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Determination of antioxidant levels in smoker men affected with polycythemia

Dakhil Ghani Omran Al-Watify[1]*, Mayadaa Abood Mahmood Ferhan[2]

University of Babylon, College of Science for Women , Department of Biology/ Iraq

Abstract : The current study was involved to investigate some oxidant–anti-oxidants parameters of smoker men affected with polycythemia.

One hundered twenty (120) men were recruited in this study ,of them, sixty (60) smoker men affected with polycythemia and the remaining number (60)men were healthy men and serves as control group in this study . all ages of the enrolled subjects were limited between 30-60 years old .According to their ages, they were classified into three groups, first group(30-39 years old),second group(40-49 years old), and third group(50-60 years old).

All patients included in this study have PCV higher than of their healthy counter parts and showed a remarkable , increase($p<0.05$) when compared with healthy control men. Moreover, levels of PCV have a positive correlation ($r=0.8$) with advanced ages. concerning lipid peroxidation, malondehyde (MDA)as markers of lipid peroxidation was used, an its results revealed a marked elevation ($p<0.05$) in all groups of polycythemic patients and also showed a positive correlation with age ($r=0.4$) and PCV($r=0.4$).

Regarding markers of antioxidants, results of reduced glutathione (GSH, non enzymatic antioxidants, were significantly decreased ($p<0.05$) in all tested groups in matching with those healthy control group. it was found that results of GSH proportionate inversely with age ($r=0.2$) and PCV($r=0.9$).about catalase activity , results of its activities indicated a marked drop ($p<0.05$) in three age groups of smoker polycythemic men in a comparison with control groups . the activities of catalase pointed out a negative correlation with both age and PCV($r=0.3$,$r=0.6$ respectively).

Serum superoxide dismutase (SOD)activity were also significantly down regulated($p<0.05$) in all age groups of patients when matched with those control group .The SOD activities were inversely proportionated with age and PCV($r=0.5$, $r=0.5$, respectively).

Finally, according to results that mentioned above the possible explanation to these findings can be showed that high mass of RBCs and toxic materials produced by smoking can be implicated with drop of antioxidant activities with increase lipo peroxidation. Moreover, aging can be exert negative action on these anti-oxidants that associated with increase lipid peroxidation marker (MDA).

Key words : polycythemia, antioxidants, lipid peroxidation.

Introduction

Polycythemia is a hematologicals disturbances results from increase number of red blood cell in unit of blood volume that principally determined by packed cell volume[1]. The word polycythemia is derived from "poly" means many and cythemia indicates blood cells[2].

Moreover, polycythemia can be classified according to specific disease that leads to incidence of polycythemia .The first type is called primary polycythemia (Polycythemia Vera or Myeloproliferative neoplasms (MPNs) [3]. this disorder occurring due to specific state which leads to abnormal heightening in the count of erythrocyte because of mutation in Janus Kinase[4]where essential erythrocyte originated in loose inner part of bone marrow where there specialized cell found there[5].

Second type of polycythemia called Secondary polycythemia can be defined as a physicological reaction that the body acts to improve the oxygen-carrying capacity of the blood. Secondary polycythemia no considermyeloproliferative neoplasm[6]. Secondary polycythemia arise from causes including (high altitude, heart and lung diseases, Overproduction of erythropoietin ,and Cigarette smoking,) [7].

Oxidative stress is a condition included the oxidation increased because the regulation between oxidative stress (free radicals) and antioxidant system in body is lost [8] .OS is state not only causes for events which can consider hazardous for example lipo-peroxidation and oxidative damage of DNA, but also can represent phenomena of physiologic adaptation and intracellular signal transduction regulation[9]. The term of free radicals emerged after the world war ll (1939-1945) by both Gershman and Gilbert in 1954 where suggested that the deadly effect of ionizing radiation may been attributed to production of free radicals or reactive oxygen species (ROS)[10].

Superoxide anion will scavenged by superoxide dismutase which represent the first enzyme for protection from reactive oxygen species, which catalyzed the superoxide radical (O2'−) to O2 and H2O2 [11] to produce H2O2. mitochondria don't contain catalase ,thus, it will avoid the toxic effect of H2O2 by glutathione peroxidase which convert H2O2 into water ,H2O2 can generate high activity OH which also results in extensive damage for DNA , lipids and protein[12]. Lipid peroxidtion is chain reaction begin with hydrogen removing or addition of oxygen radical and the result is oxidative injury of poly unsaturated fatty acids[13]. lipid peroxidation can be enzymatically occuring by lipid peroxidation enzymes such as lipoxygenases family and non enzymatically occuring by reaction of a FR molecule with poly-unsaturated fatty acids[14].it is naturally occurring product of lipid peroxidation[15].

Malondialdehyde (MDA), a normally can represent end product of membrane lipid peroxidation, which is one of the most biomarkers which used for free radical mediated injury [16]which can be defined as highly reactive three carbon aldehyde compound produced from poly unsaturated fatty acid peroxidation[17].thetripeptide glutathione is the thiol compound found in the greatest levels in the cells of all organs. Glutathione performs variant physiological functions such as defense againstfree radicals [18].

Catalase is very essential enzyme that found in all living organisms that exposed to oxygen ,whereits one of the main antioxidant enzyme [19] .catalase is an important antioxidant enzyme that dismutates hydrogen peroxide into molecular oxygen and water [20].Superoxide dismutase is enzyme that detoxifies superoxide O2− . It is essential process because superoxide is dangerous compound (reactive form of oxygen) [21]O2·- leak from electron transport chain and damage the cell through mutations in DNA , attack enzymes that make protein and other essential molecules[22].

