ESTIMATION OF SALIVARY ESTRADIOL AND SALIVARY

CALCIUM IN POST MENOPAUSAL WOMEN WITH OR WITHOUT

XEROSTOMIA

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled "ESTIMATION OF SALIVARY ESTRADIOL AND SALIVARY CALCIUM IN POST MENOPAUSAL WOMEN WITH OR WITHOUT XEROSTOMIA" is a bonafide and genuine research carried out by me under the guidance of **Dr.F.MASSILLAMANI,MDS**, Reader, Department of Oral Medicine and Radiology, Ragas Dental College and Hospital,Chennai.

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LIST OF ABBREVATIONS

ABBREVATIONS	FULL FORMS
ER	Estrogen receptors
DXA	Dual energy X-ray absorptimetry
BMD	Bone mineral density
PTH	Parathyroid hormone
DMFT	Decay missing filling teeth
DMFS	Decay missing filled teeth scores
CPI	Community periodontal index
ELISA	Enzyme linked immunosorbant assay
OSMF	Oral submucous fibrosis
PDL	Periodontal
IFN	Interferon
TGF	Tumor growth factor
TNF	Tumor necrosis factor
IL	Interleukin
RANKL	Receptor activator of nuclear factor kappa-B
OHI-S	Oral hygiene index scores
PI	Plaque index
GI	Gingival index
LOA	Loss of attachment
STRAW	Stages of reproductive aging in women
WHO	World health organization

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Introduction

INTRODUCTION

The World Health Organisation defined menopause as "The permanent cessation of menstruation due to loss of ovarian follicular activity". A female is considered to have attained menopause when there is amenorrhea with no pathology for a period of 12 months[1] It begins in the fourth to fifth decade of life and globally the overall median age at natural menopause was found to be 51.4 years[2].The average age for menopause in Indian women was identified to be 46.2 ± 4.9 years[3].

Estrogen exist in 3 physiological forms as Estrone(E1), Estradiol(E2 or 17β-Estradiol) and Estriol(E3)[4] Estradiol(E2) is considered the most potent form of estrogen. The granulosa cells of ovary uses the enzyme aromatase to convert androstenedione to estradiol. It is secreted by theca cells of ovaries and also by fat cells, particularly visceral fat. It is associated with the development of sex characteristics in females throughout the growth and development process[5] It is the key regulator of growth, differentiation and function in various tissues like female reproductive tracts, mammary glands, skeletal system and cardiovascular system[6].

There are two types of estrogen receptors-ER α and ER β. ER α receptors are present in mammary gland, ovary (theca cells), bone, liver, adipose tissue and

urogenital system. These receptors are essential for regulation of metabolism, preservation of skeletal homeostasis and normal functioning of mammary glands and genitourinary system.ER β receptors are present in bladder, ovary (granulosa cells), colon, adipose tissue, immune system, salivary gland and oral epithelium. These receptors act on central nervous system, immune system and it counteracts the cell hyper proliferation in tissue like breast and uterus.[7]

In post-menopausal period, there will be a decline in endogenous estradiol levels making it less available to bind with the estrogen receptors distributed all over the body. This scenario leads to diverse systemic and oral manifestations. The systemic manifestations exhibited by women are urogenital atrophy, osteoporosis and vasomotor symptoms.[1]Estradiol is essential for vaginal lubrication,vaginal secretion and increase mechanical compliance of vagina.Absence of the hormone leads to urogenital atrophy causing dyspareunia,urinary incontinence and urinary urgency[8].

Estradiol also causes considerable effect on maintaining the integrity of bones as estrogen receptors are present in osteoblasts and osteoclasts.It takes part in the regulation of bone calcium. The decrease in estradiol binding leads to enhanced osteoclastic and decreased osteoblastic activity. Osteoporosis is defined as a metabolic bone disease characterized by low bone mineral density that leads to fragility and susceptibility to fractures[9].The most common vasomotor

symptoms occurring in menopause are hot flushes and night sweats. It occurs due to narrowing of thermoregulatory neutral zone in women and instability of skin blood vessels [10].

Oral manifestations experienced by post-menopausal women are burning mouth syndrome, xerostomia, osteoporosis of jaw bones, periodontitis and less commonly mucosal disorders such as lichen planus, pemphigus vulgaris, benign mucosal pemphigoid and Sjogren's syndrome are also prevalent in these patients[1].In the oral cavity, estradiol binding with ERβ receptors is necessary for oral epithelial maturation. The absence of estradiol leads to atrophic oral epithelium which is one of the causes for oral discomfort. Burning mouth syndrome is characterized by a burning sensation of the oral mucosa for which no cause can be found[11].It often presents as pain and bilateral burning sensation along with dysguesia, dry mouth, alterations in breath and dysphagia. The etiology behind this condition is decreased hormone levels and small fiber sensory neuropathy of the oral mucosa[12].

Xerostomia is defined as subjective sensation of oral dryness is usually associated with hyposalivation but not always. The ER β receptors are also present in mucous acini,serous acini and ductal cells in minor salivary glands, parotid and submandibular glands and are essential for functional integrity of these glands In post-menopausal period, there is alteration in salivary flow,

salivary pH. mucin, IgA, phosphates, calcium and electrical resistance. The inorganic composition of saliva is altered both quantitatively and qualitatively because of the non availability of estradiol to bind with the receptors present on the ductal cells of the salivary glands. The above mechanism is suggested as a possible cause for xerostomia in menopause.[13].

The presence of estrogen is necessary for calcium hemostasis and regulation of bone metabolism. Estrogen receptors are present in osteoclasts and progenitor of osteoclasts. Estradiol has inhibitory effect on osteoclasts and promontory effect on osteoblasts. During menopause reduction in estradiol levels triggers osteoclastogenesis and inflammatory cytokines are released as a result of which bone resorption takes place[14].This mechanism results in osteoporosis, a skeletal systemic disorder characterized by a low bone mass and by micro architectural deterioration of bone tissue, with a subsequent increase in bone fragility and susceptibility to fracture[15].It is listed as a systemic manifestation which can also involve jaw bones. Osteoporosis and chronic periodontitis share inflammation as a common mechanism of pathogenesis. Post menopausal women with osteoporosis are at an increased risk of developing periodontitis[16].In oral cavity, teeth are embedded in alveolar process. An overall decline in bone mineral density is depicted as decreased bone mineral density of alveolar crest, subcrestal alveolar bone which leads to attachment loss and tooth loss.[17].

Saliva is an ultra filtrate of plasma, many studies have put forward that unstimulated saliva provides undiluted form of biomarkers for appropriate analysis. Estradiol is a steroid hormone and is available in free form in saliva. Measurement of salivary estradiol may reflects the ovarian functionality level in women[18].Measurement of the whole calcium (Ca^{2+}) concentration in saliva may also serves as a marker and adjuvant for prediction of osteoporosis[919].The oral hygiene index, periodontal index and xerostomia score of menopausal women should also be assessed as they may depict their oral health status. Thus, this study is aimed towards estimation of salivary estradiol and salivary calcium levels and correlating it with oral hygiene index, periodontal index and xerostomia score among post menopausal women, perimenopausal women and healthy menstruating women.

Aim and objectives

AIM AND OBJECTIVES

AIM:

The aim of our study is to evaluate salivary estradiol and salivary calcium in post menopausal women with varying degree of oral dryness.

OBJECTIVES:

- To compare salivary estradiol and salivary calcium among healthy menstruating women, premenopausal women and post-menopausal women. .

- To find out if there is any relation between salivary estradiol levels and varying degree of oral dryness in post-menopausal women.

- To find out the relation between salivary estradiol levels and periodontal status in post-menopausal women.

