

ORIGINAL ARTICLE

# Thyroid Function and some Biochemical Indices in Male Active Smokers in Calabar Metropolis

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## KEYWORDS

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Smokers;  
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**ABSTRACT:** Alterations in the homeostasis of biomolecules and multiple organ dysfunctions associated with cigarette smoke may suggest a dose dependent mechanism. This study assessed the triiodothyronine (T<sub>3</sub>), thyroxine (T<sub>4</sub>), thyrotropin (TSH), calcium (Ca), albumin and vitamin C levels in relation to smoking pack years (SPY) in male active smokers. Ninety consenting males (18-60 yr) comprising of moderate smokers (n=26), light smokers (n=24) and non-smokers (n=40) were studied. Triiodothyronine, thyroxine, TSH and cotinine were determined by ELISA, calcium, albumin and vitamin C by colorimetry. The BMI, calcium, albumin, vitamin C and TSH were higher and cotinine, T<sub>3</sub> and T<sub>4</sub> lower in non-smokers compared to moderate and light smokers. Light smokers had higher BMI, vitamin C and TSH and lower cotinine, T<sub>3</sub> and T<sub>4</sub> compared to moderate smokers. In smokers, cotinine correlated positively with SPY ( $r = 0.838, P = 0.000$ ), T<sub>3</sub> ( $r=0.339, P = 0.005$ ), T<sub>4</sub> ( $r = 0.443, P = 0.001$ ) and negatively with BMI ( $r = -0.478, P = 0.000$ ), vitamin C ( $r=-0.407, P=0.003$ ) and TSH ( $r = -0.510, P = 0.000$ ) while SPY correlated positively with T<sub>3</sub> ( $r=0.586, P = 0.000$ ), T<sub>4</sub> ( $r = 0.608, P = 0.000$ ) and negatively with BMI ( $r = -0.597, P = 0.000$ ), vitamin C ( $r = -0.599, P = 0.000$ ), albumin ( $r = -0.281, P = 0.048$ ) and TSH ( $r = -0.578, P = 0.000$ ). Cigarette smoking and increasing smoking pack year may be associated with abnormal thyroid function and altered calcium, albumin and vitamin C homeostasis.

## INTRODUCTION

Cigarette smoking is a serious public health problem, as smoking accounts for about 3.4 million deaths annually from tobacco related diseases [1]. Cigarette smoke con-

tains about 7000 chemical compounds [2] generated by a variety of processes including; hydrogenation, pyrolysis, oxidation, decarboxylation and dehydration [3].

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Cigarette smoke exerts multiple negative effects on various metabolic and biological processes leading to multiple organ malfunction, diseases and ultimately death.

The deleterious effects of cigarette smoke on the homeostasis of some biochemical indices including electrolytes, proteins, hormones and antioxidants in cigarette smokers have been independently reported [4-7]. Some have reported an increase, decrease or no effect of cigarette smoke on the metabolism and plasma levels of these indices [8-10]. These inconsistencies may be attributed to the different contrasting pathways and mechanisms by which the different constituents of cigarette smoke exert their various effects on biological systems [11]. The behavioral and pharmacological actions of nicotine (the major constituent of cigarette smoke) and smoke associated physical stress have been implicated in some of the pathological effects of cigarette smoke [11].

The pharmacological actions and central nervous system effects of nicotine has been mediated by an active metabolite of nicotine; cotinine described as the primary biomarker of nicotine exposure [12]. Cotinine has been relatively safe and has not been associated with any of the adverse health effects associated with nicotine in humans in terms of addiction [13]. However, the cumulative effects of cotinine over the years of smoking on the levels of some biochemical indices in smokers in our locality are still uncertain.

This study therefore estimated the thyroid functions, total calcium, albumin and vitamin C levels in relation to cotinine and smoking park years of active male smokers in Calabar.

## MATERIALS AND METHODS

### *Selection of subjects*

The subjects of this study were apparently healthy regular male cigarette smokers that have not been diagnosed of any smoking related illness and non-smokers aged between 18-60 years. The smokers were recruited in

drinking and smoking joints and motor parks within Calabar metropolis. The non-smokers were recruited in residential areas in the same environment. Informed consent was sought and obtained from all subjects before recruitment into the study.

