The Effect of Smoking on the Some Trace Elements and Cortisol Hormone Concentration in Serum

Ali Saad Elewi

Department of Chemistry, college of science, University of Baghdad

―――――

الخلاصة:

تم أخذ أربع مجاميع من المدخنين لدراسة تأثير التدخين على مستوى هرمون الكورتزول وبعض العناصر النادرة ومقارنة النتائج مع مجموعة من غير المدخنين.

لوحظ ارتفاع في مستوى هرمون الكورتزول لدى ألمجموعة الأولى من المدخنين (10−20 سيجارة في اليوم مع نسبه نيكوتين 0,4 مغم/سيجارة) مقارنة مع مجموعة السيطرة، بينما كان الارتفاع واضحاً جداً في ألمجموعة الثانية (17 شخص) من المدخنين (10−20 سيجارة في اليوم مع نسبة نيكوتين 0,8 مغم/سيجارة) وكذلك كان الارتفاع واضحاً جداً لدى المجموعتين الثالثة (14 شخص) والرابعة (18 شخص) من المدخنين الذين يستهلكون 40 سيجارة أو اكثر في اليوم مع نسبة نيكوتين 0.4 مغم/سيجارة و 0.8 مغم/سيجارة، على التوالي.

كان تركيز عنصر السلينيوم في جميع مجاميع المدخنين اقل من مجموعه السيطرة, والنقصان في تركيز عنصر السلينيوم يزداد مع زيادة عدد السجائر ونسبه النيكوتين في ألسيجارة.

لم يلاحظ اختلاف في تركيز عنصر النحاس لدى ألمجموعة الأولى والثانية بالمقارنة مع مجموعة السيطرة, بينما كانت هناك زيادة في تركيز عنصر النحاس لدى ألمجموعة الثالثة والرابعة.

لم يكن هناك تأثير للتدخين على تركيز عنصر الزنك لدى جميع مجاميع المدخنين ,بينما هناك ارتفاع واضح في عنصر الرصاص لدى جميع مجاميع المدخنين والزيادة في تركيز عنصر الرصاص تتناسب طرديا مع عدد السجائر ونسبه النيكوتين في ألسيجارة.

Abstract:

The effect of different doses of smoking on the some trace elements (Se, Zn, Pb, Cu) and cortisol hormone concentration have been studied.

Four groups of smokers were used in this study to investigate the effect of smoking in trace element and cortisol hormone concentration in smokers and compare the finding with nonsmokers group (control group).

Mean values of cortisol hormone concentration in group I (consumed 10-20 cigar per day with 0.4mg nicotine/cigar) were found to be significantly higher ($p < 0.01$) in comparison with the value of control group and high significantly higher ($p < 0.001$) in group II who consumed 10-20 cigar per day with 0.8 mg nicotine/cigar, and high significantly higher ($p < 0.001$) in group

III, IV (consumed 40 or more cigar per day with 0.4 and 0.8 mg nicotine/cigar, respectively).

Serum selenium concentrations in smokers were below control group among all smoker groups, and the concentrations of selenium were depression increasing with increasing doses of smoking and nicotine in cigar.

Significantly increase in concentration of Cu ($p < 0.001$) in group III, IV in comparison with control group while non significant increase ($p < 0.05$) in group I, II.

No effect of smoking in the concentration of Zn in serum of all smoker groups.

Pb serum concentration in smoker were found to be higher than control group, and the increasing in pb concentration proportion with increasing doses of smoking and nicotine in cigar.

Key Words: Smoking Effect, Cortisol Hormone, Trace Element

Introduction:

The harmful effects of smoking on health have been widely documented, although it is yet unclear whether tobacco dependence is only psychological in nature, or both psychological and physical [1]. Although smoking is a recognized risk factor for several diseases including emphysema, chronic bronchitis, cardiovascular diseases, and cancer [2,3].

Nicotine is known to release neuroendocrine substances that may subsequently reinforce smoking behavior by improving mood states. Many studies have searched for correlations between cortisol levels and smoking or nicotine dependence [2]. Most of them demonstrated elevated cortisol levels under a variety of experiment conditions, for example, in women smokers, when high doses of nicotine were given, and in heavy smokers only [1].

The biological markers of tobacco withdrawal are less well understood, such phenomena are useful as objective markers of withdrawal severity, as a potential pre doctors of smoking relapse and as mediators of treatment effects. Recent research with animals has implicated the hypothalamic-Pituitary-adrenocortical (HPA) - system in smoking relapse [4] and cortisol is the primary peripheral HPA hormone in humans.

Cortisol modulates central nervous system activity during stress, and its production has been linked to the ability to cope with stress demands. Regular cigarette smokers have been found to have elevated cortisol relative to nonsmokers [5-7] and smoking has been shown to stimulate the release of cortisol [8-10]. This effect has been attributed to nicotine exposure [8, 11] cortisol interacts with several neurotransmitters that mediate the effects of nicotine e.g. dopamine, β-endorphin, acetylcholine [13,14], and cortisol may be linked to nicotine metabolism in that sensitivity to nicotine tends to be reduced under conditions of enhanced corticosteroid activity [15].

Trace elements are normally present in very low concentrations in the body. Iron and magnesium, for example, are not trace elements because substantial quantities are normally present in the body. Many such metals are essential elements for various metabolic functions (e.g. cobalt, manganese, selenium), however they are needed and present only in minute quantities [16], and these trace elements are essential for life and their concentrations in serum vary with human ecology and different pathological conditions [17].

