Original article

Thyroid function determinants, calcium, phosphate and vitamin D in adult male smokers in Calabar, Nigeria

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Abstract

Cigarette smoke contains noxious substances associated with alteration in thyroid gland function and bone metabolism in smokers. Serum cotinine, triiodothyronine (T3), thyroxine (T4), thyroid stimulating Hormone (TSH), calcium, phosphate and vitamin D levels were estimated in 100 smokers and 50 non-smokers. The smokers were classified into light and moderate smokers based on smoking pack-years. Serum cotinine, T3, T4 and TSH were determined using ELISA, calcium and phosphate by colorimetric methods and Vitamin D by HPLC. Statistical analysis was done using analysis of variance, Student’s t-test and Pearson’s correlation. The level of significance was set at p< 0.05. The mean diastolic blood pressure (p = 0.002), cotinine (p = 0.0001), T4 (p = 0.014) was significantly higher in smokers compared to non smokers. While vitamin D was significantly lower (p = 0.0001) in smokers compared to non smokers. There was however no significant difference (p<0.05) in the mean values of systolic blood pressure, BMI, T3, calcium, phosphate and TSH between the two groups. There were significant variations in the mean systolic blood pressure (p = 0.040), diastolic blood pressure (p =0.0001), cotinine (p =0.0001), T4 (p = 0.036), vitamin D (p = 0.0001) among non smokers, light and moderate smokers. There was an increasing trend in the systolic and diastolic blood pressures and cotinine with the moderate smokers having the highest values. In contrast, there was a decreasing trend in the vitamin D with the moderate smokers having the lowest values. There were however no variations in the mean values of the other parameters among the three groups. Cigarette smoking may have no effect on serum calcium, phosphate, T3 and thyroid stimulating hormone levels. However it is associated with a decrease in serum vitamin D and an increase in T4 levels and blood pressure.

Key words: Cigarette smoking, Cotinine, Calcium, Phosphate, Thyroid function, Vitamin D

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Tobacco use is widespread globally¹ and smoking is the most common method of consuming tobacco². The effect of smoking in human health are serious and in many cases, deadly¹. Tobacco kills up to half of its users i.e. nearly 6 million people each year³. More than 5 million of those deaths are the results of direct tobacco use while more than 600,000 are the result
of non-smokers being exposed to second-hand smoke. Cigarette smoke contains up to 4000 potentially harmful and carcinogenic substances, the most addictive being nicotine. The levels of cotinine (a key metabolite of nicotine) in the blood is proportionate to the amount of exposure to tobacco smoke and therefore can be utilized as a marker for both active smoking and as an index to environmental tobacco smoke exposure.

Thyroxine (T4) and triiodothyronine (T3) are major hormones of the thyroid gland which regulate the heart rate, body temperature, blood pressure and the basal metabolic rate. They are regulated by thyroid stimulating hormone produced by the anterior pituitary hormone. Disruptions in thyroid hormone metabolism usually results in either hyperthyroidism or hypothyroidism both of which have adverse consequences on body function and metabolic rate.

Bone homeostasis is a complex process involving the following 4 key components: serum calcium; serum phosphate; 1, 25 dihydroxyvitamin D3 and parathyroid hormone (PTH). More than 99% of the total body calcium is stored in bone in the form of phosphate and hydroxide salts, predominantly as hydroxyapatite. Calcium and vitamin D have a modest effect on reducing bone loss or improving bone mineral density and decreasing fracture risk, especially in subjects with adequate calcium.

Exposure to cigarette smoke has a multiple deleterious effects on many organs of the body system resulting in heart disease, respiratory diseases, stroke, cancer and infertility. Mechanisms of damage include oxidative stress by nitrogen dioxide, carbon monoxide and deposition of metals which catalyze the oxidation of cellular proteins resulting in damage to various organs. Cigarette smoke interferes with thyroid hormones level in humans, however literature on the effects of smoking on the thyroid hormones are controversial. Both decreased and increased thyroid functions have been described in smokers. Most recent studies have shown higher thyroid hormones and lower thyroid stimulating hormone levels in smokers, while others have reported elevated TSH and lower thyroid hormones. Though nicotine has also been associated with decreasing bone density and osteoporosis resulting in increased levels of calcium and phosphate ions, is hard to determine whether a decrease in bone density is due to smoking itself or to other risk factors common among smokers such as poor dietary habits. However, in another study, smoking was associated with decreased calcium absorption and vitamin D levels resulting in low serum calcium ions and high phosphates. Most of these studies were carried out in Caucasian populations. The aim of this study therefore is to determine the levels of thyroid function determinants, calcium, phosphate and vitamin D in adult smokers and non-smokers in an African population.