- To find out the influence of salivary estradiol levels on salivary calcium levels.

Review of litreature

REVIEW OF LITERATURE

The review of literature below throws light on menopause, a physiological transition phase in women which is characterized by diverse systemic as well as oral manifestation. The study is done mainly to assess oral hygiene status, periodontal status and severity of xerostomia in postmenopausal women and compare them with premenopausal and normal menstruating women.

REVIEW ON SALIVARY ESTRADIOL:

The World Health Organisation defined menopause as "The permanent cessation of menstruation due to loss of ovarian follicular activity"[1].A menopaused female is the one who has amenorrhoea for a period of 12 months with no underlying pathological condition. A decline in the prime female sex hormones progesterone and estrogen can be noted. Estrogen exists in 3 forms Estrone (E1), Estradiol (E2 or 17β -Estradiol) and Estriol (E3) out of which estradiol is considered the most potent form. It is considered as a biomarker for ovarian aging (hormone transition).

Guyton and Hall have described 17β-estradiol as a principle steroid sex hormone secreted by ovary. Its steronic potency is 12 times that of estrone and 80 times that of estriol. The synthesis of it occurs from cholesterol derived from blood and also to a little extent from acetyl co-enzyme. Androgens and

progestrones are synthesized first during follicular phase of ovarian cycle. The estrogens diffuse out of the theca cells and enter the granulose cells where they are converted into estrogen by the enzyme aromatase. It is responsible for the development of primary and secondary sexual characteristics in females.[4]

Fig 1 – Synthesis of estrogen
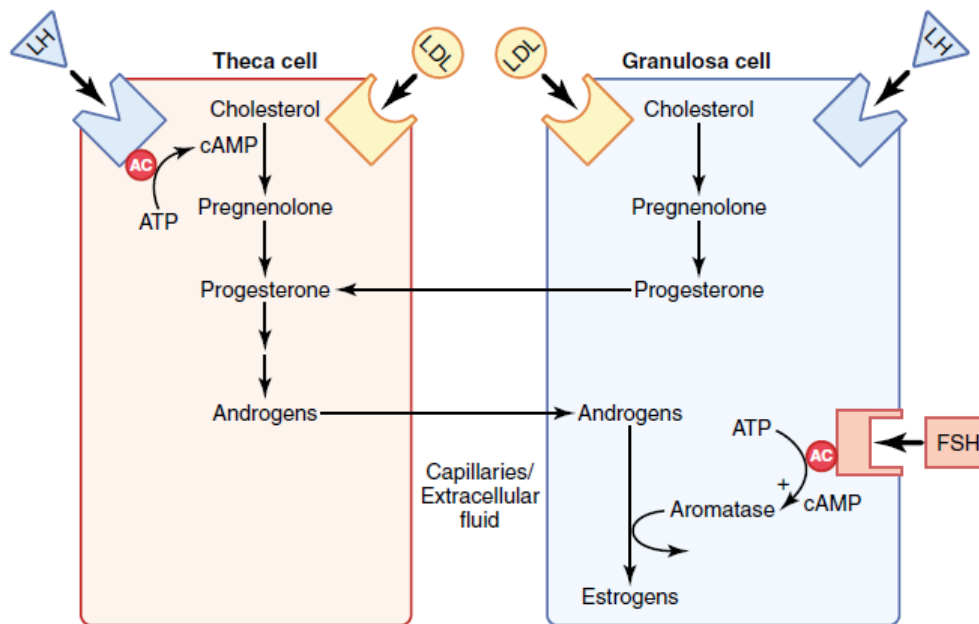
Lenne Martin et al (2001) recommended salivary hormone test for measuring steroid hormones like estradiol.In serum samples the hormone is bound to a carrier protein. After centrifugation a considerable amount of carrier protein is also removed. Hence it does not reflect true levels of steroid hormones. The hormones when not bound to carrier protein due to their smaller molecular size

diffuse into tissues and saliva. Thus, saliva reflects the biologically active (free or non bound) levels of steroid hormone[20].

Tidies et al (2005) conducted a study to determine if saliva could be used in studies of postmenopausal women in place of serum. In the study serum and saliva samples were collected from 43 post menopausal women in which 31 were under estrogen therapy and 12 were not under estrogen therapy. Saliva and serum was collected from all the patients and stored. It was concluded that concentrations of E2 in saliva reflected the serum E2 levels in estrogen therapy users. In non Estrogen users the saliva E2 was detectable only in low concentrations as the circulating serum E2 is itself low in these cases. Thus, he concluded by stating saliva E2 serves as a fairly robust predictor of serum E2 among post menopausal women under estrogen therapy. A disproportionate sample number between the two groups is stated as the main reason for the above result. It was further stated that a larger sample size may be needed to prove the same[21].

Natalia Gavrilova et al (2009) measured salivary sex hormone in a national population based study of older adults. The saliva samples were collected from men and women between 57-85 years of age. They were analysed for 17β estradiol and testosterone using ELISA test. The results of the study were 90.6% of the individuals provided self collected salivary samples out of which 95.8%

were adequate for analysis. It was found that mean testosterone levels were high in men compare to women and salivary estradiol levels were higher in women under estrogen therapy when compared to estrogen n on-users. He concluded the study by stating the datas driven are internally and externally valid. The co-operative level of the patients were also high. Thus,saliva specimens provide novel sex hormone datas of relevance for studying health and illness in later life of both men and women[22].

REVIEW ON SALIVARY CALCIUM:

Siva Reddy et al (2008) conducted a study among 45 postmenopausal women to correlate oral signs with salivary parameters as possible indicators of osteoporosis and osteopenia. In the study 45 subjects were divided into 3 groups based on their bone mineral density. Group I formed the osteoporotic women. Group II are established osteopenic women and group III non osteoporotic women. The study and control groups were assessed for mandibular cortical width, Russell's periodontal index and salivary parameters like calcium, phosphorous and alkaline phosphatase levels. The results were mandibular cortex width was less in osteoporotic and osteopenic group when compared to control group.Periodontitis comparison was insignificant among all groups. Salivary calcium, salivary phosphorous and salivary alkaline phosphatase levels were increased in osteoporotic and osteopenic group when compared to normal

controls. The number of teeth lost was also higher in group I and II compared to group III. It was concluded that salivary parameters like calcium, phosphorous and alkaline phosphatase levels along with oral signs like periodontitis and number of missing teeth may serve as indicators in the diagnosis of osteoporosis and osteopenia in postmenopausal women[23].

Ravindher Singh et al (2012) conducted a study to correlate serum estrogen and salivary calcium levels in post menopausal women with and without oral dryness. A questionnaire for identifying oral dryness was given to all the postmenopausal women those who had oral dryness formed the case group. Healthy menstruating women formed the normal controls. Saliva and serum sample were collected from both groups to measure serum oestradiol and salivary calcium levels in both the groups. The results revealed that salivary calcium level was high and the serum estrogen levels were low in case group. Pearson's correlation was performed to see if there exist any relation between serum estrogen and salivary calcium levels. A significant negative correlation was observed between the two variables. It was substantiated with the fact that a decrease in oestradiol suppresses intestinal absorption of calcium which causes increase in serum parathyroid hormone enhances bone resorption. The conclusion of the study was serum estrogen level decreases and salivary calcium level increases in post menopausal women. Post menopausal women are at a greater

risk of developing periodontitis due to alveolar bone loss as a result of osteoporosis[24].

