Al-Kindy College Medical Journal (KCMJ)

Research Article

Screening and Evaluation of Osteoporotic Biomarkers in Smokers in Basrah City, Iraq

Fatima A. Hassan[1], Falah H. Shari [2], Ausama A. Jaccob[3*]

[1] Department of Basrah health ministry, health center of Basrah university, Basrah, Iraq
[2] Department of Clinical Laboratory Sciences, College of Pharmacy, Almaaqal University, Basrah, Iraq
[3] Department of Pharmacology and Toxicology, College of Pharmacy, Basrah University, Basrah, Iraq
* Corresponding author's email: Ausama1979@yahoo.com

Article history:
Received 26 October 2024
Accepted 25 December 2024
Available online 1 April 2025

https://doi.org/10.47723/vaym3g64

Keywords: cigarette smoking; osteoporosis; RANKL/OPG ratio; pain

ABSTRACT

Background: Prolonged cigarette smoking leads to significant bone loss, an independent risk factor for osteoporosis. Currently, there are no effective prevention procedures for this, and the exact mechanisms affecting bone metabolism remain unclear.

Objectives: This study aims to (1) identify the differences in measured osteoporotic biomarkers among smokers, which are categorized based on the duration and smoking intensity, (2) identify a statistical correlation between variables and smoking intensity, (3) discuss the possibility of employing the appearance of pain sensations in healthy smokers as a reliable indicator of osteoporosis.

Subjects and Methods: A study involved 200 males in Basrah city, aged 20-60 years, who were categorized into long-term heavy smokers (LTHS), long-term light smokers (LTLS), short-term current smokers (STCS), and non-smokers (control). A self-reported questionnaire was used to collect baseline data and smoking history. Serum levels of bone-specific alkaline phosphatase (BALP), parathyroid hormone (PTH), 25-hydroxyvitamin D (25OHD), interleukin 6 (IL-6), osteocalcin (OC), Dickkopf-1 (DKK-1), osteoprotegerin (OPG), and Receptor activator of nuclear factor kappa-B ligand (RANKL) biomarkers were measured using an ELISA technique.

Results: statistical analysis for mean age 32± 8 years demonstrated a statistically significant relationship ($P<0.05$) in education levels, employment status, and type of diet among smoker groups. The prevalence of pain symptoms and pain severity showed ($P<0.0001$) among smoker groups compared to the control group. Among smokers, IL-6, vitamin D, BALP, OC, DKK-1, OPG, and PTH levels were statistically significant. The RANKL/OPG ratio shows significant differences in LTHS and LTLS.

Conclusions: The Basra community's smoking status is influenced by education, employment state, and dietary profile. The high prevalence of smoking and its negative health effects significantly on osteoporotic biomarkers, and pain prevalence is positively correlated with smoking intensity.

Introduction

Cigarette smoking is a prevalent and detrimental behavior that is strongly associated with many illnesses and health problems (1). Evidence indicates that smoking plays a substantial role in the onset of chronic diseases including respiratory disease, stroke, cardiovascular disease, and cancers (2). Furthermore, it has the potential to cause more fatalities than all deaths associated with HIV,

alcohol abuse, illegal drug use, vehicular accidents, and suicides (3). Over 70% of tobacco-related mortality occurs in developing nations (4). However, in developed countries, China has the highest prevalence of smokers at 26.5 million, followed by India at 19.8 million and Indonesia at 9.91 million (5).

Tobacco smoke contains over 7000 compounds, some of which indirectly affect the skeletal system by progression of osteoporosis and slowing down fracture healing (6). An imbalance in the bone remodeling cycle is related to an increased risk of fragility fractures (7). In addition to the negative impact of smoking on bone mass, other modifiable risk factors may induce osteoporosis and hip fracture, including low physical activity, Inadequate nutritional absorption, air pollution, and stress (8).

More recent evidence indicates that osteoporosis is significantly influenced by exposure to sunlight, serum levels of parathyroid hormone (PTH), and serum levels of both calcium and vitamin D (9). Recent investigation indicates osteoporosis, a prevalent bone disease, affects about 200 million people globally. Nevertheless, the control of bone metabolism is complex, including several signaling pathways (10). Physiologically, osteoblasts and osteoclasts collaborate to break down existing bones and generate new bones (11). Evaluation of serum levels of bone turnover markers and bone mineral density can be used to assess the homeostasis between these processes (12).

While cigarette smoking is recognized to have negative consequences on human health, smoking cessation presents a major challenge because of addiction (13). Several investigations have shown a substantial correlation between the duration and intensity of smoking and its impact on health and mortality (14). Increased nicotine demand resulting from cigarette smoking incorporates exposure to several toxic compounds, therefore causing serious health issues (15).

A strong association between smoking, pain, and inflammation has been observed. Women who regularly smoke cigarettes have an increased risk of getting rheumatoid arthritis (14). Nonetheless, proving the cause of pain resulting from bone mass loss, which is referred to as the “silent thief,” remains a challenge for physicians (16).

It is crucial to remember that southern Iraq's air pollution is an issue that can be risky to people's health since studies have revealed that gaseous pollutants like carbon monoxide, nitrogen oxides, sulfur oxides, and ozone have increased along with pollutants from energy production, dust storms, transportation, industry, and the burning of fossil fuels (17). Furthermore, tobacco consumption leads to indoor air pollution (18), emphasizing the requirement for increased research on the public health of Basra's population and predicting possible risks for chronic illnesses linked to air pollution, including osteoporosis and other conditions (8).

