COMPARISON OF BONE TURNOVER MARKERS BETWEEN MALE SMOKER AND NON-SMOKER

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ABSTRACT

Introduction: Osteoporosis is the most frequently seen metabolic bone disease. Smoking has long been defined as a changeable risk factor in life style for both bone loss and fractures. This study is aimed to compare of the bone turnover markers between smoker and non-smoker male.

Materials and methods: In this cross-sectional and descriptive study, 85 smoker males were allocated to the case group, while 85 non-smoker males were allocated to the control group. Osteocalcin (OC) and osteoprotogerin (OPG) analyzed among bone formation parameters, while RANKL (Receptor activator of nuclear factor kappa-B ligand) and CTX (C-terminal telopeptide) were studied among the bone destruction parameters alongside with TSH (Thyroid-Stimulating Hormone), Ca (Calcium), P (Phosphorus), PTH (Parathyroid Hormone), ALP (Alkaline Phosphatase), TT (Total Testosterone), and vitamin D parameters that affect bone mineral density.

Results: It was found that the smoker group’s CTX level (50.30±26.97 ng/ml) was statistically significant lower than that of the non-smoker group (65.10±42.41 ng/ml, p=0.007). The average serum PTH level of the smoker and non-smoker groups were 23.75±9.88 pg/ml and 31.35±13.15 pg/ml respectively and the related average of the non-smoker group was statistically higher than that of the smoker group (p=0.000). It was found that the smoker group’s vitamin D (16.75±8.73 ng/ml) was statistically significant lower than that of the non-smoker group (19.50±8.97 ng/ml) (p=0.044).

Conclusions: The study supports the fact that one of the risk factors for osteoporosis is smoking and it negatively affects bone formation as well. It should be noted that osteoporosis is a significant health issue not only for older men but also for middle-aged male smokers and the necessary support for smoking cessation should be offered.

Key words: Cigarette, osteoporosis, bone turnover, smoking.

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Introduction

Osteoporosis (OP) is the most frequently seen metabolic bone disease. World Health Organization (WHO) defines OP as a “systemic skeletal disorder characterized by an increase in bone fragility and bone fractures as a result of decreased bone mass and deterioration of bony microarchitectural structure of the bone tissue”(1). Another significant factor causing osteoporosis, alongside with health issues like age, diet, genetics, and hormonal factors, is smoking. Smoking has long been defined as a changeable risk factor in life style for both bone loss and fractures. Recently, studies on the relationship between smoking and low bone mineral density and osteoporotic fractures have been on the rise(2,3,4,5). Smoking is a significant risk factor for osteoporosis independent of other risk factors like age, gender, weight, and menopausal condition(1,3).

It has been stated that the rate of smoking reached significant levels today. According to the
WHO data, 1.3 billion people are smokers while 5 million people die of smoking related causes. It is expected that this figure will reach 8 million in 2030. It is also stated that at least half of the current smokers will die of causes related to smoking(7,8).

According to the data revealed by the Global Adult Tobacco Survey (GATS) conducted by the Turkish Statistical Institute (TurkStat) in 2012 in our country, the current rate of smoking rate for males is 41.4% while it is 13.1% for females(9).

Bone metabolism should be subjected to a multifaceted evaluation in order to diagnose, treat, and follow-up osteoporosis. Osteoporosis can be diagnosed by imaging methods, biochemical markers, and bone biopsy(10).

The specific biochemical bone turnover markers have gradually become more significant in the evaluation of the early diagnosis of the disorder, its clinical progress, and responses to treatment alongside with radiographic methods. It has been stated that these markers might prove to be superior to those techniques that show bone losses in the whole skeletal system as changes in bone mineral density in a single area. Biochemical resorption markers are also used in the assessment of fracture risks independent of BMD. Osteocalcin (OC), osteoprotegerin (OPG) which show osteoblastic activity, and C-terminal telopeptide and RANKL which show osteoclastic activity, are the markers show bone formation and bone destruction(11,12).

The aim of this study is to demonstrate the effects of smoking on bone formation and destruction parameters in middle-aged male smokers as revealed by biochemical parameters.