The trace element (Cu, Co, Zn, Mn, Cd, etc) which occur in body tissues and fluids have some essential activities [18]. These elements serve as cofactors is various functions of the body. They are present as component of nutrients and constituents of enzymes, vitamins, hormones and other processes and thus take part in growth development and maintenance of health. The source of these elements for human is the plants and animals taken as diet. The excess of these trace elements [19] in the body is associated with different pathological conditions such as toxic cancer, malnutrition and a variety of other disorders due to the accumulation of trace elements in the body. The increasing environmental pollution, unhygienic living conditions and food consumed has given rise to concern about the accumulation of trace elements in the body [17].

Trace element analysis reflects absorption from all sources, including occupational exposure, diet, hobbies, medication, smoking, and local soil-containing dust. It may also reflect characteristics of the host in retaining or accumulating the trace element, as in the case of patients on dialyses (aluminum), receiving parenteral nutrition (with a variety of possible deficiencies) with inborn errors of metabolism such as Wilson's disease (copper), or with impaired excretion (lead) [16].

The trace elements generally considered to be essential are cooper, cobalt, iron, manganese, molybdenum, selenium, and zinc. A balanced and regular diet generally keeps these trace elements roughly constant and consistent but there is no homeostatic mechanism that dose so. Deficiencies in the uptake or metabolism could result to sustained imbalance of trace elements. In addition, an excess intake could result in disease. Other trace elements, such as arsenic, cadmium, lead and mercury, have no known human body function and exposure to these elements could result in both immediate and delayed adverse health effects[16].

Some trace elements, such as iron, zinc and selenium were found to be deficient among healthy smokers compared to nonsmokers. However, the available dates are inconsistent regarding the effect of smoking on trace elements [20].

Selenium: the essential trace element (Se) is a component of selenoproteins, some of which have important enzymic functions. These include the glutathione peroxidases, which reduce hydrogen peroxide and harmful lipid and phospholipids hydroperoxides, and the iodothyronine deiodinases, which

catalyze the production of active thyroid hormone, tri-iodothyronine from thyroxin [21-24].

Most Se ingested by animals and humans comes from the soil, through plants[25]. Selenium is essential for humans because it protects the heart against cardiomyopathy. It may also reduce ischemic heart disease owing to its antioxidant activity [26]. Concentrations of Se in plasma and whole blood provide useful indicators of human Se intake and status. The concentration of Se in plasma is about 80% of that in whole blood [22]. Box-1- shows reference values for plasma Se concentration in specified circumstances [25].

__Box -1- Some published plasma selenium reference levels__
- 15µg/L The lowest published level: Burundi, a very low selenium country [27]
- 89µg/L Mean of previous published Australian studies (post-1990 data)
- 100 µg/L Minimum level for maximisation of glutathione peroxidase activity in plasma
- 113 µg/L Baseline selenium level below which supplemented selenium protected against
cancer in the United States Nutritional Prevention of Cancer Trial [28]
- 120 µg/L Plausible target selenium level for reduction of cancer risk
- 216 µg/L Level in a sample from Venezuela, a high-selenium country

---

Low levels of selenium are associated with a higher incidence of both lung and prostate cancer .Although, the epidemiological evidence has been inconclusive, and there has been a growing body of laboratory studies that strengthen the theoretical basis for selenium as a cancer inhibitory agent [29].

Zinc: is an essential component in many biological enzymes. Zinc plays an important role in protein synthesis, bone formation, cell-mediated immunity, endocrine function, tissue growth and wound healing. Among the trace elements, the concentration of zinc in the body is second only to iron [30].

Serum zinc is the most frequently used index for zinc statue in humans [31]. Only few studies have described the description of serum zinc in random sample of the general population [32].

Lead: it is one of the toxic elements that has known to biological functions [33]. The myocardial infarction patients have high level of lead than the normal ones[34]. The body contains about 120 mg of lead which is mainly present in skeleton and in smaller amount in hair and blood. It accumulates with age in bones, aorta, kidney, liver and spleen. Lead is a general protoplasmic poison that is cumulative, slow acting and subtle and produces a variety of symptoms [17].

Copper: is the third most abundant trace element in the human body, following zinc and iron, and is essential to all organisms. Copper participates in many biochemical processes including cellular respiration, cellular utilization of

oxygen, maintence of all cell membrane integrity, and sequestration of free radicals [35].

Serum copper measurement provides an assessment of long term copper status. Copper deficiency and toxicity (increased levels) may be acquired or inherited[36].

Materials and Methods:
All common laboratory chemicals and reagents were of analar grade and were used without purification.
Instruments:
The instruments used in this work were, LKB gamma counter type 1270 rack gamma II, atomic absorption spectrophotometer type GBC 933plus.
Samples:
Four groups of smokers were included in this study. Group I consisted of 22 smokers, who consumed (10- 20) cigar with 0.4 mg nicotine /d, (age 22-37 years). Group II consisted of 17 smokers, who consumed (10-20) cigar with 0.8 mg nicotine/d, (age 25-40 years).
Group III consisted of 14 smokers, who consumed 40 or more than cigar with 0.4 mg nicotine/d, (age 25-35 years).
Group IV consisted of 18 smokers, who consumed 40 or more than cigar with 0.8 mg nicotine/d, (age 21-33 years). The groups were matched with a group control subjects (Group IV, consisted of 25 smokers, (age 22-35 years)). All groups were in good general health and were free of known diseases at the time of study like hypertension or diabetes. The all information of smokers and control are summarized in table-1.

