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Description of Microstructure in Trabecular Jaw Quality for Patient with Smoking Habits Using Panoramic Radiograph

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ABSTRACT

INTRODUCTION: According to WHO, Indonesia country profile age-standardized estimated prevalence of smoking among those aged 15 years or more in year 2013 shows that there are currently 73.3% male adults who smoke any tobacco and 63.9% of them smoke daily. Many studies have found that smoking will be a risk factor for osteoporosis and incur only negative effects on bone, however it is unsure for young to adult active smokers will have negative effects on their microstructure of trabecular jaw quality and the rate of bone turnover **Objective**: The purpose of this research is to describe the microstructure in trabecular jaw quality for patient with smoking habits using panoramic radiograph. **Material and Method**: This research is done by using *image j* software with 50x50 pixels intensity (PI) in digital panoramic radiographs. The samples of 30 smokers and 12 non-smokers of secondary data are collected to be analysed. Analysis performed in mental foramen area in both side of the jaw. **Result**: The mean for trabecular percentage of male smokers is 21.119%; of female smokers is 21.456; male non-smokers is 29.522% and female non-smokers is 30.444%. **Conclusion:** The conclusion of this reasearch, that there is lowering of trabecular percentage for regions of interest (ROI) in mandible of male and female smokers and they are indicated for osteoporosis. All dentists can be at a very important role in preventing osteoporosis by conveying this message.

Keywords: Smoking, Osteoporosis, Panoramic Radiograph, Trabecular Percentage, Image j Software

INTRODUCTION

Recently there has been some researches done that have proven that smoking seems to incur only negative effects on bone health. According to WHO, Indonesia country profile age-standardized estimated prevalence of smoking among those aged 15 years or more in year 2013 shows that there are currently 73.3% male adults who smoke any tobacco and

63.9% of them smoke daily. There are 63.0% male cigarette smokers and 59.4% of them smoke daily. Besides, there are currently 3.8% female adults who smoke any tobacco and 2.4% of them smoke daily. There are 3.5% female cigarette smokers and 2.3% of them smoke daily[1]. This is a very important issue for Indonesia because this will have great impacts on their health conditions. If the heavier a person smoke and the earlier smoking is started, it will increase the risk of fractures and osteoporosis [2].This often causes heavy smokers in having lesser amount of teeth in their oral cavity[3]. Because there will be a drastic reduction in the mineralization process of bones in hip, hand, bone formation [4,5]. However moderate or light smokers seem that do not suffer from this harm[6]. Researchers also show that for post-menopausal woman who smoke tend to lose cortical bone like tubular and mid shaft bone 50% faster than nonsmokers[7]. Besides, the life time risk of having hip fracture if compared to non-smoker woman, smoking woman increases nearly by double; 12.5% of the hip fractures cases are estimated due to the smoking habits.Smoking not only brings disadvantages for females, however for male who smoke will also be facing bigger chance for getting the risk for fractures if compared to non-smokers [8].

Panoramic radiograph is a common imaging technique in dentistry that gives a unique image of maxillary and mandibular jaws. The advantages of this radiographic technique are it has relatively small radiation, require lesser time and it is cheap and affordable. However the main disadvantages might be due to the wrong head adjustment which will cause magnification, high distortion and possible some mistakes. Besides, parameters including imaging device, equipment and the patient's position could change the quality of image and lead to false clinical judgement. In this study, we used digital panoramic radiography and 5x5 pixel matlap software to determine the bone density of the mandibular jaw[9].

Researchers have studied more than 4,000 different components in cigarette and more than 60 of these components have been proven as potent toxins and 45 of them are chemical carcinogens. Tobacco will directly interfere with osteoblast functioning and two candidates such as benzo[*a*]pyrene (BaP) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) will stimulate bone resorption by osteoclasts [10.11]. Researchers have proposed a few mechanisms by which smoking might reduce bone mineral density and induce osteoporosis risk. The action of smoking can change the micro architecture of trabecular bone and it will induce cortical bone becoming thinner and also the bone mineral density will be reduced[12]. Smoking is likely to enhance the stress hormone cortisol which willhave the action in lowering bone mineral density. In post-menopausal woman, theaction of smoking can stimulate the increased activity of liver enzymes henceenhance the speed for the breaking down of estrogen thus this will induce theincreased of bone loss. Because female tend to have smaller, thinner bones thanmen, they will lead to the higher possibility of having osteoporosis fracture[13]. Furthermore, calcium absorptions will likely be hampered in thosesmokers. Besides, the action of smoking also blocks the hormonal function such ascalcitonin. Calcitonin is a type of thyroid hormone that forces calcium from theblood into the bones. It also blocks the breakdown of bones. If calcitonin is reduced,tobacco use encourages the breakdown of bone while preventing its mineralization[14].