This research aims to (1) identify the differences in osteoporotic biomarkers (PTH, vitamin D3, OPG, RANKL/ OPG ratio, RANKL, IL-6, BALP, DKK-1, and OC) among smokers categorized based on the duration and smoking intensity

(2) identify a statistical correlation between variables (pain severity, IL-6, OC, RANKL/OPG ratio, PTH, vitamin D3, BALP) and smoking intensity

(3) discuss the possibility of employing the appearance of pain sensations in healthy smokers as a reliable indicator of osteoporosis.

Subjects and Methods

This cross-sectional observational study was conducted from Oct 2023 to Apr 2024 in Basrah City, Iraq. Two hundred men who lived in Basrah city center or district areas and met the study requirements were involved. The study complies with the University research committee's ethical approval reference no. (EC 43.15/10/2023).

Each participant in the study had to answer questions in a questionnaire to collect baseline information. However, the study's inclusion criteria were: adult males, aged 20 to 60 years, healthy, and without chronic conditions (diabetes, asthma, hypertension, parathyroid disease, autoimmune diseases, rheumatoid arthritis, stomach cancer/ or ulcer). Older adults diagnosed with osteoporosis or low bone mass were excluded. A history of chronic medication or supplement intake was obtained from every subject to be excluded from the study conditions.

The questionnaire collected details about the subject's employment status, and level of education, categorizing participants as college graduates and college students. The variation in educational attainment was obvious in the third group (non-graduates) who were unsuccessful in finishing their academic education. To reduce the variables, those belonging to this group were classified under the same category (non-graduates), followed with slight modification.

Data concerning participants' type of diet (high-carbohydrate, low-carbohydrate, vegetarian), amount of milk consumed, and duration of sunlight exposure each day were obtained. Random blood glucose and blood pressure readings were obtained using a digital instrument. The body mass index (BMI) was calculated by dividing each subject's weight in kilograms by their height in square meters (19).

The research aspects gathered between pain symptoms appearance and physical activity. The study involved and reported all subjects with remarkable pain symptoms (pelvic pain, muscle pain, joint /or back pain), using the numeric rating scale (NRS) (20) to assess the pain severity in smokers. Furthermore, the short International Physical Activity Questionnaire (IPAQ) (21) was requested. The data were obtained from the participants about their physical activity during the last 7 days.

The primary objective of this research was to identify the particular variance among current smokers, excluding all former smokers. Two hundred participants were divided into four groups depending on duration and intensity of smoking; long-term heavy smokers (LTHS, n=50), long-term light smokers (LTLS, n=50), short-term current smokers (STCS, n=50), and non-smokers (control, n=50).

Smoking status was assessed by self-reported questionnaires that included questions on participants' smoking status, duration of smoking, and daily cigarette consumption. This study hypothesized that heavy smokers, defined as those who use more than 13 cigarettes daily, can be distinguished from light smokers, who smoke between 1 and 13 cigarettes per day, as follows (22). Meanwhile, smoking duration was defined as more than a year for long-term smoking, whereas short-term smoking duration was defined as less than a year.

Laboratory analysis

A 5 mL sample of venous blood was obtained from each participant and allowed to coagulate at room temperature for 15 minutes. The coagulated blood was subjected to centrifuge at 3000 revolutions per minute for 15 minutes, and serum was obtained. The isolated serum was stored at -20°C until it was analyzed in the laboratory. ELISA kits provided by the manufacturer (Sunlong Biotech, China) were used for measuring serum bone-specific alkaline phosphatase (BALP), parathyroid hormone (PTH), 25-hydroxyvitamin D (25OHD), interleukin 6 (IL-6), osteocalcin (OC), Dickkopf-1 (DKK-1), osteoprotegerin (OPG), and Receptor activator Of nuclear factor kappa-B ligand (RANKL). Manufacturer guidelines for sample processing, measurement, and readings were followed.

Statistical analysis

This work was statistically evaluated using the Chi-square test for analysis and Fisher exact tests with non-parametric Spearman test for correlations. One-way analysis of variance with Tukey post hoc analysis for multiple comparisons was utilized by Graph-pad Prism for Windows (version 8.0).

Results

Two hundred males were enrolled in this study with a mean age of 32 ± 8 years. (Table1) shows the comparison between smokers and control groups; there were no significant differences in age or weight**.**

Table 1: Baseline characteristics of the participants in relation to their smoking status.

variables	Control n=50	LTHS n=50	LTLS n=50	STCS n=50	p-value
Age (years)	31 ± 8	33 ± 7	35 ± 8	27 ± 8	NS
BMI (kg/m2)	27.99 ± 3.8	25.81 ± 4.1	25.92 ± 3.6	24.37 ± 4.9	NS

Data are presented as mean ± SD

(Table 2) indicates the influence of educational levels on smoking status, revealing that the proportion of LTHS who non-graduates are is almost four times more than that of their college graduates' counterparts who participated in the study. 90% of the participants in LTLS were non-graduates. The survey indicated that the greatest proportion of smokers among the STCS group were non-graduates at 64%, followed by college students at 28%, overall, a significant p-value was (0.0021). The intensity of smoking and employment status showed a significant relationship when compared to the control group, with a p-value of (0.0006).

The results of the research show no significant differences in smoking habits among Basrah City areas, and also there's no significant association between milk consumption and smoking status, as shown in (Table 2). The characteristics of the type of diet show significant differences (P-value =0.012), and most LTHS were associated with a high-carbohydrate diet (62%), furthermore, smokers with a low-carbohydrate diet were found in both LTLS and STCS with 64% and 55%, respectively.

Table 2: Demographic variables characteristics of the participants in relation to their smoking status.

