Assessing the Influence of Cigarette Smoking on Serum DNase Enzyme Levels and Antioxidant Systems among Male University of Technology Students, Iraq

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ABSTRACT

Cigarette smoking poses a major public health challenge, contributing to a substantial number of fatalities globally. It's firmly established as risk factor for various serious conditions, including oral and oesophageal carcinomas, lung cancer, and liver cirrhosis. Our research hypothesis posited that cigarette smoking induces a depletion of serum antioxidants, thereby impairing the efficacy of body's antioxidant defence mechanisms and facilitating the onset and progression of various pathological conditions. This study aimed to evaluate the impact of smoking on serum DNase I, DNase II, MDA, and GSH levels among university students. Blood samples were collected from 40 male and 40 male non-smokers over three months from November to February. Alkaline DNase activity was estimated using method modified by Retiza, Acid DNase activity was assessed using a modified version of Kunitz, MDA was measured by the thiobarbituric acid method, GSH level determined spectrophotometrically using 5,5-dithionitrobenzoic acid (DTNB). The results showed that serum alkaline DNase, Acid DNase, and MDA levels were markedly elevated among smokers relative to non-smokers, while serum GSH levels in were significantly reduced in smokers compared to their non-smokers counterparts. In conclusion, these findings suggest that the enzymes and oxidative stress biomarkers assessed in this study could serve as valuable indicators for identifying smokers at higher risk of contributing to numerous pathological conditions.

Keyword: Cigarette; Smoking; Alkaline DNase; Acid DNase; Antioxidant

INTRODUCTION

Cigarette smoking continues to be a major global health issue, accounting for numerous preventable fatalities across the world. Despite widespread awareness of its detrimental effects, it persists as a pervasive issue, with approximately 1.1 billion current smokers globally [1]. Each puff releases free radicals, some of which stimulate the generation of reactive nitrogen and oxygen species (RNS/ROS). These reactive species then initiate and advance oxidative harm by deactivating enzymatic antioxidant such as catalase, and superoxide dismutase, as well as non-enzymatic antioxidants like ascorbate and reduced glutathione (GSH). This process leads to an imbalance between oxidants and antioxidants, leading to oxidative and nitrosative stress. The consequent oxidative damage to biomolecules -lipids, protein and DNA- is recognized as a key pathological mechanism in development of smoking-related diseases[2].

Nicotine readily passes through the blood-brain barrier and is highly soluble in lipids [3]. It increases the level of oxidative stress and stress and produces free radicals. It increases the flow of free fatty acids to the liver by stimulating lipolysis, and the increased VLDL production that results from the liver's re-esterification of free fatty acids explains smoking's atherogenic effects [4]. Under normal circumstances, our bodies can handle free radicals adequately. However, when antioxidants are lacking, or there is an excess production of free radicals, it can damage tissue. Free radicals react with critical biological molecules, including like carbohydrates, lipids, proteins, and DNA, leading to metabolic disruptions and structural alterations within cells. These interactions can induce tissue damage, compromising the functionality of critical organs, including the liver, kidneys, heart, lungs, brain, and stomach[5].

Human deoxyribonucleases (DNases) are a family of enzymes that break down phosphodiester bonds in DNA. Although they represent a small fraction of all naturally occurring nucleases, DNases play a critical role in various biological processes, including DNA digestion during nutrition and regulation of apoptosis. One particular members of this enzyme family has had a profound therapeutic impact [6]. DNase is categorized into two based on its optimal pH levels and dependence on metal ions: Alkaline deoxyribonucleases (DNase I) and acid deoxyribonucleases (DNase II). DNase I, a secreted protein, is present in several body fluids, including saliva, urine, serum, and tears. This secretory glycoprotein exhibits endonuclease activity, cleaving double-stranded DNA to produce 5`-phosphorylated polynucleotides and requires divalent metal ions. Conversely, Acid DNase is a mammalian endonuclease that hydrolyzes DNA phosphodiester bounds under acidic conditions[7].

Malondialdehyde (MDA), with the formula $CH_2(CHO)_2$, serves as a biomarker for measuring oxidative stress through various chemical tests, notably the Thiobarbituric (TBA) reaction, as frequently cited by Basant Joshi in 2020 [8]. It is anticipated that elevated serum cotinine levels, indicating increased nicotine exposure and inhalation of smoke particulates, would reduce antioxidant defense molecules like glutathione [9]. To show the impact of smoking on vital biomarkers,

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this study aimed to investigate its effects on serum DNase I, DNase II, MDA, and GSH levels among university students.

