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Oxidative Stress for Smoking Persons in Suburbs Mosul City*

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ABSTRACT

The study was conducted in Mosul-suburb to show the effects of smoking on antioxidants and some biochemical parameters which include: Vitamin E, Vitamin A, β-carotene, Vitamin C, Folic acid, Ceruloplasmin (Cp), Total protein, Albumin, Calcium, Total bilirubin, Direct bilirubin, Uric acid, Creatinine, Total iron binding capacity (TIBC), Iron, Sulfate, Glutathione(GSH), Malondialdehyde (MDA), Cholesterol, Glucose, Selenium and Transferrin saturation(%). The study included (102) persons living outside city center represented into two minor groups, smokers and non smokers.

The results showed that the level decreased significantly in rural smokers when compared with rural non smokers in: vitamin C, total bilirubin, direct bilirubin and MDA, and there was a significant increase in: vitamin A and Cp., but there wasn't any significant changes in: total protein, albumin, uric acid, creatinine, TIBC, iron, sulfate, cholesterol, selenium, calcium, vitamin E, β-carotene, folic acid, GSH, glucose and transferrin saturation(%). Depending on the period of smoking, the results predicted that antioxidants and some other parameters (vitamin E, vitamin A, β-carotene, vitamin C, folic acid, total protein, albumin, calcium, total bilrubin, direct bilirubin and GSH) were decreased, while: Cp., uric acid, creatinine, TIBC, Iron, sulfate, MDA, cholesterol, glucose and selenium were increased with increased the period of smoking.

الكرب التأكسدي للمدخنين في ضواحي مدينة الموصل

الملخص

شملت الدراسة تأثير التدخين على مضادات الأكسدة وبعض المتغيرات الكيموحيوية في ضـواحي مدينة الموصل، إذ تضمنت تلك المتغيرات: فيتامين E، فيتامين A، بيتا كاروتين، فيتـامين C، حـامض الفوليك، سليروبلازمين(Cp.)، البروتين الكلي، ألبومين، كالسيوم، البليروبين الكلي، البليروبين المتـرابط، حامض اليوريك، كرياتينين، سعة الارتباط الكلي للحديد (TIBC)، الحديد، كبريتات، كلوتاثايون (GSH)، المالوندايألديهايد (MDA)، كوليستيرول، كلوكوز، سلينيوم وإشباع الترانسيفيرين(%). وتضمنت الدراسة (102) شخصاً اختيرت من خارج مركز المدينة قسموا الى مجموعتين مدخنين وغير مدخنين.

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أظهرت النتائج انخفاضاً معنوياً للمدخِنين عندما قورنت بمجموعة السيطرة للمتغيرات: فيتـامين C، البليروبين الكلي، البليروبين المترابط وMDA. فضلا عن ذلك لوحظ زيادة معنوية فـي : فيتـامين A وCp. مع عدم حدوث تغير معنوي في: البروتين الكلي، ألبومين، حامض اليوريك، كريـاتينين، TIBC، الحديد، الكبريتات، الكوليستيرول، السلينيوم، الكالسيوم، فيتامين E، بيتا كاروتين، حامض الفوليك، GSH، كلوكوز ودرجة إشباع الترانسفرين (%). كما أشارت النتائج إلى أن زيادة فترة التدخين تعمل على تقليـل مضادات الأكسدة وبعض المتغيرات الأخرى: فيتامين E، فيتامينA، بيتا كاروتين، فيتامين C، حـامض الفوليك، البروتين الكلي، ألبومين، كالسيوم، البليروبين الكلي، البليـروبين المتـرابط وGSH. وزيـادة المتغيرات مع زيادة فترة التدخين في: Cp.، حامض اليوريك، كرياتينين، TIBC، الحديـد، الكبريتـات، MDA، كوليستيرول، كلوكوز، سلينيوم وإشباع الترانسيفيرين(%).