Variables		Control (n=50)	LTHS (n=50)	LTLS (n=50)	STCS (n=50)	p-value
Education level	College graduates	6%	18%	8%	8%	* 0.0021
	College students	16%	8%	2%	28%	
	Non-graduates	78%	74%	90%	64%	
Employment status	Employee	2%	40%	46%	20%	* 0.0006
	unemployed	64%	52%	52%	52%	
	College Student	16%	8%	2%	28%	
Location	Basra city center	98%	92%	98%	94%	NS
	Basra city district	2%	8%	2%	6%	
Type of diet (%)	High-carbohydrate	42%	62%	34%	43%	* 0.021
	Low-carbohydrate	56%	30%	64%	55%	
	vegetarian	2%	8%	2%	2%	
Milk consumption (%)	Daily	30%	38%	40%	44%	NS
	Weekly	54%	40%	42%	34%	
	Never	16%	22%	18%	22%	
Exposure to sunlight (hr./day)	Three hr.	40%	20%	34%	34%	NS
	Less than three hr.	36%	46%	36%	34%	
	More than three hr.	22%	28%	26%	30%	
	never	2%	6%	4%	2%	
Physical activity (day/weak)	vigorous	10%	10%	4%	14%	NS
	Moderate	70%	82%	76%	64%	
	walking	20%	8%	20%	22%	

Data are presented as a percentage (%), * indicate significant differences versus control at P<0.05

This research categorized sunlight exposure based on the average daily hours of exposure among individuals into three classifications: more than three hours, three hours, and less than three hours. The study findings suggest no significant relationship between smoking status and sunlight exposure when compared to the control group. Smokers' physical activity characteristics showed no significant differences. (Table 3) shows the prevalence and severity of pain

symptoms through smoking groups which indicated a significant relationship (P-value <0.0001).

Table 3: Characteristics of pain symptoms of the participants and their severity in relation to their smoking status.

variables		control (n=50)	LTHS (n=50)	LTLS (n=50)	STCS (n=50)	p-value
Pain symptoms (%)	pain	0%	46%	36%	18%	*<0.0001
	No pain	100%	54%	64%	82%	
Pain severity (%)	Mild	0%	40%	35%	16%	*<0.0001
	Moderate	0%	6%	1%	2%	
	sever	0%	0%	0%	0%	

Data are presented as a percentage (%), * indicate significant differences versus control at P<0.0001 .

Nonetheless, blood pressure and random blood glucose levels were elevated in LTHS compared to the control group, with a p-value <0.05, as shown in (Table 4).

Table 4: Measurement of blood pressure and random blood glucose of the participants in relation to their smoking status.

variables	control (n=50)	LTHS (n=50)	LTLS (n=50)	STCS (n=50)	p-value
Diastolic blood pressure (mmHg)	74.7 ± 11.2	* 80.2 ± 12.8	76.8 ± 11.1	71.9 ± 9.5	0.0434
Systolic blood pressure (mmHg)	121.3 ± 8.7	* 127.5 ± 12.6	122.8 ± 13.3	121.2 ±8.9	0.0149
Random blood glucose (mg/dl)	113.6 ± 15.4	* 122.7 ± 22.9	120.7 ± 19.6	110.2 ± 11.7	0.0318

Data are presented as mean ± SD, * indicates significant differences versus control at P <0.05

The PTH serum levels were significantly increased, with a mean ± SD of 30.6 ± 5.5 for LTHS, compared to 19.39 ± 3.7 for the control group. The statistical difference in PTH levels concerning smoking intensity is seen in (Table 5, Figure 1). Meanwhile, Vitamin D3 levels were significantly decreased in LTHS and LTLS relative to the control group, with a p-value <0.0001.

The study findings in (Table5, figure 2) show Serum OPG levels were significantly increased in LTHS and LTLS compared to the control group. Serum RANKL biomarker levels, on the other hand, are not significantly different between the LTHS and LTLS groups. The RANKL/OPG ratio indicates a significant decrease in both groups (illustrated in Figures 4,5, and 6). A significant decrease in serum levels of IL-6 has been observed in LTLS and STCS compared to the control, as demonstrated in (Figure 2). The LTHS and LTLS groups had significantly increased serum levels of BALP than the control group (Figure 3); nevertheless, the p-value for both groups is < 0.0001.

Serum Dkk-1 levels were significantly increased in the smoking groups, with a p-value of <0.0001 for LTHS, as seen in (Table 5, Figure 3). In comparison to the control group (3.9 ± 2.1), serum OC levels were three times higher in the LTLS group (9.3 ± 3.4), and four times higher in the LTHS group (13.9 ± 6.8), as shown in (Table 5). At the same time, STCS exhibits no significant variation in serum OC levels, (Figure 4)

Table 5: The relation of participants' osteoporotic biomarkers levels with smoking status.

variables	Control (n=50)	LTHS (n=50)	LTLS (n=50)	STCS (n=50)	p-value
Serum PTH (pg/ mL)	19.3 ± 3.7	* 30.6 ± 5.5	* 28.8 ± 6.2	* 24.5 ± 5.1	<0.0001
Serum vitamin D3 (microgram/L)	40.1 ± 24.0	* 18.9 ± 18.9	* 19.7±18.5	38.3 ± 22.5	<0.0001
Serum OPG (ng/mL)	13.5 ± 1.6	* 20.2 ± 2.5	* 17.4 ± 2.9	13.8 ± 1.3	<0.0001
RANKL/OPG ratio	27.3 ± 85.1	* 19.4 ± 54.6	* 21.6 ± 47.3	26.7 ± 138.3	<0.0001
Serum RANKL (ng/mL)	370.2 ± 136.2	392.3 ± 136.6	375.7 ± 137.4	369.9 ± 179.8	NS
Serum IL-6 (pg/mL)	43.6 ± 21.9	37.1 ± 21.5	* 26.8 ±12.8	* 17.1 ± 7.5	<0.0001
Serum BALP (mmol /L)	7.9 ±3.1	* 13.4 ± 5.2	* 11.6 ± 3.9	8.5 ± 3.9	<0.0001
Serum DKK-1 (ng/mL)	19.5 ± 3.7	* 30.63 ± 5.5	* 28.9 ± 6.2	* 24.6 ±5.0	<0.0001
Serum OC (ng/mL)	3.9 ± 2.1	* 13.9 ± 6.8	* 9.3 ± 3.4	3.8 ± 2.3	<0.0001

Data are shown as mean ± SD; * indicates significant differences versus control (p < 0.05).

