
Measurement of Total Antioxidant Status (TAO) and Superoxide dismutase (SOD), Catalase (CAT) Enzymes in Petrol Station workers

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Abstract:

Background: Total antioxidant status, superoxide dismutase (SOD) and Catalase (CAT) are antioxidant defensive enzymes that catalyze the reduction of reactive oxygen species (ROS) to non harmful substance.

Aim: The study focuses on the serum super oxide dismutase enzyme level, CAT level and total antioxidant status in petrol station workers to detect any imbalance between aggressive and defensive factors.

Materials & Methods: the study included 40 workers and 20 healthy subjects in a comparative analysis. (TAO), serum (SOD) and (CAT) levels were measured for study subjects.

Results: Statistical analysis of serum antioxidant enzymes level and antioxidant status revealed a significant increase in SOD, CAT of worker groups ($p \geq 0.05$), and ($P \geq 0.01$) respectively. The TAO was significantly increased ($p \geq 0.001$). The increased of serum SOD, CAT levels and the TAO can be explained on the basis of alteration on enzymes activity, which may lead to disturbance in homeostasis of antioxidant/oxidant balance.

Conclusions: TAO, Catalase and Superoxide dismutase enzymes can used as a biomarker of enzymatic alteration in different diseases.

Key words: Total Antioxidant Status, Super Oxide dismutase, Catalase, Petrol Station Workers.

Introduction:

Benzene is an aromatic hydrocarbon often used for industrial purposes. It can cause serious, negative health effects in humans depending upon both the amount and duration of the exposure.

It is a clastogenic and carcinogenic agent. It may induce acute myelogenous leukemia in humans and multiple types of tumors in humans ^[1]. The main toxic effect of benzene is its myeloid effect. Because most of its metabolism occurs in the liver, observations have shown that benzene has further toxic effects after it is metabolized by the cytochrome p 450 II E1 enzyme systems ^[2].

Benzene enters into the environment because of both human and natural activities. Generally it originates from the following sources: exhaust emissions of motor vehicles, gas stations, oil refineries, cigarettes, coal mines, garages, some consumer items (sprays, synthetic rubber, adhesives and other items which contain benzene), the shoe industry, and waste products of the timber industry ^[3].

Since benzene can be conjugated, the elevation of metabolites and metabolite interactions may increase its toxicity ^[4]. Chronic benzene exposure can causes abnormalities in haematopoietic functions, including anemia, leukopenia thrombocytopenia, parcytopenia, bone marrow depression, a plastic anemia and non-Hodgkin's lymphoma and in some cases benzene causes leukemia and several types of carcinomas ^[5].

People, who work on sites where benzene concentrations are high, may have physical discomfort and changes in their blood enzyme level. In addition, some benzyldiene groups become attached to monosaccharide and disaccharides. Benzene causes energy metabolism dysfunctions in some people and may attach to microsomal proteins and hepatic enzymes ^[6]. The submetabolites, which are formed from benzene metabolism, affect on microsomal fractions, proteins and enzymes of the liver and may affect several hepatocyte functions ^[6].

Benzene affects many enzyme activities in the liver, tissues, and peripheral blood and this can lead to a decrease in the activity of those enzymes and may result in cause the oxidative stress ^[7]. Oxidative stress occurs when the critical balance between oxidants and antioxidants is disrupted due to the depletion of antioxidants or excessive accumulation of the reactive oxygen species (ROS), or both, leading to a damage of the cells ^[7].

ROS, such as superoxide anion radical ($O_2 \bullet^-$), hydrogen peroxide (H_2O_2) and highly reactive hydroxyl radical ($\bullet OH$) can react with susceptible biological macromolecules and produce lipid peroxidation (LPO), DNA damage and protein oxidation as a manifestation of oxidative stress ^[8]. Contaminant-stimulated ROS are associated with different pathologic processes in different diseases and may be a mechanism of toxicity in organisms exposed to pollutants ^[9].

Despite the potential danger of ROS, cells present a variety of defense mechanisms to neutralize the harmful effects of free radicals. The antioxidant defense system includes enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione S-transferase (GST) and other low molecular weight scavengers such as glutathione (GSH) [10].

In the present study the effects of benzene on (TAO) status and (SOD), (CAT) levels were investigated in the serum of petrol station workers and in healthy control.

Material & Methods

This study was conducted on peoples who work in petrol station in different areas of Baghdad city. This study included 40 workers and 20 healthy volunteers who served as a control group. None of volunteers exposed to the benzene and its derivatives and none of them had clinical or laboratory evidence of disease that would affect the parameter to be measured. About 5ml of venous blood was drawn from the workers and from control group. The clear serum was separated and used for estimation of SOD, CAT and TOA level.

Measurement of SOD enzyme level

Assay Principle

The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical ($O_2^{\bullet-}$) produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. The superoxide dismutase activity is than measured by the degree of inhibition of the Formazan dye. One unit of SOD is that which causes a 50% inhibition of the rate of the reaction of INT under the condition of the assay.

