

ZINC STATUS AMONG SMOKERS AND NON-SMOKERS: RELATION TO OXIDATIVE STRESS

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ABSTRACT

Objective To assess zinc status in cigarette smokers and to ascertain the relationship between the levels of serum zinc and oxidative stress.

Methods A cross-sectional study was carried out at Azadi Teaching Hospital, Duhok, Iraq, from December 2007 to June 2008. The study included 254 apparently healthy males (127 smokers and 127 non-smokers, aged 20-61 years). Exclusion criteria were: a) minerals supplements, b) medication, c) recent or chronic infections. All the participants were invited for medical health examination. Data were collected from subjects according to self-administered questionnaire. Several biochemical parameters were estimated such as, serum zinc, serum antioxidant markers (ceruloplasmin and total glutathione), serum pro-oxidant by-products (malondialdehyde and peroxynitrite), and dietary zinc intake.

Results The percent of marginal zinc deficiency in smokers was significantly higher than that of non-smokers (50.1% Vs 42.6%, $p < 0.05$). Smokers had significantly higher malondialdehyde (1.6 ± 0.5 nmol/L) and peroxynitrite (1.7 ± 0.34 mmol/L) serum levels than that of non-smokers (1.2 ± 0.1 nmol/L and 1.4 ± 0.32 mmol/L respectively, $p < 0.05$) for both parameters. Serum zinc, ceruloplasmin, and total glutathione levels, were not significantly differed between the two groups, whereas the percent of abnormally high levels of oxidative stress markers were significantly higher in smokers than in non-smokers ($p < 0.01$). In respect to dietary zinc intake for smokers and non-smokers, no significant difference was found in daily dietary zinc intake for both groups ($p = 0.45$).

Conclusion This study demonstrated that cigarette smoking is associated with marginal hypozincemia which may render smokers more susceptible to oxidative stress.

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Key words: Zinc, Smoking, Oxidative stress

Accumulating evidence suggests a link between cigarette smoking and oxidative stress.¹ Smokers are at greater risk than non-smokers of having intracellular oxidizing agents, particularly free radicals.² Tobacco smoke contains numerous compounds, many of which are oxidants and pro-oxidants capable of producing free radicals and enhancing the oxidative stress *in vivo*.³ The high production of these oxidants and pro-

oxidants associated with smoking may exceed the capacity of the oxidative defense system resulting in oxidative damage to certain proteins, lipids and DNA.⁴ Data on the other hand, suggest that zinc is a necessary factor in a variety of "antioxidant", enzymes, particularly superoxide dismutase, catalase, and peroxidase. Alteration of zinc metabolism such as adequate zinc which is unavailable for these enzymes may contribute

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to the oxidative damage observed in smoking.⁵ It has been reported that cigarette smoking decreases appetite and may decrease the amount of nutrients consumed by the smokers.⁶ Cigarette smokers may be less likely to consume micronutrient supplements and more likely to consume alcohol and other substances that interact with nutrient metabolism. Tobacco leaves contain a significant amount of cadmium which is absorbed into the body when a person smokes or chews tobacco. This cadmium can replace the bivalent metals like zinc (Zn), copper (Cu), and manganese (Mn) from superoxide dismutase (SOD) which is a powerful antioxidant.⁷ A recent study indicates that even though dietary intake of minerals in smokers was adequate, the habitual diet was not able to maintain the serum zinc concentration in the normal ranges, and thus making smokers more susceptible to oxidative stress.⁸ Therefore the present study was designed to assess zinc status in a sample of cigarette smokers in comparison with that of non-smokers and to ascertain the relationship between the levels of serum zinc, oxidant and antioxidant variables.

METHODS

This study was carried out on 254 apparently healthy males aged 20-61 years. They were recruited by consecutive sampling procedure from different areas of Duhok city. One hundred twenty seven were smokers and 127 were non-smokers. Both smokers and non-smokers underwent medical health examination at Azadi Teaching Hospital, Duhok, Iraq. None of the participants was being on mineral supplements, medication, or having recent infection or chronic disease. Verbal consent was obtained from the subjects after the nature of the study had been fully explained to them. The study protocol was approved by postgraduate committee of the University of Duhok/Medical Branch.

A pre-tested questionnaire was designed to obtain information on age, smoking habit, and habitual food consumption patterns of each participants.

