



## Effect of Smoking on Bone Mineral Density, Serum Vitamin-D, Serum Calcium, Serum Phosphate, Serum Alkaline Phosphatases and Sex Hormones (Estradiol and Estriol) on Adult Females

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Available online at: [www.isca.in](http://www.isca.in), [www.isca.me](http://www.isca.me)

Received 26<sup>th</sup> May 2015, revised 11<sup>th</sup> June 2015, accepted 4<sup>th</sup> July 2015

### Abstract

*Smoking is an important determinant of numerous diseases. This study was carried out in Kolkata to investigate the effect of smoking on Bone Mineral Density, Serum Vitamin-D, Serum Calcium, Serum Alkaline phosphatases and Sex Hormones (Estradiol and Estriol) on Adult Females. In this study 100 adult females were chosen as subjects. Among them, 50% (50 adult females) were regular smokers and rest of the 50% (50 adult females) were non smokers. After the Statistical analysis, it was found that there was a significant decrease in bone mineral density, Estradiol, Estriol and 1, 25 Dihydroxy vitamin D level in adult female as a result of smoking. Whereas increased level of serum calcium, serum phosphate and serum alkaline Phosphatases was observed as a result of the same in adult female. Data was analyzed using SPSS (version 18). One way ANOVA was used to study the influence of smoking on bone mineral density, Estradiol, Estriol and 1, 25 Dihydroxy vitamin D level, serum calcium, serum phosphate and serum alkaline Phosphatases level.*

**Keywords:** Bone mineral density, estradiol, estriol and 1, 25 dihydroxy vitamin d, serum calcium, serum phosphate and serum alkaline phosphatases.

### Introduction

There are various naturally occurring organic substances rich in alkaloids among which tobacco is one of them. It is rich in Nicotine. Other than nicotine some other toxic metals which have similar pharmacological activities like nicotine are also found in tobacco. These are arsenic, mercury, lead, cadmium, chromium, polonium, and beryllium<sup>1</sup>. The various chemical components present in cigarettes are as follows:

“Tar” which is defined as the nicotine-free, dry, particulate mass of tobacco smoke<sup>2</sup>. Along with Tar in cigarette smoke there are many harmful carcinogenic constituents, including metals, PAHs, dioxins, and some non-volatile nitrosamines. In our study, we have tried to find out the relationship between smoking and some of the physiologically important parameters like bone mineral density, serum calcium, serum phosphate, serum alkaline Phosphatases, Estradiol, Estriol and 1, 25 Dihydroxy vitamin D level. Bone mineral density testing does not diagnose fractures. Along with other risk factors it helps to predict the risk of having a bone fracture in the future. If the T-score is: (-1.0) or higher, then it is normal. Between (-1) and (-2.5) indicates early bone loss (osteopenia) and below (-2.5), indicates osteoporosis. Calcium is an important mineral mainly found in bone and teeth. The normal level of serum calcium is 8.9-10.1 mg/dl. Like calcium, phosphorus is also an important mineral. Phosphate is the constituent of bone and teeth. The normal value of serum phosphate is .24-4.1 mg/dl.

The free energy produced by metabolic reactions may be stored as high energy phosphate like-ATP. It is also the constituent of phospholipids, nucleotides/nucleic acid, lipoproteins and phosphoproteins. Serum alkaline Phosphatases remain the only useful enzyme assay for the investigation of bone diseases and liver diseases. The normal value of serum alkaline Phosphatases is 52-142 U/L. Increased level of serum alkaline Phosphatases can be seen in rickets, Osteomalacia, Paget's disease and metastasis in liver. The group of hormones which are capable of producing certain biological effects and most of the female characteristics phenomenon in mammals is estrogen which is steroid in nature. The naturally occurring estrogen in humans is  $\beta$ - Estradiol, estrone and estriol. The normal value of Estradiol is (Mid Follicular phase 27-123 pg/mL). 1,25 Dihydroxy vitamin D is the most potent metabolite of vitamin D. Deficiency of it produces rickets, Osteomalacia and renal osteodystrophy. The normal value of 1, 25 Dihydroxy vitamin D is 30.0 to 74.0 nanograms per millilitre (ng/mL).