Groups	Number	Age [year]	Cigar consumed	mg Nicotine/Cigar
I	22	22-37	10-20	0.4
II	17	25-40	10-20	0.8
III	14	25-35	40≤	0.4
IV	18	21-33	40≤	0.8
V	25	22-35	-	-

Table-1: The information of smokers and control subjects studied

The blood sample for the above mentioned groups were obtained from the men employees at the same local company in Baghdad-Iraq (Al-Mansoor fuel station, 2009). Five milliliters of blood samples were drawn from the study subjects (between 8:00 and 9:00 a.m), for the estimation of cortisol hormone and trace elements concentration by disposable syringe. Blood samples were left for 20 min at room temperature, after coagulation, sera were separated by centrifugation (at 2400 r.p.m) for 10 min, then sera were aspirated and stored in coped sterilized tubes at -20$^{°}$C until time of analysis.

Serum trace elements concentration were measured by atomic absorption spectrophotometer (atomic absorption spectrophotometer type GBC 933 plus),

and cortisol concentration was measured by LKB gamma counter type 1270 rack gamma II, with cortisol RIA kit from immunotech a Beckman Coulter Company. Normal values were (morning 260-720 nM) evening (50-350 nM). The analytical sensitivity was 10 nM.

Statistical analysis:

The results of serum cortisol hormone and trace elements concentration were analyzed statistically and values were expressed as mean $\mp$SD. The levels of significance were determined by student's t-test.

Results and Discussions:

Cortisol hormone concentration:

Cortisol levels in serum of four groups of smokers were measured by radioimmunoassay (RIA). The four groups were matched with a group of control subjects. Table-2 and figure-1 shows the results that were obtained from this study.

The mean level of serum cortisol in group I was found to be (480 $\mp$7.2 nM) whereas that of control was found to be (455.4+36.2nM). Significant increase of serum cortisol level (p$<$ 0.01) was obtained from student's t-test analyses.

While serum cortisol concentration was high significantly increase in group II who consumed 10-20 cigar with 0.8 mg nicotine per day (492.2$\mp$22.3 nM, p < 0.001) in comparison with the value of control subjects.

Group	No. of cases	No. of Cigar/d	Mg Nicotine/Cigar	Serum cortisol (nM)
I	22	10-20	0.4	480 $\mp$ 7.2
II	17	10-20	0.8	492.2 $\mp$ 22.3
III	14	40$\leq$	0.4	521.1 $\mp$ 12.8
IV	18	40$\leq$	0.8	522.7 $\mp$ 19.5
V	25	-	-	455.4 $\mp$ 36.2

Table-2: Cortisol hormone concentration (nM) in serum of different groups of smokers and control.
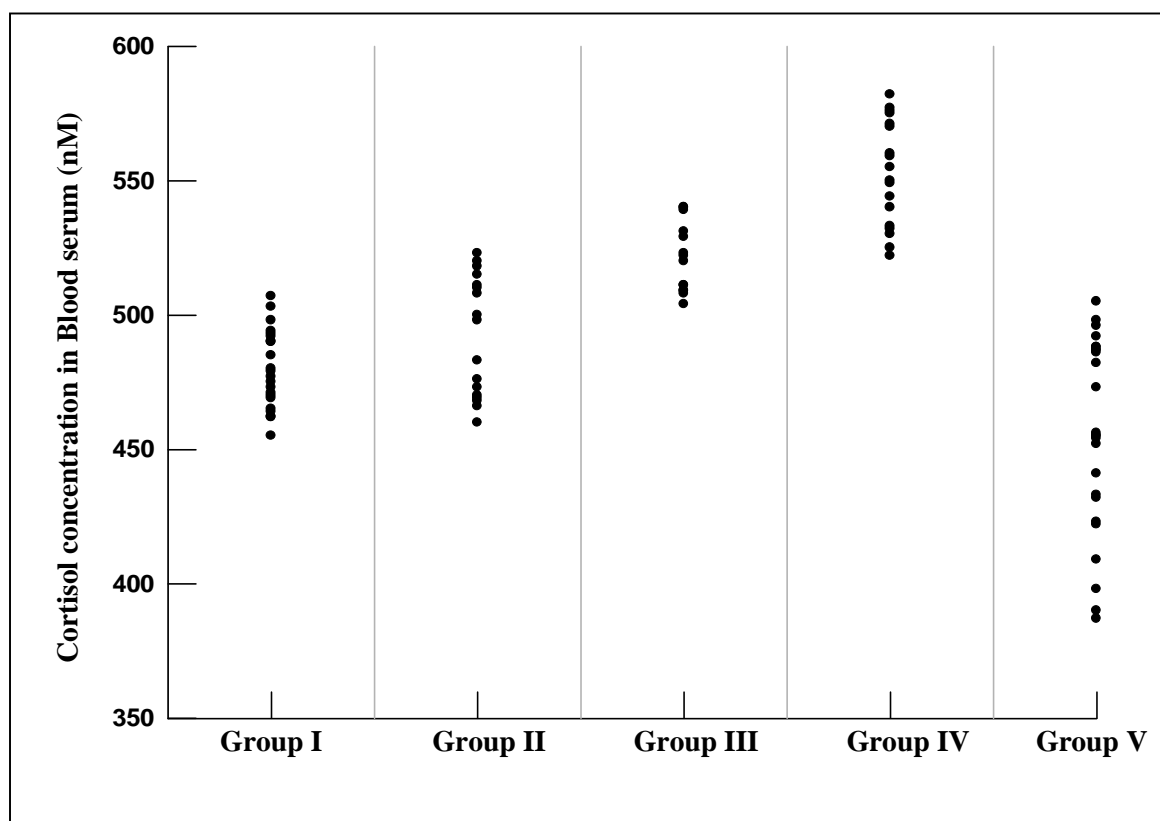
Figure-1: Distribution on serum cortisol (nM) in different groups of smokers and

