(Study of the Influence of Cigarette Smoking on Serum Oxidant and Antioxidant Status)

Safaa H. A. Al-Ghzi *

Abstract

The use of tobacco is a word wide problem and the traditional method of tobacco use is smoking cigarettes. This study was aimed to investigate the balance state in oxidation system (by measuring malondialdehyde (MDA) levels lipid peroxdation marker) - antioxidant system (by measuring ceruloplasmin (Cp) antioxidant marker) and attempted to shed a light on the possible correlation between (MDA), (Cp) and age of smokers and period of smoking.

Sample size was (49) healthy adult men smokers between the ages of 19-32. The results showed a presence of a significant increase in MDA levels in all smoker groups ($P \leq 0.05$) in comparison with control group (nonsmokers). Also, the results evinced the presence of a significant decrease in ceruloplasmin levels ($P \leq 0.05$) in comparison with control group (nonsmokers).

Our results confirmed the role of reactive oxygen species (ROS) in development of oxidative stress disorders, where serum MDA was found to be positively correlated with period of smoking. Whereas the results showed the presence a negative correlation between ceruloplasmin and period of smoking. Also proved no relationship between age of smokers and MDA levels and Cp levels.

Keywords: Somking, Oxidants, Antioxidants, Malondialdehyde, Ceruloplasmin.

*** Assistant lecturer, Biochemistry, Al-Shatra technical institute**

Introduction

Smoking, in simple words, mean drawing and exhaling smoke from a cigarette, cigar, or pipe etc. it is one of the most common forms of recreational drug use. According to world health organization tobacco smoking is today the most popular form of smoking and practiced by over one billion people in majority of all human societies and approximately 47% of men and 12% of women smoke worldwide [1, 2]. Cigarette smoking is a major public health concern worldwide and is responsible for 5 million deaths each year [3]. During the last decades, many carcinogens have been identified in tobacco smoke, and smoking is now classified as cause of cancers of lung, or pharynx, larynx, esophagus, kidney, liver, uterine cervix, stomach, bladder, pancreas, nasal cavity and paranasal sinuses, as well as leukemia, but is also associated with heart disease and vascular disease [4]. Environmental tobacco smoke has been shown to contain high amounts of polycyclic aromatic hydrocarbons (PAHs) many of which have been shown to be potent carcinogens [5]. In a rat model, exposure to PAHs rapidly induced palpable mammary tumors [6]. Indeed, cigarette smoke contains numerous toxic compounds [7]. These compounds can inter act with DNA, lipids, and proteins in multiple organs to alter their normal physiological activities and ultimately lead to adverse health effects [8]. Oxidative stress is accepted as the initial step in the biological effect after cigarette smoking [9]. Chronic cigarette smoking is a well-known risk factor for developing coronary atherosclerosis via oxidative stress [10]. Generally, oxidant stress caused by smoking may damage biologic molecules such as protein, DNA and lipid molecules, and the degree of lipid peroxidation could be investigated with malondialdehyde (MDA), which is the break down product of lipid peroxidation [11]. To cigarette smoke may cause oxidant stress and also decrease the availability of certain antioxidant nutrients [12, 13]. There are in consistent findings about the effects of smoking on dietary antioxidants and oxidant metabolites [14]. The nutritional status of smokers may become promised by an inadequate diet such that smoking might be related to a lower dietary intake of antioxidants [15].

Antioxidant is any substance that when present at a low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate [16]. This system includes the enzymes superoxide dismutase (SOD) and catalase. The body has several other important antioxidants. They are low molecular weight metabolites being either synthesized in the cell or supplied from diet. Ceruloplasmin (Cp) is a blue multi-copper oxidase which is found in the plasma of vertebrate species. The protein, which contains greater than 95% of copper (Cu) in the plasma, is synthesized mainly in the liver as a single-chain polypeptide and secreted into the plasma as a α_2-glycoprotein [17]. Ceruloplasmin is an enzyme which has a role as an oxidant or antioxidant depending on the existence of Fe ions and similar material levels in the micro base, and it also scavenges superoxide anion radical ($\bullet O^{-2}$) [2] [18]. It also stops lipid peroxidation by direct oxidation of cations. As a result, membrane lipid oxidation is controlled [19]. The catalytic cycle involving four of the six coppers associated with Cp that employ dioxygen as the terminal electron acceptor without the intermediacy of a partially reduced oxygen species such as ($^{\cdot}O_2^-$) or H_2O_2 [20,21,22].