This study was carried out in accordance with the Ethical Principles for Medical Research Involving Human Subjects as outlined in the Helsinki Declaration in 1975 (revised in 2000).

A total number of 50 male cigarette smokers (26 moderate smokers and 24 light smokers) were recruited for the study. Smokers of different brands of cigarette were recruited but these brands were not taken into consideration. Smokers were classified based on smoking pack year as either heavy smokers (>30 pack-years), moderate smokers (8-30 pack years) or light smokers (<8 pack-years), where pack-year is the number of packs of cigarette smoked per day  $\times$  number of smoking years or number of pack-years = (number of cigarettes smoked per day/20)  $\times$  number of years smoked (1 pack has 20 cigarettes) [14]. The non-cigarette smokers (control) were 40 in number. There were those that have never smoked before and do not like the smell of cigarette smoke.

Anthropometric indices as height and weight were obtained and used in calculating the body mass index (BMI). Socio-demographic data were collected by an interviewer-administered structured questionnaire aiming to determine age, educational levels, socioeconomic status, social habits such as smoking, years of smoking, number of packs of cigarette smoked per day, consumption of alcoholic beverages and drug addictions. Information on general health and history of past disease(s) were collected according to the British Medical Research Council questionnaire (BMRC, 1960). Individuals with a history of chronic organ or systemic illness and long-term medication were excluded from the study.

### **Sample collection**

Four milliliters of venous whole blood sample was taken from each adult male for laboratory estimation of  $T_3$ ,  $T_4$ , TSH, total calcium, albumin and vitamin C. The blood sample was allowed to clot, retract and the serum was separated by centrifugation at 3500 g for 5 min at room temperature. Serum samples collected in five ml plain containers and stored at  $-20^\circ\text{C}$  until analysis.

## **MATERIALS AND METHODS**

### ***Estimation of Triiodothyronine ( $T_3$ ) level by enzyme linked immunosorbent assay (ELISA) method [15].***

In the  $T_3$  enzyme immunoassay (EIA) a second antibody (goat anti-mouse IgG) is coated on a microtiter wells. A measured amount of patient serum, a certain amount of mouse monoclonal anti- $T_3$  antibody, and a constant amount of  $T_3$  conjugate with horseradish peroxidase are added to the microliter wells.  $T_3$  and the enzyme conjugated  $T_3$  compete for the unlimited binding sites on the anti- $T_3$  antibody. After 60 min incubation at room temperature, the wells are washed 5 times by water to remove unbound  $T_3$  conjugate. A solution of 3,3,5,5-Tetramethyl Benzidine (TMB) is then added and incubates for 20 min at room temperature, resulting in the development of a blue colour. The colour development is stopped with the addition of 1NHCl and the absorbance is measured spectrophotometrically at 450nm. The intensity of the colour formed is proportional to the amount of unlabelled  $T_3$  standards assayed in the same way. The concentration of  $T_3$  in the unknown sample is then calculated.

### ***Estimation of thyroxine ( $T_4$ ) level by ELISA method [16]***

Microtiter strip wells are precoated with anti- $T_4$  antibodies (solid phase).  $T_4$  in the sample competes with added horseradish peroxidase labeled  $T_4$  (enzyme-labeled antigen) for antibody binding. After incubation, a

bound/free separation is performed by solid-phase washing. The immune complex formed by enzyme-labeled antigen is visualized by adding 3, 3, 5, 5 tetramethyl benzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is inversely proportional to the amount of  $T_4$  in the sample. 1NHCl is added to stop the reaction; this produces a yellow end-point colour. Absorption at 450nm is read using an ELISA microwell plate reader.

### ***Estimation of thyroid stimulating hormone (TSH) level by ELISA method [17]***

The TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Mouse monoclonal anti-TSH antibody is used for solid phase immobilization (microtiter wells) and goat anti-TSH antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 60 min of overnight incubation at room temperature, the solid phase is washed with water to remove unbound labeled antibodies. A solution of 3, 3, 5, 5'-tetramethyl benzidine (TMB) is added and incubated for 20 min, resulting in the development of blue colour. The colour development is stopped with the addition of 1NHCl, and the resulting yellow colour is measured spectrophotometrically at 480 nm. The concentration of TSH is directly proportional to the colour intensity of the test sample.

