THE EFFECT OF SMOKING IN PARATHYROID HORMONE (PTH) AND ALKALINE PHOSPHATASE (ALP)

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ABSTRACT

Background: Smoking is one of the most common practices and believed to be associated with decreased bone density and calcium absorption therefore abnormalities of both PTH and ALP are expected in smokers. Objectives: This study was aimed to study the effect smoking in serum levels of PTH and ALP activity in comparison to healthy non-smokers. Methods: this is a cross-sectional study conducted during the period from August to December 2011. Blood samples were drawn from 50 apparently healthy cigarette smokers’ volunteers (as test group) and 50 apparently healthy non-smokers’ volunteers’ age, BMI and gender matched as control group. Serum levels of parathyroid hormone and ALP activity was analysed for both groups by using commercial reagent kits from DRG diagnostic and commercial reagent kits from Bio system Company by Selector E, respectively. Data were analysed using SPSS v 16. Results: The serum level of PTH in smokers (14.174±5.89 pg/ml) was significantly decreased than non-smokers (26.874±11.8627 pg/ml) , p.value <0.0001. The activity of ALP was significantly increased in smokers compared to non-smokers (119.24±31.2 vs 89.70±20.28 u/l , p.value < 0.0001). In test group serum parathyroid hormone was negatively correlated, while alkaline phosphatase activity was positively correlated with both the duration of smoking (per years) and the number of cigarette smoked per day. Conclusion: From this study, it is concluded that; cigarette smoking is associated with low serum parathyroid hormone and increased serum alkaline phosphates activity and more alteration is expected with both increased duration of smoking per year and number of cigarettes smoked per day.
INTRODUCTION

Tobacco smoking is the practice where tobacco is burned and the resulting smoke (consisting of particle and gaseous phases) is inhaled. Smoking is the most common method of consuming tobacco, and tobacco is the most common substance smoked. Tobacco smoking is the most prevalent and preventable cause of disease and death. Effects of smoking are estimated to kill 3 million people per year. Recent studies have shown a direct relationship between tobacco use and decreased bone density, however, it is hard to determine whether a decrease in bone density is due to smoking itself or to other risk factors common among smokers. Cigarettes’ smoking promotes osteoporosis, a condition in which bones weaken and are more likely to fracture.

Several previous reports on the relation between smoking and serum PTH, both low as well as high PTH levels have been reported as a result of smoking. PTH act to increase serum calcium concentration by increasing resorption of calcium from bone, stimulating calcium retention by the renal tubules and promoting 1-hydroxylation of 25-hydroxy vitamin D in kidney in turn, vitamin D increase calcium absorption from the intestine. PTH also increases renal excretion of phosphate. However, PTH enhances the uptake of phosphate from the intestine and bones into the blood. In the bone, slightly more calcium than phosphate is released from the breakdown of bone. Reference range is 0.29-0.85 ng/ml. In previous study, serum PTH measured in 7896 subjects and was found to be significantly lower in smokers than non-smokers.

ALP activity is present on cell surfaces in most human tissues. The highest concentrations are found in the intestine, liver, bone, spleen, placenta, and kidney. Activity in bone is confined to the osteoblasts, those cells involved in production of bone matrix. Elevated ALP may be seen in a variety of bone disorders Paget's disease, osteosarcoma, other bone disorders include Osteomalacia, rickets, hyperparathyroidism, fractured bone, vitamin D deficiency, Primary hypothyroidism and during periods of physiologic bone growth. Smoking reported to be associated with increase alkaline phosphatase. This study was designed to evaluate the effect of cigarette smoking on the serum level of parathyroid hormone and alkaline phosphatase activity compared to apparently healthy individuals (non cigarette smoker) as control group.
MATERIALS AND METHODS

This study was done in Khartoum state in Saad Geshira market and Alzaiem Alazhari University during the period from July to December 2012. Fifty long standing (one years and more) Sudanese cigarette smokers as test group and fifty apparently healthy non smoker as were included in this study. Both the test group and the control group were matched for age, gender and BMI. Those with parathyroid gland disease, acute pancreatitis, malabsorption, bone disease, liver disease, renal disease, alcohol intakes and any medication that may be affect the Parameters under study were exclude from this study. Permission of this study was obtained from to local authorities in the area of the study. An informed consent was obtained from each participant in the study after explaining objectives of the study. Interview and Questionnaire was used to collect data including.