Materials and methods

Study population

The ethics boards of Selcuk University Medical School approved this study. A total of 90 male smokers aged between 35 and 70, who had presented to the Outpatient Smoking Cessation Clinic of the Department of Family Medicine at Selçuk University Medical School, were allocated to the study group, while a total of 90 non-smoker male patients with no previous history of smoking aged between 35 and 70, who had presented to the Periodical Examination Outpatient Clinic were allocated to the control group. Participants of the study who had extreme values in TSH, ALP, and PTH parameters as shown by their analyses were excluded from the study and 85 currently smoking individuals were taken into the study group, while 85 male individuals who had never smoked were taken into the control group. Informed consent forms compatible with the Helsinki Declaration of World Medical Association were received from each participant before the study. Participants in both groups corresponded with regards to age, BMI (Body Mass Index), and level of education. Individuals with a history of alcohol abuse, those older than 70 years of age, those with a familial history of osteoporotic fracture, those with a history of fracture with minor traumas, those who had been on glucocorticoids for more than 3 months, and those who had hyperthyroidism, secondary osteoporosis, low body weight (<57 kg), rheumatoid arthritis, and those who had chronic heparin treatment and those on chronic anticonvulsants were excluded from the study.

Smoking characteristics

The Turkish version of the questionnaire, which was proposed by Prochaska et al.(13) and used in the US for the evaluation, ranking, and classification of the stages of change in cessation of smoking and its characteristics, was used for the study participants while the Fagerstrom Test for Nicotine Dependence questions were used for the scoring and classification of dependence(14).

CO (Carbon monoxide) measurement

CO measurements were done by the piCO Smokerlyzer Breath Bedfront Scientific instrument (0-100 ppm) in the expiratory airflow during the confirmation and exclusion of the study participants’ smoking statuses. Those with a CO level of 5 ppm and less were considered to be non-smokers(15).

Ca, P and ALP measurement

These measurements were conducted through the spectrophotometric method by using Abbott Architect C16000© instrument.

Total Vitamin D Measurement

The measurement was done through the chemiluminescence method by using the Roche Diagnostic E-170 instrument.

TSH, TT, PTH measurement

These were done through ECLIA (electrochemiluminescence) method by using the Roche Diagnostic E-170 instrument.
**Comparison of bone turnover markers between male smoker and non-smoker**

**CTX, RANKL, OPG, OC measurement**
These were measured through the ELISA method by Kayto rt-2100c. Eastbiopharm ELISA kits were used.

**Statistical analysis**
All the data collected were evaluated by SPSS (Statistical Package for the Social Sciences) 16.0 statistics package program. Numbers, percentages, means, and standard deviation were used in the evaluation of the data. Chi-square test and Student-t test were conducted between the groups by handing out the frequency distribution of categorical data. A p-value of <0.05 was considered significant.

**Results**
Our study covered 85 smoker and 85 non-smoker male patients. The mean age of the 170 participants of our study was 43.55±6.63 (min: 33, max: 55, median: 44), while the mean BMI was 27.08±3.16 kg/m² (min: 20.72, max: 34.89, median: 27.42). Of all the participants 1.2% (n=2) were literate, 19.4% (n=33) were elementary school graduates, 14.1% (n=24) were secondary school graduates, 17.2% (n=29) were high school graduates, and 48.2% (n=82) were vocational college or university graduates (Table 1). 4.2% (n=7) of the participants of our study resided in villages and towns, 5.3% (n=9) resided in counties, and 90.6% (n=154) resided in cities.

The mean CO level of the smoker group was 11.60±4.87 ppm, while the same figure for the non-smoker group was 1.32±0.85 ppm and there was a statistically significant difference between the two groups regarding CO levels (p=0.000).

The mean serum RANKL levels of the study group was 93.61±98.84 pg/ml, while the same figure was 117.53±8.65 pg/ml for the control group and the difference between the groups was not statistically significant (p=0.084). While the mean serum CTX levels of the study group was 50.30±26.97 ng/ml, it was 65.10±42.41 ng/ml for the control group and the CTX level was significantly lower in the smoker male group (p=0.007). The mean serum OC levels of the study group was 21.62±13.59 ng/ml, while it was 26.52±21.70 ng/ml for the control group and the mean figure for the non-smoker male group was higher than that of the smoker group although the difference was not statistically significant (p=0.080). The mean serum OPG level of the non-smoker male group (3.88±4.18 pg/ml) was significantly higher than that of the smoker male group (2.70±2.38 pg/ml) (p=0.025) (Table 2).