**Aims and Objectives:** The aim of this study was to compare the Bone Mineral Density, Serum Vitamin –D level, Serum calcium level, Serum Phosphate, Serum Alkaline Phosphatase level and Sex hormone (Estradiol and Estriol) level in the blood between adult female smokers and non-smokers. In this study, we investigated the effect of smoking by citing the blood parameters in the adult female smokers in comparison to those in the non-smokers.

## Methodology

A cross-sectional study followed was conducted in non-consecutive healthy volunteers. Participants, both smokers and non smokers adult females were taken as our case study. The duration of the study was six months. Inclusion criteria for smokers were consumption of at least 10 cigarettes /20 Bidis daily by each adult female with regular menstrual cycles. Exclusion criteria were: alcohol consumption, and diseases like osteoarthritis, chronic renal failure, chronic liver disease, malabsorption, and endocrine disorders. Bone mineral density (BMD) at the lumbar spine was measured by dual-energy X-ray absorptiometry using a Hologic 4500SL@bone densitometer (Hologic, Waltham, MA) and results were expressed in  $\text{g/cm}^2$ . Results are generally scored by the T-score which indicates the bone health of person. Negative scores indicate lower bone density, and positive scores indicate higher bone density. Serum calcium was measured by using calcium kits (OCPC method, crest bio system – a division of coral clinical system)<sup>3</sup>. Serum Phosphate was estimated by PHOSPHATE kits (colorimetric method, crest bio system – a division of coral clinical system)<sup>4</sup>. Serum alkaline phosphatase was measured by using Alkali Fosfataz kits (DGKC method, Lifechem)<sup>5</sup>. Assessment of

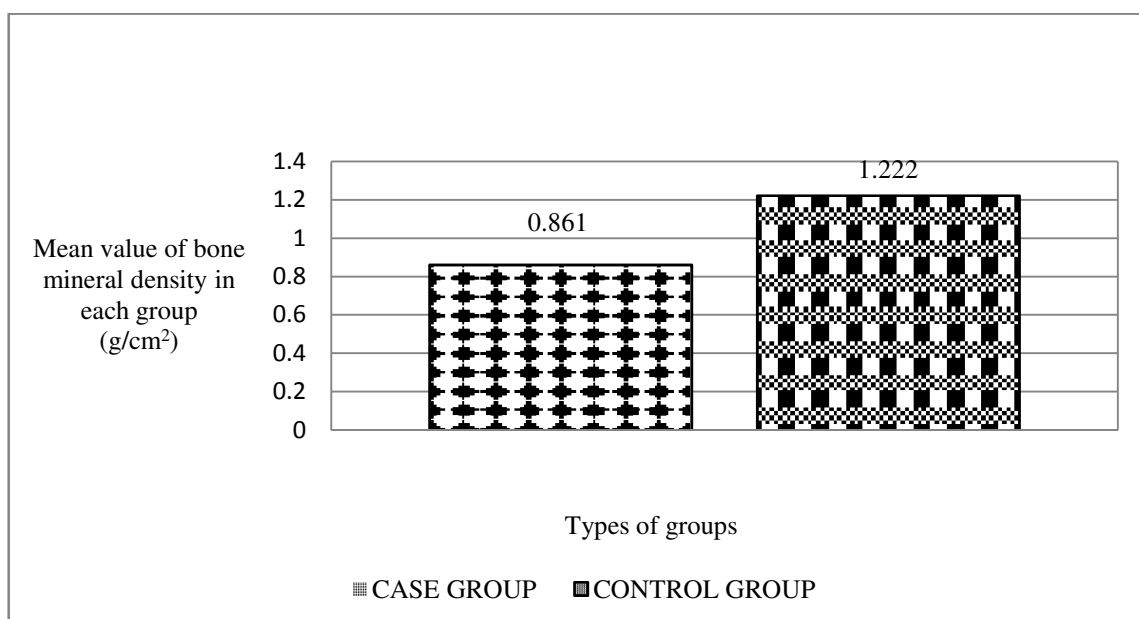
Estradiol (E2) and Estriol (E3) were measured by an ultrasensitive RIA technique (by CLIA, BRWS, Diagnostic Systems Laboratory). Serum Vitamin D level was measured by measuring 1, 25- Dihydroxy vitamin D levels. The data thus obtained were compiled and analyzed using SPSS (version 18). One Way ANOVA was used to study the influence of smoking on bone mineral density, Estradiol, Estriol and 1, 25 Dihydroxy vitamin D level, serum calcium, serum phosphate and serum alkaline Phosphatases level. P value of less than 0.05 was considered statistically significant.