The BMI was calculated for all participants as weight (kg) /Height (m)^2. Non probability sampling technique was used. Using antiseptic technique, 5 ml of venous blood was collected from each participant. Serum was separated directly from the plain container by centrifugation at (300 rpm) for 5 minutes. Serum levels of PTH was measured by PTH intact ELIZA kits from DRG diagnostics, and serum activity of alkaline phosphatase were measure using selecter E by kits from automation Biosystem Company at 37c as manufacture instructions. Analytical performance of these methods controlled using two levels control sera from DRG diagnostic for each. To evaluate the association between the duration of smoking per years and serum levels of PTH and alkaline phosphatase activity, the smokers were grouped in to three groups according to ≤ 10, 10-20, > 20 year, respectively. To assess the effect between the number of cigarette smoked per day and PTH and alkaline phosphatase activity, the smokers were grouped in to three groups according to ≤ 10, 10-20, > 20 cigarette per days respectively. The mean±SD was calculated for all quantitative variables. The data collected in this study were analyzed using SPSS computer analysis program and R software. The normality was checked by Shapiro-wilk test.

The quantitative variables between two groups were compared using student T. test or Mann Whitney U test as appropriate. To compare statistical differences between three group kruskal wallis or ANOVA test were used as appropriate. Person correlation was used to study association between variables. Chi –square was for comparison of nominal data. P.value less than 0.05 was considered significant.
RESULTS
A total of 50 male cigarette smokers and 50 apparently healthy non smokers male as controls were enrolled in this study. The mean age of the cigarette smoker was 45.35±17.89, while it was 43.00±18.036 in non-smokers. The mean BMI of the cigarette smoker was 22.1020±1.55 while it was 22.3240±1.76 in non-smokers. As shown in table (1) there was no significant differences regarding age and BMI between two groups’. All participant individuals were male, therefore gender effect was not considered.

Table (2) summarizes comparison of serum mean levels of PTH and ALP activity between smokers and non-smokers. The PTH was significantly lower in smokers than non smokers and ALP activity was significantly increased in smokers compared to non-smokers (p-value p<0.0001). The mean levels of PTH in three group of smokers according to duration of smoking per years $<10,10-20, >20$ were 16.89 , 15.06, 11.94 pg/ml respectively. The difference in the PTH between three group was statistically significant (kruskal wallis, p-value= 0.031) as indicated by box plots in figure (1-A).The mean levels of alkaline phosphatase activity in three group of smokers according to duration of smoking per years $<10,10-20, >20$ were 103.33,114.67,132.00 u/l respectively ,the difference in the ALP between three group was statistically significant (kruskal wallis ) p-value= 0.015as indicated by box plots in figure (1-B). The mean levels of PTH in three group of smokers according to $<10,10-20, >20$ cigarette per days were 15.08, 13.45 ,12.42 pg/ml , respectively. The difference in the PTH between three group was statistically significant (kruskal wallis , p-value= 0.043) as indicated by box plots in figure (2-A). The mean levels of alkaline phosphatase activity in three group of smokers according to $<10,10-20, >20$ cigarette per days were 110.04,122.00,143.89u/l ,respectively. The difference in the ALP between three group was statistically significant (kruskal wallis ) p-value= 0.014 as indicated by box plots in figure (2-B). In the correlation study, the serum level of PTH negatively correlated with duration of smoking per years (r=-0.410,p-value =0.003)figure (3-A) and with number of cigarette smoked per days (r=-0.181, p-value =0.08)figure(3-B). In the correlation study ,the serum level of ALP positively correlated with both duration of smoking per years (r=0.449,p-value =0.001)figure (4-A) and with number of cigarette smoked per days (r=0.405, p-value =0.004)figure(4-B).
Table (1) shows the comparison of age and BMI in smokers and non smokers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Smokers n=50</th>
<th>Non smokers n=50</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathyroid hormone</td>
<td>14.174±5.8965 (30.0-8.0)</td>
<td>26.874±11.8627 (63.0-13.2)</td>
<td>$7.7 \times 10^{-11}$</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>119.24±31.247 (240-74)</td>
<td>89.70±20.285 (118-47)</td>
<td>$9.2 \times 10^{-8}$</td>
</tr>
</tbody>
</table>

- The table shows the mean ± standard deviation, range in brackets (Max-Min) and P value.
- Paired sample T-test was used for comparison.
- P value less than 0.05 considered significant.