MATERIALS AND METHODS

The study design used for this research is descriptive study. The population of this research is the patients who come to Sekeloa Dental Hospital. The sample collecting of this research is by consecutive method, they are the patients who smoke and came to sekeloa Dental Hospital in one month duration time.

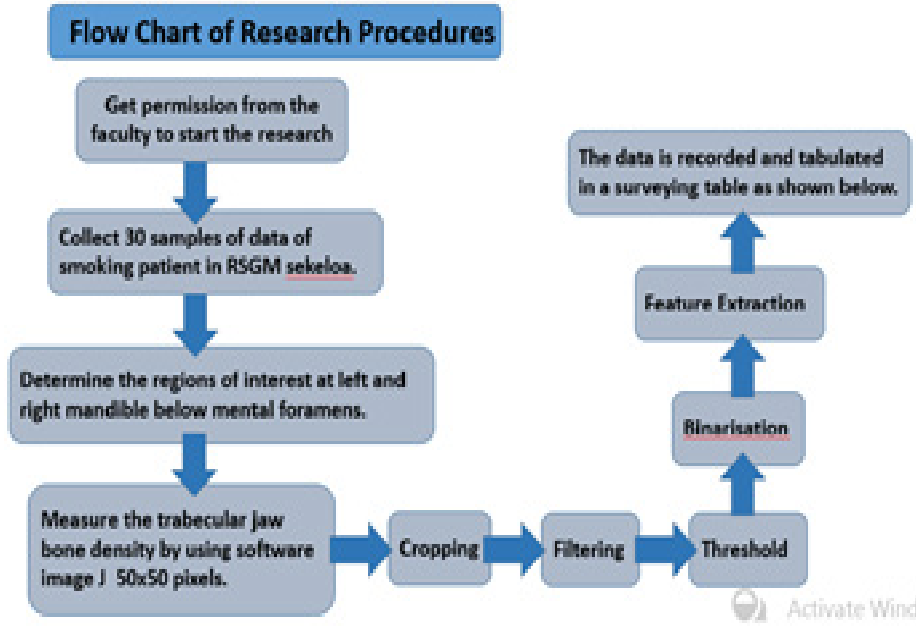

Figure 1. Chat lines of inquiry

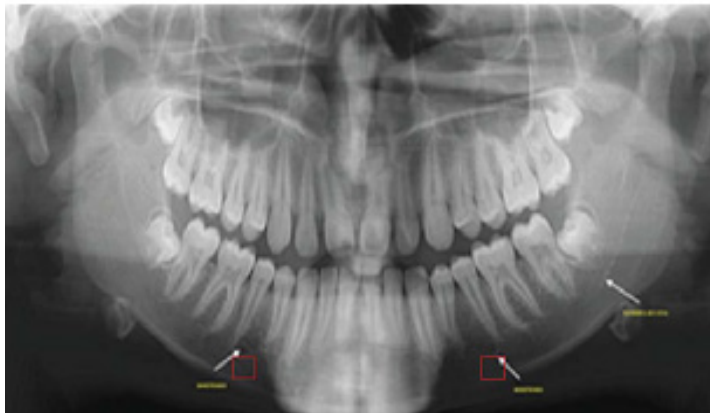

Figure 2: Panoramic Radiograph with Regions of Interest (Red Colour Boxes) with 50x50 Pixels Located Below Mental Foramens at Left and Right Mandible.

This research will only be conducted in subjects age 20-49 years who have a panoramic radiograph with good quality where sharpness, brightness and opacity clearly visible. Based on the rules above, 30 samples of radiograf panoramic data were collected. Assessment is done on the left and right mandible area with ROI 5x5mm, with position, under the mental foramen. Jaw bone density measurements carried out by assessing the trabecular using software image J. The process begins with cropping, filtering, threshold, binarisation and lasty feature extraction. The collected data will then be averaged in the form of tables and

RESULT

This research was conducted from January to April 2016 at Sekeloa Dental Hospital. A total of 30 secondary samples of panoramic radiograph for smoking patients are collected and 12 secondary samples of panoramic radiograph for normal healthy patients are collected as well. All data were then categorized based on age groups, genders and smokers or non-smokers patients.