## Results and Discussion

In this study, table-1 shows the analytical study reports of the Bone Mineral Density (BMD), Serum Calcium, Serum Phosphate, Serum Alkaline Phosphatase, Estradiol (E2), Estriol (E3) and 1, 25- Dihydroxy vitamin D based on smoking. As shown in figure-1, the mean value of bone mineral density level of adult female in case group is  $0.861 \text{ g/cm}^2$  whereas the same in that of the control group is  $1.222 \text{ g/cm}^2$  (p value <0.05). This result reveals that smoking decreases the level of bone mineral density.

**Table -1**  
**Analytical Study of the Parameters Analyzed on Smoking**

No. of Subjects	Parameter Analyzed	Unit	Case Group	Control Group	F ratio	P Value
N=50 In each group	Bone Mineral Density (BMD)	( $\text{g/cm}^2$ )	0.861	1.222	79.4232	0.1959
	Serum Calcium	(mg/dl)	8.296	6.624	3748.0216	0.1957
	Serum Phosphate	(mg/dl)	4.296	2.623	3745.5749	0.1957
	Serum Alkaline Phosphatases	(U/L)	277.876	228.348	69762.1154	1.2624
	Estradiol (E2)	(pg/ml)	34.126	58.866	11936.9801	0.1792
	Estriol (E3)	(pg/ml)	0.342	0.825	3309.5460	0.1958
	1,25- Dihydroxy vitamin D	(ng/dl)	33.54	35.16	25.4001	0.1960