Experimental

The subject of the study

The present study was essentially under taken to show oxidative stress , anti-oxidant systems. the study was initiated from October 2015 to April 2016 .the number of subjects that included in this study was 120 of those, sixty smokers' patients affected with polycythemia and subdivided according to their ages into three sub-groups ,first group 30-39 years old, second group 40-49years old, and third group 50-60 years old.

The remaining subjects(60) were used as a control group and also they were divided into the same age groups used for patients . The blood bank inHilla was the common station to take blood samples from patients attending to center to perform phlebotomy to restore their PCV within normal values . All patients and healthy subjects were diagnosed by consultant physicians that are presented in blood bank.

Blood samples collection

The blood samples were collected daily at morning between 8-9 o'clock. The anticubital vein was washed with alcohol solution (70%) and then left to dry, five milliliter (5ml) was drown and put in tubes without anti-coagulant (plain tubes) to permit the blood clotting , and left for five minutes. Plain tubes were transferred to centrifugation at 3500 for 10 minutes to ensure isolation of serum that kept within epindroff tubes at 20°C for further biochemical analysis.

Determination of Malondialdehyde (MDA) concentration:

The level of MDA(product of lipid peroxidation)was applied as mentioned by modified procedure [46].

Estimation of serum reduced glutathione (GSH) level

Determination ofGSH concentration by[47].

Measurement of Catalase Activity

The method that previously illustrated by[48]was used in this study to measure catalase activity. The principle of this method depends on the fact that Catalase in the sample can degrade hydrogen peroxide H2O2 substrate to water and molecular oxygen (O2). Degradation of H2O2 is accompanied with decrease absorbance at wave length 240 nm, and activity of catalase is measured according to differences absorptions per unit of time .

Measurement of superoxide dismutase (SOD) activity

The activity of SOD was determine according to method illustrated previously that involve catalysis of SOD to epinephrine substrate .The differences of absorption per unit of time was used to estimate of SOD.the wave length of SOD absorbance was480nm .

Statistical analysis:

The program spss of computer was used to analysis data of the present study. The explained data in this study were means ± stander deviation. The examination of the differences among groups of study was performed by using student's test and lowest significant was $P< 0.05$[49].

The Results

Packed cell volume (PCV)

The results of PCV in figures (1), (2) pointed out a progressive elevation ($p< 0.05$) in three tested groups of polycythemia with the ages(first group, second group, third group), when matched with their counter parts of healthy groups for the same ages.

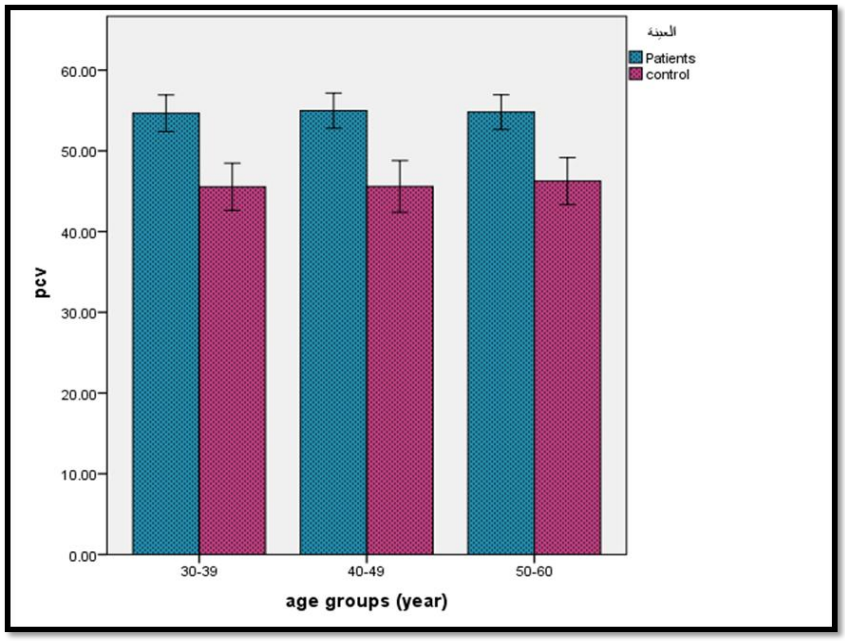


Figure (1) Means of (PCV %) of healthy and patients groups of smokers men affected with polycythemia.