Cortisol hormone concentration in smokers who consumed 40 cigar or more per day (group III and IV) was found to be (521.1 $\mp$ 12.8, 522.7 $\mp$ 19.5 nM, P < 0.001) respectively, the difference between group III and group IIII smokers was not significant (p < 0.05).

Smoking 10-20 cigars per day increase the concentration of cortisol hormone in serum about 5-8% (depending to the amount of nicotine in cigar), while in group III and IV who consumed 40 cigars or more per day increase the concentration of cortisol hormone in serum about 14%.

The results obtained from this study are nearly similar to those obtained previously by other investigators [37-39]. A number of studies have shown that cortisol levels were higher in smokers in comparison with nonsmokers [5-7], and smoking has been shown to stimulate the release of cortical [9-12].

Arbol J.L. et.al[1], reported that significant differences between smokers who consumed 20 cigar or more per day and those who consumed more than 40 cigar per day in cortisol hormone concentration. Others studies have found serum cortisol to be lowered [40] or unchanged [41] in smokers. Whereas urinary cortisol was found to be higher in smokers [42]. Smoking induces oxidative mechanisms responsible for cortisol metabolism, but excretion of 6-B-hydroxycortisol was the same in smokers and nonsmokers [43].

In conclusion, the results obtained from this study indicate that the smoking increase the cortisol concentration in serum and the level of cortsiol in smokers depends on the number of cigar consumed, and to the amount of nicotine in cigar.

Se concentration:

The concentration of Se, Cu, Zn, and Pb in serum of smokers and control are reported in table 3.

Trace element (µM0	Control (non smoker) n=25	Smoker			
		Group I (10-20 cigar with 0.4 mg nicotin /d) n=22	Group II (10-20 cigar with 0.8 mg nicotin /d) n=17	Group III (40 cigar or more with 0.4 mg nicotin /d) n=14	Group IV (40 cigar or more with 0.8 mg nicotin /d) n=18
Se	1.576∓1.62	1.41∓0.117	1.296∓0.106	1.129∓0.225	1.06∓0.198
Cu	7.1∓1.04	7.57∓1.05	7.68∓1.13	8.42∓1.124	8.08∓1
Zn	10.95∓1.1	10.95∓0.927	10.83∓1.02	10.98∓1.344	10.78∓1.117
Pb	0.518∓0.078	0.6 ∓ 0.111	0.631∓0.103	0.646∓0.112	0.63∓0.088

Tabel-3: Smoking effect in trace element concentration in serum.

As shown in these table, serum Se concentration in smokers were below control among all smokers, and the depression of Se concentration in serum was more with increasing doses of smoking and nicotine, as shown in (figure-2).
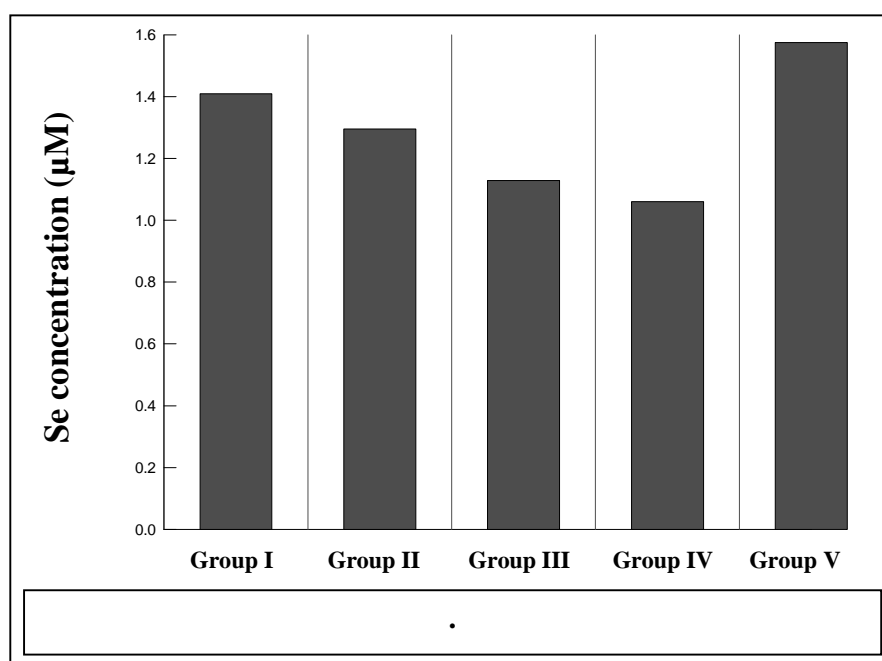

Figure-2: The concentration of Se in different groups of smokers (according to the number of cigar smoked per day and amount of nicotine in cigar) and control group.