<table>
<thead>
<tr>
<th>Bone Turnover Markers</th>
<th>Non-smoker (n=85) (mean±SD)</th>
<th>Smoker (n=85) (mean±SD)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANKL</td>
<td>117.53±8.65</td>
<td>93.61±98.84</td>
<td>0.413</td>
<td>0.084</td>
</tr>
<tr>
<td>CTX</td>
<td>65.10±42.41</td>
<td>50.30±26.97</td>
<td>-2.715</td>
<td>0.007</td>
</tr>
<tr>
<td>OC</td>
<td>26.52±21.70</td>
<td>21.62±13.59</td>
<td>-1.764</td>
<td>0.08</td>
</tr>
<tr>
<td>OPG</td>
<td>3.88±4.18</td>
<td>2.70±2.38</td>
<td>-2.27</td>
<td>0.025</td>
</tr>
<tr>
<td>RANKL/OPG</td>
<td>39.73±17.52</td>
<td>43.63±58.97</td>
<td>0.585</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Tab. 2: Comparison of bone turnover markers between male non-smoker and smoker.

The mean serum TSH levels of the study group (n=85) was 1.64±0.84 µU/L, while it was 2.00±1.01 µU/L for the control group (n=85) and there was no statistically significant difference between the smoker and non-smoker groups regarding mean serum TSH figures (p=0.021). The mean serum TT levels of the study group was 4.41±1.64 ng/ml, while it was 4.22±1.59 mg/dl for the control group and there was no statistically significant difference between the two groups with regards to mean serum TT levels (p=0.456). The mean serum Ca levels of the study group was 9.74±0.45 mg/dl, while it was 9.81±0.44 mg/dl for the control group although there was no statistically significant difference between the two groups (p=0.315).

The mean serum phosphorus levels of the study group was 3.15±0.54 mg/dl, while it was 3.18±0.49 mg/dl for the control group and there was no statistically significant difference between the smokers and the non-smokers (p=0.660). The
mean serum PTH level of the study group was 23.75±9.88 pg/ml, while it was 31.35±13.15 pg/ml for the control group and the figures for the non-smoker group was significantly higher than those of the smoker group (p=0.000). The mean serum ALP level of the study group was 80.80±21.21 u/L, while it was 72.37±21.47 u/L for the control group and there was no statistically significant difference between the groups (p=0.011). The mean Vitamin D level of the smoker group (16.75±8.73 ng/ml) was significantly lower than that of the non-smoker group (19.50±8.97 ng/ml) (p=0.044) (Table 3).

Tab. 3: Comparison of blood parameters between male non-smoker and smoker.

<table>
<thead>
<tr>
<th>Blood Parameters</th>
<th>Non-smoker (n=85) (mean±SD)</th>
<th>Smoker (n=85) (mean±SD)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>2.00±1.01</td>
<td>1.64±0.84</td>
<td>-2.337</td>
<td>0.021</td>
</tr>
<tr>
<td>TT</td>
<td>4.22±1.59</td>
<td>4.41±1.64</td>
<td>-0.748</td>
<td>0.456</td>
</tr>
<tr>
<td>Ca</td>
<td>9.81±0.44</td>
<td>9.74±0.45</td>
<td>-1.098</td>
<td>0.315</td>
</tr>
<tr>
<td>P</td>
<td>3.18±0.49</td>
<td>3.15±0.54</td>
<td>-0.441</td>
<td>0.66</td>
</tr>
<tr>
<td>PTH</td>
<td>31.35±13.15</td>
<td>23.75±9.88</td>
<td>-4.257</td>
<td>0</td>
</tr>
<tr>
<td>ALP</td>
<td>72.37±21.47</td>
<td>80.80±21.21</td>
<td>2.573</td>
<td>0.011</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>19.50±8.97</td>
<td>16.75±8.73</td>
<td>-2.028</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Discussion

Although there is only limited data on the effects of smoking on bone health for men, smoking emerges as a significant risk factor that causes bone loss(17, 18, 19).