MATERIALS AND METHODS

Study design

A case control study was conducted on 80 subjects; 40 male student volunteers who were smokers and 40 male student volunteers who were non-smoking serving as the control group. All participants were enrolled at the University of Technology and aged between 20 to 29 years. The samples were collected within three months, from November to the end of February. Informed written consent was obtained from all participants in the study.

Samples collections and preparation

Approximately 3 mL of venous blood was drawn from each participant. The blood samples were allowed to clot, after which it was centrifuged at 3000 rpm for ten minutes to isolate the serum. Then serum samples were frozen at (-20^{o}C) in a new tube until analysis.

Methods

Alkaline DNase activity was estimated using Kunitz [10] modified by Retiza [11]. Acid DNase activity was assessed using a modified version of Kunitz [10]. The MDA was quantified according to procedure outlined by Hunter et al. 1985 [12], with modifications by Bakan *et al*. 2002 [13]. GSH level estimation is done using the method developed by Margan *et al*., 2005 [14]. Anthropometric indices, including height and weight, were obtained and used to calculate body mass index (BMI) for each participant.

The distribution statistics of smokers show various characteristics related to age, weight, height, BMI, number of cigarettes smoked/day, duration of smoking. According to Age Distribution, the current study includes Smokers with ages under 22 years, 10 individuals (25%); Smokers with ages 22-24 years, 26 individuals (65%); and Smokers with ages over 24 years, 4 individuals (10%).

According to Cigarette Consumption, the participants were classified based to the number of cigarettes they smoked. Smokers consuming fewer than 20 cigarettes per day were 11 individuals (27%), the Smokers consuming between 20 and 40 cigarettes per day were 24 individuals (60%), and the Smokers consuming more than 40 cigarettes per day were 2 individuals (5%).

Based on Duration of Smoking, the participants were classified as Smokers with less than 5 years of smoking experience were19 individuals (47.5%), 19 individuals (47.5%) had 5-10years of smoking experience, and 2 individuals (5%) had >10 years of smoking experience.

Statistical Analysis

All statistical analyses in this study were conducting using Statistical Package for Social Science (SPSS) version 29.0 Windows. The results were tested for normality by Shapiro-Wilk test with a p> 0.05 that indicate the norma distribution of the data. Expressive analysis was presented as the mean and standard deviation (SD) for each variable. The significance of the difference between mean values was assessed using the student's t-test; with a p-value less than 0.05 considered statistically significant and p-value greater than 0.05 regarded as non-significant.

RESULTS AND DISCUSSION

The study comprised 40 students who were cigarette smokers in good health, and the control group included 40 healthy non-smokers. Table1 present the mean and SD of age for both control and smoker groups, indicating a successful matching with no significant difference between the two groups in the study. The current study demonstrated a significant decrease in BMI in the smokers group compared to the non-smokers. These findings, consistent with earlier research, which suggests that BMI can affect lung function, with obesity potentially impacting forced vital capacity and expiratory volume[15]. However, since the majority of our respondents had average BMI values; this factor did not significantly influence the outcomes of the study.

Table 1. Mean ± SD of age, weight, height and BMI in cigarette smokers and non-smokers

Characteristic	Group A (n=40) Smokers	Group B (n=40) Non-smokers	P value
Age (year)	22.64±2.15	23.23± 0.60	0.24
Weight (Kg) Mean ± SD	75.53 ±12.36	82.18±5.84	0.002*
Height (m) Mean ± SD	1.76±0.08	1.76±0.059	0.64
BMI	24.03± 3.16	26.52± 2.49	0.001*

Individuals who begin smoking at a very young age tend to score higher on psychological assessments of nicotine dependence and are less likely to quit as they grow order. This suggests that younger individuals are more vulnerable to developing nicotine addiction [16]. Serum Acid and alkaline DNase activities showed a significant increase in the smokers group compared to the non-smokers group ($p<0.001$), as shown in Table 2.