### ***Cotinine estimation by ELISA [18]***

The Cotinine Microplate ELISA is a competitive enzyme immunoassay for the detection of Cotinine in hu-

man serum, at a calibrator concentration of 25 ng/mL. The wells of the microtitre strips are coated with anti-Cotinine antibody. During the first incubation, the horseradish peroxidase (HRP) labelled Cotinine competes with the free cotinine in the donors sample for the anti-Cotinine antibody binding sites on the microtitre strips. The wells are washed to remove any excess enzyme material prior to the addition of the TMB substrate solution. Addition of the stop solution terminates the reaction and absorbances are read spectrophotometrically at 450 nm. The concentration of cotinine in the samples is then determined by comparing the optical density of the samples to the standard curve

***Estimation of vitamin C using the modified reduction method [19]***

Ascorbic acid is converted to dehydro-ascorbic acid by shaking with Norit activated charcoal and this is then coupled with 2, 4 – dinitrophenyl hydrazine in presence of thiourea as a mild reducing agent. In the presence of sulphuric acid, dinitrophenyl hydrazone which is a red compound is assayed colorimetrically. The absorbance of colored compound is proportional to Vitamin C concentration in the sample.

***Calcium estimation using Modified O-cresolphthalein – complexone method [20]***

Calcium in serum reacts with O-cresolphthalein complexone in an alkaline medium to form a purple colored complex. The absorbance of this complex is proportional to the calcium concentration in the sample.

***Determination of albumin using modified Bromocresol Green method [21]***

The measurement of serum albumin is based on its quantitative binding to the Indicator 3,3',5,5' tetrabromo-M cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG complex absorbs maximally at 620

nm, the absorbance being directly proportional to the concentration of albumin in the sample.

**STATISTICAL ANALYSIS**

Data analysis was done using the statistical package for social sciences (SPSS version 20.0) (Chicago, IL, USA). Analysis of variance (ANOVA) was used to test significance of variations within and among group means and Fisher's least significant difference (LSD) post hoc test was used for comparison of multiple group means. Pearson correlation analysis was employed to determine relationship between variables. A probability value  $P < 0.05$  was considered statistically significant.

**RESULTS**

The mean age, BMI, calcium, albumin, vitamin C, cotinine,  $T_3$ ,  $T_4$  and TSH levels in smokers (moderate and light) and non-smokers are shown in Table 1. Significant variations were observed in the mean BMI, calcium, albumin, vitamin C, cotinine,  $T_3$ ,  $T_4$  and TSH levels in moderate and light smokers and nonsmokers studied ( $P < 0.05$ ). No significant variation was seen in the ages of the 3 groups ( $P > 0.05$ ).

The comparison of mean BMI, calcium, albumin, vitamin C, cotinine,  $T_3$ ,  $T_4$ , TSH in smokers (Moderate and light) and non-smokers using LSD post hoc analysis are shown in Table 2. The mean BMI, calcium, albumin, vitamin C and TSH levels were significantly higher and cotinine,  $T_3$  and  $T_4$  lower in non-smokers compared to moderate and light smokers studied ( $P < 0.05$ ). Light smokers had higher BMI, vitamin C and TSH and lower cotinine,  $T_3$  and  $T_4$  levels compared to moderate smokers. No significant differences were seen in the calcium and albumin levels of both light and moderate smokers ( $P > 0.05$ ).