Although the mean serum RANKL level, which is among the bone destruction parameters, of the smoker group was lower than that of the control group, the difference between the smoker and non-smoker groups was not statistically significant. When some of the studies conducted on the subject are investigated it is seen that Lappin et al.(17) stated in a 2007 study that smokers’ RANKL level was lower than that of the non-smokers although there was no statistically significant difference. Mizrak et al.(18) found in a 2013 lab rat study that the RANKL level of the control group was higher than those of the low-dose nicotine group and the high-dose nicotine group although there was no statistically significant difference between the groups. The authors suggested that the high RANKL level in non-smokers could possibly be related to passive smoking, working in a smoking environment, or the presence of smokers in the family. A study by Özcaka et al.(19) conducted in 2010 with smoker and non-smoker patients with chronic periodontitis found that the serum RANKL level was lower in the smoker group than that of the non-smoker group although there was no statistically significant difference between the groups. The authors stated that the fact that there was no significant difference between the smoker and non-smoker groups regarding plasma RANKL levels could be related to the limited number of patients in the study, misinformation about history of smoking, presence of smokers in the family of non-smokers, and second-hand smoking. The results of our study were similar to some of the studies in literature. This situation supports the fact that RANKL, which is one of the bone destruction parameters, can be affected by factors other than smoking such as sex, age, gingival disease, rheumatoid arthritis, multiple myeloma, and diabetes and these conditions need to be investigated in detail(17, 18, 19).

The mean serum CTX level, which is another bone destruction parameter, of the study group was significantly lower than that of the control group. Supervia et al.(20) stated in their 2006 study conducted with individuals who had never smoked and current smokers that smoking and elevated urine NTX (N-terminal telopeptide) levels were related and found that the urine NTX levels in smoker female patients were significantly elevated than non-smokers. Ardawi et al.(21) pointed out in their study that the serum CTX level, which is one of the bone destruction parameters, in male individuals older than 50 years of age was significantly higher than that of those younger than 50. Khoja et al.(22) did not find a difference between the quantity of smoking and serum CTX level. In our study CTX, which is a bone destruction parameter, in smokers was found to be lower than non-smokers and this suggests that this relation was probably brought about by the fact that study participants were in the middle age group.

Serum OPG level, which is a bone formation parameter, in the smoker group was lower than the non-smoker group although the difference was not statistically significant. Lappin et al.(17) found in their 2007 study conducted with 35 smoker and 35 non-smoker patients with periodontitis that the OPG level in smokers was significantly lower than the non-smokers. Tanaka et al.(23) in their 2006 study stated that the OPG level in their smoker group was lower than the non-smoker group and this would cause bone destruction based on the increase in osteoclasts, which are destruction cells, brought
about by the interaction of nicotine and liposaccharide within the bone although the mechanism was not clearly known. Further, there are studies reporting that PGE2 decreased OPG synthesis in osteoblasts. It was stated that clinical and other specific detailed patient characteristics could create differences in the results. They pointed out that a decrease in OPG levels could cause periodontitis in smokers however since they held the groups equal with regards to sex, age, and clinical gingivitis diseases further studies with a more detailed and carefully selected long-term follow-up designs would clarify the point. Buduneli et al. (24) in their 2008 study conducted with 111 smoker and non-smoker patients with chronic periodontitis found that the OPG concentration in the saliva of smokers was significantly lower than non-smokers. It was stated that the increase in OPG levels with the increase in nicotine levels was probably related to the compensatory mechanism in the body. It was also pointed out that the serum OPG level could be affected by a systemic inflammation in the body (diabetes etc.) independent of smoking (18, 19, 24). As is seen in the literature review mentioned above, there are studies that found that the serum OPG level, which is among the bone formation parameters, in smokers was lower than non-smokers as well as studies which found that it was higher. The results of our study also support the idea that bone formation is negatively affected in smokers.

The mean figure for the RANKL/OPG rate of the smoker group in our study was higher than that of the non-smoker group although it was not statistically significant. It is known that the RANKL/OPG rate also increased in diseases which cause bone destruction like multiple myeloma and that there was a correlation between the increase in bone destruction parameters and the increase in RANKL/OPG (17, 24). The results of our study also revealed that the RANKL/OPG rate was higher in smokers than non-smokers similar to the results of some studies.

