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ORIGINAL ARTICLE

SMOKING PERTURBED LIVER MACHINERY RELEVANT TO MITOCHONDRIAL FUNCTIONS

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Summary

Once absorbed through lung tissues, smog-soluble substances are readily distributable throughout organ system reaching approximately all cells perturbing the subcellular events at mitochondrial level. The present study aimed to detect the deleterious impact of smog-containing materials on mitochondrial and thereby serum pyruvate/lactate levels coincidentally with liver proper functionality. To do so, chronic smokers were recruited and sub-classified into groups based on chronicity of smoking; control (never smoked), G1=smokers for up to 5 years, G2=smokers for up to 10 years, and G3=smokers for up to 15 years. Serum samples were collected and stored for later on analysis. Results have confirmed that serum pyruvate/lactate and liver enzymes modulated reciprocally with smoking compared to control. The results also confirmed that liver enzymes were strongly modulated, GOT elevated while GPT reduced in a way reciprocal to chronicity, while ALP elevated in first few years of smoking in G1 group compared to other groups or control group. Serum albumin was significantly elevated in studied groups compared to control group with no changes appeared in total plasma protein and the bilirubin levels were higher in G2 group compared to G1 or G3 or control groups. Serum lactate and to certain extent serum pyruvate were also significantly perturbed showing higher levels in smokers compared to control or junior smokers. In conclusion, mitochondrial subcellular machinery are strongly affected following smoking indicated by serum pyruvate/lactate measurement and this in turn strongly affect liver functionality as an important organ involved in pyruvate-lactate demarcation and pertaining to the liver functionality indicated by bilirubin and total plasma protein or albumin measurements.

Key words: smoking; liver enzymes; lactate; pyruvate; mitochondria

Introduction

Smoking tobacco has an unique combination of more than 40 distinct chemicals that may be harmful or cancerous (1). Several research revealed the risk that tobacco smoke poses to children and newborns (2). Smoking-related body's immune degradation may be the cause of severe smoker' significant risk of malignancies and respiratory system disorders (3), provoked body's immune dysfunction (3).

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Unconventionally, tobacco smoke (CS) causes rapid skin aging, liver injury disease, atherosclerosis, and cardiovascular disease (4-6). Another research showed that CS induced an instability in the elements of the connective tissue framework (7) According to certain observational studies, prolonged low-dose nicotine exposure from cigarettes has significant teratogenic and carcinogenic effects in neonatal rats (8).

Additionally, earlier research demonstrated that CS enhances ROS production both inside and outside of the mitochondrial respiratory chain. In membrane polypeptides and lipids, reactive species encourage chemical modification and conformational changes (3). Various researches have shown that tissue damage and cell death are strongly influenced by oxidative stress brought on by mitochondrial malfunction (12), among these organs is liver (13-19).

Material and methods

To conduct the present study, smokers were recruited and subdivided into three groups, those who smoke for less than 5 years (G10, those who smoke for 5-10 years (G2), those who smoke for more than 10 years (G3), compared to apparently healthy non-smokers control group. Inclusion criteria for patients were, healthy subjects, age less than 50 years old, and currently smokers. Exclusion criteria were systemic diseases, alcoholism, age older than 50 years, or patients on drug therapy well known as liver disrupting agent.

The details of patients recruited and included in the study were mentioned in table 1.

Table 1. Demography of studied groups.

Parameters	Control (n=50)	G1 (n=28)	G2(n=28)	G3(n=28)
Age (years)	20-40	23-44	30-50	30-50
BMI (kg/m^2)	28±0.4	26±0.7	27±1.1	25±0.9
Duration of smoking (years)	----	<5	5-10	>10

These smokers recruited over a period of a month and venous blood samples withdrawn from them, serum separated, collected, freezed at -20c for future analysis. Cayman's method were used for pyruvate/lactate measurement which is in principle a fluorescent technique while enzymatic colorimetric technique used for measurement of TPP, TPB, ALP, GOT, and GPT (kits supplied by Elabscience). The statistical analysis were conducted using GraphPad (Version 9.3.1) One-way ANOVA with a series of t-test conduct to identify the significant ($p<0.05$) difference group. Data expressed as mean±SD.

Results

Analysis of results of measured parameters (in U/L) has revealed a significantly ($p<0.05$) higher level of serum GOT in G1, G2, G3 groups [21±1.7, 19±1.7, and 17±1.5 respectively] compared to control group. Conversely, control group showed significantly ($p<0.05$) higher serum GPT compared to G1, G2, G3 groups [16±1.9, 14±1.8, and 15±2.6, respectively]. A significantly ($p<0.05$) higher level of serum ALP demonstrated in G1 group [116±7] and compared to control group [87±6] or G2 group [97±5.7] or G3 group [103±4.6]. A significantly higher GOT to GPT ratio were quantified in G1, G2, and G3 groups [1.6±0,2, 2.6±0.8, and 2.7±0.6, respectively] compared to control group [1.3±0.1], see figure 1.