Selection of smokers was done according to the number of cigarettes smoked per day. For a better assessment of the impact of smoking status on parameters under testing, this study included only heavy smokers who used to smoke at least 20 sticks per day and excluded mild or casual smokers to leave a buffer zone of comparison between smokers and non-smokers. All smokers included were males, because female smokers in our society are rare. Dietary intakes of selected nutrients and zinc were calculated from the computerized food-frequency questionnaire using Food Composition Tables recommended by Hands Es.⁹

A 24-hours recall procedure was used to assess the dietary intakes of these participants. The quantity of food was estimated in portions of common household measures. A known weight or volume of household measures and some weighted food items were used as model. Marginal hypozincemia was identified by serum zinc between 50-70 ug/dl.

Participants were instructed to attend the laboratory of the Department of Clinical Biochemistry at Azadi teaching Hospital at morning after overnight fasting for 12-14, avoiding smoking and heavy physical activity for a minimum of 2 hours before examinations. Blood samples were collected between 9:00-11:00 am. Ten ml of blood was withdrawn by venipuncture using vacutainer from the antecubital vein and collected in blood vacutainer system CAT-plain tubes. After 25-30 minutes, the serum was separated and divided into number of plain tubes for the estimation of serum zinc and oxidative stress biomarkers. The pro-oxidant by-products (malondialdehyde and peroxynitrite), and antioxidant markers (serum ceruloplasmin and glutathione) were

Ceruloplasmin was measured by modified method of Menden et al 1977.¹⁰ This assays measure only native, copper-containing caeruloplasmin. Serum total glutathione concentration was determined by modified method of Ellman.¹¹ Estimation of MDA was done according to the method Beuge and Aust.¹² Serum peroxy nitrite level was measured by the modified method of Vanuffelen et al¹³ Serum zinc concentration for the target group of this study was measured by atomic absorption spectrophotometer (AAS) method. Flame Atomic Absorption (Varian 220, Australia) was used as standardized procedure. The coefficient of variation (CV) for zinc in pooled serum samples was 3.5 % (n=30). Values for the internal control sera (Randox, Ltd. England) were 73.0 (SD 4.7) ug/dl compared with certified value of 69.0 (SD 6.7) ug/dl. The atomic absorption spectrophotometer was adjusted according to the instruction of the manufacture.

Differences between groups and correlations between different variables were evaluated by paired student t-test and Spearman's Correlation Coefficient. They were calculating using the statistical package for social science SPSS. P value of <0.05 was considered statistically significant. The cut off values >(mean+2SD) for non-smokers were applied to determine the percent(%) of risk in any of the parameter in smokers.

RESULTS

Among 254 eligible male participants, 115 (45.4%) had marginal zinc deficiency (serum zinc 50-70 ug/dl), a level indicated by others.¹⁶ The univariate analysis showed that marginal zinc deficiency was more frequent in smokers (50.1% Vs 42.6%) compared to non-smokers. Of 127 smokers, 10(7.9%) had high serum ceruloplasmin levels (>0.430 g/L) and 40(31.5%) had high serum total glutathione levels (>1.7 umol/L), whereas in non-smokers (n=127) the values were relatively

lower, i.e. 5 (4.2%) and 27(21.3%) respectively, though, no significant difference was observed between the two groups (serum zinc of smokers 72.7 ± 22.4 Vs non-smokers 75.9 ± 17.7 ug/dl; caeruloplasmin 0.340 ± 0.087 Vs 0.339 ± 0.045 g/L, and total glutathione 1.27 ± 0.25 Vs 1.20 ± 0.22 umol/L). Regarding pro-oxidant by-products, 30(23.6%) of smokers had elevated serum MDA (>1.4 nmol/l) and 35(27.5%) had elevated serum peroxy nitrite (>2.09 mmol/l) compared to 8(6.3%) and 11(8.7%) of non-smokers respectively (p<0.01). The mean serum values of MDA in smokers was 1.6 ± 0.5 Vs 1.2 ± 0.1 nmol/l in non-smokers (P=0.025), and for peroxy nitrite was 1.7 ± 0.34 Vs 1.4 ± 0.32 mmol/l (P=0.021), the differences were statically significant. We examined the association between hypozincemia and the oxidative stress biomarkers. In this analysis, the results of spearman's correlation coefficient(r) revealed that hypozincemia had weak, positive association with these related variables, (ceruloplasmin; r=0.176 , p=0.1; total glutathione; r=0.193, p=0.08; MDA; r=0.195, p=0.08 and peroxy nitrite; r= 0.209, p= 0.06).