Material and methods

- Blood samples collection

49 healthy university students aged 19-32 (38 smokers daily for at least 3 years) and 11 non-smokers volunteered to enter the study, with no history of diabetes, autoimmune diseases or exposure to radiation and chemotherapy. Blood samples were collected by venous puncture about 5 ml of their blood were collected in sterile, dry tubes and the plasma was separated by centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in a clean tube in the refrigerator at 2-8ºC until the time of assay.

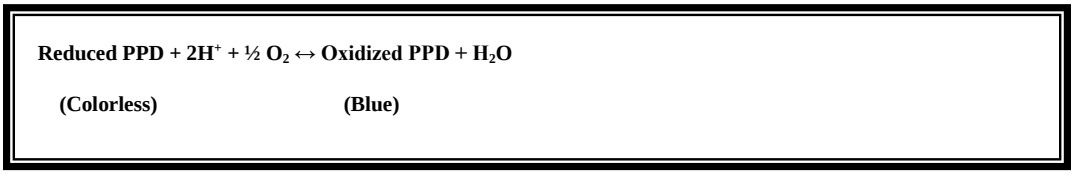
The subjects were classified into four groups as follows: (1) nonsmoker group 0 (n=11), (2) smoker group I (n=8, smoking 3_10 cigarettes per day), and (3) smoker group II (n=12, smoking 10 _ 20 cigarettes per day), (4) smoker group III (n= 18, smoking more than 30 cigarettes per day)

- Determination of serum malondialdehyde (MDA)

Lipid peroxidation was evaluated as the concentration of malondialdehyde (MDA) thus provides valuable information for evaluation of oxygen radical-induced oxidative stress. In our study, MDA was assayed by the method of published earlier [23]. The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated. The absorbance of clear supernatant was measured against reference blank at 535 nm.

- Determination of Serum Ceruloplasmin (Cp)

Plasma Cp concentration was measured by the method published earlier [24]. It is based on the ceruloplasmin-catalyzed oxidation of colorless para-phenylene diamine (PPD) to blue-violet oxidize form. The reaction is followed photometrically and the blank value is determined after inhibition of the enzyme with sodium azide at (0ºC).



A mixture of serum, substrate and acetate buffer at pH = 6.0 was incubated at 37°C for 15 min. The reaction is stopped by the addition of sodium azide, and the absorbance of the purple color formed (Oxidized PPD) in the diluted test mixture is read at 525 nm against blank solution. The corrected absorbance is directly related to the concentration of Cp [25].

- Statistical analysis

Analyses were performed using SPSS (Version19.0 software). For comparison between groups, analysis of variance followed by multiple comparisons by T-test analysis. Significance was set at $P<0.05$. All values were expressed as means ± SD.

Results and Discussion

The host information of all smokers and nonsmokers subjects is summarized in table (1).

Table (1): Data of the volunteers.

No.	age	Number of cigarette / day	period of smoking	gender
1	22	……..	……..	male
2	29	……..	……..	=
3	23	……..	……..	=
4	24	……..	……..	=
5	22	……..	……..	=
6	22	……..	……..	=
7	22	……..	……..	=
8	29	……..	……..	=
9	25	……..	……..	=
10	20	……..	……..	=
11	19	……..	……..	=
12	22	60	9	=
13	26	4	2	=
14	25	30-40	11	=
15	32	8	4	=
16	26	2	1	=
17	22	5	2	=
18	22	60	4	=
19	20	20	4	=
20	23	40-60	3	=
21	23	30	4	=
22	22	30-40	10	=
23	22	10-15	5	=
24	23	10	1	=
25	22	10-20	5	=
26	23	30-40	6	=
27	21	20	6	=
28	22	20	2	=
29	22	20	2	=
30	22	30	1	=
31	21	20	4	=
32	23	10	5	=
33	22	30	5	=
34	21	20	7	=
35	19	30	6	=
36	21	30	3	=
37	26	20	10	=
38	32	40-60	10	=
39	28	5-10	1	=
40	23	40-50	3	=
41	25	40-50	7	=
42	20	20	2	=
43	23	3	1	=
44	21	30	1	=
45	21	30	6	=
46	25	20	3	=
47	25	20	6	=
48	23	30-40	7	=
49	26	60	17	=

- Determination of serum malondialdehyde (MDA)

Results of determination of serum MDA concentration in groups (I, II, III) and control group (0) are given in table (2), illustrate a significant elevation ($P < 0.05$) in serum MDA levels in groups (I, II and III) when compared to control group (0).

The increase of MDA levels as reported in the present study; this agrees with other works [26, 27]. The rising of MDA level is directly associated with the degree of lipid peroxidation which is one of the most important measurement of oxidative stress in smokers. Also oxidative stress is accepted as the initial step in the biological effect after cigarette smoking [28]. On the other hand, the evidence is becoming increasingly strong that free radicals are involved in many of the chronic diseases that are associated with smoking. And the concentrations of radicals in smoke are so high (compared, for example, with smog) that radical-mediated reaction path ways appear certain to result from the exposure of tissue to smoke [29].