Raibei et al (2012) has conducted a study on salivary calcium concentration in post menopausal osteoporosis. It was a case control study in which 40 post menopausal women with osteoporosis formed the case group and 40 post menopausal women without osteoporosis formed the control group. The results revealed that salivary calcium levels were higher in osteoporotic women than in non-osteoporotic women. The study stated saliva is a preferred medium because of it can be easily obtained, painless, cost effective and does not require special personnel to collect. Salivary calcium can be used as an effective pre screening tool for cases in which DEXA scan is high risk in menopausal women. It also serves as an adjunct for several other serum and radiographic findings[25].

Agha Hosseini et al (2012) conducted a study to determine the relationship between serum and salivary calcium, phosphorous and alkaline phosphatase levels in postmenopausal women with or without oral dryness. Xerostomia status was assessed using a questionnaire and 30 postmenopausal women who had xerostomia formed the case group while 30 postmenopausal women who did not have symptoms of xerostomia formed that control group. Whole saliva and serum was collected from them and analyzed for calcium levels using Arsenazo III reaction. It was found that salivary calcium levels were

increased in postmenopausal women with oral dryness when compared to postmenopausal women who did not have xerostomia. While there was no difference observed in the serum calcium levels[26].

Victor R.Preedy (2015) in his book on calcium chemistry and analysis had stated that calcium is necessary to perform various biological and physiological processes. The salivary proteins secrete calcium ions, calcium levels in saliva depends on the salivary pH and salivary flow rate. In post-menopausal women there is low circulating estrogen levels, this reduces calcium absorption from intestine which leads to decrease in parathyroid hormone levels in serum. The raise in PTH level mobilizes calcium from the bones increasing the serum calcium levels this is responsible for the development of osteoporosis The fraction of salivary calcium levels contributed by salivary glands are[27],

Normal range of salivary calcium in humans	**0.2-5mM**
Whole unstimulated saliva levels	**0.5-2.8mM**
Parotid unstimulated saliva levels	**0.5-2.1mM**
Submandibular unstimulated saliva levels	**0.5-5mM**
Sublingual unstimulated saliva levels	**1.7-2.9mM**

Table 1-Salivary calcium levels in human

Pereira et al (2018), in their comparative study of oral and salivary parameters in patients with and without loss of bone mass had evaluated salivary calcium, viscosity of saliva and salivary pH and compared them with bone mineral density in 32 postmenopausal women with loss of bone mass and 32 postmenopausal women without loss of bone mass. The results of the study revealed that salivary calcium levels were elevated in patients with low BMD when compared to those with normal BMD. He has also stated that calcium is the chief element of the skeletal system and plays a significant role in bone regeneration. Salivary calcium is related to osteoporosis. It was also reported that DMFT index and tongue coating were higher in case group when compared to control group. The analysis of salivary pH, viscosity and protein concentration were not significant between the groups. The study was concluded by stating that salivary calcium can be used as a screening tool to diagnose bone mineral changes[28].

Shweta vinayak kumbhojkar et al (2019) measured salivary calcium levels among healthy, pregnant and post menopausal women. Salivary sample was chosen because it is used as a diagnostic fluid in medicine and it is in expensive, non-invasive and easy to use diagnostic aid for oral and systemic disease. Saliva contains main inorganic components like calcium and phosphorous which form the main mineral component of skeletal system. The

samples were collected from pregnant; post menopausal and healthy women.BMI and BMD using ultrasound were measured for these patients. Estrogen levels were measured in serum samples and calcium levels were measured in salivary sample and were compared.

It was concluded that in the post menopausal group the salivary calcium levels were higher when compared to other groups and it served as a potential indicator of osteoporosis. They also showed low serum estrogen level and low BMD. The salivary calcium and BMD did not differ in healthy control when compared to pregnant group. However, salivary calcium and BMD, however, did not differ in the healthy controls when compared to the pregnant group. Salivary calcium levels exhibited a correlation with BMD among all the three groups and in post menopausal group a negative correlation was seen between serum estrogen and salivary calcium. This study substantiates that salivary calcium can be used as an impeccable tool for detecting the presence and absence of osteoporosis[29].

Balwindher singh et al (2019) evaluated salivary calcium and salivary parathyroid hormone levels in postmenopausal women with and without oral dryness.80 postmenopausal women were included they were assessed for xerostomia using xerostomia XI inventory questionnaire. Women who had xersotomia formed the case group while those who did not have dryness formed the control group. Saliva was collected and assessed for calcium and parathyroid

hormone levels. Salivary parathyroid hormone was insignificant between the groups. Salivary calcium was found to increase as the symptoms of oral dryness increases[30].

REVIEW ON XEROSTOMIA IN MENOPAUSE:

Yalcin F et al (2006), did a study on oral health in postmenopausal Turkish women. A total of 348 post menopausal women were included in the study. They were interviewed and their oral hygiene statuses were examined for over a period of 2 years. They were interviewed and their oral hygiene status was examined over a period of 2 years. They were examined for DMFT index, DMFS index, RDF index and CPI. Other oral complaints and denture status were also assessed. The results were 24% of the women were using hormones and 77% were not. Oral dryness was seen in 48.8% of hormone users and 68.3% of the non users.36.3% of the hormone users and 39.5% of hormone non-users were edentulous.DMFT,DMFS and CPI were high in non-users. RDF values were not significant between both the groups. Thus, menopause significantly affect the oral health status of women.[31]

Agha Hosseini et al (2009), in his study titled relationship of stimulated saliva 17β estradiol and oral dryness feeling in menopause. 76 menopausal women aged 41-77 years were included. Based on the xerostomia XI inventory

score they were divided into 38 women who had xerostomia and 38 women who din have xerostomia. Paraffin stimulated saliva was obtained from these subjects.17β estradiol concentration in these subjects were measured using ELISA. It was found that the hormone levels were less in case group when compared to control group and there existed a negative correlation between oral dryness and estradiol concentration in menopausal women.[32]

Agha Hosseini et al (2012) conducted a study among post menopausal women to evaluate unstimulated salivary estradiol levels in subjects with or without xerostomia. He collected unstimulated saliva by spitting method and measured the salivary estradiol levels by using ELISA method. He put forth that there was a significant difference in salivary estradiol levels among the case and control group. Further, depending on the levels of this hormone severity of xerostomia varied. The case group experience severe xerostomia while the control group was devoid of it. There was a negative correlation between xerostomia and salivary estradiol levels. He concluded that during menopause a significant decrease in secretion of estradiol hormone by the ovaries were reflected by the low estradiol levels in saliva[33]

Santhosh et al (2013) conducted a study titled oral finding in postmenopausal women attending dental hospital in western part of India.365 postmenopausal women and 365 men of the same age was included in the study.

They were asked about complaints of xerostomia, taste and breath changes, facial and mucosal pain. These subjects were also examined for oral ulceration, white and red lesions. It was found 25.8% of them experienced mucosal /burning sensation/pain, dry mouth was seen in 27% of the cases. Altered breath was seen in 6.3% women,3.6% women experienced facial pain. OSMF was more common in males (5.5%) than females (1.9%).Thus, oral manifestation were common in postmenopausal women. These changes are attributed to hormonal changes so the dentist should refer these patients to gynecologist for appropriate management[34].

Minicuccici et al (2013) conducted a study to assess that impact of menopause on salivary flow and xerostomia in menarche and menopause. In this the subjects were assessed using xerostomia XI inventory questionnaire and visual analog scale questionnaire. The salivary flow rates in these subjects were assessed using chemical absorption stimulation test. Unstimulated saliva, Stimulated saliva and super stimulated saliva was obtained from these subjects. At the end in both the groups' super stimulated salivary rate was higher. The salivary flow rate was low in menopausal women. It was concluded by stating that there was a considerable reduction in salivary flow rate without oral dryness in the menopause group. So it is mandatory to normalize salivary flow for better oral health[35].