The mean serum OC level, which is a bone formation parameter, of our study group was higher in smokers than non-smokers although the difference between the two groups was not statistically significant. Tamaki et al. (25) in their 2009 study conducted with 1576 male patients aged over 65 (FOR-MEN) found no statistically significant difference in serum OC levels between smoker and non-smoker groups (25). The authors stated that since the condition and quantity of smoking of individuals were determined by face to face interviews, the information collected might have been biased and this might have given way to a difference in the results. These results and our findings were similar. Supervia et al. (26) reported that there was no significant difference in OC levels between the smoker and non-smoker young male individuals and further studies conducted with older male individuals were needed to investigate the effects of smoking on bone formation since there was limited data on the subject. While there are some studies in literature that found that OC, which is a bone formation parameter, was lower in smokers, there are also other studies that found that it was higher. The results of our study support the idea that smoking negatively affects bone formation as well (21).

The results of our study revealed that serum Vitamin D levels were significantly lower in male smokers than non-smokers. While many studies found that Vitamin D levels were lower in smokers, there are also studies in literature (21, 26, 27) which found no statistically significant difference between smokers and non-smokers with regards to Vitamin D. The reason why smokers have lower Vitamin D levels is not thoroughly known. It was argued that lower consumption and diet could cause this condition (26). Jorde et al. (28) in their 2005 study found that Vitamin D levels were significantly lower in smokers.

The authors suggested that the reason for this might be related to fatty fish consumption among dietary habits, daily sun exposure, and the period spent outdoors alongside with smoking. Szulc et al. (29) found in their study conducted with 83 active smoker, 405 ex-smoker, and 231 non-smoker male individuals that Vitamin D levels of active smokers were significantly lower than those of the other groups. The authors suggested that this mechanism could have been brought about by the possibility that smoking could affect the intestinal absorption mechanism or it could have negative effects on Vitamin D synthesis in the skin although the complete mechanism of this condition was yet to be known. Supervia et al. (26) stated that smoking probably gave way to lower Vitamin D levels by increasing enzyme activities in the liver. Smokers generally have unhealthy habit like alcohol use, lower physical activity, and lower calcium consumption (32, 33).

In our study we asked for information on alcohol use and excluded those who consumed alcohol. Moreover, smokers are less inclined to participate.
in outdoor activities and therefore it was observed that they were less exposed to sunlight\textsuperscript{(12,23)}. All these can lead to lower Vitamin D levels in smokers. The results of our study revealing lower Vitamin D levels in smokers were similar to those of many studies reported in literature.

The results of our study showed that the PTH levels in smokers were significantly lower in the smoker group than the non-smoker group. Jorde et al.\textsuperscript{(26)} also found in their 2005 study that the PTH level in smokers was significantly lower than that of the non-smokers. No statistically significant difference in serum PTH levels between the two groups was found after a year when the same group quit smoking. The authors reported that this condition could probably be explained by smoking but there was no clear mechanism that could clarify the relationship between PTH levels and smoking and that there were articles stating that the PTH levels could be lower\textsuperscript{(20)} or within normal bounds in smokers. Supervia et al.\textsuperscript{(21)} found in their study conducted with smoker male individuals and those who had quit smoking within the last month that the PTH levels were significantly lower in smokers than non-smokers. The authors concluded that the reason for this could be the negative effects of smoking on the estrogen mechanism and the serum calcium concentration. Cutillas-Marco et al.\textsuperscript{(34)} found in their 2012 study conducted with 177 healthy individuals in smokers and found a correlation between smoking and lower PTH levels.

The results of our study revealed that the PTH level was significantly lower in smokers in line with the results of many studies in literature. Studies which found higher PTH levels in smokers argued that this was related to calcium intake and dietary habits. There was no statistically significant difference between the two groups concerning dietary habits.

The limitations of our study include the exclusion of those individuals who responded positively to questions in our questionnaire regarding disorders like hypogonadism, rheumatoid arthritis, and intestinal diseases that can cause osteoporosis. It is our belief that smoking negatively affects bone formation parameters and osteoporosis can completely become attributed to smoking when a complete investigation of diseases that can lead to osteoporosis is conducted and those patients with these diseases are excluded from further studies.

Conclusion

The study at hand supports the fact that one of the risk factors for osteoporosis is smoking and it negatively affects bone formation as well. It should be noted that osteoporosis is a significant health issue not only for older men but also for middle-aged male smokers and the necessary support for smoking cessation should be offered.

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