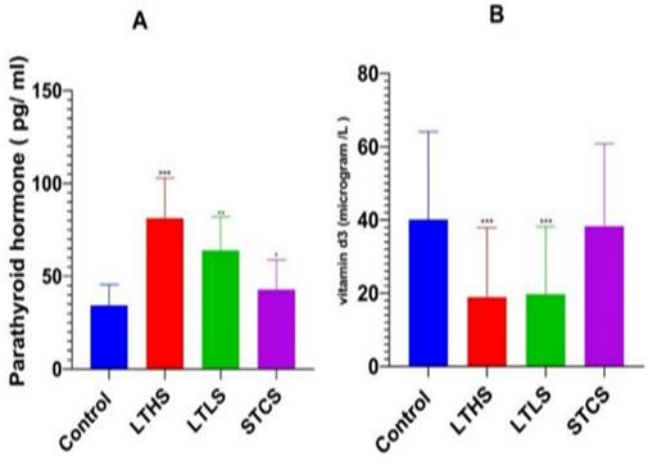

Figure 1: Serum PTH and vitamin D3 levels were compared among smokers and control groups. The three groups showed a significant increase in PTH levels compared to the control group, while LTHS and LTLS exhibited a significant decrease in vitamin D3 concentrations compared to the control group. (***) indicates a p-value <0.0001, (**) denotes a p-value <0.001, and (*) corresponds to a p-value <0.01.

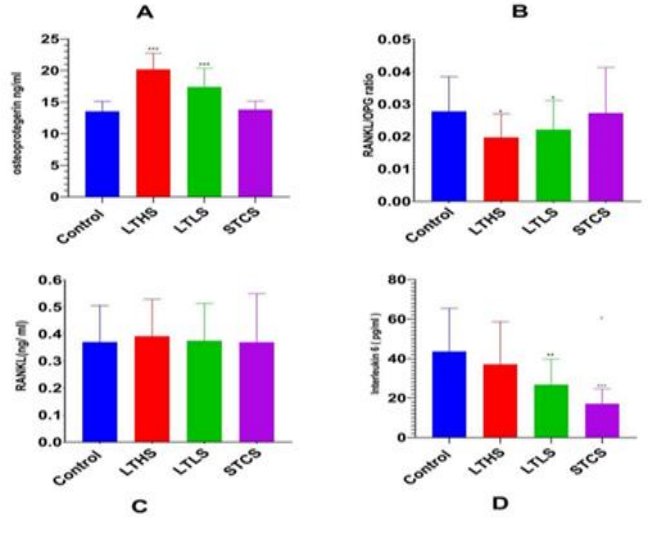

Figure 2: Evaluation of the smoker and control groups with the measurements of OPG, RANKL, the ratio of RANKL to OPG, and IL-6 serum levels.

A significant increase in OPG levels with both LTHS and LTLS groups, Meanwhile significant decrease in the RANKL/OPG ratio concerning both groups as shown (A, B). No significant differences in RANKL concentrations among smoker groups, while significant decrease in IL-6 levels concerning both LTLS and STCS (C, D). (***) indicates a p-value <0.0001, **(**)** denotes a p-value <0.001, and (*) represent a p-value <0.01.

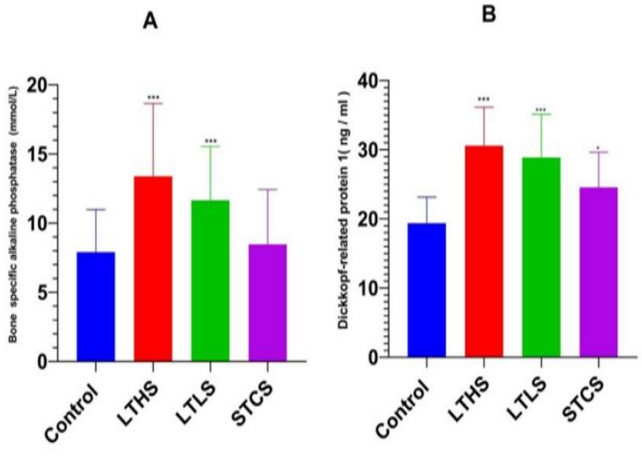

Figure 3: Serum BALP and DKK-1 levels compared among smokers and control groups. LTHS and LTLS exhibit a significant increase in BALP levels, whereas, the three groups show a significant increase in DKK-1 when compared to the control group. (***) indicates a p-value <0.0001, and (*) represents a p-value <0.01.

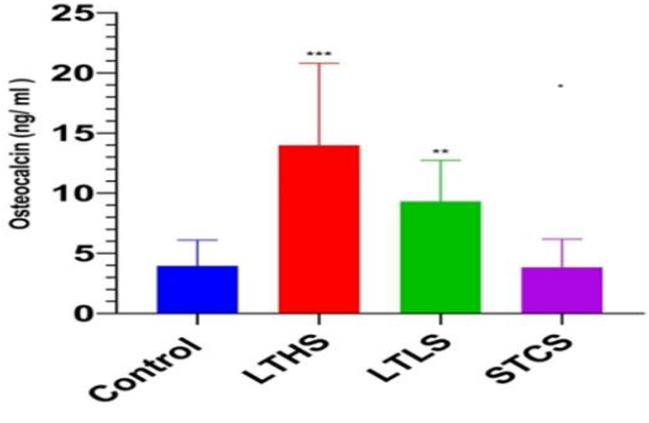

Figure 4: Measurement of OC serum level in the smoker and control groups.