Dutt P et al (2013), had reviewed various articles on menopause and oral health and concluded that periodontal health was affected in 60% of the cases, dry mouth was observed in 25% of the cases and glosssodynia was seen in 15% of the cases. These presentations were responsible for the occurrence of oral mucosal and dental diseases. Hormone replacement therapy controlled the symptoms to a certain extent but was not effective in preventing the oral symptoms. However, long term studies can provide clinical guidelines for successful management.[36]

Gill et al (2019) conducted a study among healthy postmenopausal women. Saliva was collected from them and they were assessed for oral discomfort by visual analog questionnaire. It was found that salivary flow rate was less in postmenopausal women when compared to premenopausal women and the subjective symptoms of menopause was also more in menopausal women when compared to premenopausal women. It was concluded by stating salivary flow rate and symptoms of oral discomfort were influenced by menopause[37].

REVIEW ON PERIODONTITIS AND ORAL HYGIENE STATUS DURING MENOPAUSE:

Sema Dural et al (2006) evaluated the effect of menopause on saliva and dental health. Unstimulated saliva was obtained from 18 post menopausal women and 20 regularly menstruating women. Salivary pH and salivary flow rate was

buffering capacity was measured. Oral hygiene status was assessed using OHI and DMFT respectively. It was found salivary pH of postmenopausal women were to the control group. This study showed the importance of preventive dentistry[38].

Leena Palomo et al (2013), conducted a study on the need to educate post-menopausal women on their periodontal health.94 postmenopausal women were included in the study. They were informed of their diagnosis and educated about their disease, the probable risk factors and the available preventive and treatment modalities. 97.8% of the patients had healthy periodontium and 36.2% of them had severe periodontitis. Patients associated disease with abscess and were likely to follow preventive and treatment regimens when informed to them. This study suggested a need to educated postmenopausal women on periodontal health[39].

Amit Bharadwaj et al (2012) over viewed the effect of menopause on periodontium. He put forth that estrogen and progesterone are the two important hormones influencing periodontium. In vitro studies using human premenopausal gingival fibroblasts reported that, estradiol can induce cellular proliferation while depressing protein production. This proliferation is due to specific population of cells within the parent culture that responds to physiologic concentrations of estradiol. It is also evident that human PDL cells possessed immunoreactivity for estrogen receptors which are mediated via estrogen receptors beta (ER beta).

Menopause causes a wide range of changes in women's body as well as oral cavity. Prevalance of gingivitis, periodontal disease, tooth loss and dry mouth has been reported. Estrogen is essential for growth, maturation and bone turn over and proper closure of epiphyseal plates in women. Estrogen deficiency is one of the most frequent causes of osteoporosis in menopausal women. There is cancellous as well as cortical bone loss. Enhanced endocortical resorption is the first sign of estrogen withdrawal in cortical bone loss. Intracortical porosity is also present in reduced strength. Estrogen exerts anti-resorptive effects on alveolar bone by increasing the expression of osteoprotegrin. As the hormone decreases these effects are also decreased.

The proposed mechanism for bone loss is that estrogen deficiency stimulates the immune system to produce TNF factors which are activated by T cells by complex mechanisms mediated by antigen presenting cells and involving cytokines IFN-g,IL-7 and TGF-b.TNF increases osteoclast formation and bone resorption both directly and by augmenting the sensitivity of maturing osteoclasts to the essential osteoclastogenic factor RANKL. He concluded by stating female sex hormones are alone not enough to cause gingival changes. They alter periodontal tissue response to microbial plaque and thus indirectly contribute to periodontal disease[40].

Tezal et al (2015) stated that post menopausal women are a small fraction of population. It was stated estrogen deficiency during menopause was associated with overall decrease in bone mineral density and tooth loss. The study was performed in 106 post menopausal women who were examined for clinical attachment loss, probing depth, gingival bleeding, supragingival plaque and calculus. Alveolar bone loss was measured using anterior and four posterior vertical` bite wing radiographs. These subjects were followed up and were asked the reason for tooth loss. The results were as follows 57.5% of the subjects lost one tooth during follow up 31% of them lost 3-5 teeth during follow up. The average follow up period was 11.7 years and 30% of the subjects lost the teeth due to periodontal disease 55% lost teeth due to endodontic treatment failure. and 15% lost teeth due to periodontal disease and endodontic failure. He has also stated that the alveolar bone loss in post menopausal women is higher when compared to other population which is mainly due to other population which is mainly due to loss of BMD. It was concluded by the alveolar bone loss is an independent predictor of tooth loss in post menopausal women. Thus, control of periodontal disease significantly reduce tooth loss in post menopausal women[41].

Divya Parakh et al (2016) conducted a study on 40 premenopausal women and 40 postmenopausal women. Saliva was collected from all the participants and assessed for salivary flow rate. OHI-S, DMFT, PI and GI scores

were also recorded. It was found that there was a decrease in salivary pH and salivary flow rate. While the DMFT, OHI-S, PI and GI scores were increased. It was concluded that decrease in salivary pH and flow rate were responsible for poor oral hygiene index and other physiological changes[42].

Lata Goyal et al (2017) in her review article on osteoporosis and periodontits in post menopausal women shad put forth that osteoporosis is a systemic disease which affects not only the spine and appendicular skeleton but also the alveolar bone. She also stated that both osteoporosis and chronic periodontitis are slowly progressive disease. Post menopausal women possess an increased risk of development of periodontitis and osteoporosis with a prevalent rate of 30% and 50% respectively. It was concluded by stating that there was a strong association in the literature between periodontitis and osteoporosis. Osteoporosis occurs as a result of systemic inflammation. Chronic low grade inflammation is a common mechanism for both osteoporosis and chronic periodontitis. Osteoporosis increases the rate of development of chronic periodontitis. Thus there exist a strong association between chronic periodontitis and osteoporosis in post menopausal women[16].

Rajashri et al (2017), in their study titled risk assessment of osteoporosis in premenopausal and postmenopausal periodontally healthy and chronic periodontitis women with digital panoramic radiographs. In this study 120

postmenopausal women were included they were divided into 4 groups. Group I included 30 periodontally healthy premenopausal women between 30-45 years with gingival index (GI) score as 0 and probing pocket depth (PPD) as ≤3 mm. Group II included 30 premenopausal between 30-45 years with chronic periodontitis patients exhibiting GI ≥1, PPD and clinical attachment level (CAL) ≥5 mm and there should be radiographic evidence of bone loss. Group III included 30 periodontally healthy postmenopausal women between 45-65 years exhibiting GI = 0 and PPD ≤3 mm. In group IV 30 postmenopausal with chronic periodontitis between 45-65 years who had GI ≥1 and PPD and CAL ≥5 mm and radiographic evidence of bone loss were included. Panoramic view was to calculate mental index(MI),mandibular cortical index(MCI) and panoramic mandibular index(PMI) and the scores were recorded.The results were as follows, there was a significant difference in MI between group III and group IV,between group II and IV and between group I and II. The recorded radiographic indices were significant among the groups. The study was concluded by stating panoramic radiographs can be used to determine BMD and there exists a significant correlation between osteoporosis and periodontal disease in postmenopausal women[43]

Mohammed Saeed Ayed (2018), conducted a study to evaluate the possible association between systemic osteoporosis and periodontal disease

progresssion in postmenopausal women. In his study a total of 300 postmenpausal women were included they were divided based on bone mineral density into osteoporotic group and non osteoporotic group consisting of 150 subjects in each group respectively. Periodontal status was evaluated by measuring plaque index, gingival index periodontal probing depth and clinical attachment loss.OPG was taken to assess alveolar bone height and bone mineral density. The elemental analysis of root surface of extracted teeth was done to assess the levels of calcium, magnesium, fluorine, potassium and phosphate using laser induced spectroscopy. The results of the study revealed no significant difference in plaque index, gingival index and periodontal probing dept between both the groups. A statistically significant difference were higher in osteoporotic group than non osteoporotic group whereas, fluorine and phosphate levels were not statistically significant among the groups. Thus, according to the study, osteoporosis is considered as a risk factor for the occurrence and progression of periodontal disease[44].