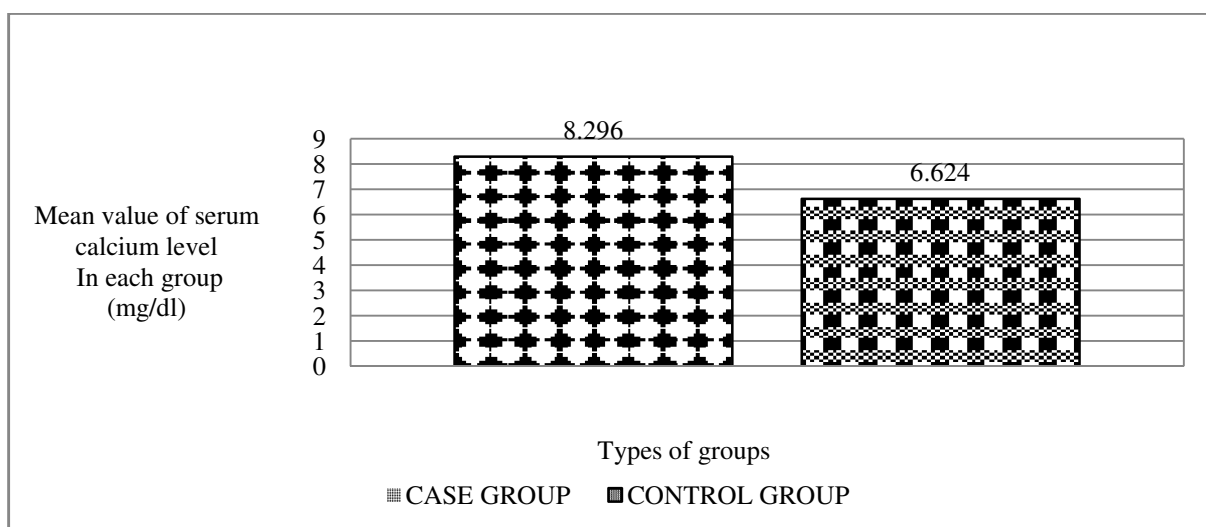
**Figure-1**

**The Mean Value of Bone Mineral Density Level of Adult Female after Study (Group Wise)**

Vitamin D binds to the chromatin to target tissue and expresses the genes for calcium binding protein as well as  $\text{Ca}^{++}$  ATPase in intestinal cells. This increases the  $\text{Ca}^{++}$  absorption by actively transporting  $\text{Ca}^{++}$  across the plasma membrane against the electrochemical gradients. The synthesis of  $\text{Ca}^{++}$  binding protein like osteocalcin is promoted which increases  $\text{Ca}^{++}$  and phosphate ions in the bone. These ions enhance the mineral deposition in the bone. In our present study we can see that, the case group shows comparatively less bone mineral density level. Hence it can be concluded that smoking either hinders the synthesis of Vitamin D or the synthesis of Calcium and phosphate ions in the bone thereby decreases the bone mineral density level<sup>6</sup>.

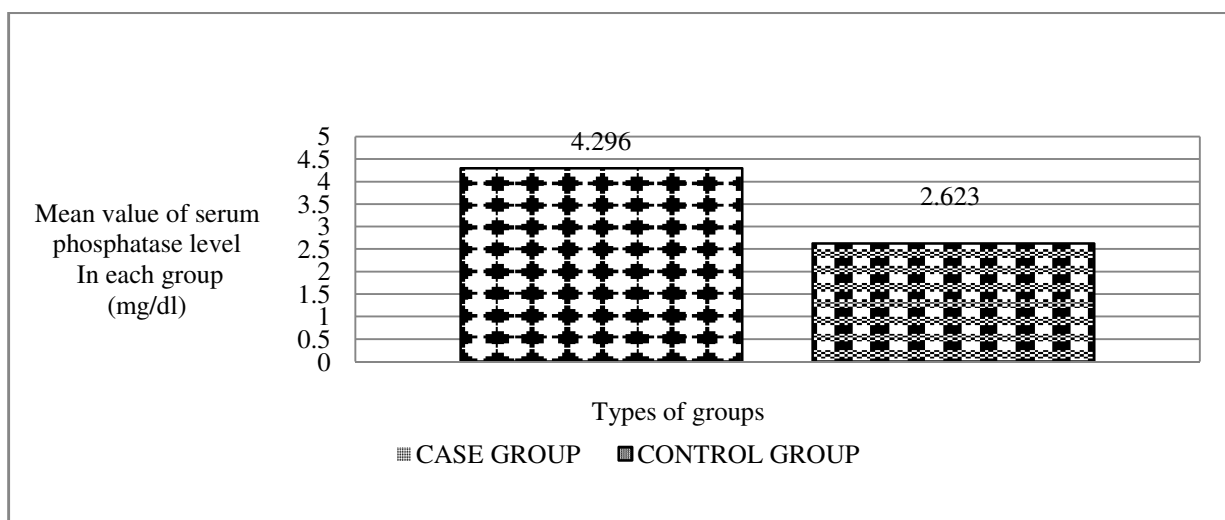
calcium level of adult female in case group is 8.296mg/dl whereas the same in that of the control group is 6.624 mg/dl (p value <0.05). This result reveals that smoking increases the serum calcium level. Rise in serum calcium stimulates the release of Calcitonin and Katakalcin hormone. Calcitonin strongly inhibits the resorption of bones by Osteoclast and thereby reduces mobilization of calcium from bones into the blood. The hormone, in addition cause a decrease in number of Osteoclast and increases the number of Osteoblasts cells, which are thought to be involved in bone laying<sup>6</sup>. In our present study we can see that smoker has comparatively high level of serum calcium level than the non smokers. This in turn affects the bone health of the smokers in the above mentioned way.