The elevated oxidative stress in this study is thought to result both directly from inhaled oxidants in cigarette smoke or pollution and in directly due to the release of reactive oxygen species (ROS) generated by various inflammatory, immune and epithelial cells [30]. These results were in agreement with previous results of [31] that exposed rabbit pulmonary alveolar macrophages to a filtered aqueous extract of cigarette smoke; they monitored lipid peroxidation by the thio barbituric acid (TBA) test and found increased levels of TBA-reactive materials in the macrophages that were exposed to the smoke [32].

The results of statistical analysis (coefficient correlation) showed no relationships between age of smokers and serum MDA for all studied groups, which were demonstrated in (Figure 1). Similar result was concluded by [33]. Who showed that the serum MDA level unrelated with age of smoker. Also, a positive correlation (Figure 2) can be depended on to support the significant increase of MDA levels with long period of smoking. This result is supported by a similar study [34] indicated that there might be a close association between the duration of smoking and oxidative stress.

Table (2): Serum MDA levels taken from healthy volunteers.

Serum MDA concentration nmol/L				
Groups	**(0) Nonsmoker/day**	**(I) Smoker (2-10)/day**	**(II) Smoker (10-20)/day**	**(III) Smoker more than 30/day**
Number of subject	**11**	**8**	**12**	**20**
MDA (mean ± SD)	**80.07 ±12.67**	**280.06 ±117.22**	**221.63 ±93.76**	**272.28 ±113.57**

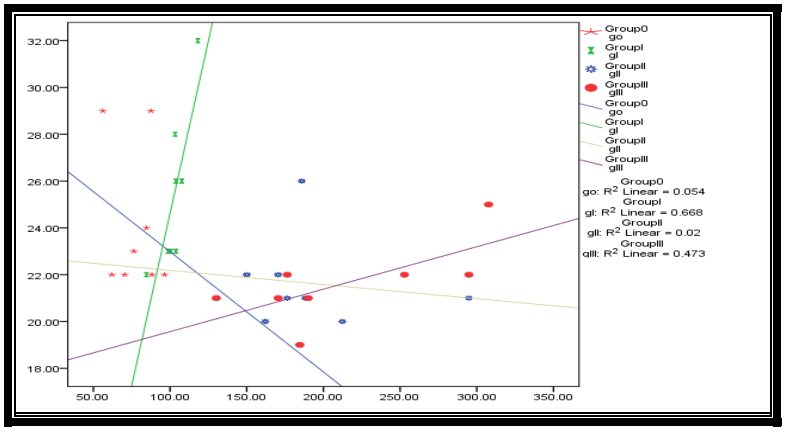


Figure (1): Correlation between age of smokers and serum MDA levels.

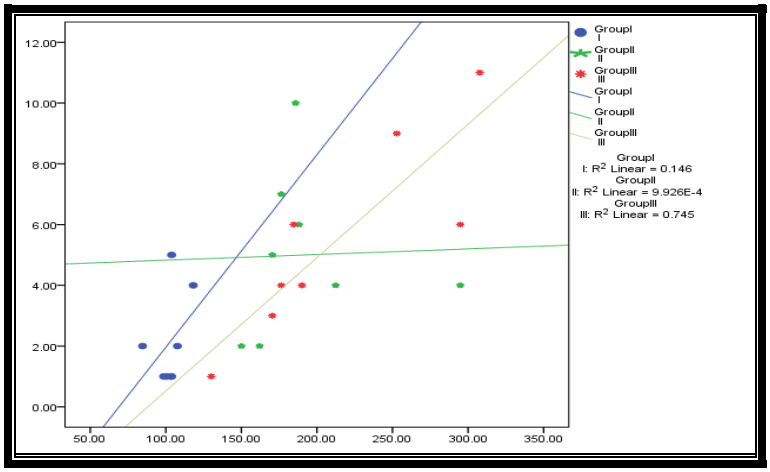


Figure (2): Correlation between period of smoking and serum MDA levels.

- Determination of Serum Ceruloplasmin (Cp)

Table (3), illustrate a significant decrease ($P \leq 0.05$) in serum Cp levels, in all smokers groups (I, II and III) when compared to control group (0).

The results in table (3) are fairly consistent with other laboratory data which shows that in vitro exposure to cigarette smoke could significantly decrease some enzymatic activities, both in plasma and in saliva [35].

