Relationship between hair nicotine levels with blood pressure, body composition, lipid profile and leptin among healthy male smokers in Kelantan

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Abstract

Objectives: Cigarette smoking has been reported to cause acute blood pressure elevation. Therefore, it is important to assess the relationships between chronic smoking and blood pressure, body composition, and the metabolic profile to gain an understanding of the long-term effects of smoking on an individual’s body weight and health. This study examined the relationships between the hair nicotine level, blood pressure, body composition, lipid profile, and leptin in healthy male smokers.

Methods: For this cross-sectional study, 107 male smokers aged between 20 and 50 years old were recruited as volunteers. The nicotine levels in the volunteers’ hair were measured using gas chromatography-mass spectrometry. Moreover, the subjects’ blood pressure, body composition (weight, height, body mass index, body fat percentage, visceral fat, waist and hip circumferences, and basal metabolic rate), lipid profile, and leptin concentration were also measured.
Introduction

It has been well-established that smoking and obesity are significant causes of preventable morbidity and mortality.\textsuperscript{1,2} For many years, smoking and obesity have posed a global growing public health burden. Deaths attributed to tobacco use have been estimated to increase annually, with low- and middle-income countries being the most affected.\textsuperscript{7} Furthermore, both smoking and obesity have been revealed as modifiable factors that promote the development of cardiovascular disease (CVD). In fact, a vast amount of evidence supports the association between cigarette smoking and CVD, and smoking trends at present can determine how heavy the health burden of CVD will be among future communities.\textsuperscript{3} Similarly, smoking has also been reported to increase blood pressure (BP) in smokers.\textsuperscript{4} However, this finding remains controversial, as results showing similar or lower BP in smokers compared to non-smokers have also been reported in some epidemiological studies.\textsuperscript{5,6}

Moreover, the relationship between smoking and obesity is complex and currently not well understood. In fact, several studies have reported that nicotine intake reduces food intake, increases energy expenditure, and decreases weight, whereas smoking cessation has been related to hyperphagia and weight gain.\textsuperscript{7} Furthermore, a previous study indicated that tobacco users have a lower body mass index (BMI), a smaller waist circumference (WC), and a lower body fat percentage (BF) compared to non-tobacco users,\textsuperscript{8} whereas other reports have indicated that smoking does not increase one’s overall fatness, but does significantly increase abdominal and visceral obesity.\textsuperscript{9,10}

Meanwhile, leptin, an adipocyte-derived signal molecule, interacts with specific receptors located in the central neural system and peripheral tissues and results in decreased food intake and increased energy expenditure.\textsuperscript{11} Nevertheless, whether leptin concentration is affected by smoking has not been conclusively determined, as previous studies have produced varying findings pertaining to the association between smoking and leptin.\textsuperscript{12–14}

Additionally, many studies in this area have used self-reporting data, such as the number of cigarettes smoked or packs per day, to identify an individual’s smoking status. In this study, however, hair nicotine levels were employed as a biomarker to represent subjects’ smoking status. Moreover, the relationships between the hair nicotine levels and the BP, body composition, lipid profile, and leptin concentration among smokers have not been previously examined. Therefore, in the present work, the relationships between the hair nicotine levels and the BP, body composition, lipid profile, and leptin concentration are examined in healthy male smokers.

Materials and Methods

Subjects

This cross-sectional study was conducted at the School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, from January to December 2012. One-hundred-seven active Malay male smokers participated in this study. All of the subjects were generally healthy: they had no history of chronic disease, were non-obese (BMI < 30 kg/m\(^2\)), were not on routine medications, and did not consume alcohol. However, subjects who had participated in weight loss and/or smoking cessation programmes were excluded from this study. The protocol of this study was approved by the Research Ethics Committee (Human) of Universiti Sains Malaysia and was conducted in accordance with the Helsinki Declaration. Additionally, written informed consent was obtained from all subjects prior to their participation in the study. Personal background information and smoking history were obtained through interview sessions.