Vaishali Narayan Mashalker et al (2018) conducted a cross sectional study to assess and correlate osteoporosis and periodontitis among postmenopausal women using a dual energy x-ray absorptiometry.94 postmenopausal woman between 45-65 years of age was included in the study. They were exposed to DXA scan to measure bone mineral density and were

graded as normal, osteopenic, osteoporotic based on their T score. Body mass index, socioeconomic status, patients educational level was recorded. Thorough periodontal examination was done and oral hygiene index, plaque index, probing depth and clinical attachment loss to check the level of periodontitis. On the basis of DXA scan it was found four women were normal, 44 were osteopenic and 46 were osteoporotic. There was no association between bone mineral density and body mass index, oral hygiene index, plaque index, probing depth, clinical attachment loss, socioeconomic status and educational status. Severe level of periodontitis was seen in 24 women, moderate periodontitis was observed in 62 patients. Slight periodontitis was observed in 8 women. There was a significant correlation between periodontitis and osteoporosis. The conclusion of the study was there exists a significant correlation between osteoporosis and severe periodontal disease in postmenopausal women. So, general practitioners and dentists should work hand in hand for the early diagnosis and treatment of these diseases[45].

Richa et al (2018), in her study titled association between osteoporosis and periodontal disease among postmenopausal Indian women between 45-55 years of age were included. Their bone mineral density was measured using ultrasonometer and divided into 300 postmenopausal osteoporotic women and 300 postmenopausal non osteoporotic women. Mean plaque index, gingival index,

bleeding score, clinical attachment loss and CPI index were measure in these subjects.It was found that mean plaque index, gingival index and bleeding score were significantly higher in osteoporotic women when compared to non-osteoporotic women. The number of sextants affected for codes 3, 4 of CPI index was more in osteoporotic women. Similarly, codes 1.2.3 for attachment loss were affected more in osteoporotic women. As a result statistically significant association was present between osteoporosis and menopausal duration, attachment loss, bleeding and gingivitis scores. Thus,it was concluded by stating there is an association between osteoporosis and periodontal diseases in postmenopausal women[46]

Deepa et al (2018), did a study to assess periodontal health status in postmenopausal women visting dental hospital in and around Meerut. The study comprised of 90 post-menopausal women. Periodontal status of the subjects were assessed by measuring plaque index, gingival index ,bleeding on probing, periodontal pocket and Russell's periodontal index. In the study, it was found 11 patients had initial stages of destructive periodontal disease,34 patients had established destructive periodontal disease and 30 patients had terminal periodontal disease.It is suggested that menopausal women are at a risk of developing destructive periodontal disease if oral hygiene in not maintained properly[47].

Rukmini et al (2018) conducted a study to evaluate the effect of menopause on saliva and dental health.40 post menopausal women were included in the study. Stimulated saliva was obtained from the participants. Salivary flow rate and salivary pH were measured. The OHI, DMFT, CPI and LOA indices were assessed clinically. Finally, it was found that salivary pH and salivary flow rate were lower in post-menopausal group. The OHI,DMFT,CPI and LOA were higher in case group. Thus, preventive dentistry is of significance in post-menopausal women[48].

Naijlaa.S.Al.Obaidi et al (2019) conducted a study titled oral hygiene status in relation to salivary estradiol hormone level among pre-menopausal women and postmenopausal Iraqi women.90 women were included in the study they were between 48-52 years old. The control group consists of 45 post-menopausal women and 45 pre-menopausal women the post-menopausal women were examined for gingival index, plaque index and calculus index.Unstimulated saliva was obtained from both the groups to obtain salivary estradiol levels. The mean gingival index, plaque index and calculus index were higher in post-menopausal and their salivary estradiol levels were considerably lower. The outcome of the study was salivary estradiol and oral hygiene status had a negative correlation. Oral hygiene status was influenced by salivary estradiol in post menopausal women[49].

Materials and methods

MATERIALS AND METHODS

MATERIALS

This is a hospital based study designed to evaluate salivary estradiol and salivary calcium in post menopausal women. Patients were selected from the Department of Oral medicine and Radiology, Ragas dental college and Hospital, Chennai.

STUDY DESIGN: Prospective study.

STUDY SAMPLING: Random sampling.

STUDY SETTING:

- Department of Oral medicine and Radiology, Ragas dental college and Hospital, Chennai
- ARRA Clinical laboratory,Royapettah, Chennai

ETHICAL CONSENT:

The study was approved by the institutional ethical committee of Ragas Dental College and Hospital,Chennai. Also consent was obtained from all subjects participating in the study.

SELECTION CRITERIA:

60 subjects between were included in the study. The subjects were divided into 3 groups.

STUDY GROUPS:

Group I: This group includes 20 healthy menstruating women between 25-35 years of age.

Group II: This group includes 20 healthy menstruating women between 35-45 years of age.

Group III: This group includes 20 post menopausal women between 45-60 years of age.

INCLUSION CRITERIA:

Group I:

- Female subjects who are having a regular menstrual cycle.
- Subjects between 25-35 years of age.
- Subjects with good oral hygiene.

Group II:

- Female subjects who are having a regular menstrual cycle.

- Subjects between 35-45 years of age.

Group III:

- The female subjects who have not had a menstrual cycle for more than a year.

- Subjects between 45-60 years of age.

EXCLUSION CRITERIA:

Group I:

- Patients under hormone replacement therapy.

- Patients with any systemic illness.

- Patients under any xerogenic medical agents.

- Patients with any deleterious habits.

- Patient taking contraceptive pills.

Group II:

- Patients under hormone replacement therapy.

- Patients under any xerogenic medical agents.

- Patients with any systemic illness.

- Patients with any deleterious habits.

- Patient taking contraceptive pills.

Group III :

- Patients under any xerogenic medical agents.

- Patients with any systemic illness.

- Patients with any deleterious habits.

- Patient who acquired surgical menopause.

RESEARCH TOOL:

Presence or absence of xerostomia was assessed using xerostomia XI inventory questionnaire by Thomas et al[50].

PROCEDURE FOR EXAMINATION: A detailed case history was taken and the findings were recorded in the case sheets.

ARMAMENTARIUM USED:

For clinical evaluation:

- Dental chair with light source

- Disposable gloves and mask

- Kidney tray

- Mouth mirror and plain probe

- William's periodontal probe

- Shepherd hook explorer

- Tweezer

- Cotton and gauze pieces

For collection of unstimulated saliva:

- Graduated sterile container

- Disposable graduated pipette (3 ml)

- Sterile storage vials (5 ml)

For assay procedures:

- Salivary estradiol ELISA kit (Diametra,Italy)

- Calcium reagent for Arzenaso III reaction (Accucare labcare diagnostics)

METHODOLOGY:

A proper clinical history was obtained from all subjects. The ethical committee of Ragas Dental College and Hospital approved the study protocol. All the patients were explained about the study and written consent was obtained.