Smoking 40 cigar or more decrease the Se concentration (39% in group III and 48% in group IV), while smoking 10-20 cigar per day decrease these concentration (11% in group I and 21% in group II) compared with controls.
These results are inconsistent with earlier finding of decreased serum concentration of selenium among smokers [44,46].
Kafai et al. finding that Se concentration in smokers may be 25%-30% lower than those in adult control subjects [47].
Several studies documented that smoking may increase oxidative stress and impair oxidant defense system [48].

Zn concentration:

Serum zinc concentration in (μM) were measured in four groups of smoker. The results represented in table 3 revealed non significant differences ($p < 0.05$) in zinc concentration of smokers in comparison with that of the control group.

Sam et al. [20] did not observe any significant effect in smokers who consumed 10-20 cigar per day on zinc concentration, but serum zinc concentration decreased among smokers how consumed more than 20 cigar per day. (Figure-3) show the effect of smoking in Zn concentration in serum.
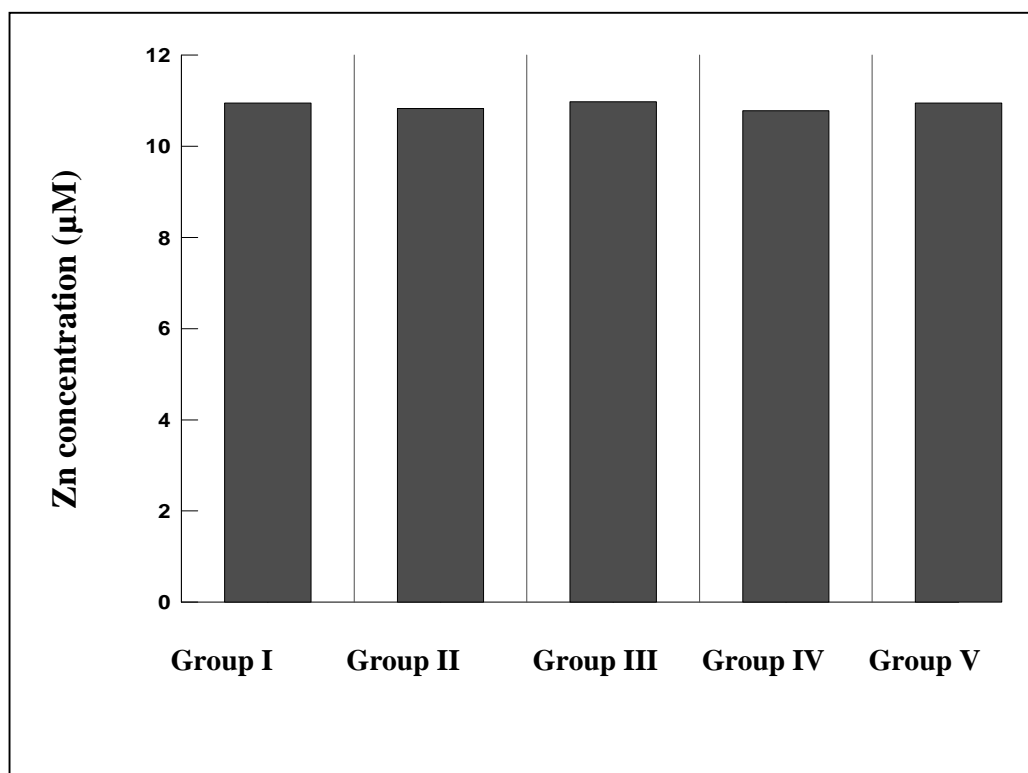

Figure-3: The concentration of Zn in different groups of smokers (according to the number of cigar smoked per day and amount of nicotine in cigar) and control group.

Pb concentration:

Serum concentration of Pb in smokers and control has been given in table-3. Figure-4 shows a significant increase (p<0.01) in serum Pb concentration in group I of smokers in comparison with that of the control group, and high significant increase (p< 0.001) in group II, III and IV in comparison with control group.

Pb concentration in group II who consumed 10-20 cigar per day (0.631μM) higher than control by about 21%, while group III and IV who consumed 40 cigars or more per day (0.646 - 0.63 μM) higher than control by about 24-21%.
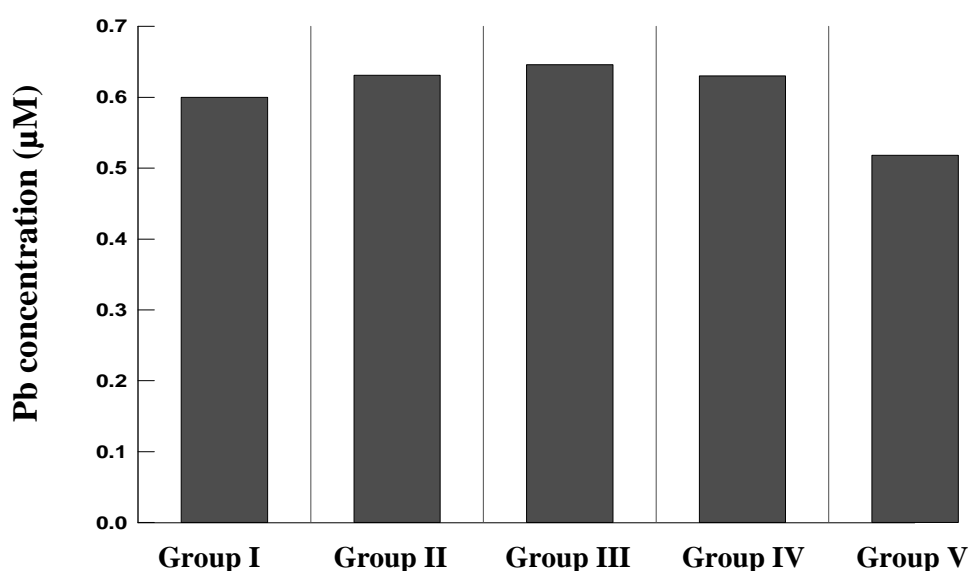