In this study, as shown in figure-2, the mean value of serum



**Figure-2**

**The Mean Value of Serum Calcium Level of Adult Female after Study (Group Wise)**



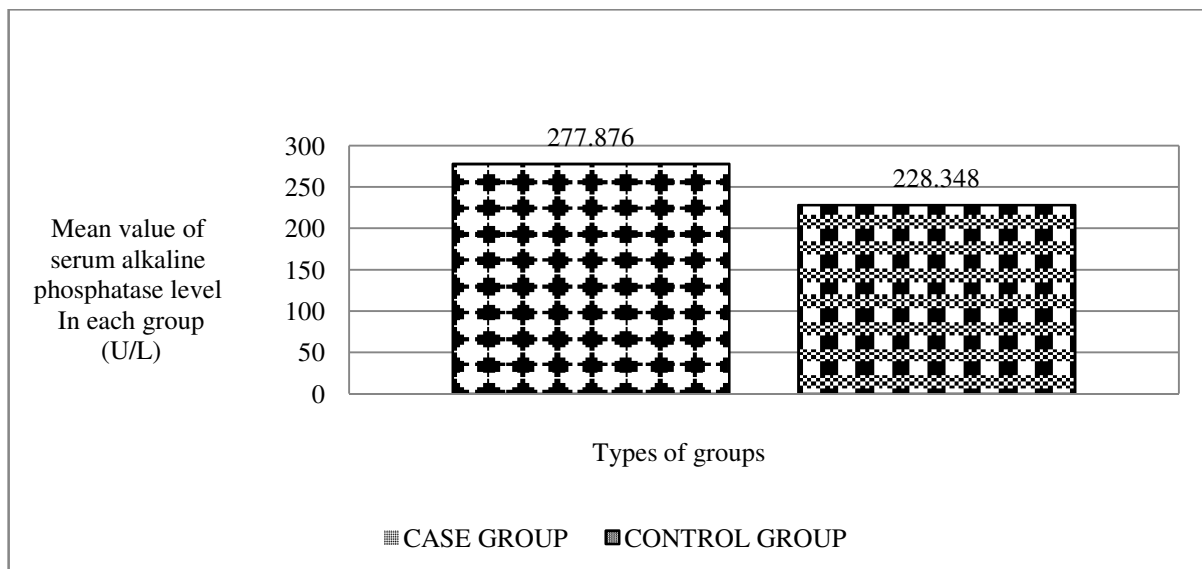
**Figure-3**

**The mean value of serum phosphate level of adult female after study (Group Wise)**

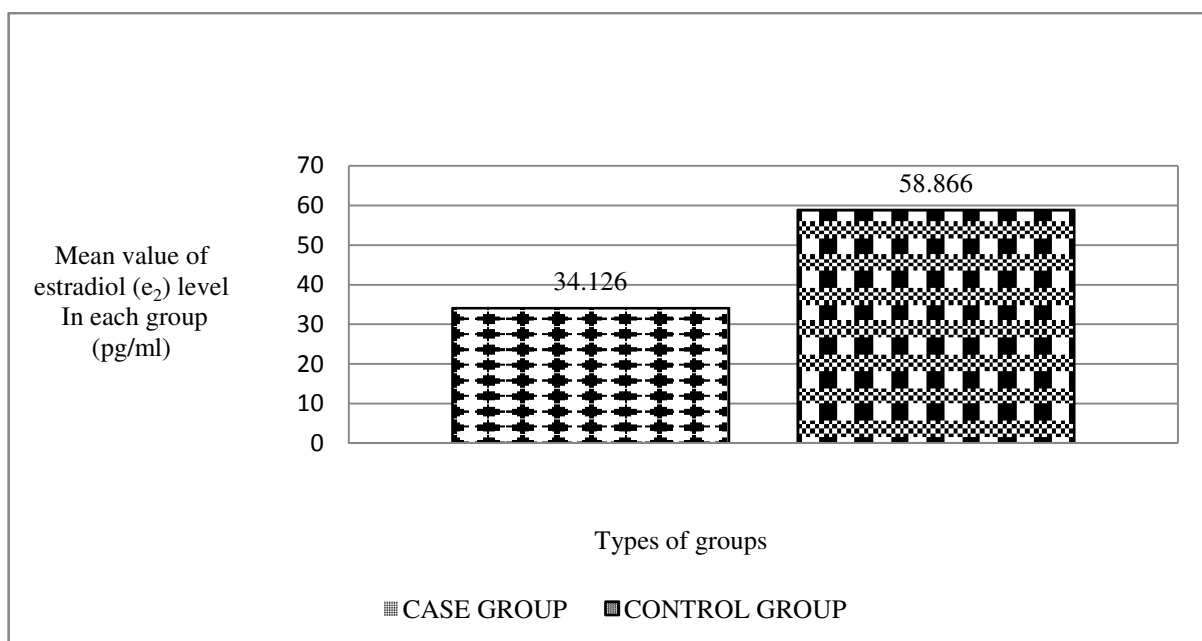
Now, as shown in figure-3, the mean value of serum phosphate level of adult female in case group is 4.296mg/dl whereas the same in that of the control group is 2.623 mg/dl (p value <0.05). This result reveals that smoking increases the serum phosphate level. Serum phosphate affects the resorption of bone by stimulating the osteoclast activity, with the increase in serum phosphate level as well as an increase in the rate of bone resorption<sup>6</sup>.

As shown in figure-4, the mean value of serum alkaline Phosphatase level of adult female in case group is 277.876 U/L

whereas the same in that of the control group is 228.348 U/L (p value <0.05). This result reveals that smoking increases the serum alkaline Phosphatase level. There is an significant increase of serum Alkaline Phosphatase level in case of obstructive jaundice, bone diseases like rickets, Paget's disease, hyperparathyroidism , slight to moderate increase in acute liver diseases , metastatic carcinoma, SOL of liver , renal dysfunction and osteoblastic sarcoma<sup>6</sup>. As in our present study there is an increased level of alkaline Phosphatases in the case group than that of the control group, it is apparent that the case group may suffer from the above mentioned diseases.