The low concentrations of ceruloplasmin which was reported in the present study agree with the study of [36] that demonstrated the extent of ROS-induced oxidative damage can be exacerbated by a decreased efficiency of antioxidant defense mechanisms. On the other hand the fall in ceruloplasmin level which was reported in the present study agree with the study of [37]. Which demonstrated that ceruloplasmin concentration was decreased in the advanced stage of oxidative strees during smoking. Results of our study support the data presented by [38] who have reported decreased activity of ceruloplasmin in the smokers. In agreement with previous findings [39, 40]. On the other hand, the results of statistical analysis (coefficient correlation) showed no relationships between age of smokers and serum CP for all studied groups, which were demonstrated in (Figure 3). This result is consistent with results of study conducted by ministry of health (MOH) which found that the age of smokers do not relate with reduction of cp level [41].

Our results that were reported in the present study, illustrated that reactive oxygen species production increased during all studied types of smoking. Therefore, the slightly decrease in whole serum Cp may be due to its work in scavenging of hydrogen peroxide (H_2O_2) and hydroperoxide (ROOH). This is supported with negative correlation between serum Cp and period of smoking, which was shown in figure (4).

Table (3): Serum CP levels taken from healthy volunteers.

Serum Cp concentration mg/L				
Groups	**Nonsmoker/day**	**Smoker (2-10)/day**	**Smoker (10-20)/day**	**Smoker more than 30/day**
Number of subject	**11**	**8**	**12**	**20**
Cp (mean ± SD)	**9.29±1.68**	**4.52±2.59**	**4.73±2.19**	**3.57±1.72**

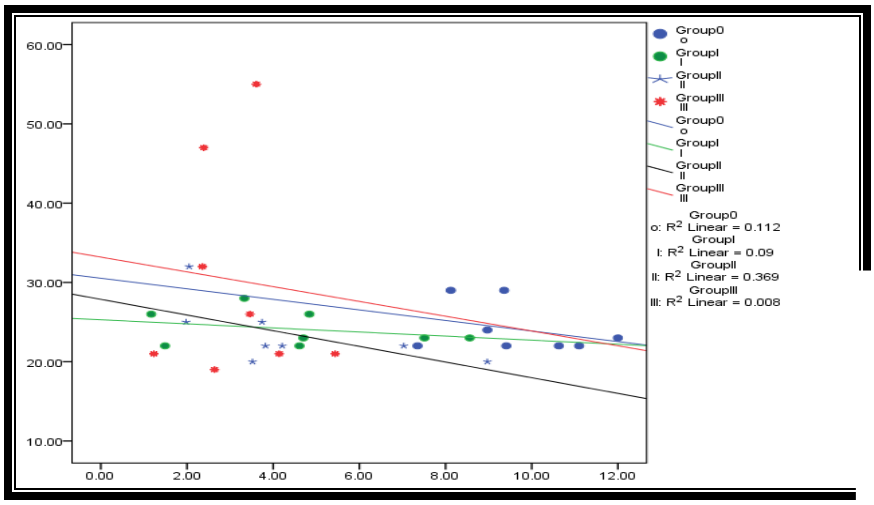


Figure (3): Correlation between age of smokers and serum CP levels.

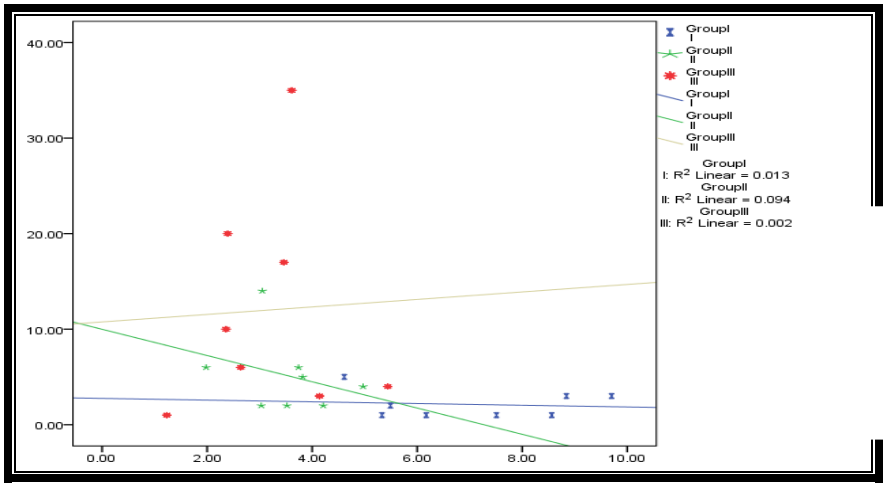


Figure (4): Correlation between period of smoking and serum CP levels.