First group Second group Third group

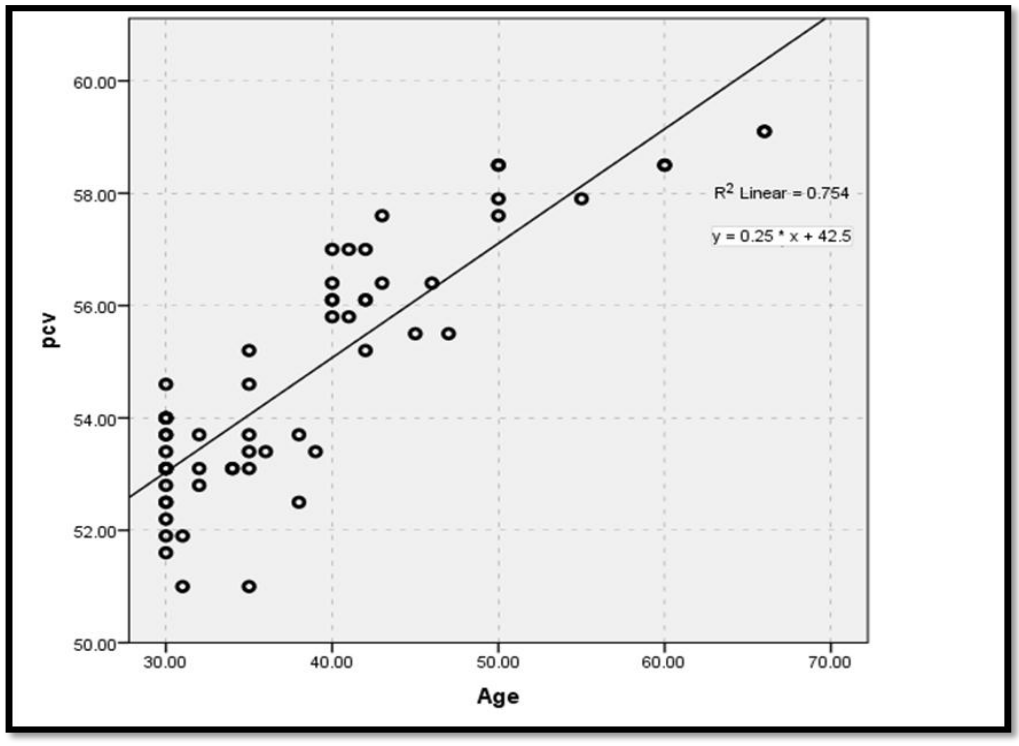
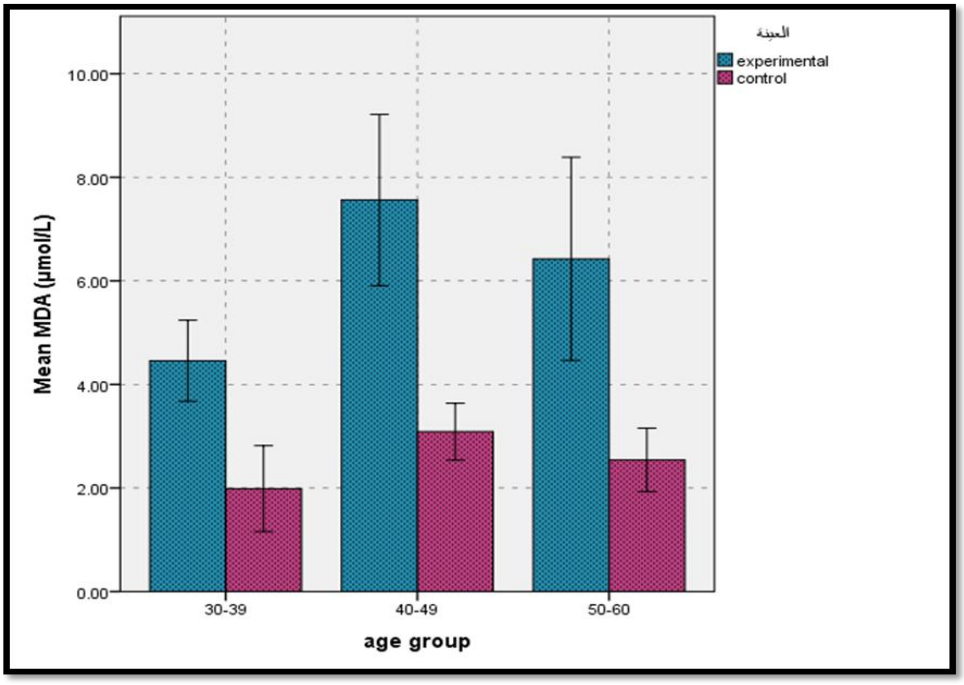


Figure (2) :Correlation coefficient between packed cell volume (PCV%) with the age in smokers men affected with polycythemia.

Levels of serum Malondehyde (MDA)

The results of malondehyde concentration in figure (3)appeared markedly increase ($p< 0.05$) in three age groups of smokers' polycythemia (first, second, third group), when compared to that of healthy control groups .



First group Second group Third group

Figure (3)Means of Malondehydeconcentrations (µmol/L) of healthy and patients groups of smokers men affected with polycythemia.

Results of MDA showed a positive correlation with age groups (R=0.4) and illustrated in figure (4).