Blood collection, blood pressure and body composition

All eligible subjects were requested to fast overnight before the measurement day. That morning, peripheral blood samples were collected and allowed to clot at room temperature before undergoing a serum separation process. The systolic and diastolic blood pressures (SBP & DBP) were measured on the right arm in the sitting position using an automatic digital blood pressure monitor (HEM-780, Omron, Japan) after 10 min of rest. Height was measured from the standing position using a portable body meter (206, Seca, Germany), and weight, body mass index (BMI), body fat percentage (BF), visceral fat (VF), and basal metabolic rate (BMR) were measured using a digital body composition analyser (SC-330, Tanita, Japan) that applied the principles of bioelectrical impedance analysis (BIA). Furthermore, waist and hip circumferences (WC & HC) were measured using body tape at the midway point between the lower rib margin and the iliac crest and at the maximal circumference over the buttocks, respectively, from the standing position.
Lipid profile and leptin

Serum samples and aliquots for determining the lipid profile and leptin concentration were separated from clotted blood via centrifugation and stored at −80 °C until analysed. Total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were analysed using an automated biochemistry analyser (Vitalab Selecta E, Netherlands). The TC level was measured via the cholesterol oxidase-peroxidase (CHOD-PAP) colorimetric method (Randox, UK), whereas TG was estimated according to the glycerol oxidase-peroxidase (GPO-PAP) colorimetric method (Randox, UK). HDL-C was estimated using a direct clearance method (Randox, UK), and low-density lipoprotein cholesterol (LDL-C) was calculated according to the following equation: [TC (mmol/l) − HDL-C (mmol/l) − TG (mmol/l)/2.2].

Serum leptin was measured in duplicate using commercial 96-well enzyme-linked immunosorbent assay (ELISA) kits (Cusabio, China), which were based on the direct sandwich ELISA technique. Moreover, the optical density was determined using a microplate reader (Varioskan Flash, Thermo Scientific, USA) at 450 nm, whereas a standard curve of four parameter logistic (4-PL) curve-fits was generated using SkanIt software (SkanIt Software 2.4.3, Thermo Scientific, USA).

Hair nicotine

Hair nicotine levels were examined at the National Poison Centre (PRN), Universiti Sains Malaysia, Pulau Pinang, using gas chromatography-mass spectrometry (GCMS) analysis, as previously mentioned. The vortex posterior hair samples were cut as close as possible to the scalp, and only 10 cm (maximum length) of each hair sample (measured from the scalp end) was used for the hair nicotine analysis. The analysis involved hair sampling, washing, digestion, extraction, and measurement.

Statistical analysis

Statistical analysis of the data was performed using IBM® SPSS® Statistics software (Version 20, IBM, USA). The data are shown as the means (SD), and a p value less than 0.05 was considered statistically significant. The relationships between the hair nicotine level and the serum leptin, BP, body composition, or serum leptin and lipid profile were determined using a simple linear regression analysis.

Results

The mean age and BMI for the one-hundred-seven male smoker subjects were 37 (9.42) years old and 24.81 (3.73) kg/m², respectively, and the subjects had an average smoking duration of 16.91 (7.74) years. Forty-three subjects (40.2%) smoked 10 or fewer cigarettes per day; fifty-five subjects (51.4%) smoked 11–20 cigarettes per day; seven subjects (6.5%) smoked 21–30 cigarettes per day, and two subjects (1.9%) smoked more than 30 cigarettes per day. The personal background descriptions of the subjects are portrayed in Table 1, and Figure 1 illustrates the relationships between hair nicotine levels and TC and TG. Significant positive relationships were revealed between hair nicotine levels and TC (r = 0.314, r² = 0.099, p = 0.028) and TG (r = 0.351, r² = 0.123, p = 0.013). Nonetheless, no significant relationships were found between hair nicotine levels and BP, body composition, or serum leptin.

Discussion

The present study demonstrated that hair nicotine levels were related to TC and TG. However, there were no significant relationships between hair nicotine levels and BP, body composition, or serum leptin.