- Conclusion and recommendations:

From these findings, it's possible to conclude that effects of cigarette smoking is associated with lower levels of antioxidants in smokers serum, suggesting the use of this antioxidant for the purpose of limiting lipid peroxidation. However, this study will need to be replicated with a greater number of subjects in order to establish conclusive reference values. A larger study would also allow researchers to look for different antioxidants.

References

1- **Freire-de-Lima CG, Xiao YQ and Gardai SJ (2004) Monographs on the evaluation of the carcinogenic risk to humans, Tobacco Smoke and Involuntary Smoking, J Biol Chem 281: 386.**

2- **Bonarius HP, Brandsma CA, Kerstjens HA, Koerts JA, Kerkhof MC and Nizankowska-Mogilnicka EA (2010) Antinuclear auto antibodies are more prevalent in COPD in association with low body mass index but not with smoking history. J Cell Biol 155: 649.**

3- **Lubin JH, Purdue MB, Kelsey KN, Zhang ZF, Winn DH, Wei QL, Talamini RL, Szeszenia-Dabrowska GN, Sturgis EM and Smith EY (2009) Total exposure and exposure rate effects for alcohol and smoking and risk of head and neck cancer: a pooled analysis of case-control studies. Epidemiology, 170: 937.**

4- **Kinchen JM and Ravichandran KS (2008) Phagosome maturation: going through the acid test. Nat Rev Mol Cell Biol 9: 781.**

5- **Egan KM, Stampfer MJ, Hunter DA, Hankinson TS, Rosner BA, Holmes FM, Willett WC and Colditz GA (2002) Active and passive smoking in breast cancer: prospective results from the Nurses' Health Study. Epidemiology, 13: 138.**

6- **Hecht SS (2002) Tobacco smoke carcinogens and breast cancer. Environ Mol Mutagen, 39: 119.**

7- **De Barros Silva SS, De Carvalho Rondo PH and Erzinger GS (2005) β-Carotene concentrations in maternal and cord blood of smokers and non-smokers. Early Hum Dev81: 313.**

8- **Pouniotis DS, Plebanski M and Apostolopoulos V (2006) Alveolar macrophage function is altered in patients with lung cancer. Clin Exp Immunol 143: 363.**

9- **Tao FD, Gonzalez-Flecha BT and Kobzik LB (2003) Reactive oxygen species in pulmonary infammation by ambient particulates. Free Radic Biol Med. 35: 327.**

10- **Schindler TH, Nitzsche EU, Munze lT, Olschewski FM, Brink NI and Jeserich MC (2003) Coronary vaso regulation inpatients with various Risk factors in response to cold pressor testing: contrasting myocardial blood flow responses to short-and long-term vitamin C administration. Jam Coll Cardiol; 42: 814.**

11- **Niki EM, Yoshida YG, Saito ZY and Noguchi NI (2005) Lipid peroxidation: mechanisms, inhibition, and biological effects. Bio chem Res Commun 338: 668.**

12- **Tang JJ, Wang MW, Jia EZ, Yan JJ, Wang QM, Zhu JK, Yang ZJ, Lu XR and Wang LS (2010) The common variant in the GSTM1 and GSTT1genesis related to markers of oxidative stress and inflammation in patients with coronary artery disease: acase-only study. Mol Biol Rep 37: 405.**

13- **Northrop-Cleves CA and Thurnham DI (2007) Monitoring micro- Nutrients in cigarette smokers. ClinChimActa377: 14.**

14- **Kiely MC, Cogan QP, Kearney PJ and Morrissey PA (1999) Relationship Between smoking, dietary in takes and plasma levels of vitamin and β-carotenein matched maternal-cord pairs. Int J Vitam Nutr Res 69: 262.**

15- **Cogs well ME, Weisberg PH and Spong CL (2003) Cigarette smoking, Alcohol use and adverse pregnancy out comes: implications for Micronutrient supplementation. J Nutr 133: 1722.**

16- **Halliwell BE (2001) Roles of free radicals in neurodegenerative disease: Therapeutic in antioxidant treatment. Drugs Aging; 18: 685.**

17- **De Domenico IH, Ward YD, Patti MC, Jeong SY, David US, Musci AG and Kaplan WJ (2007) Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin. EMBO J. 26: 2823.**

18- **Sarma PD, Barrett MO and Chavez LM (2008) Safety of green tea extracts: a systematic review by the US Pharmacopeia. Drug Safety. 31: 469.**

19- **Healy JR and Tipton KC (2007) Ceruloplasmin and what it might do. J Neural Transm114: 777.**

20- **Frieden ED (1980) Caeruloplasmin: amulti-function almetallo Protein of vertebrate plasma. Ciba Found Symp79: 93.**