LTHS and LTLS groups show a significant increase in serum OC levels compared to the control, represented by (***) for p-values <0.0001, and (**) for a p-value <0.001.

Correlations

A positive significant correlation was seen in BALP, OC, and PTH investigated biomarker levels and smoking intensity (p<0.0001), as shown in (Table 6), and a negative significant correlation associated with vitamin D3 levels (p<0.0001). Pain severity shows a significant positive correlation in responding to smoking intensity, however, both IL-6 and RANKL/OPG statistical analysis shows no correlation with smoking intensity.

Table 6: The statistical correlation among smoking intensity, pain severity, and biomarkers (IL-6, OC, RANKL/OPG ratio, PTH, vitamin D3, BALP) of smokers.

	Smoking intensity vs. IL-6	Smoking intensity vs. OC	Smoking intensity vs. RANKL/ OPG ratio	Smoking intensity vs. PTH	Smoking intensity vs. Vitamin D 3	Smoking intensity vs. BALP	Smoking intensity vs. Pain severity
The correlation coefficient (r)	-0.0558	0.6768	-0.0606	0.7232	-0.3963	0.4703	0.2714
P-value	0.4322	*<0.0001	0.3933	*<0.0001	*<0.0001	*<0.0001	*0.0008

*Indicate significant correlation at p<0.001

Discussion

The prevalence of smoking differs throughout various global locations. Our study results reveal that smoking status within the Basrah community is significantly correlated with variables such as level of education and employment state. Further research with Iraqi students showed a significant association between smoking habits and social and cultural characteristics, suggesting that smoking prevalence maybe two to three times higher among uneducated youth populations (23,24). A significant relationship has been shown between unstable employment and an increased probability of being a heavy smoker among current smokers who have a lower tendency to quit smoking (25).

A statistically significant association was seen between a high-carbohydrate diet and smoking intensity, with the LTHS group representing the largest percentage (62%) and showing a p-value of 0.012.

Earlier studies reveal that unhealthy food consumption was significantly increased among smokers, typically including excessive levels of calories, salt, and detrimental fats, therefore leading to chronic diseases (26). Due to the potential unreliability of self-reported data and clinical evaluations by healthcare professionals, biomarkers are becoming increasingly important in the diagnosis of diseases. We found that osteoporotic biomarkers were significantly modified due to smoking state, which could influence bone health. It has previously been established that smoking cigarettes for a long duration of time is associated with an increase in the incidence of osteoporosis and osteoporotic fractures among individuals of both genders (27).

The PTH-vitamin D axis plays a crucial role in bone mass density and calcium homeostasis. Parathyroid hormone (PTH) is a hormone that controls blood levels of ionized calcium by bone resorption and renal absorption, while the active form of vitamin D, 1,25-dihydroxy vitamin D (1,25-OH2-D), controls intestinal calcium absorption (7). Radiographic and cephalometric techniques have shown that a diet deficient in vitamin D and calcium may disrupt osteogenesis at growth sites in rats (28). Previous Investigation in vitro studies and animal models indicate that cigarette smoke can potentially decrease the local anti-inflammatory effects of vitamin D (29). Consistently with smoking intensity, we observed a significant decrease in vitamin D3 levels in LTHS and LTLS groups, with an increase in serum PTH levels. Due to the dual effects of parathormone on osteoblasts and osteoclasts. PTH appears to promote bone formation at low levels, but at high levels, it may cause bone resorption and result in overall bone loss (30).

The membrane protein RANKL is mostly produced by osteoblasts. Upon binding of RANKL to its specific receptor on osteoclast precursor cells, it promotes the differentiation of these precursors into active mature osteoclasts and enhances bone resorption(31). Alterations in RANKL among smokers are believed to result from the direct biological impact of smoking on osteoblast (32). As a decoy receptor, OPG, a soluble receptor also generated by osteoblasts, neutralizes RANKL, stops RANKL from interacting with RANK, and so suppresses osteoclast survival, activity, and proliferation (31).

The present study results indicated no statistically significant elevation in RANKL levels among the smoking groups. Meanwhile, OPG levels increased among LTHS and LTLS groups suggesting that these smokers may have a higher susceptibility to bone loss compared to non-smokers. Nevertheless, increased bone turnover and enhanced osteoclast activity are believed to result in higher OPG levels (33). Epidemiological evidence demonstrates that osteoporosis, an age-related condition, is affected by pro-inflammatory cytokines (IL6) in both disease prevention and development (34,35). However, Increased levels of IL-6 are seen throughout the continual stages of aging and menopause, as shown by the stimulation of osteoclasts and progressive loss of bone mass (36). Our findings demonstrate that the levels of IL-6 concentration were relatively decreased in LTHS, but a significant decrease was seen in both the LTLS and STCS groups compared to non-smokers. It seems that a decrease in IL6 levels in smokers may be associated with alveolar macrophages that may release a suppressor of the inhibitor factor IL-6. Furthermore, this secretion is enhanced by smoking by a mechanism still unknown (36). Other studies have shown that cigarette smoke contains a biologically active endotoxin that induces the reprogramming of macrophages (AM) which alters their responses to TLR2 and TLR4 stimuli, similar to what is seen in endotoxin tolerance (37). Consequently, smoking exerts localized immunosuppression of AM interaction with TLR2 and TLR4 stimuli manifested by impaired gene expression and secretion of pro-inflammatory cytokines (TNF, IL-1, IL-6) and chemokines (IL-8, RANTES) (38).