Materials and Methods

Selection of subjects

This was a case-control study. One hundred male cigarette smokers and 50 male non-smokers (controls) aged between 19 and 45 years were consecutively recruited for this study. They were all Nigerians living in the Calabar metropolis of Cross River state. The non-smokers were recruited in residential areas in the same environment. Ethical clearance was obtained from the Cross River State Ministry of Health (Ref No.: RP/REC/2015/311). They were informed of the nature of the research and their consent obtained. Informed consent was obtained from all the subjects after which sociodemographic information was collected using a well structured questionnaire. Blood pressure and anthropometric indices were measured and body mass index calculated. Based on smoking pack years they were classified as Light smokers (<8 pack years), Moderate smokers (8-30 pack years) and Heavy smokers (>30 pack years). The smoking pack-year was calculated as the number of packs smoked/day multiplied by the number of smoking years.

Inclusion criteria

The smokers had to be male regular smokers i.e. smokers who smoked between 5-20 cigarette sticks per day. The controls had never smoked cigarettes in their life.

Exclusion criteria

Smokers who had been diagnosed of any smoking-related disease (such as lung cancer, coronary heart disease), terminal disease or on drugs, were excluded from the study.

Sample collection

Five milliliters of venous whole blood sample was aseptically collected from each adult male for laboratory investigation of T3, T4, TSH, calcium, inorganic phosphate, vitamin D and cotinine. Serum samples were obtained and stored at -20°C until analyzed.

Sample analysis

Thyroxine was estimated using T4 ELISA kits (DRG International Inc., USA). Triiodothyronine
was estimated using T₃ ELISA kits (DRG International Inc., USA)²⁰. Thyroid stimulating hormone was estimated using TSH ELISA kit (DRG International Inc., USA)²¹. Serum vitamin D was analyzed using high performance liquid chromatography (HPLC) 1200 series (HPLC Agilent, USA) at the Nigerian Institute of Medical Research, Ibadan. Cotinine was estimated using a solid-phase competitive ELISA kit (BQ kits Diagnostics, San Diego, USA). Serum calcium (Ca) level was estimated by modified Ortho-cresolphthalein complexone methodology using inorganic phosphor commercial kit (AGAPPE, Switzerland). Serum inorganic phosphate (pi) level was estimated by phosphomolybdate methodology using inorganic phosphorus commercial kit (AGAPPE, Switzerland).

Data was analyzed using the PAWStat 18, a statistical package from SPSS Inc, Chicago, USA. Results were expressed as Mean±SD. Comparisons of groups were made using analysis of variance and post hoc analysis using Least significant difference (LSD). The level of significance was set at 95% confidence interval, where p-value less than 0.05 (p<0.05) was considered as statistically significant. Correlation was done using Pearson’s correlation. Graphs created with Microsoft excel 2007 version.

**Results**

A comparison of age, body mass index, blood pressures, cotinine, thyroid function hormones, calcium, phosphate and vitamin D in male smokers and non-smokers is shown in Table 1. The mean diastolic blood pressure (p = 0.002), T4 (p = 0.014) was significantly higher in smokers compared to non-smokers. While Vitamin D was significantly lower (p = 0.001) in smokers compared to non-smokers. There was however no significant difference (p<0.05) in the mean values of systolic blood pressure, BMI, T₃, calcium, phosphate and TSH between the two groups.

