Oxidative stress in smokers and non-smokers healthy subjects

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Abstract

The purpose of this study was to examine the effect of cigarette smoking on serum oxidative damage , antioxidant status in healthy subjects . Subjects were randomly chosen from Baquba community. Smoker(n=86) was defined as a person who had smoked 10 or more cigarettes per day continually for at least one year(1) , while non-smoker(n=39) was a person who had no previous smoking experience . The results show that the serum MDA levels were significantly higher ($p<0.05$) in smokers compared with non smokers and lower significantly($p<0.01$) serum glutathione , superoxide dismutase and catalase activities were in smokers than in non smokers. Thus increase levels of serum MDA and decreased antioxidant activities may be important in determining the oxidant / antioxidant imbalance in smokers

Keywords: MDA , Smokers Antioxidant

فرط الأكسدة عند المدخنين و غير المدخنين الأصحاء

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المستخلص

الهدف من الدراسة لمعرفة تأثير الضرر التأكسدي الحاصل بسبب التدخين على بعض مضادات الاكسدة في مصل دم المدخنين الاصحاء ،ضمت هذه الدراسة 86 مدخن (الشخص الذي يدخن 10 سكائر او أكثر يوميا لمدة سنة واحدة على الاقل 0وقورنت بمجموعة سيطرة مكونة من 39 شخص (ليس له تأريخ سابق بالتدخين)0

أضهرت نتائج الدراسة وجود فروقات معنوية في مستوى (MDA) حيث إرتفعت عند المدخنين مقارنة بغير المدخنين($p<0.05$), بينما إنخفضت قيم مستويات الكلوتاثايون والسوبر أوكساید ديسموتيز والكاتاليز ($p<0.01$) في مصل دم المدخنين مقارنة بغير المدخنين0 إن إرتفاع مستوى (MDA) و انخفاض مضادات الأكسدة في المصل دليل على الضرر الحاصل بسبب التدخين.

كلمات مفتاحيه: فرط الأكسدة , مضادات الأكسدة , المدخنون.

Introduction

Oxidative stress plays an important role in the pathogenesis of some disease , and atheroscleorosis. Smoking may enhance oxidative stress not only through the production of reactive oxygen radicals in smoke but also through a weak of the antioxidant defense systems. Cigarette smoke may promote atherogenesis by producing oxygen – derived free radicals that damage lipids(2).

Two major phases were identified in cigarette smoke : A tar phase and a gas phase ; both phases are rich in oxygen – centered, carbon- centered andnitrogen –centered. Free radicals as well as non- radical oxidants. From theanalysis of each phase ,it was estimated that a single cigarette puff contains approximately 10 free radicals in the tar phase , and 10 radicals in the gas phase . These include various compounds , which are capable of causing an increase in the generation of various reactive oxygen species (ROS) like superoxide (O2) , hydrogen peroxide (H2O2) , hydroxyl (OH) and peroxyl (ROO) radicals. These reactive oxygen species in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation (3).

Reactive oxygen species (ROS) are involved in many cellular metabolic and signaling processes and are thought to have a role in aging and smoking (4,5). Therefore , their detoxification and elimination are necessary for normal physiologic cell activity and survival. Critical sites of ROS attack are the numbers of intercellular organelles , e.g. the phospho - lipid –rich lysosomal membranes . Lipid peroxidation involves oxidative destruction of lipids, localized mainly in cell membranes. Lipid peroxidation ,well correlated with oxidative stress intensity , is a chain reaction , in which polyunsaturated fatty acids are degraded to small,

more reactive particles such as conjugated dienes , lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS) (6).

To defend themselves against these free radical attacks, cells have developed various antioxidant systems .Several enzymatic systems can detoxify free radicals : copper/zinc superoxide dismutase (SOD) catalyzes the conversion of the superoxide anion to hydrogen peroxide and works concomitantly with hydroperoxide ,removing enzymes such as catalase and aselenoprotein , glutathione peroxidase (GPX) (14).

The present study was conducted to determine the effect of cigarette smoking on change in lipid peroxidation and antioxidant status (glutathione , superoxide dismutase and catalase) in smoker healthy subjects and compared with non smokers healthy subjects.

Subject and methods

Subjects : The study was carried out in a group of 125(39 non smoker and 86 smokers) healthy subjects aged from 16 – 42 years who had no acute or chronic diseases .

Blood sampling:

10 ml of venous blood samples were drawn (2.5 ml in to EDTA tubes for blood pictures by cells coulter, and 7.5 ml of blood samples were drawn in plain tubes), the blood samples centrifuged within 1-3 hours after blood collection at 3000rpm for 10 minutes. The samples were then transferred to 1.5 ml Eppendrof tubes and stored at -20C for less than one month before subsequent analysis.

Estimation of lipid peroxidation

Estimation of MDA in the serum , which is an index of lipid peroxidation , is based on the reaction with the thiobarbituric acid (TBA) to form a pink colored chromophore (TBA2-MDA adduct) , according to the method of stocks and dormandy(7). The pink colored chromophore formed was measured spectrophotometrically at 532nm. The concentration of MAD was measured using the molar absorbtivity coefficient of 1.56x 10 L/mol. cm . The results were expressed as µmole MDA /L .