RANSOD (Randox) CAT NO. SD 125

Calculation

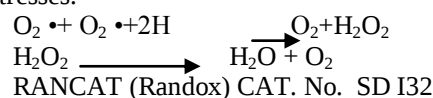
The SOD enzymatic activity is expressed as the percentage of inhibition of INT reduction. Where one unit of SOD is defined as the amount

of sample that causes 50% decreases in the SOD inhibition.

Measurement of Catalase Activity

Assay Principle

Catalase enzyme catalyzed the divalent reduction of H_2O_2 (at high concentration) to water. As for SOD, its activity could be induced by oxidative stresses.



Measurement of Total Antioxidant Status (TOA) Level

Assay Principle

(2,2-Azino-bis[3-ethylbenzthiazoline sulphnate] is incubated with a peroxidase (Metmyoglobin) and H_2O_2 to produce the radical cation. This has a relatively stable blue-green color, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree which is proportional to their concentration.

Total Antioxidant Status (RANDOX) Cat. No. NX 2332

Statistical Analysis

Statistical analysis was performed using analysis of variance (ANOVA) following the Mann-Whitney U-test to determine the difference in level of the enzymes studies. Student's *t*-test was used. $P \leq 0.05$ was considered significant.

Result

Serum superoxide dismutase (SOD) activity

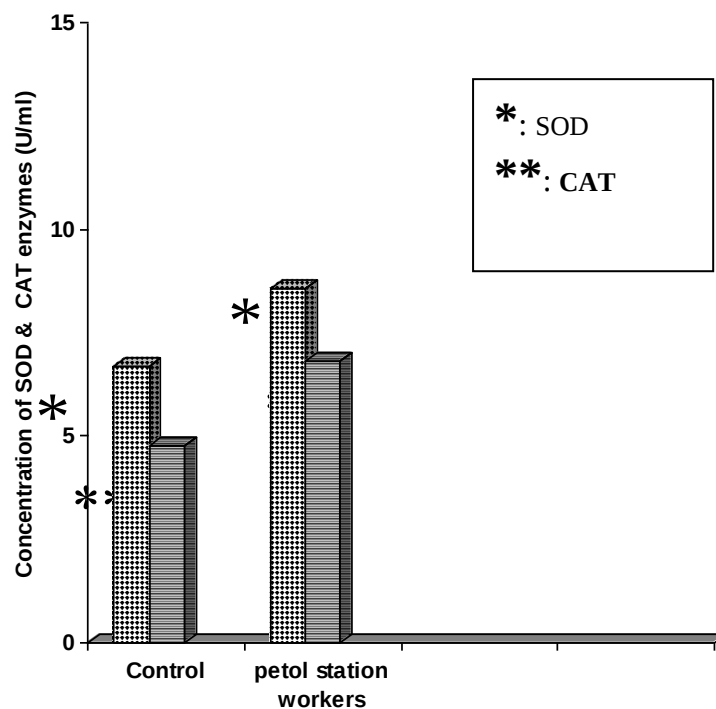
The total serum SOD activity in this study is shown in **table (1) and Figure (1)**. The results illustrated that the total serum SOD activity has a significant increase ($p < 0.01$) in workers when compared with healthy controls. The mean value in workers is (8.67 ± 0.2 U/ml), while, the mean of controls is (6.92 ± 0.153 U/ml).

Table (1): level of SOD in petrol workers and in control

Groups	Control	Worker	P-Value
Mean	6.92	8.67	$P \leq 0.01$
S.D.	0.153	0.255	
S.E.	0.09	0.24	

Table (2): Serum of catalase levels in petrol station workers and Control

Groups	Control	Petrol station workers	
Mean	4.76	6.81	$P < 0.01$
S.E	1.98	2.2	



Figure(1): The SOD and CAT enzymes concentraion
Serum Catalase (CAT) activity
 The total serum catalase activity is illustrated in table (2) and figure (1). The results show a significant increase in the total catalase activity ($P < 0.05$) in petrol station workers when compared with controls. The mean of workers is $(6.81 \pm 2.2 \text{ U/ml})$, while, the mean of controls is $(4.76 \pm 0.02 \text{ U/ml})$.