Table 1. Characteristic Samples

Age	Male Smokers	Female Smokers	Total	Male Non-smokers	Female Non-smokers	Total
20-29	8	2	10	2	2	4
30-39	8	3	11	3	2	5
40-49	7	2	9	1	2	3
Total	23	7	30	6	6	12

Table 2. Percentage of Trabecular and Marrow in Male and Female Smokers to Male and Female Non-Smokers in Region 3 and 4.

	Mean for Male and Female Smokers		Mean for Male and Female Non-Smokers	
Age	Trabecular (%)	Marrow (%)	Trabecular (%)	Marrow (%)
20-29	19.916	80.084	29.321	70.679
30-39	25.652	74.348	30.625	69.375
40-49	18.322	81.678	30.003	69.997
Mean	21.297	78.703	29.983	70.017

Table 3. Percentage of Trabecular and Marrow in Male Smokers to Male Non- Smokers in Region 3 and 4.

	Male Smokers		Male Non-Smokers	
Age	Trabecular (%)	Marrow (%)	Trabecular (%)	Marrow (%)
20-29	19.435	80.565	27.692	72.308
30-39	25.656	74.344	30.694	69.306
40-49	18.265	81.735	30.181	69.819
Mean	21.119	78.881	29.522	70.478

Table 4. Percentage of Trabecular and Marrow in Female Smokers to Female Non-Smokers in Region 3 and 4.

	Female Smokers		Female Non-Smokers	
Age	Trabecular (%)	Marrow (%)	Trabecular (%)	Marrow (%)
20-29	20.396	79.604	30.950	69.050
30-39	25.593	74.407	30.556	69.444
40-49	18.378	81.622	29.826	70.174
Mean	21.456	78.544	30.444	69.556

Table 5. Percentage of Trabecular and Marrow in Male Smokers to Female Smokers in Region 3 and 4.

	Male Smokers		Female Smokers	
Age	Trabecular (%)	Marrow (%)	Trabecular (%)	Marrow (%)
20-29	19.435	80.565	20.396	79.604
30-39	25.656	74.344	25.593	74.407
40-49	18.265	81.735	18.378	81.622
Mean	21.119	78.881	21.456	78.544

Table 4.6. Percentage of Trabecular and Marrow in Non-Smokers Male to Female Non-Smokers in Region 3 and 4.

	Male Non-Smokers		Female Non-Smokers	
Age categories	Trabecular (%)	Marrow (%)	Trabecular (%)	Marrow (%)
20-29	27.692	72.308	30.950	69.050
30-39	30.694	69.306	30.556	69.444
40-49	30.181	69.819	29.826	70.174
Mean	29.522	70.478	30.444	69.556

Table 7. Percentage of Trabecular for Male and Female Smokers to Male and Female Non-Smokers according to Age Categories at Region 3 and 4.

Age	Male Smokers	Female Smokers	Mean	Male Non-smokers	Female Non-smokers	Mean
20-29	19.435	20.396	18.498	27.692	30.950	28.605
30-39	25.656	25.593	25.398	30.694	30.556	30.533
40-49	18.265	18.378	17.797	30.181	29.826	28.451
Mean	21.119	21.456	20.564	29.522	30.444	29.196

Table 1. shows the characteristics of total sample collected for this research. There are total 30 smokers and 12 non-smokers who are according to age group 20- 29, 30-39 and 40-49. There are 23 male smokers and 7 female smokers, whereas there are 6 male and 6 female non-smokers. If according to age 20-29, there are 8 male smokers, 2 female smokers, 2 male non-smokers and 2 female non-smokers. For age 30-39, there are 8 male smokers, 3 female smokers, 3 male non-smokers and 2 female non-smokers. For age 40-49, there are 7 male smokers, 2 female smokers, 1 male non-smoker and 2 female non-smokers.