The correlation of cotinine and smoking pack years with other indices in smokers are shown in Table 3. Cotinine levels correlated positively with smoking pack years ( $r = 0.838$ ,  $P = 0.000$ ),  $T_3$  ( $r = 0.339$ ,  $P = 0.005$ ) and  $T_4$  ( $r =$

0.443,  $P = 0.001$ ) and negatively with BMI ( $r = -0.478$ ,  $P = 0.000$ ), vitamin C ( $r = -0.407$ ,  $P = 0.003$ ) and TSH ( $r = -0.510$ ,  $P = 0.000$ ) respectively ( $P < 0.05$ ). Significant positive correlations were also observed between smoking pack years and cotinine ( $r = 0.0838$ ,  $P = 0.000$ ),  $T_3$

( $r = 0.586$ ,  $P = 0.000$ ) and  $T_4$  ( $r = 0.608$ ,  $P = 0.000$ ) and negative with BMI ( $r = -0.597$ ,  $P = 0.000$ ), vitamin C ( $r = -0.599$ ,  $P = 0.000$ ), albumin ( $r = -0.281$ ,  $P = 0.048$ ) and TSH ( $r = -0.578$ ,  $P = 0.000$ ) respectively ( $P < 0.05$ ).

**Table 1.** Mean Age, BMI, Calcium, Albumin, Vitamin C, Cotinine,  $T_3$ ,  $T_4$ , TSH in Smokers (Moderate and light) and Non smokers.

| Index                     | Moderate smokers n=26 | Light Smokers n = 24 | Non smokers n=40 | F-ratio | P - value |
|---------------------------|-----------------------|----------------------|------------------|---------|-----------|
| Age (yr)                  | 43.27+2.19            | 40.29+1.72           | 41.90+1.11       | 0.72    | 0.489     |
| BMI(kg/m <sup>2</sup> )   | 19.31+0.45            | 21.67+0.43           | 23.22+0.45       | 18.43   | 0.000*    |
| Ca <sup>2+</sup> (mmol/l) | 2.37+0.01             | 2.38+0.015           | 2.45+0.010       | 8.89    | 0.000*    |
| Albumin (g/l)             | 38.69+0.99            | 38.75+1.01           | 43.40+0.64       | 11.50   | 0.000*    |
| Vit C (mg/dl)             | 0.53+0.02             | 0.62+0.01            | 0.73+0.02        | 17.31   | 0.000*    |
| Cotinine(ng/ml)           | 117.69+7.40           | 34.73+3.92           | 0.83+0.17        | 213.26  | 0.000*    |
| T <sub>3</sub> (ng/ml)    | 1.78+0.09             | 1.31+0.02            | 1.19+0.04        | 25.67   | 0.000*    |
| T <sub>4</sub> (µg/dl)    | 11.44+0.54            | 9.55+0.33            | 8.25+0.28        | 18.32   | 0.000*    |
| TSH (µIU/ml)              | 1.93+0.21             | 2.72+0.14            | 2.89+0.17        | 7.29    | 0.001*    |

\* = Significant at  $P < 0.05$ , BMI: Body mass index,  $T_3$ : Triiodothyronine,  $T_4$ : Thyroxine, TSH: Thyroid stimulating hormone

**Table 2.** Comparison of mean BMI, Calcium, Albumin, Vitamin C, Cotinine,  $T_3$ ,  $T_4$ , TSH in Smokers (Moderate and light) and Non smokers using LSD post hoc analysis.

| Index                     | Groups              |                       | Mean diff.   | P-value |
|---------------------------|---------------------|-----------------------|--------------|---------|
|                           | Non Smokers, n = 40 | Mod. Smokers, n= 26   |              |         |
| BMI(kg/m <sup>2</sup> )   | 23.22±0.45          | 19.31±0.45            | 3.90±0.64    | 0.000*  |
| Ca <sup>2+</sup> (mmol/l) | 2.45±0.010          | 2.37±0.01             | 0.08±0.02    | 0.000*  |
| Albumin (g/l)             | 43.40±0.64          | 38.69±0.99            | 4.70±1.15    | 0.000*  |
| Vit C (mg/dl)             | 0.73±0.023          | 0.53±0.02             | 0.19±0.03    | 0.000*  |
| Cotinine(ng/ml)           | 0.83±0.18           | 117.69±7.40           | -116.63±5.70 | 0.000*  |
| T <sub>3</sub> (ng/ml)    | 1.20±0.045          | 1.78±0.09             | -0.58±0.08   | 0.000*  |
| T <sub>4</sub> (µg/dl)    | 8.25±0.28           | 11.44±0.54            | -3.19±0.52   | 0.000*  |
| TSH (µIU/ml)              | 2.89±1.12           | 1.93±0.20             | -0.95±0.25   | 0.000*  |
|                           | Non Smokers, n = 40 | Light Smokers, n = 24 |              |         |
| BMI(kg/m <sup>2</sup> )   | 23.22±0.45          | 21.67±0.43            | 1.55±0.65    | 0.021*  |
| Ca <sup>2+</sup> (mmol/l) | 2.45±0.010          | 2.38±0.02             | 0.07±0.02    | 0.001*  |
| Albumin (g/l)             | 43.40±0.64          | 38.75±1.01            | 4.65±1.18    | 0.000*  |
| Vit C (mg/dl)             | 0.73±0.023          | 0.62±0.02             | 0.09±0.03    | 0.006*  |
| Cotinine(ng/ml)           | 0.83±0.18           | 34.73±3.92            | -33.90±5.80  | 0.000*  |
| T <sub>3</sub> (ng/ml)    | 1.20±0.045          | 1.31±0.02             | -0.12±0.08   | 0.048*  |
| T <sub>4</sub> (µg/dl)    | 8.25±0.28           | 9.55±0.33             | -1.30±0.54   | 0.018*  |
|                           | Mod. Smokers, n= 26 | Light Smokers, n = 24 |              |         |