Table (3-2) shows the comparison between level of Parathyroid hormone and alkaline Phosphatase in smokers and non smokers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Smokers</th>
<th>Non smokers</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>45.35±17.890 (80-20)</td>
<td>43.00±18.036 (80-20)</td>
<td>0.517</td>
</tr>
<tr>
<td>BMI</td>
<td>22.1020±1.55235 (25.00-18.00)</td>
<td>22.3240±1.76376 (25.00-18.30)</td>
<td>0.508</td>
</tr>
</tbody>
</table>

- The table shows the mean ± standard deviation, range in brackets (Max-Min) and P value.
- Paired sample T-test was used for comparison for the calcium ,and Mann –whitney U test used for PTH, phosphate and ALP.
- P value less than 0.05 considered significant.

Figure (1) : Box and wisher plots showing the serum levels of PTH (A) and ALP (B) in different smoking groups according to duration of smoking. The horizontal lines represents median. The boxes covers the 25~ 75% percentile results and the maximum and length of each wishker is 1.5 times the interquartile range. Points outside this indicate outliers.
Figure (2): Box and whisker plots showing the serum levels of PTH (A) and ALP (B) in different smoking groups according to number of cigarette per day. The horizontal lines represent median. The boxes cover the 25~75% percentile results and the maximum and length of each whisker is 1.5 times the interquartile range. Points outside this indicate outliers.

Figure (3): Scatter shows the correlation between parathyroid hormone and duration of smoking (A) and number of cigarette per day (B).

Figure (4): Scatter shows the correlation between alkaline phosphatase and duration of smoking (A) and number of cigarette per day (B).
DISCUSSIONS

In the present study the effects of smoking on PTH, and alkaline phosphatase activity in healthy male smoker compared to healthy male non smoker was evaluated. In Sudanese society it hard to found female smokers ,females are reluctant to discuss the issue of smoking in open manner .the authors impression is that a considerable number of young female do smoke .

Several author have reported an association between smoking and fracture risk as well as low bone mineral density\(^{(3,4)}\), however the underlying mechanism remains to be clarified. To our knowledge there appears to be no relevant published data on the effect cigarette smoking on PTH and ALP among Sudanese. In this study we demonstrated that level of PTH is decreased in Sudanese healthy smoker when compared to non smoker. This finding is consistent with other previous studies. *Landin-Wilhelmsen* et al in a study on 347 men and women\(^{(5)}\) *Brot* et al. in a study on 510 women aged 45–58 years\(^{(8)}\). *Need et al.* in a study on 405 postmenopausal women\(^{(9)}\) found serum PTH to be significantly lower in smokers. On the other hand, *Rapuri et al.* in a study on 444 elderly women\(^{(10)}\) found serum PTH to be significantly higher in smokers than non-smokers. A considerable number of chemicals have been found in cigarette smokers\(^{(11,12)}\). One or more of these chemical may interfere with action these could be inhibiting it is release from the parathyroid gland, it is action on bone or it is action on renal tubules. The reduced serum PTH among smokers might therefore be explained by a decreased secretion or an increased degradation of the hormone.

In this study serum PTH was negatively correlated with both ;the number of cigarette smoked per day and the duration of smoking and this agree with study done in USA . Serum alkaline phosphatise was found to be significantly increased in the cigarette smoker and this agree with study done by *Woitge et al.*\(^{(13)}\) and had positive correlation with both ;the duration of smoking and number of cigarette smoked per day. Increase activity of ALP could be due to increase activity of osteoblast that play a role in bone turnover and hence try to compensate for the low plasma calcium. This effect could be accumulative effect of one or more of the chemicals in cigarette smoke that is reflected as lowering of serum level of PTH and increasing of serum ALP with positive correlation of both the duration of smoking and the number of cigarette smoked per day.
CONCLUSION
It is obvious from the results of this study that the cigarette smoking reduces the levels of serum PTH and increases the level this ultimately will lead disturbances of calcium and phosphorous and may bone health and may represent another potential mechanism for the deleterious effects of smoking on the skeleton, and may contribute to the reported risk of osteoporosis and low bone mineral density among smokers.

ACKNOWLEDGMENT
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