**Table 1**: Comparison of age, body mass index, blood pressures, cotinine, thyroid function hormones, calcium, phosphate and vitamin D in male smokers and non-smokers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Smokers n = 100</th>
<th>Non-smokers n = 50</th>
<th>Calc. t</th>
<th>Crit. t</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.3±10.22</td>
<td>30.9±10.46</td>
<td>1.329</td>
<td>1.98</td>
<td>0.186</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.3±4.48</td>
<td>23.9±2.58</td>
<td>0.808</td>
<td>1.98</td>
<td>0.420</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>130.8±16.49</td>
<td>128.2±11.52</td>
<td>1.018</td>
<td>1.98</td>
<td>0.310</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82.7±12.22</td>
<td>76.42±9.17</td>
<td>3.182</td>
<td>1.98</td>
<td>0.002</td>
</tr>
<tr>
<td>Cotinine (ng/ml)</td>
<td>30.90±32.39</td>
<td>1.09±1.35</td>
<td>9.188</td>
<td>1.98</td>
<td>0.0001</td>
</tr>
<tr>
<td>T3 (µg/dl)</td>
<td>2.51±3.40</td>
<td>3.35±2.76</td>
<td>1.513</td>
<td>1.98</td>
<td>0.108</td>
</tr>
<tr>
<td>T4 (µg/dl)</td>
<td>6.11±1.51</td>
<td>5.45±1.54</td>
<td>2.500</td>
<td>1.98</td>
<td>0.014</td>
</tr>
<tr>
<td>TSH (IU/L)</td>
<td>2.22±1.60</td>
<td>2.12±1.14</td>
<td>0.368</td>
<td>1.98</td>
<td>0.714</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.37±0.15</td>
<td>2.36±0.14</td>
<td>0.444</td>
<td>1.98</td>
<td>0.657</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.21±0.24</td>
<td>1.20±0.19</td>
<td>0.014</td>
<td>1.98</td>
<td>0.989</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>23.69±6.11</td>
<td>61.83±12.35</td>
<td>20.607</td>
<td>1.98</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table 2 shows a comparison of body mass index, blood pressures, cotinine, thyroid function hormones, calcium, phosphate and vitamin D in non-smokers, light and moderate smokers. There were significant variations in the mean systolic blood pressure (p = 0.040), diastolic blood pressure (p =0.0001), cotinine (p =0.0001), T4 (p = 0.036), vitamin D (p = 0.0001) among the three groups. There was an increasing trend in the systolic and diastolic blood pressures and cotinine with the moderate smokers having the highest values. In contrast, there was a decreasing trend in the vitamin D with the moderate smokers having the lowest values. There were however no variations in the mean values of the other parameters among the three groups (Table 2). There was also a significant positive correlation between smoking pack year (p = 0.281; r = 0.005) and diastolic blood pressure in the smokers (Fig 1). A power analysis was carried out for the 100 male cigarette smokers and 50 non-smokers using repeated measures of the general linear model on PAWstat 18 (SPSS), using the 7 analytes measured. The observed power for using α = 0.05 was 1.000 (p =0.0001), the observed power of within tests effects was 1.000 (p = 0.0001) and the observed power for between subjects effect was 0.410 (p = 0.083).
Table 2: Comparison of body mass index, blood pressures, cotinine, thyroid function hormones, calcium, phosphate and vitamin D in light, moderate and non smokers using smoking pack years

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non smokers (n = 50)</th>
<th>Light smokers (n = 78)</th>
<th>Moderate smokers (n = 22)</th>
<th>Calc. f</th>
<th>Crit. f</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9± 2.58</td>
<td>23.3±3.87</td>
<td>23.4±6.29</td>
<td>0.339</td>
<td>3.083</td>
<td>0.713</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>128.2±11.52</td>
<td>129.0±13.11</td>
<td>137.4±24.39</td>
<td>3.279</td>
<td>3.083</td>
<td>0.040</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>76.4±9.17</td>
<td>81.32±9.69</td>
<td>88.3± 17.80</td>
<td>8.979</td>
<td>3.083</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cotinine (ng/ml)</td>
<td>1.09±1.35</td>
<td>21.81±22.46*</td>
<td>63.11±41.26*</td>
<td>57.969</td>
<td>3.083</td>
<td>0.0001</td>
</tr>
<tr>
<td>T3 (ng/ml)</td>
<td>3.35±2.76</td>
<td>2.54±3.44</td>
<td>2.40±3.33</td>
<td>1.156</td>
<td>3.083</td>
<td>0.317</td>
</tr>
<tr>
<td>T4 (µg/dl)</td>
<td>5.45±1.54</td>
<td>6.17±1.57*</td>
<td>5.88±1.28</td>
<td>3.415</td>
<td>3.083</td>
<td>0.036</td>
</tr>
<tr>
<td>TSH (IU/L)</td>
<td>2.12±.1.14</td>
<td>2.19±1.63</td>
<td>2.31±1.52</td>
<td>0.201</td>
<td>3.083</td>
<td>0.818</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.36±0.14</td>
<td>2.37±0.15</td>
<td>2.38±0.15</td>
<td>0.100</td>
<td>3.083</td>
<td>0.905</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.20±0.19</td>
<td>1.20±0.23</td>
<td>1.23±0.28</td>
<td>0.110</td>
<td>3.083</td>
<td>0.896</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>61.83±12.35*</td>
<td>23.93±6.16</td>
<td>22.87±5.96</td>
<td>319.691</td>
<td>3.083</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*significantly higher than that of non-smokers, # significantly higher than that of light smokers, ¥ significantly higher than that of moderate smokers

Fig 1. Correlation plot of smoking pack years against diastolic blood pressure

Discussion

This research was carried out to determine the effects of cigarette smoke on serum thyroid function hormones, calcium, phosphate and vitamin D levels in male smokers compared to non-smokers.