DISCUSSION

For female, a previous study done by Australian cross-sectional study in pre-menopausal women (118 current smokers, 158 non-smokers; mean age 33 years) found a 4–5% deficit in BMD at the femoral neck, lumbar spine and total body in smokers. This association was more pronounced in women with a BMI (body mass index) <25 kg/m2 and who had breastfed at least one child [15]. Sporting activity appeared protective against bone loss. Another study of healthy community dwelling young women found that, at 2 years of follow-up, smokers aged 20–39 years had a lower spinal BMD than non-smokers. This can be shown in table 7, in which the mean for trabecular percentage of the female smokers is 21.456%, whereas the mean for trabecular percentage of the female non-smokers is 30.444%. This shows about 8-9 % deficit in trabecular percentatge in ROI for our mandibular jaws.

For male, a previous cross-sectional data collected as part of the Framingham Study, a population-based cohort study with over 40 years of follow-up, found a 4–15.3% lower BMD in male smokers at all skeletal sites [16]. Further analysis of longitudinal data over 4 years from this cohort found that men (but not women) who smoked lost more BMD at the hip than men who had never smoked [17]. A French study of 719 men aged 51–85 years reported that former smokers had a higher BMD at the forearm than current smokers [18]. However, following adjustment for age, body mass, alcohol intake and caffeine intake, the two groups

had a similar BMD at the lumbar spine, hip and whole body. This also can be shown in table 7, in which the mean for trabecular percentage of the male smokers is 21.119%, whereas the mean for trabecular percentage of the male non-smokers is 29.522%. This shows about 8-9% deficit in trabecular percentage in ROI for mandibular jaws.

The mean percentage of trabecular in male and female smokers in table 2, is 21.297% which is lower than the mean percentage of trabecular in male and female non-smokers which is 29.983. A 2003 study also found that smoking can significantly reduce the protective effect of calcium on the bones. The inhibition of calcium may be an offshoot of the effect of tobacco use on vitamin D levels. But this observation also indicates that smoking may affect the body's ability to properly utilize calcium. This is not surprising considering the fact that the hormones and vitamins needed to mineralize the bone with calcium are all inhibited by tobacco use.

Traditionally, vitamin D has been considered almost exclusively in the context of its role in calcium homeostasis. Whether ingested or synthesized, vitamin D is transported to the liver, which is then transported to target tissues, where it functions like a steroid, binding to the vitamin D receptor (VDR). When there is a need to increase blood calcium levels (e.g., during growth or pregnancy), 1,25 (OH)2D3 acts in the intestine to increase calcium absorption. If this increased intestinal absorption is insufficient to restore normal calcium levels, 1,25 (OH)2D3 works in concert with the parathyroid hormone (PTH) in the kidney to promote calcium reabsorption from the distal tube, and in the skeletal system to release calcium from bones.

As a result the results in table 3 between male smokers and male non-smokers, table 4 between female smokers and female non-smokers as well as table 7 between male and female smokers and male and female non-smokers shows significant different in their trabecular percentage as well. For table 3, the mean percentage of trabecular for male smokers is 21.119 % and 29.522 % in male non-smokers. The different is 8.403 % between these values and the result is significant. One of the reasons behind this value is that smoking will increase the nicotine level in human blood circulatory system. Nicotine is one of the addictive components of tobacco, is a highly toxic alkaloid and has been the focus of several studies evaluating the relationship between specific cigarette components and bone. The effect of nicotine on bone remains controversial, with some studies finding adverse effects by found an important effect of tobacco smoking on BMD of lumbar spine and femur in rat which is compatible with the results from previous studies by [19, 20]. The experiment is about the rat being exposed to smoke and the BMD of lumbar spine and femur was lower in 4-month in controls. This is not seen in the 2-month and 3-month. This might be due to differences in time of passive smoking[19,20]. Proved that the 5-month cigarette smoke inhalation induced the decrease in BMD, demonstrated that 4 months of nicotine treatment was detrimental to bone by causing an increase in the bone resorbing cytokines and cotinine levels and nicotine also exerted negative effects on the dynamic trabecular histomorphometric parameters. In a rabbit model of bone graft revascularization, elevated systemic levels of nicotine impaired vascularization of a cancellous bone graft implanted

into the distal femur [21,22]. Others have reported direct toxic effects of smoking on bone mass in rodents *in vivo* (Broulik P. D. and Jarab J., 1993) (Epping-Jordan et al.) reported the mean blood nicotine concentration for smokers who smoked 30 cigarettes daily was 40-42 ng/ml. The blood nicotine concentrations in the 4-month smoke-exposed rats were 40.6 ng/ml, indicating that the average blood nicotine concentration was similar to the average for heavy smokers. The blood nicotine concentration did not differ among 2, 3 and 4 months, but there may be a harmful effect of longer smoke exposure for 4 months. Thus, we

considered that the timing of BMD decrease may be associated with the dose level and the duration of smoke exposure.