The study consisted of 3 groups. In Group I 20 female subjects between the age group of 25-35 years of age were selected based on the following criteria like, they should have good oral hygiene and should have a regular menstrual cycle. They should not have and systemic illness or any deleterious habits or take any xerogeneic medication and should not be under contraceptive pills. Group II 20 female subjects between the age group of 35-45 years of age were selected based on the following criteria like; they should have good oral hygiene and should have a regular menstrual cycle. They should not take any xerogenic medication and should not have any systemic illness or any deleterious habits and should not be under contraceptive pills. Group III is formed by 20 female subjects between 45-60 years of age who have not had a menstrual cycle for more than a year (<12 months).These subjects should not be under any xerogenic medical agents.

<u>**SIMPLIFIED ORAL HYGIENE INDEX (1964):**</u>

The oral hygiene statuses of the subjects were analysed using Oral hygiene index proposed by **John.C.Greene and Jack R.Vermillion** (1964). The scores were recorded and interpreted.

$$DI\text{-}S\ score = \frac{Total\ score}{No.\ of\ surfaces\ examined}$$

$$CI\text{-}S\ score = \frac{Total\ score}{No.\ of\ surfaces\ examined}$$

OHI – S score = DI – S score + CI – S score

Interpretation of Oral hygiene index:

OHI – S Score	Oral hygiene status
0.0 -1.2	Good
1.3 – 3.0	Fair
3.1 – 6.0	Poor

RUSSELL'S PERIODONTAL INDEX (1956):

The periodontal status was assessed by using Russell's periodontal index proposed by **Russell A.L** (1956).The scores were recorded and interpreted.

$$\text{Periodontal index score per person} = \frac{\text{Sum of individual scores}}{\text{Number of teeth present}}$$

Interpretation of Russell's periodontal index:

Individual PI score	Clinical conditions
0- 0.2	Clinically normal supportive tissues
0.3-0.9	Simple gingivitis
1.0-1.9	Beginning destructive periodontal disease
2.0- 4.9	Established destructive periodontal disease
5.0-8.0	Terminal disease

UNSTIMULATED SALIVA COLLECTION METHOD:

The unstimulated whole saliva (mixed saliva) which is a mixture of oral fluids and includes secretion from major and minor salivary glands is collected

from the selected participants by draining method. In this method, the patient is asked to sit quietly with the head bent down. They should be trained to pool the saliva in the floor of the mouth without applying any stimulus. They were asked to open the mouth and allow the saliva to slowly drip from the lower lip into the sterile container. The ideal time for collection is between 9 a.m. and 12 p.m., and at least 2 h after the last intake of food or drink.

ANALYSIS OF SALIVARY ESTRADIOL:

The collected salivary samples were centrifuged at 2000 rpm for 10 min and immediately stored at -20°C for later determination of estradiol concentration using ELISA kit (Diametra, Italy).

Principle:

The estradiol (antigen) in the sample competes with the antigenic Estradiol conjugated with horseradish peroxidase (HRP) for binding to the limited number of antibodies anti-estradiol coated on the microplate (solid phase).After incubation, the bound/free separation is performed by a simple solid-phase washing. After which the enzyme HRP in the bound-fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blue color that changes into yellow when the stop solution (H_2SO_4) is added. The colour intensity is inversely proportional to the estradiol concentration of in the sample. The values were expressed in pg/ml.

Reference level (by ELISA):

	PHASE	Pg/ml
	Follicular phase	1 – 20
WOMEN	Ovulatory peak	10 – 40
	Luteinic phase	5 – 25
	Menopause	< 10

ANALYSIS OF SALIVARY CALCIUM:

In whole calcium Ca^{2+} analysis the saliva was centrifuged to 3600 rpm and the supernatant saliva was separated. The whole calcium concentration was determined using Arsenazo reaction (Accucare Calcium, Labcare diagnostics).

Principle:

Calcium with Arsenazo III at neutral pH yields a blue colored complex. The intensity of the color is directly proportional to the salivary calcium levels. The procedure was done in a fully automated machine which displays the values digitally. The values were expressed in mg/lathe normal whole stimulated saliva calcium level is 0.5-2.8mM or 4-6 mg/dl.

STATISTICS USED: SPSS WINDOW VERSION 21.0

Figures

Fig 2-Salivary estradiol ELISA kit (Diametra, Italy)
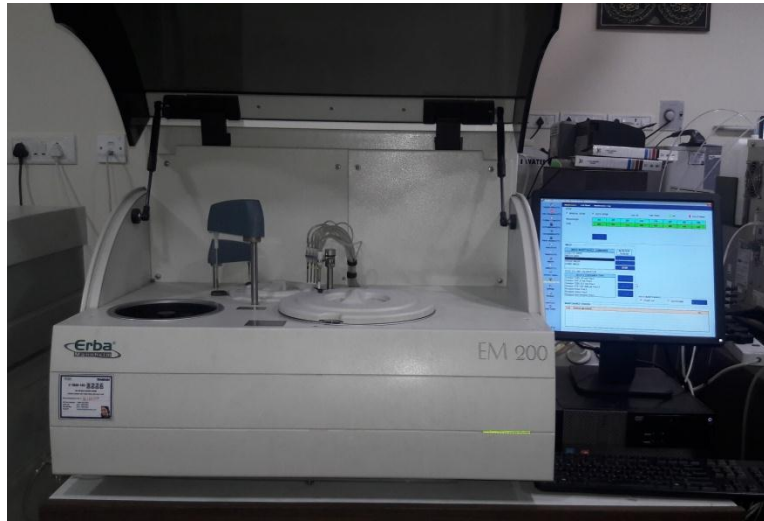

Fig 3-Armamentarium

Fig 4-Fully automated equipment for Salivary calcium analysis (Erba

Mannheim,USA)

Fig 5-Fully automated equipment for Salivary estradiol analysis by

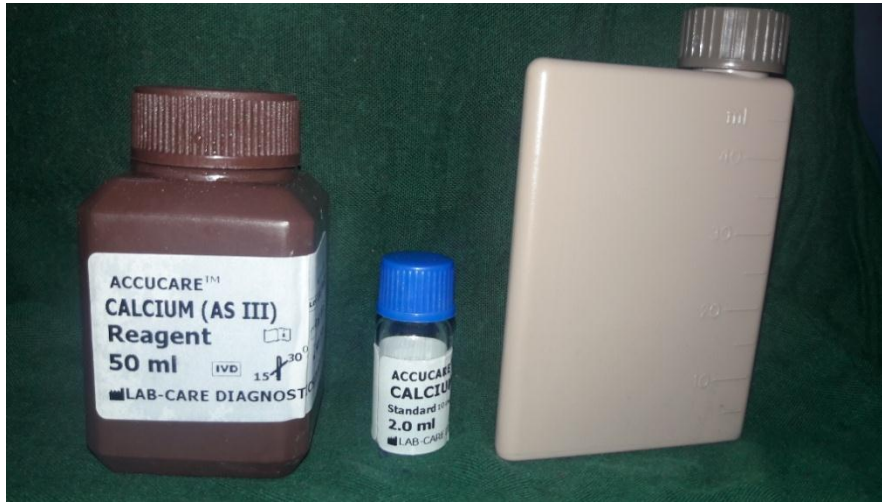**ELISA method (Erba Mannheim,USA)**

40

Fig 6-Accucare calcium reagent (Arzenaso III reaction)

Fig 7-Disposable pipette (3ml) and storage vial (5 ml)

Results

RESULTS

The study was conducted among healthy menstruating women and post menopausal Ragas dental college and hospital. They were evaluated for the presence of xerostomia using xerostomia XI inventory questionnaire, periodontitis using Russell's periodontal index. Their debris index score, calculus index and oral hygiene index scores were also assessed. Unstimulated saliva sample were obtained from the subjects and measured for salivary estradiol and salivary calcium levels.