**Figure-4**  
**The Mean Value of Serum Alkaline Phosphatases Level of Adult Female after Study (Group Wise)**

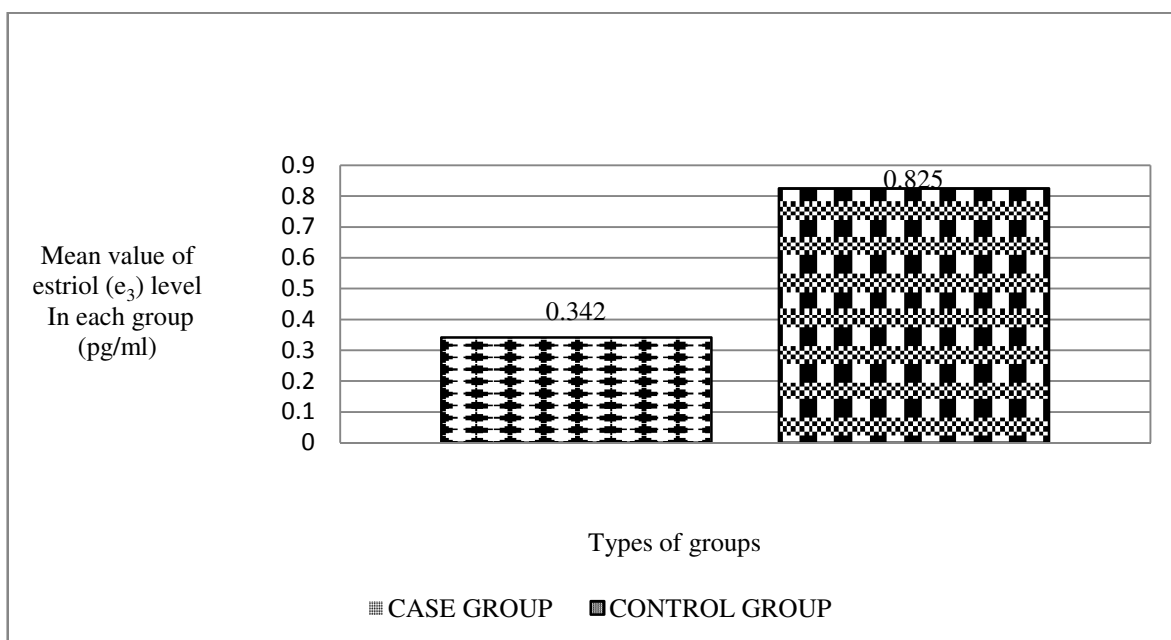


**Figure – 5**  
**The mean value of estradiol level (e<sub>2</sub>) of adult female after study (group wise)**

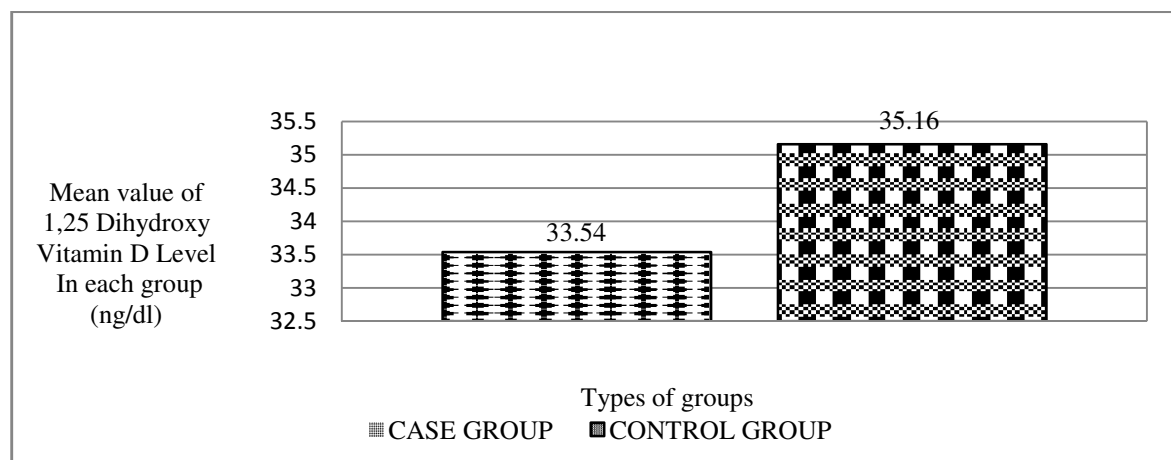
As shown in figure-5, the mean value of Estradiol level of adult female in case group is 34.126pg/ml whereas the control group shows 58.866 pg/ml (p value <0.05). This result reveals that smoking decreases the estradiol level. Estradiol favors the retention and elevation of calcium as well as phosphorous and skeletal deposition of calcium as a result of calcification and ossification of bones. Thus  $\beta$  Estradiol prevents osteoporosis which is frequently seen in menopausal women when estrogen decreases. Menopausal women are liable to get fractures due to the weakness of bones to osteoporosis. Besides this, Estradiol also has a cholesterol lowering effect which reduces the "Bad cholesterol"-LDL. (Low Density Lipoprotein). There by Estradiol has a protective function against myocardial infarction<sup>6</sup>. In our present study we have found see that the case group shows a comparatively low level of serum Estradiol than

the control group. Hence, it can be concluded that the case group is more prone to osteoporosis and myocardial infarction.