Besides, this can be seen in table 2 between male and female smokers to male and female non-smokers, table 4 between female smokers to female non-smokers as well as table 4.7 between male and female smokers and male and female nonsmokers. The different in percentage of trabecular shows significant results. For table 7, we know that the mean of percentage of trabecular is 30.444% but the mean for the female smokers is 21.456% only. Tobacco smoke affects the metabolism of sex hormones and its most profound effect is on the level and activity of estrogen. By stimulating increased activity of liver enzymes, tobacco smoke promotes the destruction of estrogen. Decreased estrogen levels due to natural or smoking habits might induced menopause lower bone mineral density in humans. (Eastell, 2006). Because of the nature of its association with various hormonally related diseases, smoking has been considered potentially anti-estrogenic. There was a small (8%) reduction in oestriol excretion in smokers among premenopausal woman. MacMahon et al. (1982) reported similar excretion rates of oestrone, oestradiol and oestriol in smokers and non-smokers in the follicular phase, but about 30% lower excretion of all three oestrogens in smokers in the luteal phase, whereas Michnovicz et al. (1988) reported 31% lower excretion of oestriol in the follicular phase. Estrogen plays a role in bone metabolisme. Low circulating

estrogen is an important risk factor for post-menopausal osteoporosis (Sambrook and Cooper, 2006). Estrogen has been shown to induce apoptosis in bone-resorbing osteoclasts (Kameda *et al*, 1997; Kousteni *et al*, 2002). Estrogen is anti-apoptotic in osteoblasts, leading to an overall building of bone (Kousteni *et al*, 2002). Besides, another hormone affected by smoking is cortisol. Cortisol is known as the stress hormone and smokers have higher levels of this hormone than nonsmokers.

High levels of cortisol is also directly responsible for higher stress levels among smokers. Cortisol promotes the breakdown of bones. Therefore, when tobacco use increases its level and its duration of usage, this will cause the percentage of bone to be reduced. Furthermore, smoking blocks the actions of the calcitonin hormone. Calcitonin is a thyroid hormone that drives calcium from the blood into the bones. It also blocks the breakdown of bones. By reducing its level, tobacco use encourages the breakdown of bone while preventing its mineralization. Therefore, this will cause the percentage of bone to be reduced. In addition, smokers had lower levels of the hormone, DHEAS (dehydroepiandrosterone sulfate), a testosterone metabolite which later causes the testosterone level to be lower as well. The androgen found in blood is testosterone (Williams et al., 2013). Androgens

protect men from the onset osteoporosis through the maintenance of cancellous bone and expansion of cortical bone (Kini & Nandeesh, 2012). Androgens have multiple effects on the skeleton. It affects bone size, bone mass and bone remodeling. In adolescence, it promotes skeletal growth by stimulating osteoblast and suppressing osteoclast function, activity and lifespan. It also increases periosteal apposition, providing men with a bigger and thicker cortical bone (Kung, n.d.).

As a result for the female smokers, they tend to have lower in percentage of trabecular due to the reduction in estrogen, testosterone and calcitonin as well as increase in cortisol. The different in percentage of bone can be seen also in table 2, 3, 5 and 7. This is most obvious to be seen in table 4 which is comparing the female smokers and the female non-smokers, having the different of 8.988% of trabecular.

For table 7, the mean for percentage of trabecular for male and female smokers, increases from 18.498% at age 20-29 to 25.398% at 30-39 and decrease again to 18.378% at age 40-49; the mean for percentage of trabecular for male and female non-smokers increases from 28.605% at age 20-29 to 30.533% at age 30-39and decreases to 28.451% at age 40-49.