With the data obtained the results of the present study is as follows,

Table 2(Graph 1): Shows distribution of subjects by age

In the present study age of the participants was between 25 to 60 years. In Group I the mean age was 29.25, in group II 38.1 years and group III 56.25 years. Among the total of 60 subjects; the mean age was 41.20 years.

Table 3 (Graph 2): Shows salivary estradiol levels of subjects

The mean salivary estradiol level in normal controls (group I) is 5.61.In perimenopausal women (group II) is 7.86 and in postmenopausal women (group I) the mean salivary estradiol levels are 3.22.

Table 4:Multiple comparison of salivary estradiol level among the groups by post hoc turkey test.

Table 5 (Graph 2): Shows salivary calcium levels of subjects

The mean calcium level in normal controls (group I) is 4.39.In premenopausal women (group II) is 3.55 and in postmenopausal women (group I) the mean salivary calcium levels are 5.9.

Table 6: Multiple comparison of salivary calcium levels among the groups by post hoc turkey test.

Table 7 (Graph 4): Shows xerostomia score of subjects

The mean calcium level in normal controls (group I) is 11.0.In premenopausal women (group II) is 13.80 and in postmenopausal women (group I) the mean salivary calcium levels are 35.80.

Table 8: Multiple comparison of salivary calcium levels among the groups by post hoc turkey test.

Table 9 (Graph 5): Shows Russell's periodontal score of subjects.

The mean Russell's periodontal score in normal controls (group I) is 0.21.In premenopausal women (group II) is 0.68 and in postmenopausal women (group I) the mean Russell's periodontal score is 3.60.

Table 10: Multiple comparison of Russell's periodontal score among the groups by post hoc turkey test.

Table 11 (Graph 6): Shows Oral hygiene index score of subjects.

The mean OHI-S scores in normal controls (group I) is 1.08.In premenopausal women (group II) is 1.35 and in postmenopausal women (group I) the mean OHI-S score is 4.11.

Table 12:Multiple comparison of OHI-S score among the groups by post hoc turkey test.

Table 13(Graph 7): Shows correlation between salivary estradiol levels and xerostomia score.

Pearson's correlation between salivary estradiol levels and xerostomia score reveals a strong negative correlation between the two variables.

Table 14(Graph 8): Shows correlation between salivary calcium levels and periodontal score.

Pearson's correlation between salivary calcium levels and periodontal score reveals a strong positive correlation between the two variables.

Table 15(Graph 9): Shows correlation between salivary calcium levels and salivary estradiol levels.

Pearson's correlation between salivary calcium levels and periodontal score reveals a weak negative correlation between the two variables.

Tables and graphs

	N	Mean	Std. Deviation
Group I	20	29.25	3.307
Group II	20	38.10	2.634
Group III	20	56.25	2.447

Table 2: Shows distribution of subjects by age.
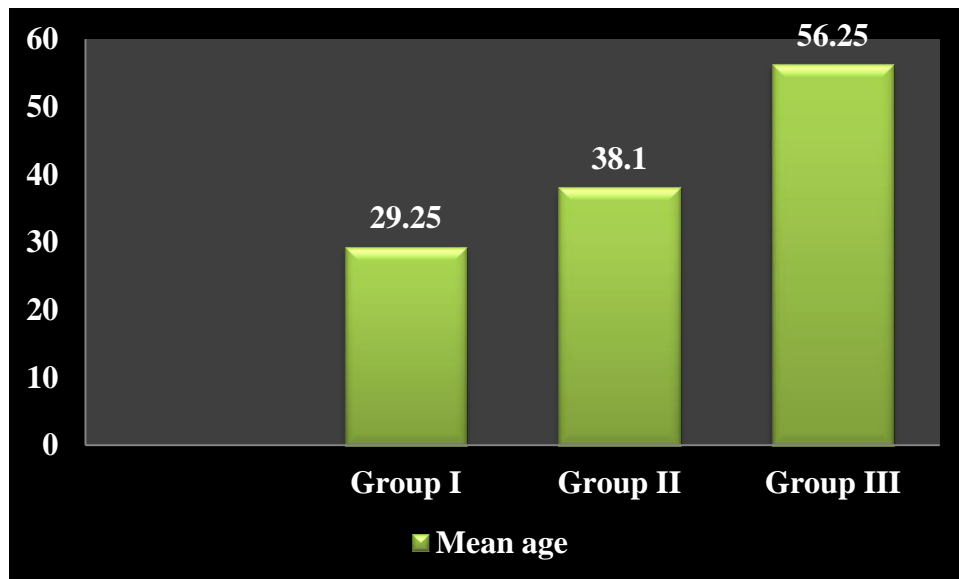
Graph 1: Shows distribution of subjects by age.

	N	Mean	Std. Deviation	p value
Group I	20	5.61	5.02	
Group II	20	7.86	5.60	**0.01**
Group III	20	3.22	2.87	

Table 3: Mean salivary estradiol levels among 3 groups.
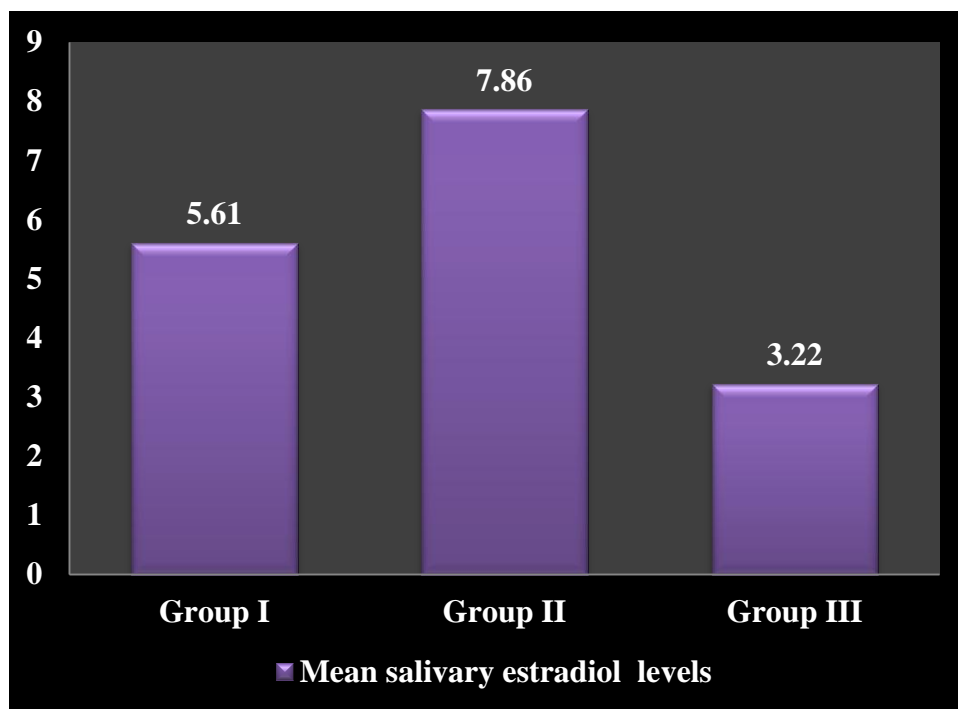
Graph 2: Mean salivary estradiol levels of subjects

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(I) Group	(J) Group	Mean Difference (I-J)	p value
Group I	Group II	-2.24	0.2863
	Group III	2.393	
Group II	Group I	-2.245	0.2428
	Group III	4.638	
Group III	Group I	2.393	0.0072
	Group II	4.638	

Table 4: Post hoc test for multiple comparison of salivary estradiol levels among 3 groups