Figure-4: **The contrition of Pb in different groups of smokers (according to the number of cigar smoked per day and amount of nicotine in cigar) and control group.**

Cu concentration:

The results represented in table 3 revealed a non-significant increase (P < 0.05) in serum copper concentration in group I and II smokers, in comparison with that of the control group. While significant increase (P<0.01) in serum copper concentration in group III and IV (8.42 μM and 8.08 μM) when compared with that of control group (7.1μM) as shown in figure (5).

Kim et al. (2003)[48] did not observe any effect of smoking on serum copper concentration. In a later study, kim et al (2004) further evaluated the influence of long and short term cigar smoking among Korean teenage girls (14-18years) and adult males (36-51 years), serum copper concentrations were higher only among long term smokers compared to non smokers [49].
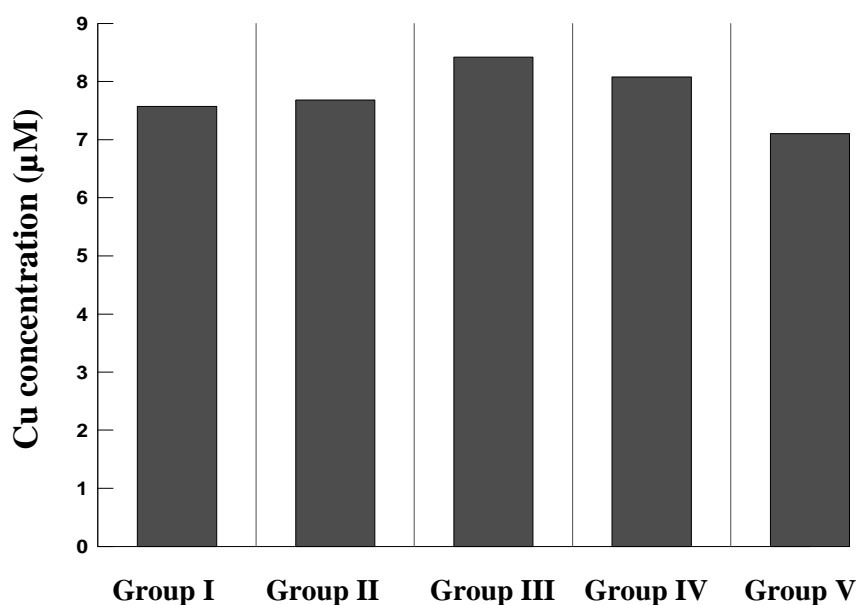
Figure-5: The concentration of Cu in different groups of smokers (according to the **number of cigar smoked per day and amount of nicotine in cigar) and control group.**

In our study, we observed increase serum concentration of copper among group III and IV (those who smoked 40 or more cigar per day), But not among group I and II (those who smoked 10-20 cigar per day), as compared to non-smokers.

Both the studies suggest that probably an increasing dose of smoking modify serum copper status more compared to those who smoke less or do not smoke at all.

Manthey et al [50] in a study of men with chest pain found a significant association between serum copper and smoking habits. Cigar smokers had copper concentration 13% higher than nonsmokers and this compares with our (12-18%) increase.

Kromhout et al [51] studying a random sample of men aged 57-76 years, of which 64.9% were smokers, found a significant relationship between cigar smoking and serum copper. A dose- response relationship was present but only in men smoking 10 or more cigar per day. The increase was also 13%.

Refernces:

1- Arbol, J.L.; Munoz, J.R.; Ojeda, L.; Cascales, A.L.; Irles, J.R.; Miranda, M.T.; Ruiz Requena, M.E. and Aguirre, J.C. (2000). *Plasma concentrations of beta − endorphin in smokers who consume different numbers of cigarettes per day*. pharmacology, Biochemistry and Behavior. 67: 25-28

2- Snyder, L.D. and Eisner, M.D. (2004). *Obstructive lung disease among urban homeless*. Chest. 125: 1719-1725.

3- Bain, C.; Feskanich, D.; Speizer, F.; Thun, M.; Hertzmark, E.; Rosner, B.A. and Colditz, G.A. (2004). *Lung cancer rates in men and women with comparable histories of smoking*. J Natl Cancer Inst. 96: 826-834.

4- Buczek, Y.; Le, A.D.; Wang, A.; Stewart, J. and Shaham, Y. (1999). *Stress reinstates nicotine seeking but not sucrose solution seeking in rats*. Psychopharmacology. 144: 183–188.