**Table 2.** Continued.

|                              |             |            |            |        |
|------------------------------|-------------|------------|------------|--------|
| <b>BMI(kg/m<sup>2</sup>)</b> | 19.31±0.45  | 21.67±0.43 | -2.30±0.72 | 0.002* |
| <b>Vit C (mg/dl)</b>         | 0.53±0.02   | 0.62±0.02  | -0.09±0.03 | 0.011* |
| <b>Cotinine(ng/ml)</b>       | 117.69±7.40 | 34.73±3.92 | 82.95±6.39 | 0.000* |
| <b>T<sub>3</sub> (ng/ml)</b> | 1.78±0.09   | 1.31±0.02  | 0.47±0.09  | 0.000* |
| <b>T<sub>4</sub> (µg/dl)</b> | 11.44±0.54  | 9.55±0.33  | 1.88±0.95  | 0.002* |
| <b>TSH (µIU/ml)</b>          | 1.93±0.20   | 2.72±0.14  | 0.78±0.28  | 0.008* |

**Table 3.** Correlation of Cotinine and smoking pack year with other indices in Smokers

| <b>Index</b>      | <b>Index</b>   | <b>R – value</b> | <b>P – value</b> |
|-------------------|----------------|------------------|------------------|
| <b>Cotinine</b>   | BMI            | -0.478           | 0.000**          |
|                   | Pack year      | 0.838            | 0.000**          |
|                   | T <sub>3</sub> | 0.390            | 0.005**          |
|                   | T <sub>4</sub> | 0.443            | 0.001**          |
|                   | TSH            | -0.510           | 0.000**          |
|                   | Vitamin C      | -0.407           | 0.003**          |
| <b>Pack years</b> | BMI            | -0.597           | 0.000**          |
|                   | Vitamin C      | -0.599           | 0.000**          |
|                   | Albumin        | -0.281           | 0.045**          |
|                   | Cotinine       | 0.838            | 0.000**          |
|                   | T <sub>3</sub> | 0.586            | 0.000**          |
|                   | T <sub>4</sub> | 0.608            | 0.000**          |
|                   | TSH            | -0.578           | 0.000**          |

\*\* Significant at P<0.001

### DISCUSSION

In this study, the BMI of light and moderate smokers were significantly lower than those of non-smokers studied. BMI also correlated negatively with cotinine levels and smoking pack years of smokers. Smokers have lower BMI compared to non-smokers [22, 23]. Consistent with our findings, other studies have also demonstrated an inverse relationship between cigarette smoking and BMI and smoking cessation has been linked to weight gain [24, 25]. A nearly linear diminution in BMI associated with increasing duration of cigarette smoking has also been demonstrated [5]. These effects have been attributed to effects of nicotine on the appetite-regulating regions of the hypothalamus and on metabolism [23, 26]. Nicotine has been associated with appetite suppressor effects, decreased food intake, in-

creased gastrointestinal motility and hence increased weight loss [27]. These nicotinic effects may translate to smokers consuming decreased meal size or using smoking as substitute for dessert or entire meal [28]. Besides the physiologic effects of nicotine, smokers have been associated with poor nutritional status as resources required for balanced meals may be channeled towards satisfaction of cravings for tobacco [5].