Table 2. Activities of serum Acid DNase and Alkaline DNase in cigarette smokers and non-smokers

Characteristic	**Smokers (n=40) Smokers (mean ±SD)**	**Non-smokers (n=40) (mean ±SD)**	**P value**
Alkaline DNase Activities x10^{3} [U/L]	0.409 ±0.23	0.209 ±0.1	0.001
Acid DNase Activities x10^{3} [U/L]	12.05±5.3	4.71±2.1	0.001

The mean levels of MDA were significantly elevated, while serum GSH was decreased considerably in cigarette smokers compared to the non-smokers group, as shown in Table 3.

Table 3. Mean ± SD of serum MDA and GSH in cigarette smokers and non-smokers

Characteristic	Smokers [n=40] Mean ± SD	Non-smokers [n=40] Mean ± SD	P value
Serum MDA (µmol/L)	16.02 ±6.84	10.31 ±0.37	0.001
Serum GSH (µmol/L)	5.78±3.08	7.87±1.85	0.001

Our research hypothesis posited that the reduction in serum antioxidants due to cigarette smoking might play a crucial role in diminishing the protective capacity of antioxidant systems, contributing to numerous pathological conditions.

To our knowledge, no previous studies have shown to compare serum DNase I and II in smoker and non-smoker individuals. Elevated serum levels of DNase enzyme may be due to the effect of smoking on cells, which can damage cells by generating ROS and lead to DNA damage and other cellular components.

DNase I has significant physiological functions. This major nuclease, found in the blood and other body fluids, is essential for preventing autoimmune reactions. At the same time, DNase II, located in the lysosome and active at acidic pH, is critical for metabolism and organism production [17]. Recent research indicates that DNase II plays a role in activating the inflammatory response, making this enzyme necessary for activation toll-like receptor 9(TLR9) by bacterial genomic DNA [18,19].

Our findings align with older and recent research, highlighting MDA as a well-established marker lipid peroxidation. There is a presumption that MDA plays a role as a co-carcinogenic agent and tumour promoter and due to its pronounced cytotoxic effects and its ability to suppress antioxidant enzymes. Serum MDA levels indicates cell damage caused by free radicals, as noted by Zahid et al. in 2020 [20].

Studies by Kashinakunti et al., (2011) [21], Ali et al., (2015) [22], and Basant et al., (2020)[8] have shown a significant increase in MDA levels among smokers compared to non-smokers. Our study's results support these findings, indicating heightened lipid peroxidation in smokers, as observed by other researchers.

Oxidative stress in smokers can manifest in two main ways. The first is direct exposure to oxidants including volatile organic compounds and polycyclic aromatic hydrocarbons, as noted by Guleken et al. in 2020 [23] and Viola et al. in 2023[19]. The second mechanism is indirectly, arising from inflammation processes. This indirect oxidative stress is driven largely by macrophages and dendritic cells as they work to dissolving, absorbing, or detoxifying the inhaled particulate matter [18].

Our findings are consistent with both older and recent research. GSH, a significant endogenous antioxidant, plays a crucial role in defending against oxidative stress in various tissues such as the lungs, as discussed by Mons et al. (2015) [24]. Studies have shown that smoking leads to reduction in plasma and lung epithelial GSH levels, partially due to its conversion into its oxidized form (GSSG). Interestingly, smokers exhibit higher GSH concentrations in erythrocytes compared to non-smokers, as noted by Muscat et al. (2004) [25], likely reflecting an adaptive response to chronic exposure to tobacco smoke's pro-oxidants.

Recent studies have found that GSH levels rise significantly in smokers who quit compared to those who continue smoking. Additionally, plasma GSH levels are comparable between non-smokers and smokers consuming fewer than twenty cigarettes/day but significantly lower in those smoking more than 20 cigarettes daily, according to Ana-Maria et al. (2023) [9]. Furthermore, Begum et al. (2018) [26] observed significantly lower in saliva GSH levels of individuals using forms of chewing tobacco compared to non-users.

CONCLUSION

Considering the study's findings, it is plausible to suggest that these antioxidant and oxidative stress biomarkers could serve as valuable indicators for identifying smokers at higher risk of developing cardiovascular diseases and inflammation in pulmonary induced by smoking. Therefore, an awareness campaign highlighting the health risks of smoking and its impact on the respiratory system should be conducted for university students, especially those at the University of Technology.

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