Since the presence of nicotine was first discovered in human hair in 1983, many studies have employed hair as a research medium, thus proving the usefulness and reliability of the hair nicotine parameter for measuring nicotine exposure in both active and passive smokers. Due to the shorter half-lives of the biomarkers measured from urine, saliva or serum, hair nicotine analysis provides better information on long-term (up to several months) active or passive smoking exposure. Moreover, hair can be easily collected and immediately stored without deterioration at a minimal cost. Thus, hair nicotine analysis appears to be reliable and efficient for the measurement of nicotine exposure in humans.

In this study, no significant relationships were discovered between hair nicotine levels and SBP or DBP. Similarly, another research group found that SBP was not significantly different in a younger (16–45 years old) smokers’ group compared to non-smokers, nor were there differences detected in DBP between younger and older (≥45 years old) smokers’ groups compared with non-smokers. Meanwhile, a study of Japanese smokers found no significant correlation between BP and smoking amount, particularly in normal weight smokers. Our subjects’ backgrounds were quite similar to those of the Japanese subjects in this study: our subjects were healthy and non-obese, with a mean BMI of 24.81 (3.73), and the majority were less than 45 years old.

![Table 1: Basic characteristics of subjects.](image)
These factors may possibly determine the BP status of our smoker subjects given the lack of a significant association between BP and the chronic smoking habit. On the contrary, however, chronic smoking has been reported to increase aortic SBP in chronic smokers compared to non-smokers, and it was also associated with uncontrolled hypertension among previously diagnosed hypertensive patients. On the other hand, SBP and DBP were also found to be lower in smokers than in non-smokers. In fact, similar findings have been reported by several groups, which reveals an inconsistent association between smoking and BP among smokers with different classes of BMI (normal, overweight, and obese). In addition to age and BMI, factors such as alcohol consumption, habitual exercise, and dietary intake may contribute to the conflicting results from studies on smoking and BP. Although, the effects of smoking on BP seem rather contradictory, it has been well-established that smoking is a major risk factor in the development and progression of CVD, and harmful effects of smoking on other cardiovascular risk factors, such as arterial stiffness, have also been reported.

In the present study, no correlation was identified between hair nicotine levels and body composition, which includes factors such as weight, BMI, WC, HC, WHR, BF, and VF. This finding is in accordance with many others from previous studies. For example, in a population-based survey of middle-aged Caucasians, Clair et al. (2011) found an insignificant association between smoking (defined as a cigarette smoked daily) and BMI. Likewise, a study of male Korean smokers found that there were no dose-dependent associations observed with BMI and BF. Yet another study reported no difference in the mean BMI between smokers and non-smokers, regardless of gender. However, the relationship between smoking and obesity indices, especially in terms of body composition, is more complex. Contrary to our results, Canoy et al. (2005) demonstrated that smokers had a lower BMI, WC, and BF than non-smokers; on the other hand, a study of Japanese men showed that obese smokers had a higher WC compared to obese non-smokers. Furthermore, the associations between smoking amount and BMI, WC, WHR, BF and VF have been reported to be dose-dependent insofar as an increased smoking amount contributes to increases in the levels of these obesity indices among smokers.

Although, there was a lack of association between hair nicotine levels and body composition in this study, significant positive relationships were revealed between hair nicotine levels and TC and TG. In accordance with these findings, previous studies have discovered higher concentrations of TC, TG, and LDL-C and a lower concentration of HDL-C in smokers compared to non-smokers. Moreover, Joshi et al. (2013) found that in male Indian smokers, the concentrations of TC, TG, and LDL-C paralleled the severity or level of smoking; whereas TC and LDL-C were reported to be strongly correlated with the smoking levels of Greek smokers. These findings suggest that smoking may contribute to dyslipidaemia in smokers, thus leading to the development of atherosclerosis and an increased risk of CVD.