The average percentage contribution of different food sources of dietary zinc intake of participants reveled that cereal products were the major source of dietary zinc, 46.3% of all participants depend on cereal products as a source of dietary intake. Among cereal products, wheat bread contributed to 16.5% of the daily dietary zinc intake of male smokers and non-smokers. No difference in daily dietary zinc intake for male smokers and non-smokers was observed (smokers % zinc intake Vs non-smokers: cereal products 43.0 % Vs 47.0%; vegetables and vegetables products 14.0% Vs 19.0%; Legumes, seeds and related products 15.0% Vs 13.6%; Meat 15.0% Vs 7.8%; milk and diary products 5.0% Vs 4.6%; eggs 5.0% Vs 4.0%; fruits and fruit products 2.3% Vs 2.8%; and miscellaneous items 0.7 Vs 1.2%.

DISCUSSION

There have been reports of marginal zinc deficiency in Iraqi population.¹⁴⁻¹⁵ In our sample, marginal zinc deficiencies do exist, even among non-smokers. Although there have been several studies investigating the effects of cigarette smoking on zinc status, there are few published data on zinc status in our population. This study revealed low concentration of zinc in our sample compared with other population. For example, in adults a study performed on Saudi Arab males in 2006, serum zinc concentrations were markedly higher than the levels reported in this locality (i.e. 84.5 ± 11.8 vs. 74.2 ± 19.0)⁵ as well as in western populations.¹⁶ A low serum zinc concentration in Iraqi population was also observed in an earlier study of adults where mean serum zinc concentration for adult males was 78.0 ± 11.7 $\mu\text{g/dl}$ ¹⁷ Such a difference is especially note worthy because several factors are known to impact negatively on biochemical zinc status.¹⁸ Of these, dietary factors have the most marked negative effect on serum zinc concentration. The present study shows that around half of total subjects had marginal hypozincemia and a relatively higher frequency in smokers compared to non-smokers. The higher frequency of marginal zinc deficiency among smokers may have resulted from deficient absorption of zinc caused by a tobacco chelating effect. In view of the confounding effect of dietary zinc intake on biochemical zinc status, no significant difference in this parameter for male smokers and non-smokers was observed. Thus, our results suggest that smoking-induced hypozincemia is an additive factor to low dietary bioavailability among subjects living in Duhok city.

Several studies documented that cigarette smoking may increase oxidative stress and impair oxidant defense system, particularly antioxidant enzymes, and

some trace elements such as selenium, zinc and copper, which protect the body against reactive oxygen species.¹⁹ Our data shows that a high frequency of smokers had low serum zinc levels and high oxidative stress biomarkers, Thus our results suggest that cigarette smoking is associated with marginal hypozincemia, which renders smokers more susceptible to oxidative stress. However, it is unlikely that the changes in serum zinc were caused solely by dietary imbalance. Oxidative stress, a common disorder associated with smoking habit, can disturb zinc metabolism, this withstanding 51.1% of the smokers were at high risk of oxidative stress (increased levels of MDA and/or peroxynitrite). This may reflect the high prevalence of marginal zinc deficiency in smoker males. There may be a complex interaction between zinc, antioxidant (ceruloplasmin and glutathione) and pro-oxidant (MDA and peroxynitrite) markers that increases oxidative stress risk in smokers. However, it is not exactly clear that plasma zinc plays the major role as antioxidant against oxidative damage induced by cigarette smoking.

The study has few limitations. Firstly, the data from males living in Duhok city may not represent all subjects living in other parts of the Duhok governorate. Secondly, the serum zinc analyses were not indicative of zinc status for all subjects. Finally the number size of the participant was relatively low.

Even though, this study demonstrated a significance association between cigarette smoking and zinc status which may render smokers more susceptible to oxidative stress. The effects of zinc have received great deal of interest, through effects on oxidant defense system; zinc may decrease the risk of oxidative stress. Larger prospective studies are needed to confirm our observations, and experimental data may further elucidate the biological mechanisms of the associations.