INTRODUCTION

Smoking and related diseases account for approximately 440,000 American deaths every year. This number includes both the direct effects of smoking on the smoker, but also indirect effects of smoking, such as premature births and the effects of second hand smoke .The costs are enormous. Estimates of costs of smoking, both in terms of health care costs and related to lost productivity, are between 100-150 billion dollars each year (Zheng, 2003).

Cigarettes smoke contains carbon monoxide and free radicals which are generated because of high temperatures (up to 900 ºC) at the burning tip (Gillham *et al.*,2000).

The free radicals found in the tar phase are different to those in the gas phase with respect to stability and half-life. The tar phase contains long-lived and fairly stable radicals. It also contains transition metal ions, which can drive the formation of the reactive hydroxyl radical. The gas phase contains short-lived reactive radicals such as nitric oxide ($^{\cdot}NO$). In the presence of oxygen, this is oxidized to nitrogen dioxide radicals, which can damage lung tissue (Gillham *et al.*, 2000):

$$^{\cdot}NO + 1/2O_2 \longrightarrow {}^{\cdot}NO_2$$

and contain reactive olefins and dienes.

A major exogenous source of free radicals is the cigarette smoke. The cigarette smoke is a complex mixture of approximately 5000 chemical compounds, including high concentrations of free radicals and other reactive oxygen species. These oxidants are contained in both the tar and gas phases of cigarette smoke. The gas-phase reactive oxidants are both inorganic and organic in nature, and include epoxides, peroxides, nitric oxide (NO), nitrogen dioxide, peroxynitrite (ONOO-), perinitrates, carbon monoxide, ammonia, dimethylnitrosamine, formaldehyde, hydrogen cyanide, acrolein and a myriad of other free radicals. Indeed, it has been reported that gas-phase cigarette smoke contains approximately one quadrillion radicals per puff. These represent an enormous oxidant load to body tissues (Zheng, 2003).

Damage to DNA appears to involve a complex of polyphenolic tar components with DNA, followed by production of the hydroxyl radical that nicks DNA (Zheng, 2003).

Health disease and lung cancer comprise the vast majority of deaths caused by smoking, followed by chronic bronchitis, strokes, peripheral vascular disease and other cancers.

Materials and Methods

Kits for determination of Total Protein No.(0303), Albumin No. (0801), Calcium No.(2403),Total Bilirubin and Direct Bilirubin No.(0401),Iron No. (0502) ,Total Iron Binding Capacity No. (0512), Glucose No.(0903), Cholesterol No.(0603), were obtained from Syrbio kits, Syria.

But other biochemical parameter (Vitamin A, β-carotene, vitamin E, vitamin C, Folic acid, Malondialdehyde, Glutathion, Selenium, Uric acid and Creatinine), determination used manual methods (Table 1).

Table 1:Methods used to determination of biochemical parameters.

No.	Parameters measured	Method used	Source
1	**Vitamin A**	Needld-Pearson method	Neeld and Pearson, 1963
2	**β-carotene**	Needld-Pearson method	Neeld and Pearson, 1963
3	**Vitamin E**	Emmerie-Engel reaction	Emmerie and Engel, 1938
4	**Vitamin C**	2,4-dinitrophenylhydrazine derivatization method	Roe and Kuther, 1943
5	**Folic acid**	Microbiologyical assay	AOAC, 1950
6	**Glutathion**	Modified procedure utlilizing Ellman`s reagent.	Sedlak and Lindsay, 1968
7	**Malondialdehyde**	Thiobarbituric acid method	Lunec, 1990
8	**Uric acid**	Phosphotungstic acid method	Varley, 1967
9	**Total bilirubin**	Diazo method	Toro and Ackermann, 1975
10	**Direct bilirubin**	Diazo method	Toro and Ackermann, 1975
11	**Total protein**	Biuret methods	Kingsley, 1942
12	**Albumin**	Bromocresol green mthod (dye binding method)	Doumas *et al.*, 1971
13	**Ceruloplasmin**	p-Phenylenediamine oxidase method	Sunderman and Nomoto, 1970
14	**Glucose**	Glucose oxidase method	Trinder, 1969
15	**Cholesterol**	Cholesterol estrase methods	Richmond, 1973
16	**Creatinine**	Jaffĕ method	Jaffĕ, 1886
17	**Total Iron Binding Capacity**	Ramsay method	William *et al.*, 1977
18	**Transferrin saturation**	Transferrin saturation(%)= Serum Iron /TIBC X 100	Burtis and Ashwood, 1999
19	**Iron**	Bathophenanthroline method	Burtis and Ashwood, 1999
20	**Calcium**	Methylthymol blue method	Rbertson and Marshall., 1979
21	**Selenium**	Selenium-orthophenylenediamine compex	Snell, 1981