The highest levels of BALP are associated with the LTHS and LTLS groups in the present study. However, The production of bone alkaline phosphatase (BALP) is believed to contribute to the calcification of bone matrix, via a unclear process (39). Further study indicates that women showing higher levels of BALP are associated with rapid bone mass loss (40). DKK1 is generated by osteocytes and mature

osteoblasts, suppressing the canonical Wnt signaling pathway, which is a putative mechanism involved in regulating the RANKL/OPG balance (31). The Previous research serves to validate our results, by suggesting that smokers had higher levels of this protein DKK-1, which is responsible for inhibiting bone formation (41). During the bone formation phase of bone remodeling, osteoblasts release, also referred to as bone gamma-carboxy glutamic acid-containing protein (BGLAP). Since osteocalcin is a calcium-dependent biomarker and has a significant affinity for the bone matrix (hydroxyapatite), which is responsible for bone mineralization, osteoporosis is often associated with a deficit of calcium and phosphorus. Osteoporosis occurs in decreased hydroxyapatite crystal formation, thereby elevating blood osteocalcin levels(31,42). It was believed that Cigarette smoke contributes to modulating serum osteocalcin and bone metabolism (40). Our findings established that higher OC serum levels were significantly related to LTHS and LTLS groups. Earlier research on postmenopausal osteoporotic women revealed that smokers had substantially increased OC levels, which could suggest bone mass loss (42). Meanwhile, one study conducted on patients with periodontitis found that the mean saliva OC levels were significantly lower in smokers than non-smokers (43).

To determine the correlation between smoking and pain symptoms, Our findings demonstrate a statistically significant relationship, consistent with another research that establishes smoking as a risk factor for chronic pain (44). While it is expected that pain would be rare among healthy individuals, our study revealed a positive correlation between smoking intensity and the prevalence of pain, even among those with STCS. The type of pain appearance was not determined in this study. However, we are highlighting the possibility of employing the appearance of pain sensations in healthy smokers as a reliable indicator of osteoporosis. Nevertheless, the low bone mass has been associated with the higher density of bone sensory nerve fibers and their pathological alterations, along with an upregulation of nociceptors that are particularly sensitive to the decrease in pH induced by osteoclastic activity (16). Prior investigations have shown that sympathetic nerve fibers within the bone can control many processes such as bone destruction and generation (44). Therefore, it is evident that these nerve fibers have a crucial function in osteoporosis pathophysiology (45). However, Chronic exposure to nicotine causes widespread adaptive changes in the endogenous opioid system which may affect the processing of nociceptive stimuli in general (16).

The clinical evidence suggests that smokers are at increased risk of developing back pain and other chronic pain disorders. however, The relationship between smoking and chronic pain is complex (44). Due to the addictive and analgesic properties of nicotine, smoking intensity may increase nicotine demand, resulting in increased exposure to several toxic substances included in cigarette smoke, such as carbon monoxide, which contribute to the induction of neuropathic pain (15).

Further investigation is needed to determine the primary factors that may contribute to the onset of pain in healthy smokers and to examine the effect of smoking intensity on pain perception in this population.

Conclusion

The smoking status in the Basra community is affected by factors such as level of education, employment status, and dietary profile, which demonstrate the widespread prevalence of smoking habits and their negative health consequences. Although STCS were smoking within a year, statistically significant differences in the concentrations and alterations of IL-6, DKK-1, and PTH biomarkers relative to non-smokers were observed. Meanwhile, LTHS and LTLS exhibited significant differences in PTH, Vitamin D3, DKK-1, OC, OPG, BALP, and the RANKL/OPG ratio.

Through a mechanism that is currently unclear, this study reveals the prevalence of pain among smokers who seem to be in health condition is positively correlated with the intensity of smoking.

Acknowledgments

The presented study was abstracted from an M.Sc. thesis submitted to the College of Pharmacy, Basrah University, Department of Clinical Laboratory Sciences. The authors thank the college for its continuous support**.**

Ethical statement

The Human Ethics Committee (EC) of the College of Pharmacy at the University of Basrah obtained research permission, with approval reference number and date (EC 43.15/10/2023). The research participants agreed to collect blood samples after being fully informed of the study's objectives

Conflict of Interest

Fatima Amer Hassan, Falah Hassan Shari, and Ausama Ayob Jaccob declare no conflict of interest.

ORCID

Fatima Hassan 0009-0005-9955-6299
Ausama Jaccob 0000-0001-9989-5470
Falah Shari 0000-0002-1433-6731

References

[1] Tarantino U, Cariati I, Greggi C, Gasbarra E, Belluati A, Ciolli L, Maccauro G, Momoli A, Ripanti S, Falez F, Brandi ML. Skeletal system biology and smoke damage: from basic science to medical clinic. International Journal of Molecular Sciences. 2021 Jun 21;22(12):6629. https://doi.org/10.3390/ijms22126629

[2] Heydari G, Heidari F, Yousefifard M, Hosseini M. Smoking and diet in healthy adults: a cross-sectional study in tehran, iran, 2010. Iranian journal of public health. 2014 Apr;43(4):485.