Cotinine is a major nicotine metabolite that may be used as a marker for both active smoking and as an index to environmental tobacco smoke exposure. In this study it was observed that the smokers had higher levels of exposure to tobacco smoke (as shown by their cotinine levels) compared to the non-smokers. Mean cotinine of moderate smokers were also higher than those of light smokers showing higher levels of exposure in the former. It was observed that the mean serum TSH and T3 levels in smokers were comparable to that of the non-smokers even when their T4 levels were higher; however the T4 levels of both groups were within the normal range. The light smokers had higher T4 compared to the non-smokers. The reason for this is unclear, but it has been reported that the noxious effect of smoking on the thyroid gland seems to become apparent when thyroid function is slightly compromised, while in euthyroid patients the pool of circulating thyroid hormones is adequate to compensate for the smoking-induced defect of thyroid hormone action. Thus, in normal adults smoking has either no effect on thyroid function or a weak pro-thyroid effect, causing small, thyrotrophin-independent increases in thyroid function. This probably explains the reason for the elevated T4 in the smokers. This may account for the observations made in the thyroid function of both smokers and non-smokers in our study. Both decreased and increased thyroid functions have been described in smokers. Most recent studies have shown higher thyroid hormones and lower thyroid stimulating hormone levels in smokers while others have reported elevated TSH and lower thyroid hormones which differ from our findings. A study by Sepkovic et al (1984) did not show any changes in TSH and free T3 and which agrees with our findings.
The unchanged plasma calcium and phosphate among smokers could be due to a combination of factors, the first being as a result of a decreased calcium and phosphate uptake in bone. In vitro studies have shown a direct toxic effect of tobacco on osteoblasts which is detrimental to bone formation because it inhibits osteoblast differentiation. Nicotine has also been associated with decreasing bone density and osteoporosis. This should contribute to reduced calcium and phosphate uptake by the bone and hence increased levels of these ions in plasma. However, decreased calcium absorption and vitamin D levels have also been associated with smoking. The mean vitamin D level in the smokers in our study was decreased by almost 50% compared to the non-smokers irrespective of their cotinine levels or if they were light or moderate smokers. In a study using animal models, authors reported that some components of cigarette smoke cause damage to the intestinal villi. The vasoconstrictive effect of nicotine on intestinal blood supply results in moderate reductions in intestinal circulation causing a decrease in the absorption of calcium and hence lower serum calcium levels. A balance between these two opposing phenomena may be responsible for the unchanged levels of calcium and phosphate. Similar observations were also made by Brot and colleagues.

In this study, the diastolic blood pressure was significantly higher in smokers than non-smokers. This finding is similar to those in an earlier study by Abtalic et al (2011) who reported that diastolic hypertension is more prevalent in smokers than non-smokers. He suggested that this may be due to the acute vasoconstrictive effect of smoking on blood pressure and heart rate as a result of the release of sympathetic neurotransmitters, epinephrine and norepinephrine, which are potent vasoconstrictors.

Ito et al (2013) reported similar findings. They noted that smoking is a risk factor which can cause hypertension. The explanation behind this is that nicotine has been found to transcriptionally upregulate the expression of LDL receptor gene revealing possible mechanism by which cigarette mediates abnormality in lipid metabolism which may lead to development of cardiovascular related disease.

It was also observed in this study, that systolic and diastolic blood pressures in moderate smokers (8-30 pack years) was significantly higher than those of light smokers (< 8 pack years). This finding is in line with earlier studies by Rosen and colleagues who reported that the incidence of hypertension is increased among those who smoke 15 or more cigarettes per day and the co-existence of hypertension and smoking decreases left ventricular function in asymptomatic people. Abtahi et al (2011) also reported that smokers with higher pack-years smoking were more likely to have systolic hypertension than occasional smokers with less than 5 pack years smoking. This also is in accordance with this study.

Conclusion

This research suggests that cigarette smoking may have no effect on serum calcium, phosphate, T3 and thyroid stimulating hormones. However it is associated with a decrease in serum vitamin D and an increase in T4 levels and blood pressure. It recommended that in further studies where effect of smoking on bone metabolism is to be carried out it may be more beneficial to use other more sensitive markers in conjunction with calcium and phosphate.

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References

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