5- Field, A.E.; Colditz, G.A.; Willett, W.C.; Longcope, C. and McKinlay, J.B. (1994). *The relation of smoking, age, relative weight, and dietary intake to serum adrenal steroids, sex hormones, and sex hormone-binding globulin in middle-aged men*. J Clin Endocrinol Metab. 79: 1310–1316.

6- Baron, J.A.; Comi, R.J.; Cryns, V.; Brinck-Johnsen, T. and Mercer, N.G. (1995). *The effect of cigarette smoking on adrenal cortical hormones*. J Pharmacol Exp Ther. 272: 151–155.

7- Al'Absi, M.; Hatsukami, D.; Davis, G.L. and Wittmers, L.E. (2004). *Prospective examination of effects of smoking abstinence on cortisol and withdrawal symptoms as predictors of early smoking relapse*. Drug Alcohol Depend. 73: 267–278.

8- Wilkins, J.N.; Carlson, H.E.; Van Vunakis, H.; Hill, M.A.; Gritz, E. and Jarvik, M.E. (1982). *Nicotine from cigarette smoking increases circulating levels of cortisol, growth hormone, and prolactin in male chronic smokers*. Psychopharmacology. 78:305–308.

9- Kirschbaum, C.; Wust, S. and Strasburger, C.J. (1992). *Normal cigarette smoking increases free cortisol in habitual smokers*. Life Sci. 50:435– 442.

10- Reuter, M.; Netter, P.; Rogausch, A.; Sander, P.; Kaltschmidt, M.; Dorr, A. and Hennig, J. (2002). *The role of cortisol suppression on craving for and satisfaction from nicotine in high and low impulsive subjects*. Hum Psychopharmacology. 17:213–224.

11- Newhouse, P.A.; Sunderland, T.; Narang, P.K.; Mellow, A.M.; Fertig, J.B.; Lawlor, B.A. and Murphy, D.L. (1990). *Neuroendocrine, physiologic and behavioral responses following intravenous nicotine in nonsmoking healthy volunteers and in patients with Alzheimer's disease*. Psych neuroendocrinology. 15: 471–484.

12- Gilbert, D.G.; Meliska, C.J. and Plath, L.C. (1997). *Noise stress does not modulate effects of smoking/nicotine on beta-endorphin, cortisol, ACTH, glucose, and mood*. Psychopharmacology. 130:197–202.

13- Pomerleau, O.F. and Pomerleau, C.S. (1991). *Research on stress and smoking: progress and problems*. Br J Addict. 86:599–603.

14- Reuter, M. and Hennig, J. (2003). *Cortisol as an indicator of dopaminergic effects on nicotine craving*. Hum Psychopharmacology. 18:437– 446.

15- Pomerleau, O.F. and Pomerlau, C.S. (1990). *Dexamethasone attenuation of the cortisol response to nicotine in smokers*. Psychopharmacology. 101:284 –286.

16- Gwidotti, T.L.; Mc Namara, J. and Moses M.S. (2008). *The interpretation of trace element analysis in body fluids*. I ndian J Med Res. 128: 524-532.

17- Bukhari, I.H.; Hassan, M.N.; Haleem, A. and Bhatti, M.M. (2005). *Role of metals (cadmium and lead) in patients of hypertension and their relationship with Ischemic heart disease*. Res. J. Agric. Biol Sci. 1(2): 190–194.

18- Bakos, S.N.; Ahmed, H.K. and Nasser, T.A. (1988). *Changes in the serum copper, agnesium, zinc, 13 calcium and potassium following acute myocardial infraction*. Ngiology. 39[5]: 413-416.

19- Saltman, P. (1983). *Trace elements and blood pressure*. Ann Intern Med. 98(5): 823-827.

20- Bashar, S.K. and Mitra, A.K. (2004). *Effect of smoking on Vitamin A, Vitamin E, and other trace element in patients with cardiovascular disease in Bandladesh: a cross – sectional study*. Nutrition. (3) 18: 215-221.

21- Rayman, M.P. (2000). *The importance of selenium to human health*. Lancet. 356: 233-241.

22- Combs, G.F. (2001). *Selenium in global food systems*. Br J Nutr. 85: 517-547.

23- Rayman, M.P. (2002). *The argument for increasing selenium intake*. Proc Nutr Soc. 61: 203-215.

24- Lyons, G.H.; Stangoulis, J.R. and Graham, R.D. (2003). *High-selenium wheat: biofortification for better health*. Nutr Res Rev. 16: 45-60.

25- Lyons, G.H.; Judson, G.J.; Stangoulis, J.C.; Palmer, L.T.; Jones, J.A. and Graham, R.D. (2004). *Trends in selenium status of south Australians*. MJA. (180) 19 : 383 – 386

26- Dhindsa, H.S.; Bermingham, M.A.; Mierzwa, J. and Sullivan, D.(1998). *Plasma selenium concentration in a sikh population in sydeny, Australia*. Analyst. 123: 885-887

27- Benemariya, H.; Robberecht, H. and Deelstra, H. (1993). *Daily dietary intake of copper, zinc and selenium by different population groups in Burundi, Africa*. Sci Total Environ. 136: 49-76.

28- Duffield-Lillico, A.J.; Reid, M.E. and Turnbull, B.W. (2002). *Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention of Cancer Trial*. Cancer Epidemiol Biomarkers Prev. 11:630-639.