21- **Deibel MX, Ehmann WH and Markesbery WS (1996) Copper, iron ،and zinc imbalances in severely degenerated brain regions in Alzheimer's disease: possible relation to oxidative stress. J. Neurol .Sci., 143: 137.**

22- **Kessler HB, Pajonk FN and Meisser AP (2006) Cerebrospinal fluid diagnostic markers correlate with lower plasma copper and cerulo-plasmin in patients with Alzheimer's disease. Neural Transm.326: 177.**

23- **Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Meth Enzymol 52: 306.**

24- **Merle US, Eisenbach CA, Weiss KG, Tuma SM and Stremmel WG (2009) Serum ceruloplasmin oxidase activity is a sensitive and highly specific diagnostic marker for Wilson disease. J Hepatol.51: 925.**

25- **Ravin AT (1961). Oxidants and antioxidants in clinical medicine. J. Lab. Clin. Med, 58: 161.**

26- **Doll RE and Hill BS (1950) Smoking and carcinoma of the lung: preliminary report. Br MedJ2: 739.**

27- **Shaheen AD and Al-Fattah AF (1995) Effect of dietary zinc on lipid peroxidation, glutathione, protein thiols levels and superoxide dismutase activity inrattissues. Int J Biochem Cell Biol 27: 89.**

28- **Tao FZ, Gonzalez-Flecha XB and Kobzik LI (2003) Reactive oxygen species in pulmonary infammation by ambient particulates. Free Radic Biol Med. 35:327.**

29- **Fayol LO, Gulian MY, Dalmasso CE, Cala PR, Simeoni UJ and Millet VM (2005) Antioxidant status of neonates exposed inuteroto tobacco smoke. Bio l Neo nate87:121.**

30- **Petruzzelli SY, Tavanti MO, Pulera AN, Fornai ED, Puntoni RL and Celi AC (2000) Effects of nicotine replacement therapy on markers of oxidative stress in cigarette smokers enrolled in a smoking cessation program. Nicotine Tob Res; 2:345.**

31- **Ghio JB, Stonehuerner JZ and Quigley CR (1994) Humic-like substances in cigarette smoke condensate and lung tissue of smokers. Am J Physiol. 266 :382.**

32- **Fong KV, McCay PN and Poyer WJ (1973) Oxidative stress. Free Radic J. Biol. Chem. 248: 7792.**

33- **Coldiron AF, Sanders RG, and Watkins QJ (2002) Effects of combined quercetin and Coenzyme Q10 treatment on oxidative stress in normal and diabetic rats. *J. Biochem.* Mol.Toxicol. 16, 197.**

34- **Hoffmann DB, Hoffmann IK and El-Bayoumy KF (2001) Less harmful cigarette: acontroversialissue. Attribute to Ernst L. Wynder. Chem. Res. Toxicol. 14, 767.**

35- **Nagler RM, Lischnisky CS, Diamond OE, Drigues NC, Klein AI, and Reznick ZL (2000) Effect of cigarette smoke on salivary proteins and enzyme activities. ArchBiochem Biophys; 379, 229.**

36- **Reynolds PJ, Hurley SR, Goldberg EM, Anton-Culver HB, Bernstein LZ, Deapen DC, Horn-Ross LB, Peel DU, Pinder RY and Ross KT (2004) Active smoking, household passive smoking, and breast cancer: evidence from the California Teachers Study. J Natl Cancer Inst, 96: 29.**

37- **Senra AG, Alvarez MH, Lopez JN, and Quintela ES (2008) Ceruloplasmin antioxidant activity. Neoplasia13: 25**

38- **Holvoet PI, Collen DB, and Werf FY (1999) Malon dialdehyde – modified LDL as a marker of acute coronary syndromes. JAMA281: 1718.**

39- **Ortega RX, Lopez-Sobaler AM, Martinez MK, Res PL, and Quintas EC (1998) Influence of smoking on vitamin E status during the third trimester of pregnancy and on breast-milk tocopherol concentrations in Spanish women. AmJ Clin Nutr 68: 662.**

40- **Cao GC and Chen DL (1991) Effects of dietary zinc on free radical generation, lipid peroxidation, and superoxide dismutase in trained mice. Arch Bio chem Biophys291: 147.**

41- **Candan FN, Gultekin FD and Candan FE (2002) Effect of vitamin C and zinc on osmotic fragility and lipid peroxidation in zinc-deficient haemo dialysis patients. Cell Bio chem Funct20: 95.**

دراسة تأثير تدخين السجائر على حالة الأكسدة مضادات الأكسدة

صفاء حسين علي *

الخلاصة

يعد التدخين مشكلة عالمية، ويمثل تدخين السكائر الطريقة الاكثر شيوعا. تهدف هذه الدراسة الى تحقق من حالة التوازن بين عمليات الأكسدة بواسطة قياس مستوى المالون داي الدهايد (MDA) كمقياس لعمليات الأكسدة ومضادات الأكسدة بواسطة قياس مستوى السيروبلازمين (Cp) كمقياس لمضادات الأكسدة، ومحاولة تسليط الضوء على العلاقة المحتملة بين المالو داي الدهايد(MDA) والسيروبلازمين (Cp) وكل من عمر المدخن وفترة التدخين.

