking (year) (r = -.25, P = 0.048)

Table 1: comparison of superoxide dismutase activity in cigarette smokers and non smokers control group

Variable	Smoker (N=60)	Non smoker (N=60)	p-value
Superoxide dismutase	907.45 ± 134.476	1159.80 ± 93.447	0.00
activity mean(U/g Hb)± SD	907.43 ± 134.470	1139.80 ± 93.447	0.00

The table shows the mean  $\pm$  SD, and the probability (p), T-test was used for comparison, P-value  $\leq$  0.05 is considered significant



superoxide dismutase activity (U/g Hb)

Fig-1: correlation between the level of superoxide dismutase and number of cigarette per day (r = -.044, P = 0.00)

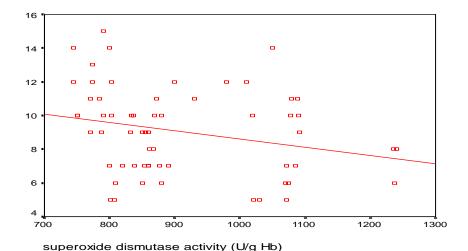


Fig-2: correlation between the level of superoxide dismutase and duration of smoking (year) (r = -.25, P = 0.048)

### DISCUSSION

Cigarette smoking is a serious health problem and most important avoidable causes of death in world. The risk of disease increases with increasing intensity and duration of smoking. In this study the level of superoxide dismutase was found to be significantly decreased in cigarette smokers compared with non smokers (907±134.47 versus 1159±93.44). Justification of this study results that SOD is a naturally occurring enzyme that protects the body against active oxygen free radicals by scavenging excess superoxide. Cigarette smoke contains abundant amount of oxidants and superoxide (O<sub>2</sub>-) anion from cigarette smoke may rich the vascular endothelium and can then react with nitric oxide (NO) to form peroxynitrite anion, a highly reactive intermediate with strong cytotoxic potency. Thus the damaging free radicals in cigarette smoke may cause either direct arterial wall injury or protein peroxidation and activation of phagocyte platelet endothelial cell interaction [5].

This finding within the same line with those obtained by which found decreased in SOD activity in smokers compare with control (714.25 $\pm$ 72.42 versus 952.58 $\pm$ 92.25) [1] which found decreased in SOD activity in smokers compare with control(2.71 $\pm$ 0.29 versus 4.30 $\pm$ 0.40) , and [5] which also found that SOD activity in smokers is 1.26  $\pm$ 0.24 compare with 144.2 $\pm$ 15.2 in control.

Also study result showed negative moderate correlation between the level of superoxide dismutase and number of cigarette per day(r = -.044, P = 0.00). decrease enzyme activity due to increase number of cigarette. also showed significant weak negative correlation between the level of superoxide dismutase and duration of smoking (r = -.25, P = 0.048) increase smoking duration lead to decrease superoxide dismutase activity. No recent studies support this finding.

### CONCLUSION

From this study it is concluded that: The level of superoxide dismutase is significantly decreased in cigarette smokers. There is significant negative correlation between the level of superoxide dismutase and number of cigarette per day. There is significant negative correlation between the level of superoxide dismutase and duration of smoking (year). Periodic follow up of the enzyme because smoking is consider as risk factor. Using of SOD enzyme as a biomedical marker for oxidative stress.

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