[3] Singh CR, Kathiresan K. Effect of cigarette smoking on human health and promising remedy by mangroves. Asian Pacific journal of tropical biomedicine. 2015 Feb 1;5(2):162-7. https://doi.org/10.1016/S2221-1691(15)30337-3

[4] Jemal A, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. Cancer

epidemiology, biomarkers & prevention. 2010 Aug 1;19(8):1893-907. https://doi.org/10.1158/1055-9965.EPI-10-0437

[5] Kumboyono K, Chomsy IN, Hakim AK, Sujuti H, Hariyanti T, Srihardyastutie A, Wihastuti TA. Detection of vascular inflammation and oxidative stress by cotinine in smokers: measured through interleukin-6 and superoxide dismutase. International journal of general medicine. 2022 Sep 16;15:7319. https://doi.org/10.2147/IJGM.S367125

[6] Ratajczak AE, Szymczak-Tomczak A, Rychter AM, Zawada A, Dobrowolska A, Krela-Kaźmierczak I. Impact of cigarette smoking on the risk of osteoporosis in inflammatory bowel diseases. Journal of clinical medicine. 2021 Apr 5;10(7):1515. https://doi.org/10.3390/jcm10071515

[7] Al-Bashaireh AM, Haddad LG, Weaver M, Chengguo X, Kelly DL, Yoon S. The effect of tobacco smoking on bone mass: an overview of pathophysiologic mechanisms. Journal of osteoporosis. 2018;2018(1):1206235. https://doi.org/10.1155/2018/1206235

[8] Pouresmaeili F, Kamalidehghan B, Kamarehei M, Goh YM. A comprehensive overview on osteoporosis and its risk factors. Therapeutics and clinical risk management. 2018 Nov 6:2029-49. https://doi.org/10.2147/tcrm.s138000

[9] Jorde R, Stunes AK, Kubiak J, Grimnes G, Thorsby PM, Syversen U. Smoking and other determinants of bone turnover. PLoS One. 2019 Nov 25;14(11):e0225539. https://doi.org/10.1371/journal.pone.0225539

[10] Ikeda K, Takeshita S. Factors and mechanisms involved in the coupling from bone resorption to formation: how osteoclasts talk to osteoblasts. Journal of bone metabolism. 2014 Aug 31;21(3):163. https://doi.org/10.11005/jbm.2014.21.3.163

[11] Gao Y, Patil S, Jia J. The development of molecular biology of osteoporosis. International journal of molecular sciences. 2021 Jul 30;22(15):8182. https://doi.org/10.3390/ijms22158182

[12] Dawood H, Albadran MM, Aulraman NM, Abdul-Wahhab RD, Shari FH. Effects of Melatonin on Some Bone Mineralization Biomarkers for Overweight or Obese Perimenopause Women; A Possible Role in Osteoporosis Treatment. International Journal of Drug Delivery Technology. 2020;10(2):222-226

[13] Hong SW, Teesdale-Spittle P, Page R, Ellenbroek B, Truman P. Biologically active compounds present in tobacco smoke: potential interactions between smoking and mental health. Frontiers in Neuroscience. 2022 Apr 26;16:885489. https://doi.org/10.3389/fnins.2022.885489

[14] Grignon M, Renaud T. Duration and intensity when estimating smoking-attributable mortality: Development of a new method applied to the case of lung cancer in France. Population. 2020;75(4):561-89.

[15] Shi Y, Weingarten TN, Mantilla CB, Hooten WM, Warner DO. Smoking and pain: pathophysiology and clinical implications. Anesthesiology. 2010 Oct 1;113(4):977-92. https://doi.org/10.1097/aln.0b013e3181ebdaf9

[16] Mattia C, Coluzzi F, Celidonio L, Vellucci R. Bone pain mechanism in osteoporosis: a narrative review. Clinical Cases in Mineral and Bone Metabolism. 2016 Oct 5;13(2):97. https://doi.org/10.11138/ccmbm/2016.13.2.097

[17] Al-Hassen SI, Sultan AW, Ateek AA, Al-Saad HT, Mahdi S, Alhello AA. Spatial analysis on the concentrations of air pollutants in Basra province (southern Iraq). Open Journal of Air Pollution. 2015 Aug 19;4(3):139-48.

[18] Al-Easawi N. The exposure effect of water pipe smoke (wps) on the respiratory tract of swiss mice. Iraqi Journal of Science. 2010;51(4):543-9. https://doi.org/10.24996/ijs.2010.51.4.%25g

[19] Gloria Kang GJ, Ewing-Nelson SR, Mackey L, Schlitt JT, Marathe A, Abbas KM SS. HHS Public Access. Physiol Behav. 2018;176(1):139–48.

[20] Ali S, Athar M, Ahmed SM. Basics of CPB. Indian J Anaesth. 2019;49(4):257-62.

[21] Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund UL, Yngve A, Sallis JF, Oja P. International physical activity questionnaire: 12-country reliability and validity. Medicine and science in sports and exercise. 2003 Aug 1;35(8):1381-95. https://doi.org/10.1249/01.mss.0000078924.61453.fb

[22] Gazibara T, Milic M, Parlic M, Stevanovic J, Mitic N, Maric G, Tepavcevic DK, Pekmezovic T. What differs former, light and heavy smokers? Evidence from a post-conflict setting. African Health Sciences. 2021 Apr 16;21(1):112-22. https://doi.org/10.4314/ahs.v21i1.16

[23] Juslina O, Leelavathi M, Khairani O, Iryani T. Prevalence of smoking among secondary school students in Sarawak. Malaysian family physician: the official Journal of the Academy of Family Physicians of Malaysia. 2011 Aug 31;6(2-3):66.

[24] Al-Delaimy AK, Al-Ani WA. Prevalence of hookah smoking and associated factors among male high school students in Iraq. BMC public health. 2021 Dec;21:1-9. https://doi.org/10.1186/s12889-021-11386-4

[25] Jung Y, Oh J, Huh S, Kawachi I. The effects of employment conditions on smoking status and smoking intensity: The analysis of Korean Labor & Income Panel 8th–10th wave. PloS one. 2013 Feb 20;8(2):e57109. https://doi.org/10.1371/journal.pone.0057109

[26] Alribdi FF, Almutairi S, Jo M, Teixeira-lemos E. Dietary Patterns among Smokers and Non-Smokers : Findings. 2024;2017–8.