For determination of Body Mass Index (BMI) calculated using the formula as weight (kg)/height2(m2) (Al-Abbad and Al-Sowielem, 1998).

The study included (102) persons (59 nonsmokers, 43 smokers) healthy subjects, with comparable age, all were males with minimal or no risk of exposure to a polluted

atmosphere. They were farmers living in Al-Khather village (30 km east of Mosul city center).

For venipuncture, 10 ml sterile syringes equipped with (22G x 1.25) syringe needles were used and put in dry and clean plain tube. After coagulation, it was centrifuged at 4000 x g for 15 minute. Serum was transferred into plain tube equipped with tight–fitting caps by disposable tips, then stored at –20 ˚C (Liang *et al.*, 1989).

RESULTS AND DISCUSSION

The results of smokers and non-smokers groups in suburbs group were listed in Table (2).

Table 2: The biochemical parameters of suburbs non-smokers and smokers

Parameters	Suburbs non-smokers (n=59)				Suburbs smokers (n=43)			
	mean	SD	Min.	Max.	mean	SD	Min.	Max.
Age(year)	35.7	10.9	17.0	74.0	32.4*	8.77	17.0	65.0
Weight(kg)	76.31	14.5	55.0	108.0	80.34*	14.6	52.0	118.0
Height(cm.)	169.0	10.5	149.0	198.0	171.2	8.5	146.0	196.0
B.M.I(k.g./m^2)	26.71	2.45	24.77	27.54	27.41*	2.08	24.39	30.72
Vit.E(mg/dl)	1.0	0.21	0.05	1.78	0.97	0.45	0.10	1.69
Vit.A(µg/dl)	46.42	12.4	18.42	84.2	44.9*	8.1	15.1	68.5
β-carotene(µg/dl)	80.49	21.5	69.8	253.2	65.05	12.2	59.45	247.2
Vit.C(mg/dl)	0.85	0.17	0.04	1.87	0.61*	0.15	0.04	1.69
Folic acid(ng/ml)	6.85	1.7	3.03	12.76	6.68	1.1	4.84	10.06
Cp.(mg/l)	151.1	39.4	15.0	534.0	186.3*	37.2	15.04	534.0
T.p.(gm/dl)	5.5	0.38	5.02	7.35	6.02	0.69	5.07	7.87
Alb. (gm/dl)	4.36	0.12	4.08	6.1	5.22	1.0	4.13	6.3
Calcium(mg/dl)	11.78	0.24	7.89	11.2	11.78	0.82	8.3	12.93
Total Bilir. (mg/dl)	0.41	0.27	0.0	1.195	0.25*	0.07	0.0	1.25
Direct Bilir. (mg/dl)	0.19	0.03	0.0	0.3	0.13*	0.02	0.0	0.28
Uric acid(mg/dl)	5.05	1.58	1.4	6.69	5.16	2.0	3.15	7.1
Creatinine(mg/dl)	0.82	0.05	0.42	1.33	0.91	0.27	0.48	1.29
TIBC(µg/dl)	186.8	32.1	85.54	510.2	228.9	29.9	88.8	482.2
Iron(mg/l)	1.6	0.19	0.03	1.69	1.7	0.12	0.3	1.64
Sulfate(mmol/l)	2.5	1.05	2.21	6.78	3.3	1.09	2.0	7.8
GSH(µmol/l)	13.7	0.89	4.73	22.7	12.7	2.0	3.73	20.7
MDA(µmol/l)	6.5	2.97	2.31	45.6	5.7*	2.4	2.44	44.34
Cholesterol(mg/dl)	180.8	40.1	102.8	257.1	184.0	18.0	122.5	251.0
Glucose(mg/dl)	68.1	35.8	57.2	123.5	61.69	5.6	67.2	128.7
Selenium(µg/dl)	35.7	11.0	20.39	79.8	41.26	0.89	24.37	67.9
Tansferrin saturation%	36.65	13.5	25.14	39.21	34.26	4.8	23.41	38.24