The results of the present study confirmed significantly higher level of serum albumin in G1, G2, G3 groups [3.9±0.1, 3.7±0.1, and 3.7±0.1, respectively] compared to control group [3.3±0.1]. Total serum bilirubin in G2 group [2.6±0.4] was shown to be higher than the control, G1, and G3 groups [1.4±0.2, 1.2±0.1, and 1.1±0.1 respectively]. In the other hand, a non-significant differences was reported between studied groups and control regarding plasma total protein and values were all close to control group [6±0.2].

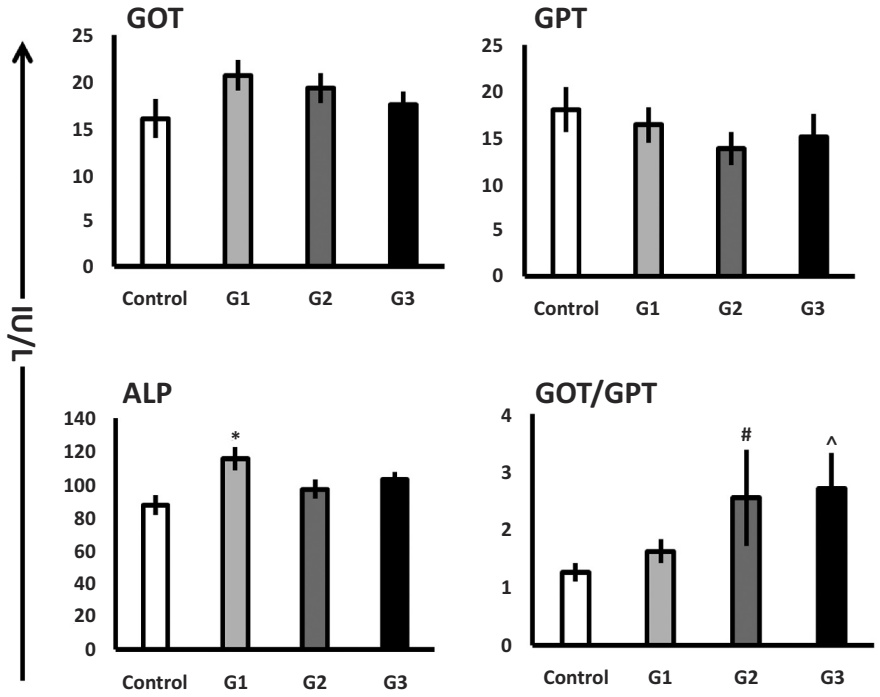

Figure 1. Liver function test in chronic smokers for up to 5 years (G1), 5-10 years smokers (G2), smokers for more than 10 years (G3). Data expressed as mean±SD, *#$P<0.05 significant difference. *$^control versus other groups.

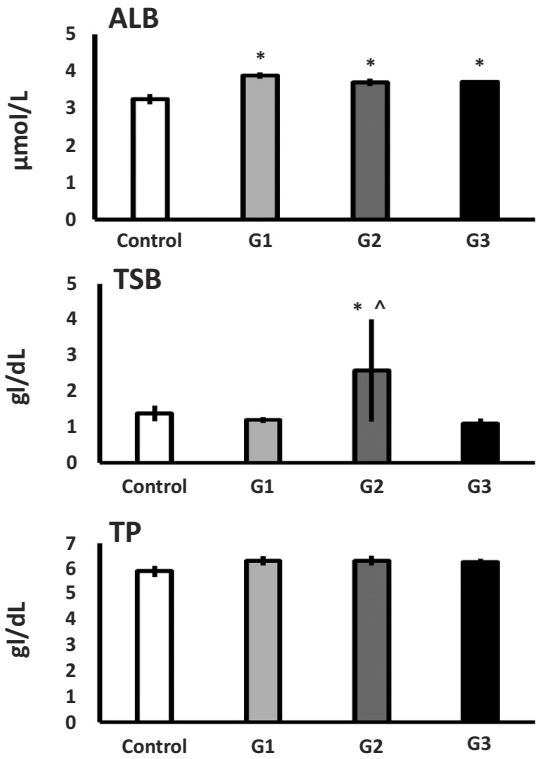

Figure 2. Plasma parameters confirmative for normal vital organ functionality in chronic smokers for up to 5 years (G1), 5-10 years smokers (G2), smokers for more than 10 years (G3). Data expressed as mean±SD, *P<0.05 significant difference as a compared to control group. ^significant difference as a compared to other groups.