Furthermore, several mechanisms for the modification of lipid levels in smokers have been described. First, nicotine can increase the secretion of catecholamine by the sympathetic adrenal system. This condition is followed by the synthesis and secretion of hepatic free fatty acids, TG, and very low-density lipoprotein cholesterol (VLDL-C) into the bloodstream. Additionally, smoking also induces the reduction of lipolysis enzymes, such as lipoprotein lipase (LPL) activity, in adipose and muscular tissue, thus reducing TG hydrolysis and clearance. Moreover, previous studies have proven that there is a TG metabolism disturbance in adipose tissue and reduced activity of LPL in the skeletal muscle of chronic smokers compared to non-smokers. Finally, smoking also reduces LPL activity through hyperinsulinemia, thus decreasing TG hydrolysis and leading to an increase of TG levels in smokers.

Nicotine administration has been reported to reduce food intake, increase energy expenditure, and decrease weight, whereas smoking cessation has been linked to hyperphagia and weight gain. Nicotine may act directly on the hypothalamus, thus influencing the regulation of energy intake and expenditure. However, the effect of chronic smoking or nicotine itself on leptin, an appetite-suppressing factor, may also offer an explanation for the influence of smoking on body weight. In the present study, no significant relationship was identified between hair nicotine levels and leptin concentration. This finding is in accordance with previous studies, which reported no relationship between smoking and leptin in smokers, non-smokers, or ex-smokers.
regardless of gender.\textsuperscript{13,33} Moreover, previous studies of smokers and non-smokers have shown no significant difference in leptin levels for either group.\textsuperscript{13,34}

Confusingly, two studies found higher leptin levels in smokers compared to non-smokers,\textsuperscript{14,35} whereas other studies portrayed lower leptin levels in smokers than in non-smokers.\textsuperscript{12,36,37} Therefore, it may be concluded that chronic smoking may affect the leptin concentration in smokers in several ways. Smoking may increase leptin secretion from adipose tissue and decrease leptin clearance, thus resulting in a higher leptin concentration.\textsuperscript{38} Smoking may also cause leptin insensitivity in the hypothalamus, thus disturbing the leptin feedback loop and leading to a subsequent compensatory increase in leptin production.\textsuperscript{35} However, reduced leptin levels among smokers may be due to an indirect effect of nicotine increasing the catecholamine concentration, which is believed to inhibit leptin expression and secretion.\textsuperscript{37} Due to these mixed findings, there is currently no single claim that can adequately represent the relationship between smoking and leptin levels. The actual relationship may vary based on factors related to dose, time, administration route, study design, and population.\textsuperscript{37}

Nonetheless, we found no significant relationship between smoking and leptin levels among our subjects. The reasons for this are unclear, but it is worthwhile to consider that smoking may not have direct effects on leptin in such a way that can easily explain the association between smoking and decreased weight or reduced food intake among smokers.\textsuperscript{13}

It must be noted that some of the limitations in this study might have influenced the findings. First, the number of subjects was small and might not reflect the exact makeup of smokers in the population. This also may contribute to the lack of association between hair nicotine levels and blood pressure, body composition, and leptin. Second, other factors might affect the obesity indices and leptin concentration among smokers, such as physical activity (PA), diet, hormones, and inflammatory markers.\textsuperscript{38} These factors were not measured. For example, PA has been reported to decrease body weight and inversely correlate with the leptin concentration.\textsuperscript{39,40} On the other hand, the strength of this study is based on employing a smoking biomarker, hair nicotine levels, rather than using self-reported information, such as cigarettes smoked per day or packs per day, to represent smoking activity. The hair nicotine level is likely a more reliable marker in determining the smoking amount. This study choice eliminated any possible biased information in terms of smoking activity.

Conclusion

In conclusion, the positive relationship between hair nicotine levels and TC and TG suggested harmful effects of chronic smoking even in generally healthy male subjects. Therefore, the promotion of and assistance with smoking cessation are among the best methods for reducing the harmful effects of smoking.

Conflict of interest

The authors have no conflict of interest to declare.

Authors’ contributions

ZS participated in the data collection, analysis and interpretation and in the manuscript preparation. MZS contributed to the study design and participated in the data collection, analysis and interpretation. CNM provided assistance with the hair nicotine analysis. AHGR was involved in the data interpretation and manuscript preparation. HMY conceived the idea and the study design and coordinated the study. All of the authors read and approved the content of the manuscript.

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