Lower albumin levels were seen in light and moderate smokers compared to non-smokers. Negative correlations were also observed between albumin and smoking pack years in smokers studied. Similar observations have been made by other studies [9, 29, and 30]. Cigarette smoking is associated with increased oxidative stress. Albumin has been described as extracellular anti-

oxidants where albumin constitutes up to 49% of total plasma antioxidant status [29, 31]. It acts by inhibiting the generation of free radicals [32], by binding copper ions and scavenging hypochlorous acid (HOCl). The scavenging of HOCl by albumin may be due to the rapid reaction with sulfur hydriyl group (SH-group) in the circulation and their degradation [33, 34]. Albumin also contains one reduced cysteine residue (Cys34) which constitutes the largest pool of thiols in circulation. Through the reduced Cys34, albumin is able to scavenge hydroxyl radicals [9]. Thus, significantly lower levels of plasma albumin in cigarette smokers could be a consequence of their increased consumption to buffer the effect of increased free radicals generation associated with smoking. Albumin transports bilirubin, albumin-bound bilirubin has been attributed with lipid peroxidation inhibitory effects [35]. Lower plasma albumin concentrations in smokers compared to non-smokers may also suggest the induction of an acute-phase response to smoking, since albumin is a negative acute-phase protein. Moreover, there may be dietary effects on albumin synthesis, since smokers have been associated with poor nutritional status [5].

The calcium levels of light and moderate smokers were significantly lower than those of non-smokers studied. Calcium absorption and vitamin D levels have been lower in smokers [36]. Damage to the intestinal villi by some components of cigarette smoke has been demonstrated in an animal study [37]. Nicotine has vasoconstrictive action on intestinal blood supply. Modest reduction in intestinal blood flow may result in decrease calcium absorption and hence lower serum calcium levels seen in smokers [38]. Contrary to our findings, smoking increases the serum calcium levels [39]. Significant increase was demonstrated in Ca levels in chronic smokers compared to controls [40]. Nicotine increases ionized calcium levels  $[Ca^{2+}]_i$  [41, 42 and 43]. It modulates calcium metabolism and regulate adhesion and motility of respiratory epithelial cells by increasing the

expression of alpha 3, 4, 5 and 7 nicotinic receptors [43]. Long-term exposure to milimolar nicotine levels had been resulted in a steady increase in ionized calcium  $[Ca^{++}]_i$ , that may lead to cell damage [43]. Disparity between the findings of these studies and this present study may be related to the smoking pack year, since only light and moderate smokers without heavy smokers (>30 pack-years) were studied.

Light and moderate smokers have lower vitamin C levels compared to non-smokers studied and serum vitamin C levels correlated negatively with the smoking pack year. Associations between smoking and lower levels of serum ascorbic acid have been documented [6, 44, and 45]. Lower serum levels of ascorbic acid observed in smokers may reflect inadequate dietary intake. Smoking has been contributed to a significant reduced intake of fruits rich in antioxidants [6]. Altered metabolism of vitamin C because of effects of nicotine may also be a contributing factor to lower vitamin C status in smokers. Factors that might influence the metabolic fate of ascorbic acid and result in consistent observation of lower serum ascorbic acid levels in the smoking population may include decreased absorption which may be related to vasoconstrictive effects of nicotine on intestinal blood flow, increased metabolic demand and increased rate of elimination among others [46].

Several mechanisms have been proposed for hypovitaminosis C in smokers. Increased oxidative stress has been described as the major mediator of these effects. One puff of cigarette smoke has been estimated to contain as many as  $10^{15}$  of gas-phase radicals and  $10^{14}$  of tar-phase radicals [47]. Oxidative stress arising directly from the toxicity of the smoke itself can increase vitamin C turnover [48]. In addition, smoke-induced inflammatory responses may indirectly increase oxidative stress, thereby contributing to the turnover of vitamin C [49, 50]. Directly or indirectly, cigarette smoke has resulted to an increased vitamin C oxidation ratio among smokers [51, 52]. This may be related to the activity of

vitamin C as a free radical scavenger in smokers, but could also be because of an impairment of the enzymatic recycling of vitamin C [52]. Differences in vitamin C status between smokers and nonsmokers may be related to altered pharmacokinetics [52]. From these observations, we can then deduce that increasing smoking pack years will therefore be associated with a corresponding deficit in vitamin C status.