As shown in figure-6, the mean value of estriol level of adult female in case group is 0.342 pg/ml whereas the same in that of the control group is 0.825 pg/ml (p value <0.05). This result reveals that smoking decreases the estriol level. Estriol is a derivative of estrogen hormone. Estrogens like androgens promote calcification and bone growth. It is proved that decalcification of bone in the post menopausal women leading to osteoporosis is due to lack of estrogen<sup>6</sup>. As per our present data, the case group shows a comparatively low level of estrogen than that of the control group. Hence, the case group is more prone to osteoporosis than that the control group.



**Figure-6**  
The mean value of estriol ( $e_3$ ) level of adult female after study (group wise)



**Figure-7**  
The mean value of 1, 25 dihydroxy vitamin d level of adult female after study (group wise)

Figure-7 shows that the mean value of 1, 25 Dihydroxy vitamin D level of adult female in case group is 33.54ng/dl whereas the same in that of the control group is 35.16 ng/dl (p value <0.05). This result reveals that smoking decreases the 1, 25 Dihydroxy vitamin D level. Besides smoking, diet therapy and sunlight exposure has a marked effectiveness in elevation of Vitamin D. The change in 1, 25 Dihydroxy Vitamin D level also depends upon the demographic factors like age, sex, caste and socioeconomic status<sup>7</sup>. Vitamin D helps the body to absorb calcium. It binds with calcium helps build bones and keep bones strong and healthy. On the other hand, it also helps to block the release of parathyroid hormone. This hormone reabsorbs bone tissue, which makes bones thin and brittle. Vitamin D may also play a role in muscle function and the immune system. Thereby it helps to protect us against infections and other illnesses<sup>8</sup>. In our present study it is observed that the case group shows a comparatively low level of serum Vitamin D than the control group. Hence, it can be concluded that the case group is more susceptible to bone deformities as well as may suffer from poor immune system than that of the control group.

**Discussion:** In our present study, it is found that the level of serum calcium, serum phosphate and serum alkaline phosphatases are comparatively high among the case group than that of the control group. It indicates that somehow smoking influences the level of the above mentioned parameters. On the other hand, it is also found that the level of bone mineral density, 1, 25 Dihydroxy vitamin D and important sex hormones Estradiol (E2) and Estriol (E3) are comparatively low in the control group. Like our study, the study of Hollenbach KA, Barret-Connor E, Edelstein SL, Holbrook T<sup>9</sup> and the study of Ortego Centeno N, Muñoz Torres M, Jfidar, E, Hernández Quero J, Jurado Duce A, de la Higuera, Torres Puchol J<sup>10</sup> also present the same result about the influence of smoking on the bone mineral density level of the adult females. Whereas, in contrast to the study done by A. Supervía, X. Nogués, A. Enjuanes, J. Vila, L. Mellibovsky, S. Serrano, J. Aubía, A. Díez-Pérez we found a significant differences in between serum calcium, serum phosphate and serum alkaline Phosphatases level. Brot C, Jorgensen NR, Sorensen OH<sup>11</sup> also found the same relation between serum calcium level and smoking. There are many conflicting published results showing either increased normal or decreased levels of estrogens (both Estradiol and Estriol) for smoking women<sup>12-15</sup>. Although in our present study we found a significant decrease in both Estradiol and Estriol in the case group as compared to control.

Unlike the study done by A. Supervía, X. Nogués, A. Enjuanes, J. Vila, L. Mellibovsky, S. Serrano, J. Aubía, A. Díez-Pérez<sup>16</sup> we found a significant decrease in 1,25 Dihydroxy vitamin D level in the case group.

## Conclusion

From this study, we conclude that there was a significant decrease in bone mineral density, Estradiol, Estriol and 1, 25

Dihydroxy vitamin D level in adult females as a result of smoking. Whereas increased level of serum calcium, serum phosphate and serum alkaline Phosphatases was observed for the same in adult female.

**Recommendations:** From the very beginning it is essential either to stop smoking or smoking in a limited level (which is yet not determined) by the adult females to prevent Osteoarthritis and Liver dysfunction.

**Limitations:** Our study is based on the adult females only. Similar study may be done on adult males also with suitable parameters.

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