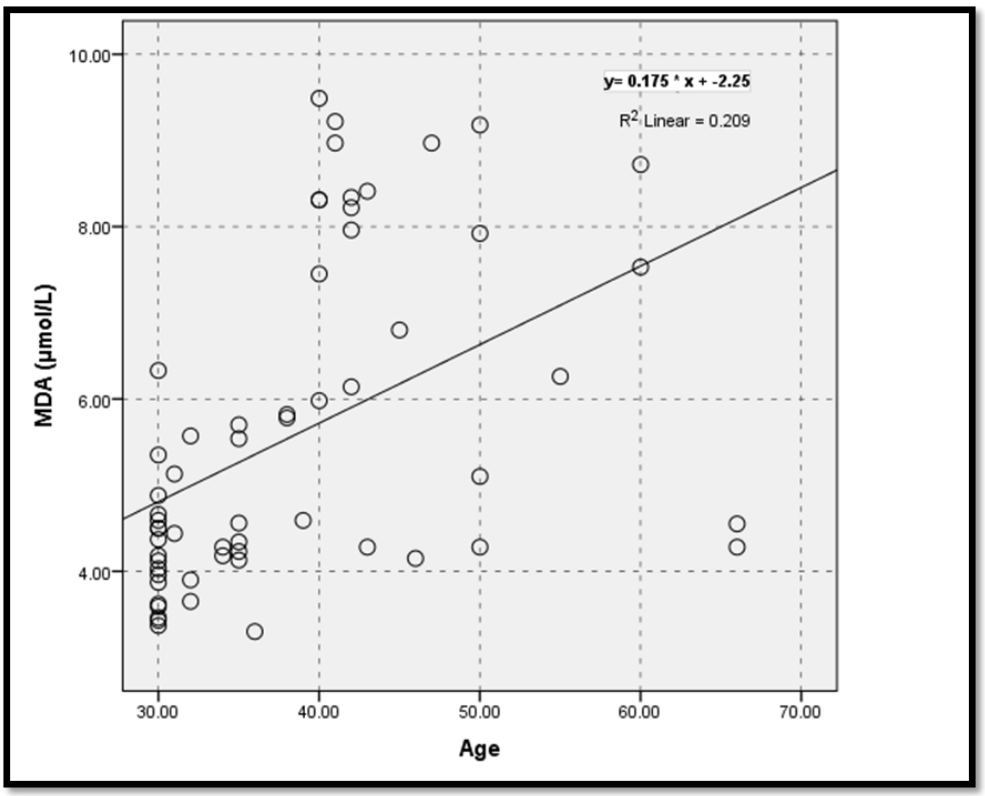


Figure (4)Correlation coefficient between Malondehyde concentrations (MDA) with the age in smokers men affected with polycythemia.

The results of correlation coefficient between PCV values and malondehyde concentration showed positive correlation (R = 0.4) as described in figure**(5)**

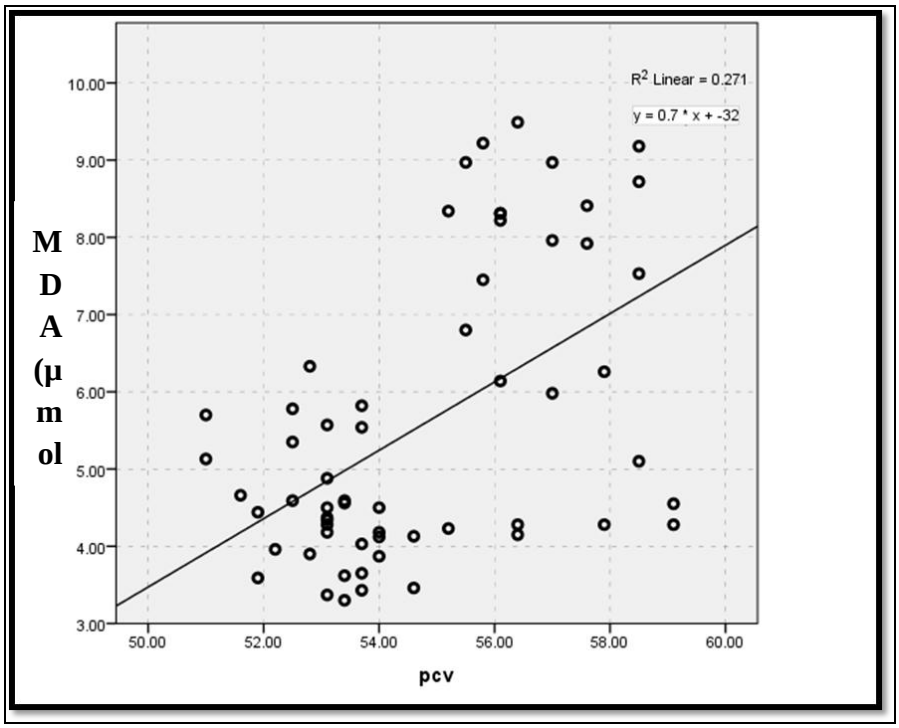
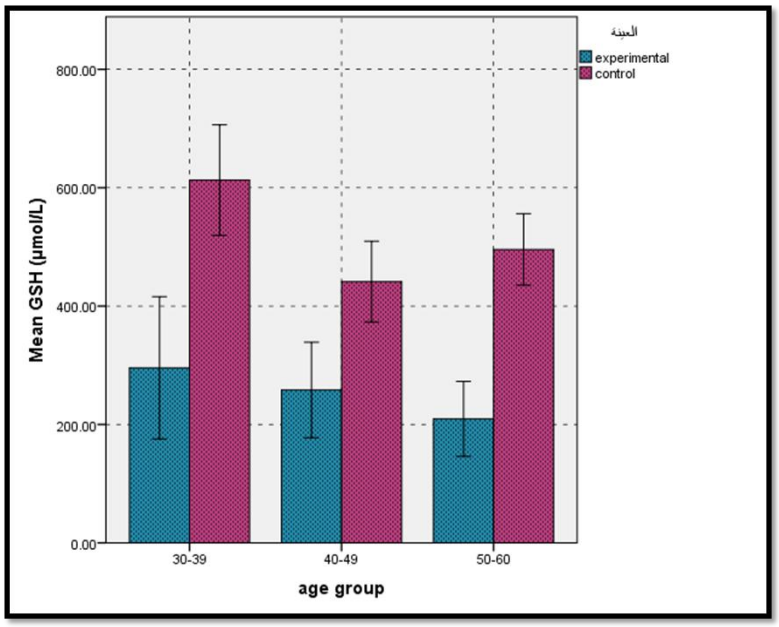


Figure (5)Correlation coefficient between Malondehyde concentrations (MDA) with the packed cell volume (PCV%) in smokers men affected with polycythemia