29- Goodman, G.E.; Schaffer, S.; Bankson, D.D.; Hughes, M.P. and Omen G.S. (2001). *Predictors of serum selenium in cigarette smokers and the lack of Association with Lung and prostate cancer risk*. Cancer Epidemiology Biomarkers and prevention. 10: 1069 - 1076.

30- Cousins, R. J. (1985). *Absorption, transport and hepatic metabolism of copper and zinc: special reference of metallothionein and cerulopasmin.* Physiol. Rev 65: 238-309.

31- Solomons, N. W. (1979). *On the assessment of zinc and copper nutriture in man.* Am J., Clin. Nutr. 32: 856-871.

32- Pilch, S. M. and Senti F.R. (1985). *Analysis of zinc data from the second National Health and Nutrition Examination Survey (NHANES II).* J. Nutr. 115: 1393-1397

33- Telisman, S.; Pient, A.; Jurasovic, J. and Cvitkovic, P. (2004). *Lead effect on blood pressure in moderately lead exposed workers.* Am. J. Ind. Med. 45 (5): 446-454.

34- Speich, M. (1982). *Concentrations of lead, cadmium and Zinc in human heart muscle and aorta after acute myocardial infraction.* J. Am. Coll. Nutrition. 1(3): 255-262.

35- Henry, J.B. (2001). Clinical diagnosis and management by laboratorymethods . 20th ed., W.B. saunders company, Philadelphia, pp.180,210.

36- Andersons, (1994). Mechanisms of injury in progressive renal disease. Exp.Nephrol.;4:34-40

37- Gilbert, D.G.; Meliska, C.J.; Williams, C.L. and Jensen, R.A. (1992). *Subjective correlates of cigarette - smoking- induced elevations of peripheral beta − endorphin and cortisol.* Psychopharmacology. 106:275-281.

38- Meliska, C.J. and Gilbert, D.G. (1991). *Hormonal and subjective effects of smoking the first five cigarettes of the day: a comparison in males and females.* Pharmacol Biochem Behav. 40:229-235.

39- Seyler, L.E.; Fertig, J.B.; Pomerleau, O.F.; Hunt, D. and Parker, K. (1984). *The effects of smoking on ACTH and cortisol secretion.* Life Sci. 34:57- 65.

40- Handa, K. S., Kono, H.; Ishii, K.; Shinchi, K. and Imanishi Arakawa, K. (1974). *Relationship of alcohol consumption and smoking to plasma cortisol and blood pressure.* J. Hum. Hypertens. 8:891–894.

41- Sparrow, D.; O'Connor, G. T.; Rosner, B.; Demolles, D. and Weiss, S.T. (1993). *A longitudinal study of plasma cortisol concentration and pulmonary function decline in men.* Am. Rev. Respir. Dis. 147:1345–1348.

42- Eliasson, M.; Hagg, E.; Lundblad, D.; Karlsson, R. and Bucht, E. (1993). *Influence of smoking and snuff use on electrolytes, adrenal and calcium regulating hormones.* Acta Endocrinol. 128:35–40.

43- Vestal, R.; Cusack, B.J.; Mercer, G.D.; Dawson, G.W. and Park B.K. (1987). *Aging and drug interactions: I. Effect of cimetidine and smoking on the oxidation of theophylline and cortisol in healthy men.* J. Pharmacol. Exp. Ther. 241:488–500.

44- Kocyigit, A.; Erel, O. and Gur, S. (2001). *Effects of tobacco smoking on plasma selenium, zinc, copper and iron concentrations and related antioxidative enzyme activities.* Clin Biochem. 34:629-633.

45- Bates, C.J.; Thane, C.W.; Prentice, A. and Delves H.T. (2002). *Selenium status and its correlates in a British national diet and nutrition survey: people aged 65 years and over.* J Trace Elem Med Biol. 16: 1-8.

46- Luty-Frackiewicz, A.; Jethon, Z. and Januszewska L. (2002). *Effect of smoking and alcohol consumption on the serum selenium level of Lower Silesian population.* Sci Total Environ 285: 89-95.

47- Kafai, M.R. and Ganji V. (2003). *Sex, age, geographical location, smoking, and alcohol consumption influence serum selenium concentrations in the USA: third National Health and Nutrition Examination Survey, 1988-1994.* J Trace Elem Med Biol. 17: 13-18.

48- Kim, S.H.; Kim, J.S.; Shin, H.S. and Keen, C.L. (2003). *Influence of smoking on markers of oxidative stress and serum mineral concentrations in teenage girls in Korea.* Nutrition. 19:240-243.

49- Kim, S.H.; Ensunsa, J.L.; Zhu, Q.Y.; Kim, J.S.; Shin, H.S. and Keen C.L. (2004). *An 18- month follow-up study on the influence of smoking on blood antioxidant status of teenage girls in comparison with adult male smokers in Korea.* Nutrition. 20:437-444.

50- Manthey, J.; Stoeppler, M. and Morgenstern, W. (1981). *Magnesium and trace metals: risk factors for coronary heart disease.* Circulation. 64:722-729.

51- Kromhout, D.; Wibowo, A.E. and Herber, R.F. (1985). *Trace metals and coronary heart disease risk indicators in 152 elderly men.* Am J Epidemiol. l22:378-385.