*Different Significantly at $P \leq 0.05$.

The results showed that there was a significant decrease of suburbs smokers when compared with suburbs non-smokers in vitamin C(P=0.03). Similar results were published by other investigators (Riemersma *et al.*, 1991; Sakai *et al.*, 1998; Pincemail *et al.*, 2000; Lykkesfeldt *et al.*, 2000; Nuttall *et al.*, 2002). In vivo vitamin C has been suggested to be a biomarker of oxidant stress, and the level of vitamin C was

decreased under the oxidant stress of smoking (Turnlund *et al.*, 2004). Low vitamin C levels found in smokers are considered to be a direct consequence of vitamin C utilization as an antioxidant due to the smoke-related pro-oxidant load (Nuttall *et al.*, 2002).

Other biochemical parameters were also decreased significantly as: total bilirubin (P=0.01), direct bilirubin (P=0.005) and malondialdehyde (P=0.019). These results were in a good agreement with the previously reported data (Al-Timimi and Al-Khayat, 2001).

Vitamin E, β-carotene and folic acid were not significantly decreased. These results meet with those of (Riemersma *et al.*, 1991; Durand *et al.*, 1997; Hippeli *et al.*, 2003). The decreased in vitamin A, found in smokers is considered to be a direct consequence of its utilization as an antioxidant due to the smoke-related pro-oxidant load (EBSCO, 2005) similar to other reported results (Kim *et al.*, 2004).

Glutathion wasn't decreased significantly and this finding was in a good agreement with others (Lykkesfeldt *et al.*, 2000; Nuttall *et al.*, 2002; Polidori *et al.*, 2003; Detroit *et al.*, 2003; Kim *et al.*, 2004). Similarly glucose and transferrin saturation (%) were decreased non significantly.

Moreover, it has been observed that there was a significant increase in: ceruloplasmin (P=0.004) which were similar to other reported results (Al-Timimi and Al-Khayat, 2001; Sürmen-Gür *et al.*, 2003).

The result of this study might suggest that the increase of Cp. levels in smokers is a part of the total antioxidant status protecting tissues from the effects of free radicals (Al-Timimi and Al-Khayat, 2001;Sürmen-Gür *et al.*, 2003), or might merely reflect increase copper intake from the tar component of the cigarettes (Duthie *et al.*, 1991).

The results also showed non significant increase in: total protein, albumin, uric acid, creatinine, total iron binding capacity, iron, sulfate, cholesterol, selenium and calcium. This finding was in a good agreement with other (Wingerd and Sponzilli, 1977; Ryback *et al.*, 1985; Chopra *et al.*, 2000; Valkonen and Kuusi, 2000; Orth, 2002; Awadallah, 2003). Nephrologists "rediscovered" were reported that smoking as a major renal risk factor in 1997 (Orth *et al.*, 1997).

Effects of the smoking period on the biochemical parameters in smoking suburb group.

Depending on the period of smoking, suburb smokers group was divided into three subgroups, which were listed in Table (3).

The results showed that vitamin E showed no changes with the increase of the period of smoking (Table 3). Notice that vitamin A significant decreased in (0-10 year) group and in (21year and over) group, but there were no significant differences between them.

β-carotene, vitamin C and folic acid were decreased significantly with the increase of the period of smoking. While no significant difference was observed in β-carotene. This result was in a good agreement with other reported results (Helen and Vijayammal, 1997).