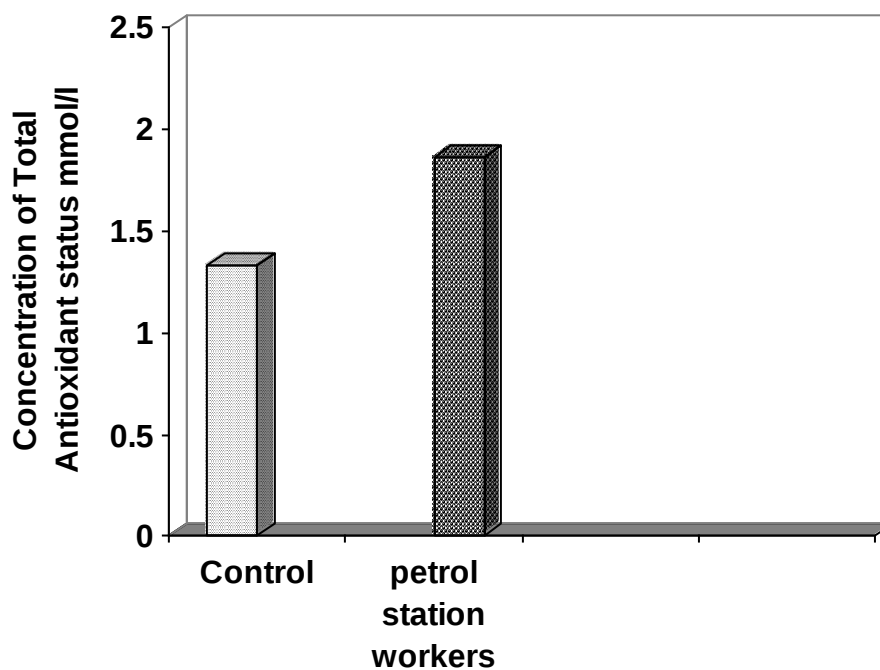


Figure (2): Total Antioxidant Status in Petrol Station Workers and Control Subjects

Serum Total Antioxidant Status (TAO) level activity

The total antioxidant status is illustrated in **table (3) and figure (2)**; the results show a significant increase in the total antioxidant

status ($P < 0.01$) in petrol station workers when compared with controls. The mean of workers is : (mean 1.86 ± 0.07 mmol/l). While, the mean of controls is (mean 1.33 ± 0.04 mmol/l).

Table (3): Total Antioxidant Status in Petrol Station Workers and In Control Subjects

Groups	Control	Petrol station workers	P-Value
Mean	1.33	1.86	P < 0.001
S.E	0.04	0.06	
S.D.	0.15	0.23	

Discussion:

The present study showed that benzene affected some serum enzymes activities in the petrol station workers. Cells have elaborated protective mechanisms to cope with potentially damaging molecules such as reactive oxygen species by synthesizing antioxidant enzymes^[11]. Superoxide dismutase (SOD) is a key enzyme in cellular defense systems that disproportionate O^{2-} into oxygen and H_2O_2 , with the latter being detoxified by glutathione peroxidase or catalase to H_2O molecules and O_2 . The present study demonstrated that petrol derivatives pollution resulted in high oxidative stress in the petrol station workers. A long period exposure to petrol derivatives was reported to produce significant alteration in antioxidant enzymes such as (SOD) and (CAT) and on the total antioxidant status that result from oxidative stress^[11].

Benzene may directly affect organelles at the cellular level in various tissues, which will indirectly influence enzyme activities such as SOD and CAT. Thus, it was shown that some properties of membrane phospholipids were changed^[12]. The main hepatic metabolites of benzene are phenol, catechol and hydroquinone. Microsomal metabolism of benzene plays a critical role in benzene toxicity^[13]. The above mentioned and other intermediate products reach target tissues and cause much of direct or indirect damage. Such damage occurs either by attachment to molecules such as DNA, protein, carbohydrates and their smaller components or different types of enzymatic activity. The reason for the high enzyme activities in our experimental group may be that benzene affects the metabolism through some metabolites such as phenol and benzene oxide^[14], and that cause an increase in the formation of reactive oxygen species and as a response to increased formation of free radical, the formation of antioxidant enzyme is increased.

Cu-Zn SOD referred to as SOD 1 and Mn SOD as SOD 2 are the two eukaryotic SOD's.

Cu-Zn SOD located in the cytosol is expressed constitutively and is considered as a house keeping enzyme; in contrast to Mn SOD which is located in the mitochondrion. This enzyme links directly with CAT enzyme. When the superoxide anion increase as a response to oxidative stress, the concentration of superoxide dismutase enzyme is increased. As a result to this process the formation of H_2O_2 was increase. CAT enzyme converts hydrogen peroxide to H_2O and O_2 molecule. So as a result to increase formation of H_2O_2 , the formation of CAT is increased. So these two enzymes works together to eliminate the reactive oxygen species (ROS) by converting this substance to non harmful molecules.

In this study, both SOD and CAT enzymes activities were increased in workers in comparison with control. This is because these two enzymes have related functions. SOD catalyzes the dismutation of superoxide anion radical to H_2O_2 and H_2O . The H_2O_2 is detoxified to H_2O and O_2 molecule by CAT. Due to the inhibition effect against oxyradical formation the SOD-CAT system provide the first defense line against oxygen radical toxicity^[15] and usually used as a biomarker or indicator for ROS production^[16]. Our study suggest that SOD, CAT plays an important role as an antioxidant against ROS that generate during the long period of exposure to the petrol derivative, and subsequently, in the maintenance of cell turnover in petrol station workers.

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