Higher levels of  $T_3$  and  $T_4$  were seen in moderate and light smokers and lower TSH only in moderate smokers compared to non-smokers. Moderate smokers also had higher  $T_3$  and  $T_4$  and lower TSH compared to light smokers studied. Similar observations have been made by other studies [8, 53]. Lower thyrotropin (TSH) levels have been reported in both current and former smokers compared to never smokers, while higher levels were also seen in moderate smokers compared to heavier smokers. [54]. Lower TSH and higher FT3 have also been demonstrated in a cohort of pregnant women who smoke compared to their non-smoking counterparts [55]. Lower serum TSH concentration was also observed in smokers compared to ex-smokers and non-smokers [56]. Nicotine has been a thyroid stimulant. Nicotine is also a potent activator of the hypothalamic - pituitary thyroid axis. It mimics the effects of acetylcholine at selected central nicotine acetylcholinergic receptors, thereby causing sympathetic activation, which in turn activates the thyroid-stimulating hormone to stimulate the thyroid gland to cause increased thyroid hormones secretion. The lower serum level of thyroid stimulating hormone in smokers is due to its suppression by the elevated levels of serum thyroid hormones because of negative feedback regulation [2, 57, and 58]. However, contrary to our findings, a decrease in the levels of both  $T_4$  and  $T_3$  has been reported in smokers [10]. The serum concentrations of thyrotropin, free thyroxine, and triiodothyronine were similar in the smokers and non-smokers among women with hypothyroidism [59]. In another study, smoking habits did not seem to affect the

serum triiodothyronine ( $T_3$ ) concentrations [56]. Accompanying TSH levels were lower in most [60, 61] and normal in some studies [62].

Smoking pack year, which is a product of quantity of cigarette, smoked and duration of smoking correlated positively with  $T_3$  and  $T_4$  and negatively with BMI, vitamin C and TSH levels in the smoking population studied. A similar correlation trend was also observed between cotinine levels and  $T_3$ ,  $T_4$ , BMI, vitamin C and TSH. Associations between dose of cigarette smoke exposure and levels of thyroid hormones have been documented [63]. Increasing concentration of nicotine and hence cotinine (biomarker of nicotine exposure) in the blood is directly proportional to the quantity of cigarette smoked [64]. High concentration of nicotine in the blood will therefore be associated with a corresponding increase in  $T_3$  and  $T_4$  levels and a corresponding decrease in TSH through negative feedback effect. Effects of cigarette smoke on thyroid functions may therefore be dose dependent. The risk of Grave' disease has been heightened for smokers who smoked more than 28 sticks of cigarette per day [65]. Increase in cotinine levels of 10ng/mL has been associated with a 1.4% decrease of odds ratio of having TSH level above 4.5 mU/L after adjustment for age, gender, iodine status and race/ethnicity. "Moreover, every 10 ng/mL increase in serum cotinine was reported to be associated with an approximately 2% increased odds of having TSH level of 0.1–0.4 mU/L" [66]. "Heavier smoking has been associated with lower TSH concentration, but smoking 12 or more cigarettes per day did not cause any further decrease of TSH concentration" [6, 57]. Contrary to these findings, no correlation between the numbers of cigarettes smoked daily and serum TSH concentration in smokers was reported by another study [67].

## CONCLUSIONS

Cigarette smoke and increasing smoking pack years may be associated with changes in thyroid functions and the



homeostasis of calcium, albumin and ascorbic acid as demonstrated by decreased TSH, total calcium, albumin and ascorbic acid and increased T<sub>3</sub> and T<sub>4</sub> levels seen in smokers studied. Cigarette smoke may therefore predispose to oxidative stress and development of thyroid disorders.

### Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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