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پوخته

باری کهرستی زنگ لدهف که سین جگار کیش و جگار نه کیش: هه فبهندی دگهل ئه رکی ئوکسید کرنی

ئارمانج: هه لسه نگاندا باری کهرستی زنگ لدهف نمونه کا جگار کیشان ب بهروردی دگهل جگار نه کیشان ژبو دهستنی شان کرنا کارتیکرنا زنگی وه کهرسته کی پاریزکه ر دژی زیانا ئوکسید کرنی.

شیراز: فی شه کوینی (254) کهس بخوفه گرتن کو هه می نیتر بوون و د ساخلم بوون و ل جهین جوره و جور ل باژیری دهوکی دژین. 127 ژوان جگار کیش بوون و 127 ین دی جگار نه کیش بوون. هه می هاتنه داخو زکرن بو به شداری بوون د تاقیکر نه کا نوژداری ل نه خوشخانا نازادی یا فیکر نی لدهوکی/عیراق. پیژانین هاتنه وه رگرتن ژ به شداران بریکا پرسه نامه یین که سایه تی و ژماره کا تاقیکر نیین کیمیاوی لسهر خوینا وان هاتنه کرن سه بارهت کهرستی زنگ و نیشانین دژه ئوکسید که ران و ئه نجامین ئوکسید ه ران، هه روه سا چه ندییا زنگی د خوراکی روژانه یی به شدار بوویان.

ئه نجام: ریژا ژسه دی یا ئاستی کیمیا زنگی لدهف جگار کیشان بلندتر بوو ب بهروردی دگهل جگار نه کیشان ($P < 0.05$ Vs 42.6% % 50.1). سه بارهت ئاستی دژه ئوکسید که ران چ جیاوازی نه هاته دیتن دناقبه را هه ردوو گروپان دا، به لی ریژه کا بلند ژ جگار کیشان ئاسته کی بلند ژ نیشانین ئه رکی ئوکسید کرنی هه بوون ب بهروردی دگهل جگار نه کیشان ($P < 0.01$). ئه نجامان دیارکر کو چ جیاوازیا گرنگ نه بوو د چه ندییا زنگی د خوراکی روژانه یی هه ردوو گروپان دا.

دهر نه نجام: فه کولینی گریدانه ک دیت دناقبه را جگار ه کیشانی و کیماتییا کهرستی زنگ و ئه فه یه کو جگار کیشان پتر بهر ره فدهکته ت بو توشبوونی ب زیانین ئه نجامین ئه رکی ئوکسید کرنی.

الخلاصة

حالة الخارصين في الأشخاص المدخنين وغير المدخنين : العلاقة مع جهد الأوكسدة

الهدف: تقييم حالة الخارصين في عينة من المدخنين بالمقارنة مع غير المدخنين وكذلك لاكتشاف تأثير الخارصين كعنصر وقائي ضد الضرر التاكسدي .

طريقة العمل: شملت الدراسة على (254) شخصا من الذكور الأصحاء الذين يعيشون في مناطق مختلفة من مدينة دهوك ومنهم (127) شخص مدخن و (127) غير مدخن. وقد تم دعوة هؤلاء المشاركين إلى الفحص الطبي في مستشفى ازادي التعليمي

في محافظة دهوك / العراق . تم تجميع البيانات في الأشخاص المشاركين طبقا للاستبيانات الذاتية. عدد من الاختبارات الكيماوية تم إجرائها في مصل الدم شملت على الخارصين, دلالات مضادات الأوكسدة , نواتج المؤكسدات

كما تم احتساب كمية الخارصين الغذائية اليومية للمشاركين .

النتائج: أظهرت النتائج ان النسبة المئوية لمستوى نقص الخارصين في الأشخاص المدخنين كان اعلى بالمقارنة مع غير المدخنين (50.1 % Vs 42.6% , $P < 0.05$). أما بخصوص مستوى دلالات مضادات الأوكسدة لا تباين معنوي وجد بين تلك المجموعتين, بينما نسبة عالية من المدخنين كان عندهم مستوى عالي من دلالات جهد الأوكسدة بالمقارنة مع غير المدخنين ($P < 0.01$).

تشير النتائج إلى عدم وجود تباين معنوي في كمية الخارصين الغذائية اليومية لكلتا المجموعتين .

الاستنتاج: بينت الدراسة وجود ترابط بين تدخين السكائر ونقصان الخارصين وهذا ما قد يعد المدخنين أكثر عرضة للضرر الناتج عن جهد الأوكسدة .