Levels of serum reduced glutathione (GSH) concentration

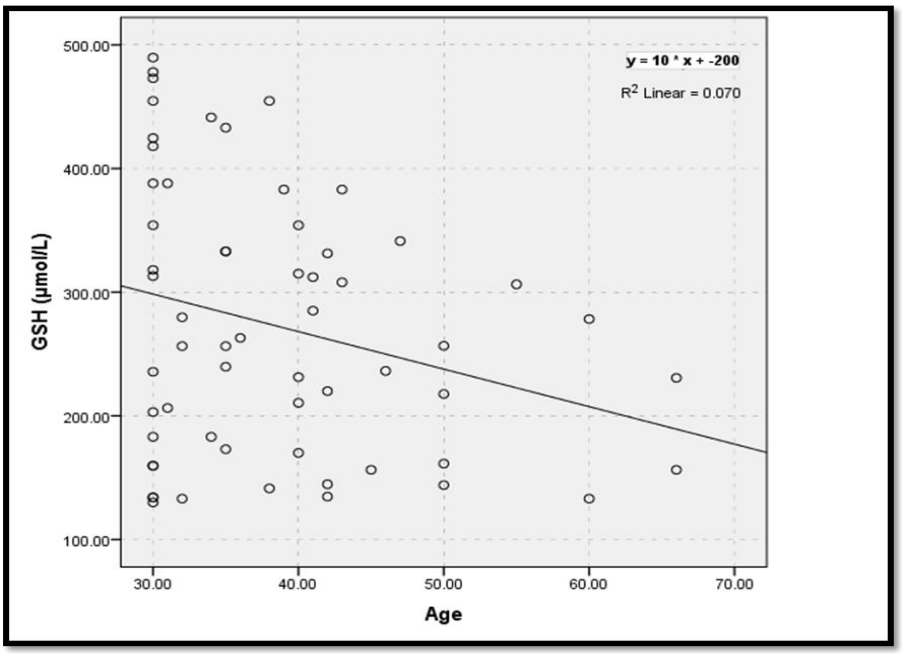
The results of GSH concentration in figure (6)appeared markedly drop ($p < 0.05$) in three groups of smokers' polycythemia (first group, second group, third group), when compared to that of healthy control group.



First group Second group Third group

Fig(6) Means of GSH concentrations of healthy and patients groups of smokers men affected with polycythemia.

There is a negative (r=0.2) correlation coefficient, as illustrated below in figure (7) between GSH and age groups of polycythemic smoker' men.



Age group (years)

Figure (7)Correlation coefficient between Glutathione concentration with the age(years) in smokers men affected with polycythemia

The results of correlation coefficient between PCV values and GSH concentrations indicate a significant negative correlation (r = 0.9) as indicated in figure(8).

GSH (µmOl / L)

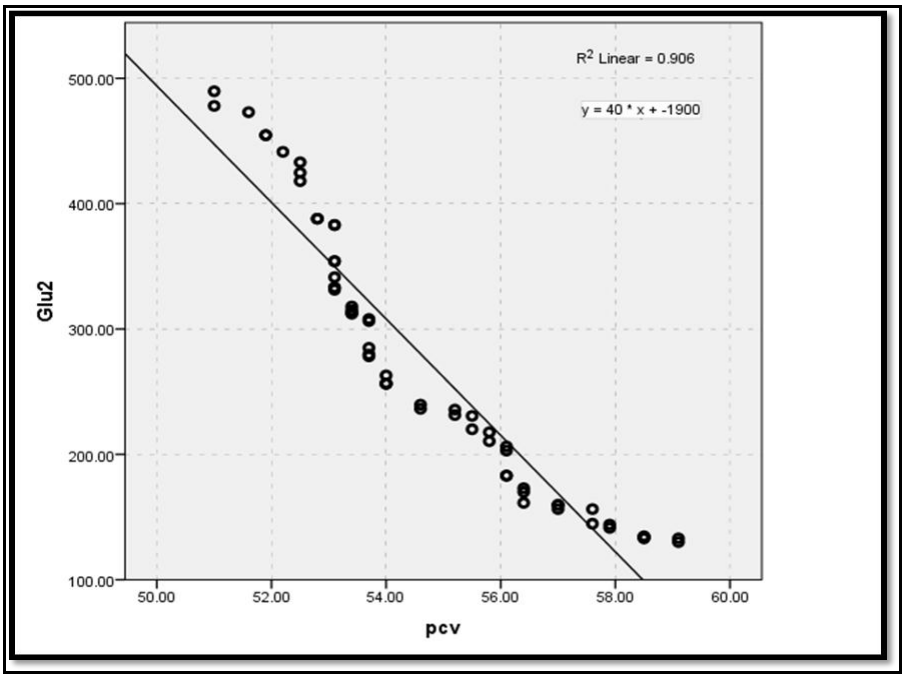


Figure (8)Correlation coefficient between GSH concentrations with the packedcell volume (PCV%) in smokers men affected with polycythemia

Levels of serum catalase activity

Catalase enzyme activity values illustrated in figure(9) showed significant drop ($p< 0.05$) in three age groups of smokers' polycythemia (first group, second group, third group), when compared to that of healthy control groups .