بلغ حجم العينة (49) مدخن من الرجال البالغين ممن تراوحت اعمارهم بين (32-19). بينت النتائج وجود زيادة معنوية ($P \leq 0.05$) في مستويات المالون داي الدهايد (MDA) في كل مجاميع المدخنين عند مقارنتها مع مجموعة السيطرة (غير المدخين). وكذلك برهنت النتائج وجود انخفاض معنوي ($P \leq 0.05$) في مستويات السيروبلازمين (Cp) في كل مجاميع المدخنين عند مقارنتها مع مجموعة السيطرة (غير المدخين).

اثبتت نتائجنا دور جذور الاوكسجين الحرة في تطوير عمليات فرط الاكسدة حيث وجد علاقة موجبة بين مستويات المالون داي الدهايد (MDA) وفترة التدخين. بينما اثبتت النتائج وجود علاقة سالبة بين مستويات السيروبلازمين (Cp) وفترة التدخين. ومن ناحية اخرى اثبتت النتائج عدم وجود ارتباط بين مستويات المالون داي الدهايد (MDA) و مستويات السيروبلازمين (Cp) نسبة الى عمر المدخنين.

*** مدرس مساعد، كيمياء حيوية، المعهد التقني الشطرة**

Table (1): Data of the volunteers.

No.	age	Number of cigarette / day	period of smoking	gender
1	22	……..	……..	male
2	29	……..	……..	=
3	23	……..	……..	=
4	24	……..	……..	=
5	22	……..	……..	=
6	22	……..	……..	=
7	22	……..	……..	=
8	29	……..	……..	=
9	25	……..	……..	=
10	20	……..	……..	=
11	19	……..	……..	=
12	22	60	9	=
13	26	4	2	=
14	25	30-40	11	=
15	32	8	4	=
16	26	2	1	=
17	22	5	2	=
18	22	60	4	=
19	20	20	4	=
20	23	40-60	3	=
21	23	30	4	=
22	22	30-40	10	=
23	22	10-15	5	=
24	23	10	1	=
25	22	10-20	5	=
26	23	30-40	6	=
27	21	20	6	=
28	22	20	2	=
29	22	20	2	=
30	22	30	1	=
31	21	20	4	=
32	23	10	5	=
33	22	30	5	=
34	21	20	7	=
35	19	30	6	=
36	21	30	3	=
37	26	20	10	=
38	32	40-60	10	=
39	28	5-10	1	=
40	23	40-50	3	=
41	25	40-50	7	=
42	20	20	2	=
43	23	3	1	=
44	21	30	1	=
45	21	30	6	=
46	25	20	3	=
47	25	20	6	=
48	23	30-40	7	=
49	26	60	17	=

Table (2): Serum MDA levels taken from healthy volunteers.

Serum MDA concentration nmol/L				
Groups	**(0) Nonsmoker/day**	**(I) Smoker (2-10)/day**	**(II) Smoker (10-20)/day**	**(III) Smoker more than 30/day**
Number of subject	**11**	**8**	**12**	**20**
MDA (mean ± SD)	**80.07 ±12.67**	**280.06 ±117.22**	**221.63 ±93.76**	**272.28 ±113.57**

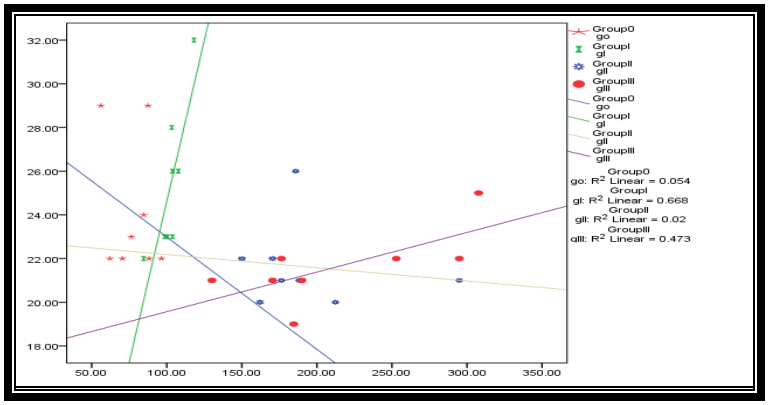


Figure (1): Correlation between age of smokers and serum MDA levels.