Additionally, pyruvate and lactate concentration were significantly higher in smokers versus control groups (Figure 3). Smokers in G1, G2, and G3 has shown significantly higher plasma concentration of lactate and pyruvate compared to control group.

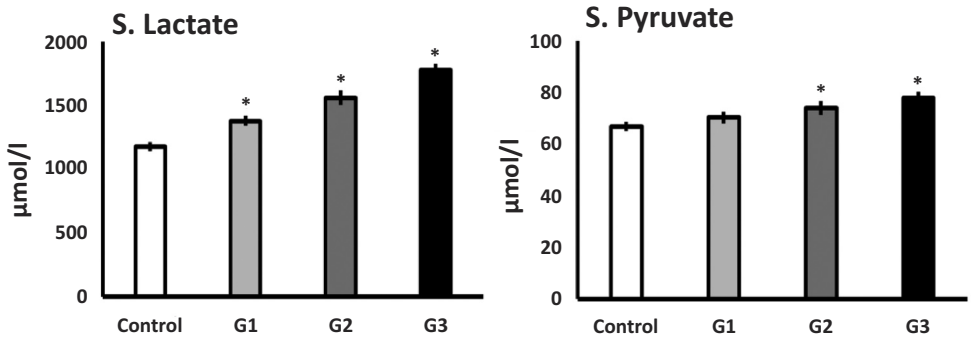

Figure 3. Lactate and pyruvate plasma concentration in chronic smokers for up to 5 years (G1), 5-10 years smokers (G2), smokers for more than 10 years (G3). Data expressed as mean±SD, *P<0.05 significant difference as a compared to control group.

Discussion

The present study confirmed that smoking has disrupted mitochondrial function represented by alteration in mitochondrial functionality indicated by increase in serum levels of lactate and pyruvate. These changes in mitochondrial functions might emphasizes the malfunctioning of vital organs; including liver. We do measured liver enzymes and the outcome confirmed that smoking has increased the liver enzyme functions especially in those smokers of less than 5 years, since the result shown that enzyme levels were stabilized in smokers for more than 5 years indicating cellular adaptation to these events. These effects of smoking on liver enzymes has been reported in some other studies which were collectively confirmed a link between smoking and its effects on liver enzymes (13-19).

To exclude presence of any hepatic diseases other than smoking; we do also measured total plasma protein, bilirubin, and albumin. Albumin has significantly increased with smoking. Total serum bilirubin in smokers older than 5 years was higher compared to other groups. Overall plasma protein levels has shown no changes in smokers versus non-smokers control group. These effects of smoking on albumin, total protein, and bilirubin has been reported in some other studies which were collectively confirmed a link between smoking and its effects on liver architecture (20-22).

Cigarette smog contain different toxic materials, among them acrolein; the material which fundamentally induce oxidative stress at subcellular level via binding to sulfhydryl group (23). In vitro studies on the toxic effects of acrolein on mitochondrial function have been conducted using PC12 cells, CHO cells, isolated mitochondria from the brain and spinal cord, lung, and heart, as well as pure mitochondrial enzymes pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase (24-29). Acrolein and its precursor allyl alcohol have been shown to have hepatotoxicity in both in vitro and in vivo studies, and treatment of acrolein to rats for 45 days has been shown to affect some hepatic mitochondrial characteristics (23).

Hypoxia has been reported to impact intracellular events via modulating mitochondrial DNA content. Correspondingly, the elevation of glycolysis and LDHA expression during the earlier stages of hypoxia, where HIF-1a contributes as a component of metabolic tolerance, promotes lactate accumulation at the latter phase of hypoxia (30). Hypoxia affect cells behaviors in term of changing cell secretome, exosome, and even the cell architecture (31-33).

The healthcare providers should hold campaign warning the individual and society against the deleterious impact of smoking on individual and community in general. This will be helpful for those who decided but not totally abandoned the smoking. On the other hand, conferences, meetings, seminars, and warning posters should be stacked on public places with gentle warning regarding smoking habits. Identified places should be allocated for smokers and smoking should be prohibited in general public.

Conclusion

Smoking is deleterious for tissue and cells of human body due to tissue hypoxia leaving a great burden on glycolytic pathways resulting in excess lactate and pyruvate this impact subsequently followed by findings of elevated level of liver enzymes and confirmed by plasma protein and albumin level with bilirubin to confirm that the hepatocyte architecture affected by smoking. The study could be further extended to study the liver structural finding by ultrasonography. We recommend screening test for liver in chronic smokers.

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Conflict of interest

The authors declare no conflict of interest concerned in the present study.

Adherence to Ethical Standards

The study was approved by the Research Ethical Committee and Scientific Committee (Study Approval Letter Number 1583 on 17.09.2019.

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