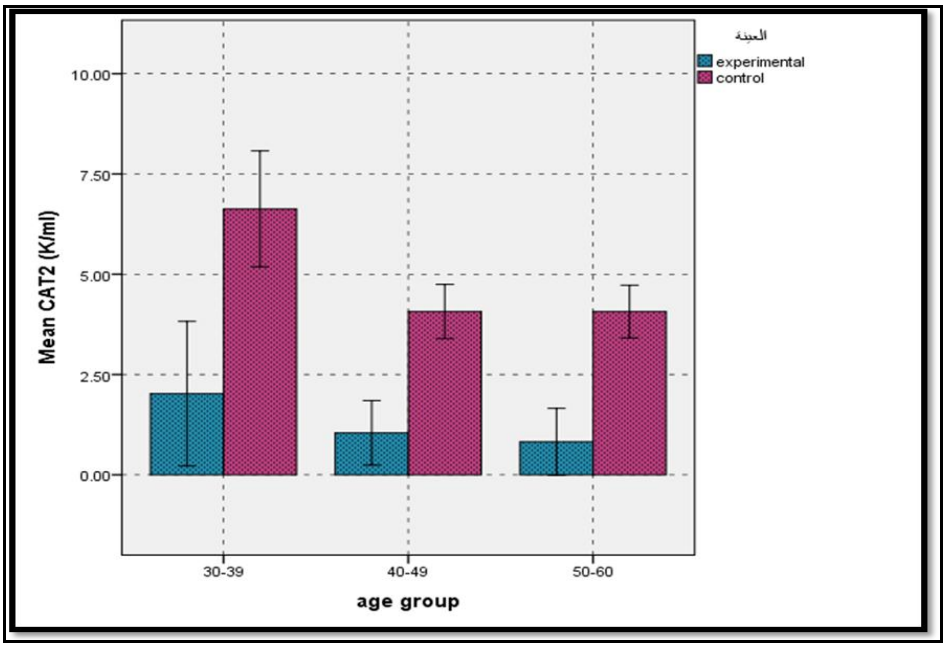


Fig (9)Means of catalase activity (K/ml) of healthy and patients groups of smokers men affected with polycythemia.

First groupSecond groupThird group

The figure (10) illustrated below indicated that values of catalase were correlated negatively (r=0.3) with age groups of polycythemic patients

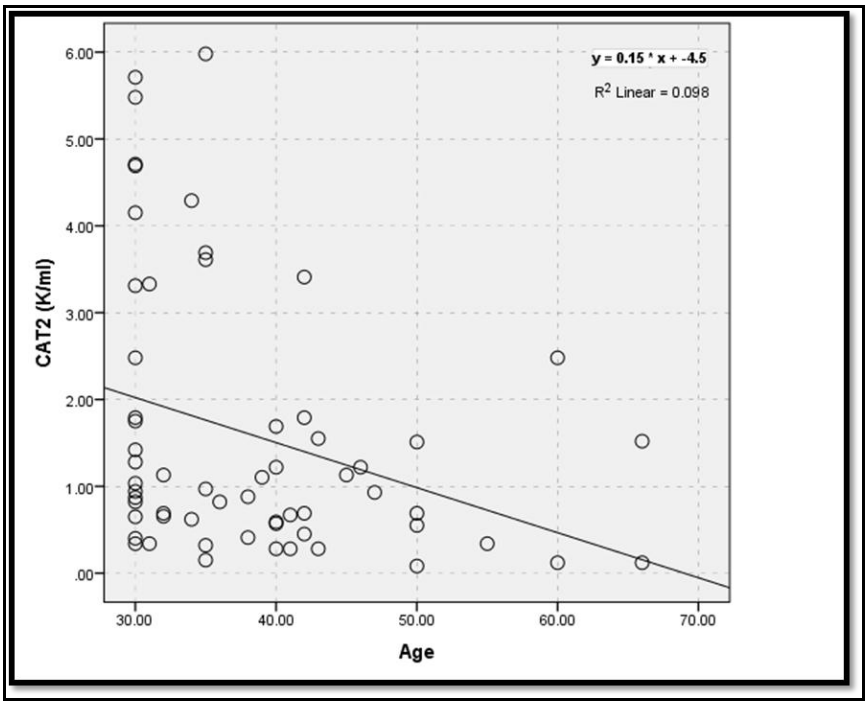


Figure (10)Correlation coefficient between catalase activity with the age in smokers men affected with polycythemia.

The results of correlation coefficient between PCV values and catalase activity indicate a significant negative correlation (R = 0.6) as indicated in figure (11)

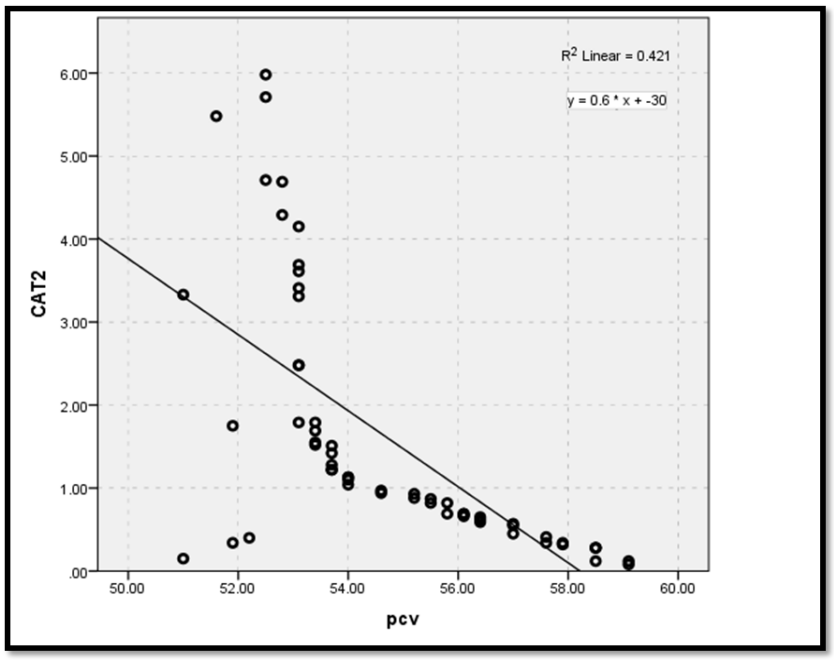
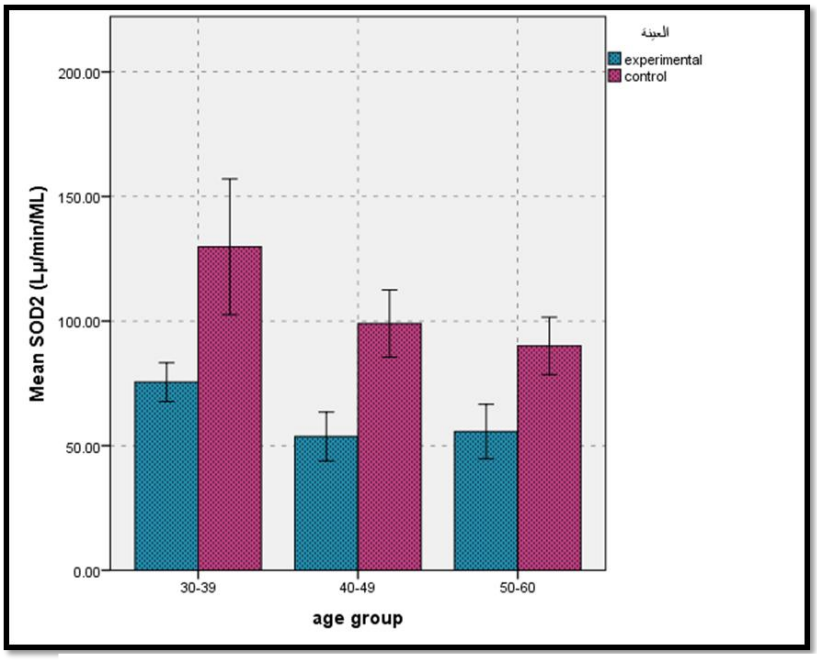