From the age of a newborn, the bone is undergoing growing phase untilapproximately age 35-39, these ages are the ages people will reach their peak bonemass. Peak bone mass is the time at which their bones are highest in density andstrongest moment in this current life. (Edelson and Kleerekoper 1995). Howeverafter the age of 35-39, the average bone mass will start declining and women tendto lose 0.5 percent to 1 percent of their bone mass every year. During menopause,when the ovaries cease creating estrogen, the rate of bone loss increases. Withoutthe use of estrogen replacement therapy, most women tend to lose 3-7 percentagein BMD per year. This will result in the loss of BMD up to 15-35 percentage of lossin bone mass in the first 5 years after menopause (Bonnick 1994). This osteoporosisprocess will further increase when they going older. This will normally starts from6% at the age of 50 year and will up to 50% after the age of 80 (Prevalence of lowfemoral bone density in older U.S. adults from NHANES III. By *Looker AC, 1997*).

A gross prediction states that 10% female who are older than the age of 50 will sufferfrom osteoporosis. However, only 2% of men older than the age of 50 will sufferfrom osteoporosis, according to the U.S. Centers for Disease Control andPrevention. An estimation of 50% of women and 20% of men if they are more thanthe age of 50 will tend to suffer from osteoporosis-related fracture (Osteoporosis,by Sambrook P, 2006). Besides, the different in percentage of bone between male and female smokers and male and female non-smokers which shown by table 2, 3, 4 and 7 might be due to tobacco smoking which contain toxic compounds. Toxins in smoke will have negative influence on bone remodelling process. Previous study had investigated into two likely candidates which are benzo[*a*] pyrene (BaP) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). These two components will stimulates bone resorption by osteoclasts. When used at low doses BaP and TCDD were able to stimulate osteoclast formation *in vitro* and *in vivo*, an effect that was due to interaction with Ahr. Cyp1 inhibitors reduced osteoclastogenesis in cells treated with RANKL, TCDD or

BaP. Furthermore, cells lacking Cyp1a1/1a2 or Cyp1a1/1a2/1b1 treated with TCDD showed lower levels of osteoclast differentiation than cells in which the genes encoding these proteins were functional, demonstrating that the Cyp enzymes are downstream of Ahr.

Furthermore, cigarette smoking can also be directly toxic to the bone. Besides nicotine, the primary addictive compound in tobacco, the other known and suspected toxic compounds can directly kill off osteoblasts. Osteoblasts are the cells that give rise to the bone. They produce the protein known as osteocalcin which is absolutely essential for the proper mineralization of the bones. With the reduction of osteoblasts, the percentage of bone will be reduced as well. Smoking also damages blood vessels and nerves. This means that tobacco use can reduce blood flow and sensations in the limbs. This damage to the surrounding muscles and nerves increases the risks of injuries and bone fractures. In addition,the damage to the blood vessels means that repair to the bones take much longer.

For table 6, when comparing the trabecular percentage of male non-smokers and female non-smokers, the data shows that in age 20-29, female non-smokers has higher percentage of mean value of trabecular, but male non-smokers has higher percentage of mean value of trabecular in age 30-39 and 40-49. Male non-smokers should have greater trabecular percentage, but the difference was dependent on skeletal site for example higher 9% than women in lumbar spine (1.05+0.25 g/cm2 for man and 0.96 ± 0.11 g/cm2); femoral neck in men (0.85 ± 0.13 g/cm2) was 6% higher than in women (0.80 ± 0.11 g/cm2). The age achieving pBMD was reached in women was younger than in men. For example, at the femoral neck, age of pBMD in women was 22.4 years (95% CI: 19 - 24) which was earlier than in men (26; 95% CI: 24 - 29). This trend was also observed at the lumbar spine (25 in women and 27 years in men).

Smoking is associated with increased concentrations of free radicals, which may contribute to bone resorption. (Duthie G. G., 1991) A prospective cohort study involving 66651 women, aged 40–76 years of age, found that current smokers with a low vitamin E and C intake had an increased risk of hip fracture. (Melhus H., et al 1999).

CONCLUSION

It can be concluded, based on the panoramic radiograph using image j software at ROI below mental foramen at region 3 and 4, it was found that the trabecular percentage decreases. Based on three age groups; gender as well as between smokers and non-smokers.

REFERENCES

1. Indonesia Country Profile WHO Report on the Global Tobacco Epidemic. 2015. James, R. 2004. Panoramic Radiograph. Pp. 2-4.
2. Hollenbach, K.A.; E. Barrett-Connor; S.L. Edelstein; T. Holbrook. 1993. Cigarette smoking and bone mineral density in older men and women. Am J public Health83. Pp.