Estimation of serum glutathione(GSH) Levels Glutathione contents in serum (measured as total Sulfhydryl group were measured according to the method of Godin et al.(8), which is based on the reaction of GSH with dithionitro benzene at pH 8 to form yellowish color chromophore which absorbs light at 412nm. The concentration of GSH was calculated using standard curve prepared for this purpose.

The assay for superoxide dismutase (SOD) was based on the ability of an enzyme to accelerate the dismutation of superoxide produced by the xanthine /xanthine oxidase system(9) .

Estimation of serum catalase activity

Serum catalase activity was assayed according to the method of Aebi (10), based on spectrophotometric follow – up for the decomposition of 1 ml (30mM) H2O2 after rapid mixing with 100Ul of serum at pH 7.0, after is and 30 seconds relative to control sample containing I ml of phosphate buffer instead of H2O2.

Statistical analysis

All data were expressed as mean ± SD . The statistical significance was elevated by student s test using statistical package for the social sciences (SPSS Cary, NC , USA) Version 18.

Result and discussion

Heamatology : Table (1)shows an increase in the neutrophils in smoking compared with the non smoking (control group) , these highly mobile cells spend most of their lives wandering in the connective tissues killing bacteria. They do this in two ways –by phagocytosis and digestion , and by reaction called the respiratory burst. The later process begins when lysosomes migrate to the neutrophil surface and degranulate , or discharge their contents in to the tissue fluid . The lysosomal enzymes catalyze the respiratory burst in which the cell take up oxygen and reduces it to highly toxic superoxide anion (O2).

Superoxide reacts with hydrogen ions to form hydrogen peroxide (H2O2). Neutrophil also release an enzyme that synthesizes hypo- chlorate [HCLO] , the active ingredient in chlorine bleach, from chloride ions in the tissue fluid. Superoxide, hydrogen peroxide , and hypochlorite are highly toxic to bacteria ; they form a chemical killing zone around the neutrophil can destroy by phagocytosis . The killing zone is also deadly to the neutrophils themselves . which die in the course of the attack . These oxidizing agents also damage connective tissues and contribute to rheumatoid arthritis (11). There were no significant differences in red blood cells count, heamoglobin , heamotocrit , whiteblood cells count and blood platelets

Table (1) Heamtological indices of smoking and non smoking subjects

	Non- Smoking	Smoking
Parameters	Mean ± SD	Mean ± SD
Numbers	39	86
Age (year)	24.67 ± 7.23	24.76 ± 4.46
RBC (x10/ul)	5.35 ± 0.71	5.54 ± 0.44
Heamoglobin(g/dl)	14.93 ± 1.42	14.56 ± 1.1
Heamatocrit(%)	50.07 ± 6.24	49.82 ± 3.72
White blood cells x10/ul	7.9 ± 1.7	8.17 ± 2.26
Neutrophil %	55.23 ± 8.54	59.98 ± 9.73*
Lymphocytes %	28.43 ± 8.11	28.64 ± 9.04

*significant ($P<0.05$)

Lipid peroxidation ,Antioxidant enzyme activities and glutathione

As shown in Table (2), The serum MDA levels were significantly higher ($p< 0.05$)in smokers compared with non-smokers which indicate the oxidative damage of cigarette smoking .

SOD along with CAT preventive antioxidants , plays a very important role in protection against lipid peroxidation . In this study, SOD and CAT activities were

significantly lower smokers than in non smokers. SOD is the first enzyme in antioxidant defense that scavenges superoxide radicals to form H2O2 and hence diminishes the toxic effects of the radical. Decreased activity of SOD has been reported in pathological conditions. The quinine-semiquinone radical from the tar phase of cigarette smoke are capable of reducing molecular oxygen to superoxide radicals whose excessive generation inactivates this enzyme .

Hence , a decrease in SOD activity upon smoke exposure could have resulted from its inactivation by tar phase oxidants. CAT is involved in the detoxification of high concentrations of H2o2 . CAT has been suggested to play an important role in the protection against oxidative stress (1 2).The presence and production of the free radicals from smoke lower this enzyme , leading to accumulation of H2o2 . A marked decrease in the activity of CAT in smokers suggests the inability of host antioxidant defense to meet the oxidative stress following chronic ±GSH, a widely distributed cellular reduce is a metabolic regulator and putative indicator of health. Serum glutathione levels are believed to be predicators of morbidity and mortality (13) . GSH plays a key role in protecting cells against electrophiles and free radicals.GSH can act directly as a free radical scavenger by neutralizing hydroxyl radicals, or indirectly by repairing initial damage to macromolecules inflicted by hydroxyl radicals. It is essential in the maintenance of protein and non protein SH group in reduced form (15).

Table (2) Levels of serum lipids and antioxidant status in non smokers and smokers subjects

Parameters	Non smokers	Smokers
MDA (u mol/L)	1.13± 0.45	2.11± 0.7*
GSH (u mol / L)	66.64± 18.61	39.18 ± 16.38**
SOD (u mol / L)	75.54 ± 11.34	27.0 ± 10.63**
CAT (u mol / L)	123.39 ± 26.99	39.84 ± 20.79**

Significant ($p<0.05$), significant ($P < 0.01$)*

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