Several studies have also examined the effect of vitamin E in cigarette smokers. Cigarette smoking stimulates the formation of highly reactive molecules. Serum levels of vitamin E (as well as vitamin C and β-carotene) react as antioxidant to these molecules. Therefore the level of vitamins were significantly lower in the smokers compared to the

non-smokers, and antioxidant decreased too when the period of smoking increased (Hughes, 2000).

Table 3: The biochemical parameters of suburb smokers at different period of smoking and control using Duncan test.

Parameter	Control group (n=59)		(0-10 year) n=18		(11-20 year)n=16		(21 > year) n=9	
	mean	SD	mean	SD	Mean	SD	mean	SD
Age(year)	35.7 **c**	10.9	27.98**d**	8.1	34.13 **b**	6.67	42.0 **a**	6.17
Weight(kg)	76.31 **a**	14.5	77.81**a**	13.7	82.7 **b**	14.07	81.9 **b**	18.33
Height(cm.)	169.0 **a**	10.57	170.48 **a**	8.3	173.1 **a**	8.07	169.0 **a**	9.5
B.M.I(k.g./m^2)	26.71 **b**	2.45	26.92 **b**	2.45	27.6 **a**	2.08	28.67 **a**	1.98
Vit.E(mg/dl)	1.0 **a**	0.21	0.99 **a**	0.14	0.96 **a**	0.12	0.92 **a**	0.15
Vit.A(µg/dl)	46.42 **a**	12.4	42.76 **c**	18.84	48.2 **b**	10.3	42.05 **c**	15.3
β-carotene(µg/dl)	80.49 **a**	21.52	63.0 **a**	9.6	70.6 **a**	8.7	73.59 **a**	11.9
Vit.C(mg/dl)	0.85 **b**	0.17	0.88 **a**	0.17	0.42 **c**	0.08	0.33 **d**	0.024
Folic acid(ng/ml)	6.85 **a**	1.7	6.96 **a**	1.18	6.61 **a**	1.6	5.34 **b**	1.71
Cp.(mg/l)	151.1 **d**	39.4	150.64 **c**	32.6	204.3 **b**	27.7	261.7 **a**	30.46
T.p.(gm/dl)	5.5 **a**	0.38	6.02 **a**	0.69	5.24 **a**	0.74	5.14 **b**	0.89
Alb. (gm/dl)	4.36 **a**	0.12	4.22 **b**	0.88	4.33 **a**	0.73	4.27 **b**	0.34
Calcium(mg/dl)	11.78 **a**	0.24	11.85 **b**	0.9	11.84 **b**	0.66	11.44 **b**	0.92
Total Bilir. (mg/dl)	0.41 **a**	0.27	0.16 **b**	0.08	0.12 **b**	0.01	0.11 **b**	0.07
Direct Bilir. (mg/dl)	0.19 **a**	0.03	0.14 **b**	0.06	0.11 **a**	0.08	0.13 **b**	0.07
Uric acid(mg/dl)	5.05 **b**	1.58	5.16 **b**	1.10	5.64 **a**	1.23	5.98 **a**	0.52
Creatinine(mg/dl)	0.82 **a**	0.05	0.94 **a**	0.1	0.84 **a**	0.12	0.87 **a**	0.13
TIBC(µg/dl)	186.8 **a**	32.16	179.0 **a**	50.2	192.2 **a**	36.6	186.0 **a**	37.3
Iron(mg/l)	1.6 **a**	0.19	1.27 **a**	0.09	1.1 **a**	0.2	1.32 **a**	0.2
Sulfate(mmol/l)	2.5 **b**	1.05	2.63 **b**	1.14	2.66 **b**	0.10	3.97 **a**	0.27
GSH(µmol/l)	13.7 **a**	0.89	14.57 **b**	8.2	11.68 **c**	3.2	10.06 **d**	1.3
MDA(µmol/l)	6.5 **a**	2.97	5.66 **a**	2.66	5.89 **a**	2.59	5.14 **a**	0.93
Cholesterol(mg/dl)	180.8 **d**	40.13	184.0 **b**	58.02	194.51 **c**	68.42	223.54 **a**	66.21
Glucose(mg/dl)	68.1 **c**	35.8	61.69 **c**	24.69	72.45 **b**	42.13	85.64 **a**	29.58
Selenium(µg/dl)	35.7 **c**	8.06	37.1 **c**	9.69	54.4 **a**	9.01	40.41 **b**	8.42
Tansferrin saturation%	36.65 **a**	13.5	32.94 **a**	10.32	31.23 **b**	10.59	33.96 **a**	12.51