1265-1266.
3. Johnston D.W. 1994. Current status of professionally applied topical fluorides. community dent oral epidemiol. Pp. 265–269
4. Fang, M.A.; P.J. Frost; A. Iida-Klein; T.J. Hahn. 1991. Effects of nicotine on cellular function in UMR 106–01 osteoblast–like cells. bon. Pp. 283-286
5. Yuhara, S.; S. Kasagi; A. Inoue. 1999. Effects of nicotine on cultured cells suggest that it can influence the formation and resorption of bone.European Journal of Clinical Pharmacology. Pp. 387-393
6. Daniel M., A. D. Martin , D. T Drinkwater. 1992. Cigarette smoking, steroid hormones, and bone mineral density in young women. Calcif. Tissue Int. Pp. 300–305.
7. Hannan, M.T.; D.T. Felson; B. Dawson-Hughes; K.L. Tucker; L.A. Cupples; P.W. Wilson; D.P. Kiel. 2000. Risk factors for longitudinal bone loss in elderly men and women: The Framingham osteoporosis study. Journal of bone and mineral research. Pp. 485–492
8. Elizabeth Weiss. 2014. Paleopathology in Perspective: Bone Health and Disease through Time, Amazon Prime. pg 51
9. Hoseini Zarch, S.H.; A. Bagherpour; A. Javadian Langaroodi; A. Ahmadian Yazdi; A. Safaei. 2011. Evaluation of the accuracy of panoramic radiography in linear measurements of the jaws. Pp. 950-968.
10. Jamel A et all. 2013.Smoke carcinogens cause bone loss through the aryl hydrocarbon receptor and induction of Cyp1 enzymes.PNAS.V.110. Pp. 27
11. Levin, Schwartz, Arad, 2005. The effect of cigarette smoking on dental implants and related surgery. Implant Dent J. Vol. 14(4).Pp:357-61.
12. Hollenbach, K.A.; E. Barrett-Connor; S.L. Edelstein; T. Holbrook. 1993. Cigarette smoking and bone mineral density in older men and women. Am J public Health 83. Pp. 1265-1266
13. Kiel, D.P.; J.A. Baron; J.J. Anderson; M.T. Hannan; D.T. Felson. 1992. Smoking eliminates the protective effect of oral estrogens on the risk for hip fracture among women. Pp. 716-721.
14. Krall & Dawson-Hughes, 1991. Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. Ann Intern Med. Vol. Oct 15.Pp.505-12.
15. Jones G., F. S Scott ., 1999. A cross-sectional study of smoking and bone mineral density in premenopausal parous women: effect of body mass index, breast feeding, and sports participation. J. Bone Miner. Res. Pp. 1628–1633
16. Kiel, D.P.; J.A. Baron; J.J. Anderson; M.T. Hannan; D.T. Felson. 1992. Smoking eliminates the protective effect of oral estrogens on the risk for hip fracture among women. Pp. 716-721
17. Hannan, M.T.; D.T. Felson; B. Dawson-Hughes; K.L. Tucker; L.A. Cupples; P.W. Wilson; D.P. Kiel. 2000. Risk factors for longitudinal bone loss in elderly men and women: The Framingham osteoporosis study. Journal of bone and mineral research. Pp. 485–492.
18. Schulze, R.; F. Krummenauer; F. Schalldach; B. d'Hoedt. 2000. Precision and accuracy

of measurements in digital panoramic radiography. Dentomaxillofac Radiol. Pp. 52–56
19. Hollinger JO et al. 2004. Recombinant human bone morphogenetic protein-2 is superior to demineralized bone matrix in repairing craniotomy defects in rats. Journal of Biomedical Materials Research. Vol 28(10):1127-38
20. César-Neto JB et al. 2011.Smoking and periodontal tissues: a review,Internasional Prociding in International Symposium, held at the 16th Congress of the Brazilian. Association for Oral Health Promotion (ABOPREV)
21. Hadipin et all.2013.Bone Resorption Marker Satus of Postmenopausel Malay Women in Kelantan and Its Coresponding Riks Factors, Sains Malaysiana Journal.Vol 42(8), Pp : 1191-1200
22. Riebel GD et all.1995. The effect of nicotine on incorporation of cancellous bone graft in an animal model. Spine 20: PP.2198-2202