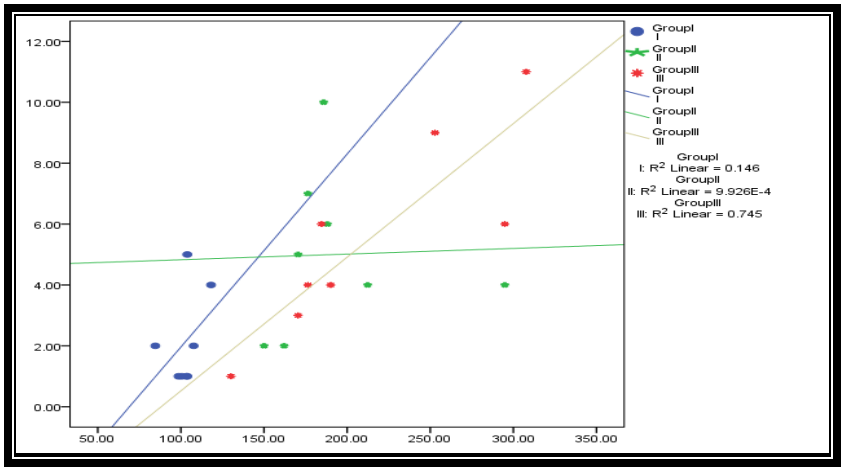


Figure (2): Correlation between period of smoking and serum MDA levels.

Table (3): Serum CP levels taken from healthy volunteers.

Serum Cp concentration mg/L				
Groups	**Nonsmoker/day**	**Smoker (2-10)/day**	**Smoker (10-20)/day**	**Smoker more than 30/day**
Number of subject	**11**	**8**	**12**	**20**
Cp (mean ± SD)	**9.29±1.68**	**4.52±2.59**	**4.73±2.19**	**3.57±1.72**

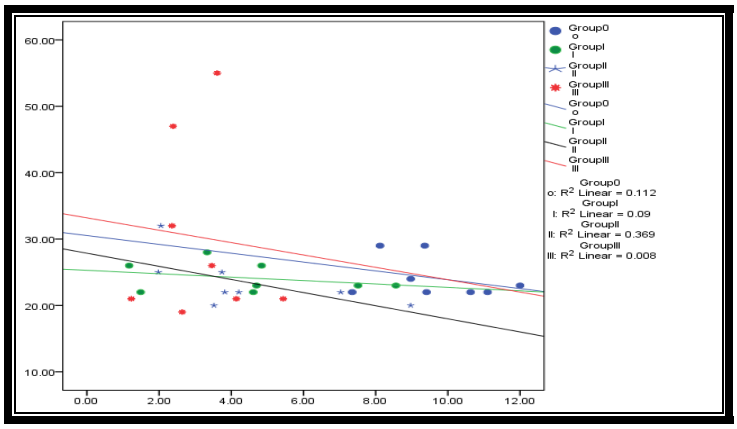


Figure (3): Correlation between age of smokers and serum CP levels.

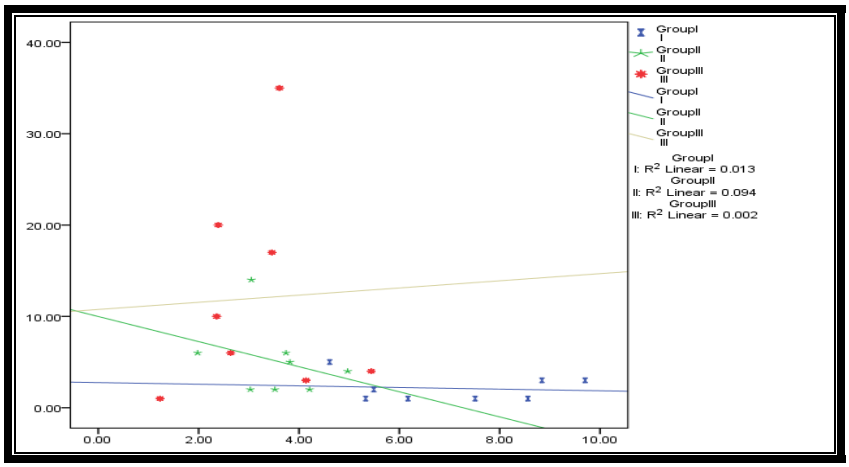


Figure (4): Correlation between period of smoking and serum CP levels.