Figure (11)Correlation coefficient between catalase activity (CAT) with the packed cell volume (PCV%) in smokers men affected with polycythemia.

Level of Serum Superoxide Dismutase activity (SOD) The results which are explained in figure (12) showed a significant decrease ($p < 0.05$) of SOD in three groups of smokers' polycythemia (first group, second group, third group), when compared to healthy control group .



Figure(12) Means of SOD activity of healthy and patients groups of smokers men affected with polycythemia.

There is a negative (r=0.5) correlation coefficient, as illustrated below in figure (13) between SODand age groups of polycythemic smoker' men.

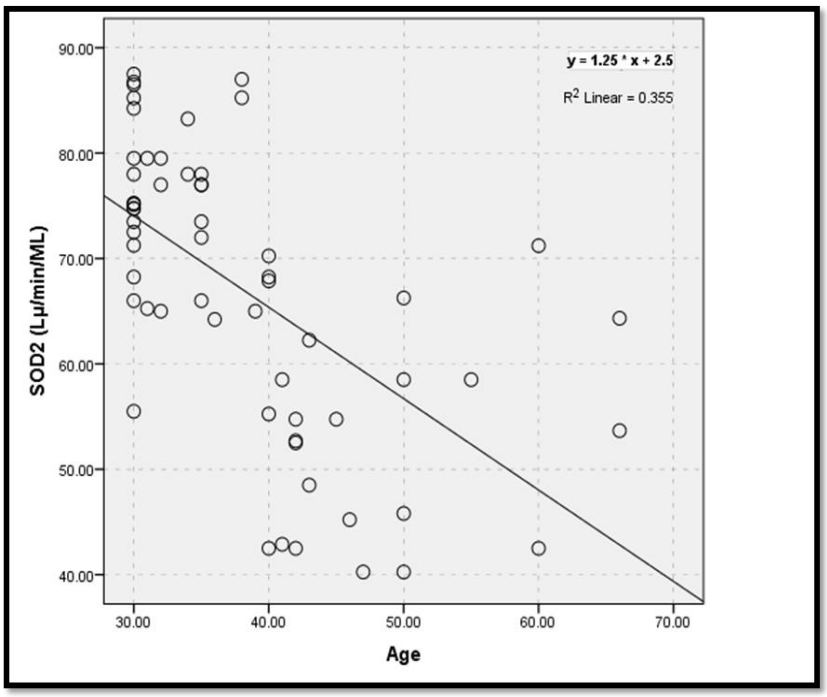


Figure (13)Correlation coefficient between superoxide dismutaseactivity with the age in smokers men affected with polycythemia.

The results of correlation coefficient between PCV values and SODconcentrations indicate a significant negative correlation (R = 0.5) as infigure (14)

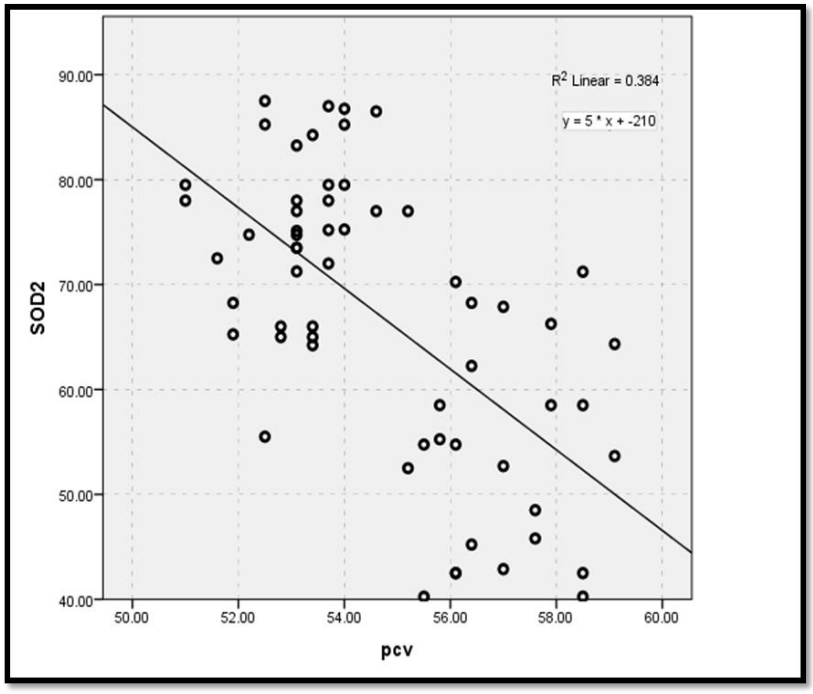


Figure (14)Correlation coefficient between SOD activity with the packed cell volume (PCV%) in smokers men affected with polycythemia.