Different letters horizontally a, b, c, d, indicate that the mean are different significantly at $P \leq 0.05$.

Ceruloplasmin, on the other hand, increased with increasing the period of smoking and there was a significant difference within each one of the groups.

Total protein and albumin were significant difference in last groups (Table 3).

Calcium, total bilrubin and direct bilirubin were not affected with increasing the period of smoking and this result was agreement with literature (Chan-Yeung *et al.*, 1981; Vogt, 1999). The mechanism of action of smoking on these parameters is unclear (Vogt, 1999).

Uric acid, creatinine, TIBC and Iron increased significantly with the increase of the period of smoking, while TIBC and iron showed non a significant differences. Similar results were published by others (Chan-Yeung *et al.*, 1981; Ahmad and Al-Helaly, 2002; Mannino *et al.*, 2004).

Sulfate was increased with increasing period of smoking, where a significant difference among the groups was observed. The increase in sulfate might be due to the different compounds present in the smoking, especially SO_2 ,H_2S..etc.

Glutathion was decreased with the increase of the period of smoking and there was a significant difference among them. Nitric oxide in cigarettes might contribute to the GSH levels by directly reacting with glutathione and inhibiting antioxidants enzymes like glutathione reductase (Fechner *et al.*, 2001).

Malondialdehyde wasn't affected, and this finding agrees with other observation that was found earlier (Helen and Vijayammal, 1997).

Cholesterol increased with increasing the period of smoking and showed significant difference in all groups as found in the literatures (Ahmad and Al-Helaly, 2002; Walker, 2003). The increase might be due to increase secretion of adrenaline hormone stimulated by the nicotine in cigarettes (Cryer *et al.*, 1976). It is Known that adrenaline would increase the lipolysis and cholesterol level would be increased too (Burtis and Ashwood, 1999).

Glucose was increased with increasing the period of smoking and a significant difference among the groups was present. This finding agrees with the other observation found (Ko *et al.*, 2001). It was reported that insulin concentration in smokers was decreased, and hence glucose would be increased (Burtis and Ashwood, 1999).

The results also showed that selenium was increased significantly with increasing the period of smoking and a significant difference among the groups was observed.

Transferrin Saturation (%) on the other hand was decreased significantly and a significant difference among them was observed.

RECOMMENDATIONS

1. Smoking might be a contributing factor in increasing lead absorption among exposed people.
2. Avoidance of direct and indirect exposure to tobacco smoke is of primary importance not only for healthier lungs, but as a preventative measure for the other diseases: Cardiovascular disease, cancer, and diabetes, as well as the clinical importance of the present results suggesting that free radicals inhaled in cigarette smoke are highly toxic, and impaired oxidant-antioxidant balance is a risk factor in degenerative diseases. Therefore, may God help to prevent anything that affects the human body and lead to death:

In the name of Allah Most Gracious Most Merciful.

(The heifer) (195) {And spend of your substance in the cause of Allah and make not your own hands contribute to your destruction but do good; for Allah loveth those who do good}

بسم الله الرحمن الرحيم

{وَأَنْفِقُوا فِي سَبِيلِ اللهِ وَلَا تُلْقُوا بِأَيْدِيكُمْ إِلَى التَّهْلُكَةِ وَأَحْسِنُوا إِنَّ اللهَ يُحِبُّ الْمُحْسِنِينَ} (195)(البقرة)

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