[27] Benson BW, Shulman JD. Inclusion of tobacco exposure as a predictive factor for decreased bone mineral content. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2004 Feb 1;97(2):266-7. https://doi.org/10.1080/14622200500259119

[28] Crincoli V, Cazzolla AP, Di Comite M, Lo Muzio L, Ciavarella D, Dioguardi M, Bizzoca ME, Palmieri G, Fontana A, Giustino A, Di Cosola M. Evaluation of vitamin d (25OHD), bone alkaline phosphatase (BALP), serum calcium, serum phosphorus, ionized calcium in patients with mandibular third molar impaction. An Observational Study. Nutrients. 2021 Jun 4;13(6):1938. https://doi.org/10.3390/nu13061938

[29] Lange NE, Sparrow D, Vokonas P, Litonjua AA. Vitamin D deficiency, smoking, and lung function in the Normative Aging Study. American journal of respiratory and critical care medicine. 2012 Oct 1;186(7):616-21. https://doi.org/10.1164/rccm.201110-1868OC

[30] Zahmatkesh M. Impact of parathyroid hormone on osteoporosis; a short look at the current knowledge. Journal of Parathyroid Disease. 2023 Jan 1;11(1). https://doi.org/10.34172/jpd.2023.11215

[31] Weng W, Li H, Zhu S. An overlooked bone metabolic disorder: cigarette smoking-induced osteoporosis. Genes. 2022 Apr 30;13(5):806. https://doi.org/10.3390/genes13050806

[32] Kadri ZH. Effect of cigarette and water-pipe smoking on osteocalcin and RANKL serum levels among Iraqi university students. EurAsian Journal of BioSciences. 2020;14(2):3025-30.

[33] Pandey A, Khan YA, Kushwaha SS, Mohammed F, Verma A. Role of serum osteoprotegerin as a diagnostic indicator of primary osteoporosis in perimenopausal and postmenopausal women: an Indian perspective. Malaysian orthopaedic journal. 2018 Mar;12(1):31. https://doi.org/10.5704/MOJ.1803.006

[34] Li X, Zhou ZY, Zhang YY, Yang HL. IL-6 contributes to the defective osteogenesis of bone marrow stromal cells from the vertebral body of the glucocorticoid-induced osteoporotic mouse. PLoS One. 2016 Apr 29;11(4):e0154677. https://doi.org/10.1371/journal.pone.0154677

[35] Khosla S, Atkinson EJ, Dunstan CR, O'fallon WM. Effect of estrogen versus testosterone on circulating osteoprotegerin and other cytokine levels in normal elderly men. The Journal of Clinical Endocrinology & Metabolism. 2002 Apr 1;87(4):1550-4. https://doi.org/10.1210/jcem.87.4.8397

[36] Twigg L. Cigarette smoking decreases bioactive secretion by alveolar macrophages. 2018;

[37] Hasday JD, Bascom R, Costa JJ, Fitzgerald T, Dubin W. Bacterial endotoxin is an active component of cigarette smoke. Chest. 1999 Mar 1;115(3):829-35. https://doi.org/10.1378/chest.115.3.829

[38] Chen H, Cowan MJ, Hasday JD, Vogel SN, Medvedev AE. Tobacco smoking inhibits expression of proinflammatory cytokines and activation of IL-1R-associated kinase, p38, and NF-κB in alveolar macrophages stimulated with TLR2 and TLR4 agonists. The Journal of Immunology. 2007 Nov 1;179(9):6097-106. https://doi.org/10.4049/jimmunol.179.9.6097

[39] Roudsari JM, Mahjoub S. Quantification and comparison of bone-specific alkaline phosphatase with two methods in normal and paget's specimens. Caspian journal of internal medicine. 2012;3(3):478.doi

[40] Dr. Ross PD, Knowlton W. Rapid bone loss is associated with increased levels of biochemical markers. Journal of Bone and Mineral Research. 1998 Feb 1;13(2):297-302. https://doi.org/10.1359/jbmr.1998.13.2.297

[41] Peng J, Dong Z, Hui Z, Aifei W, Lianfu D, Youjia X. Bone Sclerostin and Dickkopf-related protein-1 are positively correlated with bone mineral density, bone microarchitecture, and bone strength in postmenopausal osteoporosis. BMC Musculoskeletal Disorders. 2021 May 25;22(1):480. https://doi.org/10.1186/s12891-021-04365-8

[42] Singh S, Kumar D, Lal AK. Serum osteocalcin as a diagnostic biomarker for primary osteoporosis in women. Journal of clinical and diagnostic research: JCDR. 2015 Aug 1;9(8):RC04. https://doi.org/10.7860/JCDR/2015/14857.6318

[43] Al-Bashaireh AM, Alqudah O. Comparison of bone turnover markers between young adult male smokers and nonsmokers. Cureus. 2020 Jan 27;12(1). https://doi.org/10.7759/cureus.678

[44] Zhen G, Fu Y, Zhang C, Ford NC, Wu X, Wu Q, Yan D, Chen X, Cao X, Guan Y. Mechanisms of bone pain: Progress in research from bench to bedside. Bone Research. 2022 Jun 6;10(1):44. https://doi.org/10.1038/s41413-022-00217-w

[45] Mantyh PW. Mechanisms that drive bone pain across the lifespan. British journal of clinical pharmacology. 2019 Jun;85(6):1103-13. https://doi.org/10.1111/bcp.13801

To cite this article:
Hassan FA, Shari FH, Jaccob AA. Screening and evaluation of osteoporotic biomarkers in smoking individuals in Basrah city, Iraq. Al-Kindy Col. Med. J. 2025;21(1).
https://doi.org/10.47723/vaym3g64