Discussion

It is well documented that MDA represents the common marker of lipo-peroxidation[23]Previous data which obtained by study of Waseem *et al*., (2012) who indicated that serum levels of MDA were progressively greater in patients complained from chronic obstructive disease which leads to drop of PO2 in body tissues . Also, MDA concentration were estimated to be elevated both pulmonary cancer and tuberculosis disease[25].Recent study of Jain *et al*., (2015)which noted that elevation in MDA happened because high energy requirments causes to consume greater of oxygen molecules for metabolic activities. Excess oxygen can cause several abnormalities and result in oxidative stress(OS) . Dakrory*et al*. , (2015)indicated that elevation in MDA level associated to the injury happen in erythrocytes because of generation of free radicals via mitochondria.Shohag *et al* .,(2012)who found that serum MDA concentration were progressively greater in Obsessive-Compulsive Disorders (OCD)than of controls and who suggested greater reactive oxygen species in Obsessive -Compulsive disease (OCD) and therefore show some range of tissue injury because of oxidative stress.

Our data are consistent withAl Salhen and Abdalslam , (2014)who revealed that the concentration of MDA in smokers group were remarkedly elevated when matched with control group . other study estimated MDA concentrations in normal healthy smokers and non-smoker subjects. and noted that MDA concentrations were progressively elevated in normal smokers than of non-smokers[28].the significant increase of MDA concentration noted in smoker subjects in this research, can been attributed to one or more of the causes are : one, smoker subjects are generate too oxidation from inspiration of abundant volumes of gas-phase and other free radicals causing elevate oxidative injury[29].

The present findings consistent with the data of Naga and Manohar, (2013)who have documented the effect of smoking on enzymatic antioxidant and who established markedly lowered glutathione peroxidase activity in smoker subjects and documented that red blood cell GPXpotency is higher sensitive and a prominent marker of smoking-generated free radicals. Also reduce glutathione (GSH), which refers to dysregulation between antioxidant and oxidant system in the hepatic tissue. As GSH represents the common significant components of antioxidants[31].

In previous studies [32]explained that thereis reversible association between reduced glutathione level and lipo- peroxidation .Also, it is documented that there is significantly lowering in GSH with elevation MDA level in advanced age of both sexes [33] and the down regulation of GSH concentration to 20%-30% causes abnormalities of the cell by free radicals[34].

Recent study of Li *et al*., [35]which documented that there is a marked fall of GSH concentration which explains happen of free radicals which is implicated as one of the most pathological processes that produces in propagation and development of different hepatic tissue and hematological diseases, such as virus affecting liver (hepatitis), alcohol – generating disease . alsoestablished that oxidative stress elevate with aging and this result in drop of activity of antioxidants levels[36]. Our data agrees with other studies which, pointed out a drop in catalase efficiency which acts on free radicals that produced abnormalities affecting cardiovascular system .also, established that catalase roles down regulation with the advanced age in males[37 ,38].

Recent study of Metta*et al*. [39]which indicated a decrease in catalase activity (CAT) and superoxide dismutase reactivity in the red blood cell of smokers in a compared to nonsmokers.it is well established that, reduction CAT role, which is most modulator of hydrogen peroxide(H2O2)degradation (enzymatically degrades hydrogen peroxide compound into oxygen (O2)and water (H2O) and thus depress it effects). Overproduction of hydrogen peroxide compound may leads to remarkable injuries to cellular proteins, nucleic acids DNA, RNA, and lipids. Low CAT activity is consistent with the suggesting that mean long-term exposure to OS may take part to the progression of a different of late-onset disease.

Lowering SOD levels were established in tumor disease[25].Our data agree withGiergiel, and Kankofer, (2015)who reported that SOD level become down regulation with the aging .the present data are consistent agree withOrhan *et al*. , (2005) , who confirmed statistically drop levels of GPX and SOD in the red blood cells of smokers. the enzymatic antioxidant activities including glutathione peroxidase (GPX) , superoxide dismutase (SOD), and glutathione (GSH) were reduced markedly in serum with the progress of age[33].the urothelial tumor of the urinary bladder significantly decrease in catalase and Cu, Zn-SOD levels in tumor tissue

versus normal urothelium[42]. the enzymes stimulation of antioxidants such as catalase and SOD may be a morepotent process than non- enzymatic antioxidants (such as vitamins C and E), at best, stoichiometrically remove a very little level of total oxidant synthesis[43] .

There are correlation coefficient recognized between SOD values , hemoglobin (Hb) levels , RBCs count, and percentage of reticulocyte. In all condition , these levels are not significant ($p > 0.05$)[44]. These finding confirm that the SOD level is not affected by secondary agents and therefore conclude that the SOD value explains in each group of affected patients an intrinsic elevation of SOD expression in the erythrocyte.

It is well documented that the SOD activity is well heightened significantly in middle and aging group, On the other hand, the catalase activity appeared more significantly in young age[45-50].there is a markedly elevation GPX activity of liver in the group of children affected with chronic hepatitis whereas non-markedly elevation was noted in SOD and CAT expression of liver[21].

The our possible explanation to these changes based on the facts that the activity of SOD is progressively decrease because of exhaustion of catalytic activity of enzyme to scavenge and remove free radicals generated by smoking.

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