	N	Mean	Std. Deviation	p value
Group I	20	4.39	2.75	
Group II	20	3.55	2.29	0.03
Group III	20	5.91	3.47	

Table 5: Shows salivary calcium levels of subjects
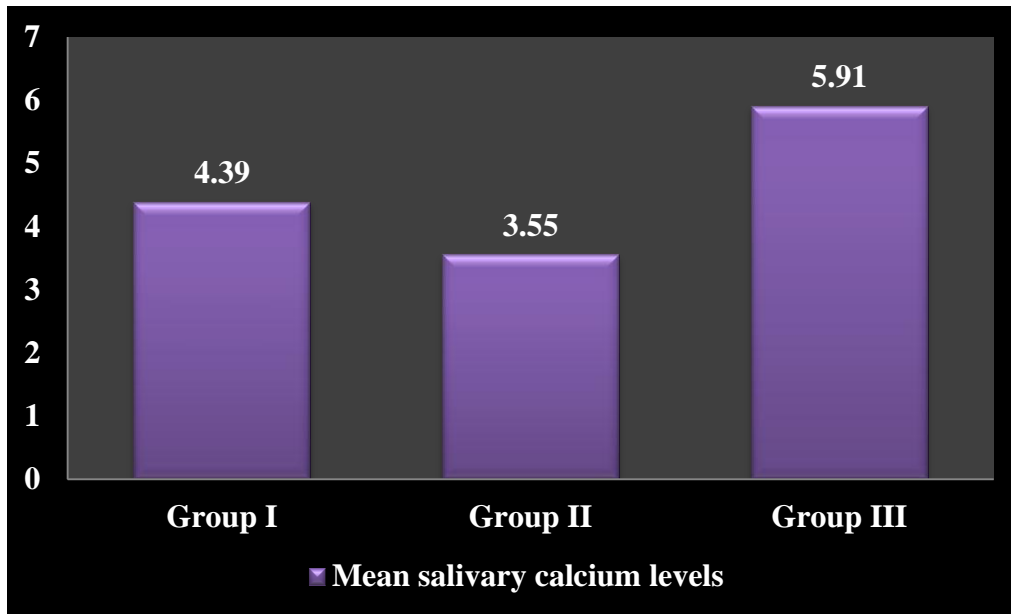
Graph 3: Mean salivary calcium levels of subjects

(I) Group	(J) Group	Mean Difference (I-J)	p value
Group I	Group II	0.8350	**0.634**
	Group III	-1.525	
Group II	Group I	0.8350	**0.2242**
	Group III	-2.360	
Group III	Group I	-1.525	**0.0322**
	Group II	-2.360	

Table 6: Post hoc test for multiple comparisons of salivary calcium

levels among 3 groups

	N	Mean	Std. Deviation	p value
Group I	20	11.00	.000	
Group II	20	13.80	1.735	**0.0001**
Group III	20	35.80	8.076	

Table 7: Mean Xerostomia score among 3 groups
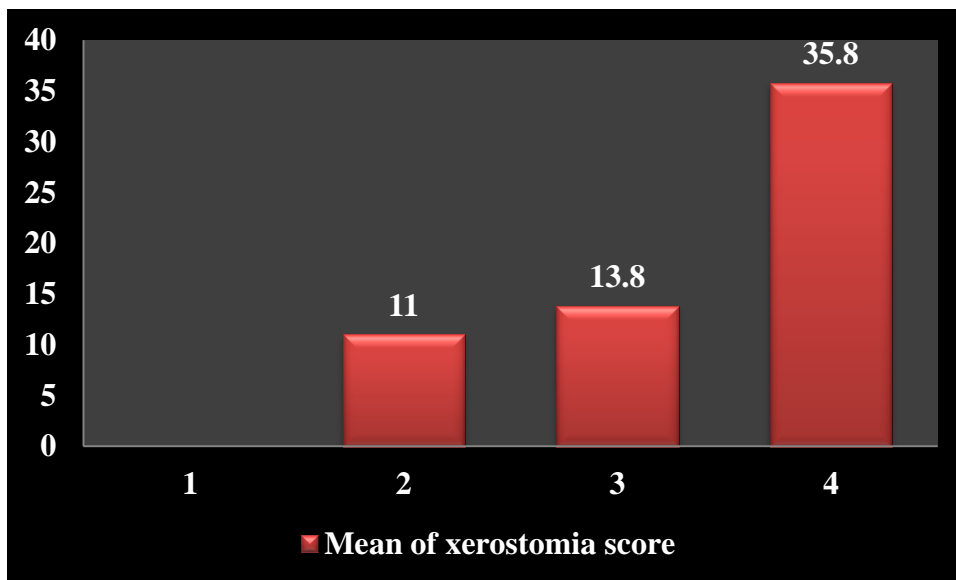
Graph 4: Mean xerostomia score levels among 3 groups.

(I) Group	(J) Group	Mean Difference (I-J)	p value
Group I	Group II	-2.800	0.1608
	Group III	-24.80	
Group II	Group I	2.800	0.0001
	Group III	-24.80	
Group III	Group I	24.80	0.0001
	Group II	-22.00	

Table 8: Post hoc test multiple comparison of xerostomia score among 3 groups

	N	Mean	Std. Deviation	p value
Group I	20	.21	.32	
Group II	20	.68	.63	0.000
Group III	20	3.6	1.2	

Table 9: Mean Russell's periodontal score levels among 3 groups.
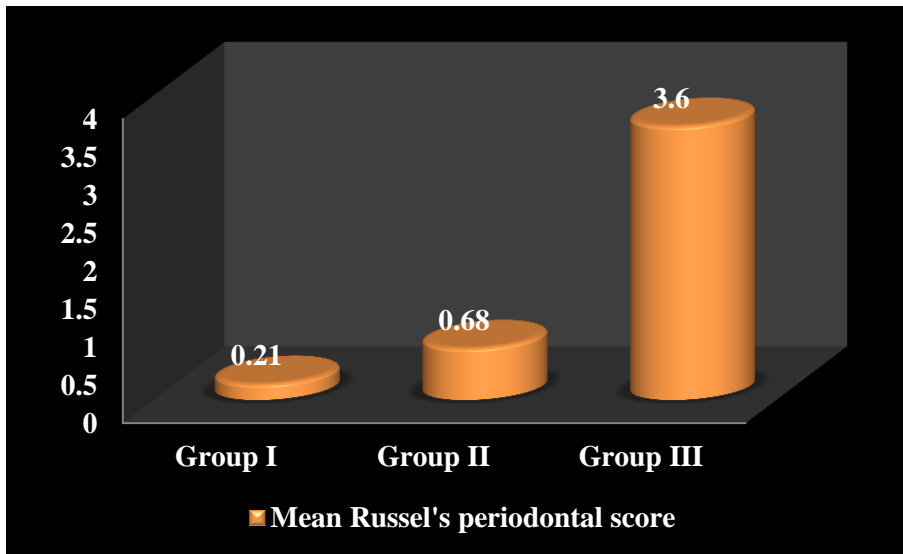
Graph 5: Mean Russell's periodontal score levels among 3 groups.

(I) Group	(J) Group	Mean Difference (I-J)	p value
Group I	Group II	-0.4687	**0.1518**
	Group III	-3.390	
Group II	Group I	-0.4687	**0.0001**
	Group III	-2.922	
Group III	Group I	-3.390	**0.0001**
	Group II	-2.922	

Table 10: Post hoc test for multiple comparisons of Russell's periodontal score among the groups.

	N	Mean	Std. Deviation	p value
Group I	20	1.08	1.02	
Group II	20	1.35	.95	**0.0001**
Group III	20	4.11	1.096	

Table 11: Mean OHI-S score among 3 groups.
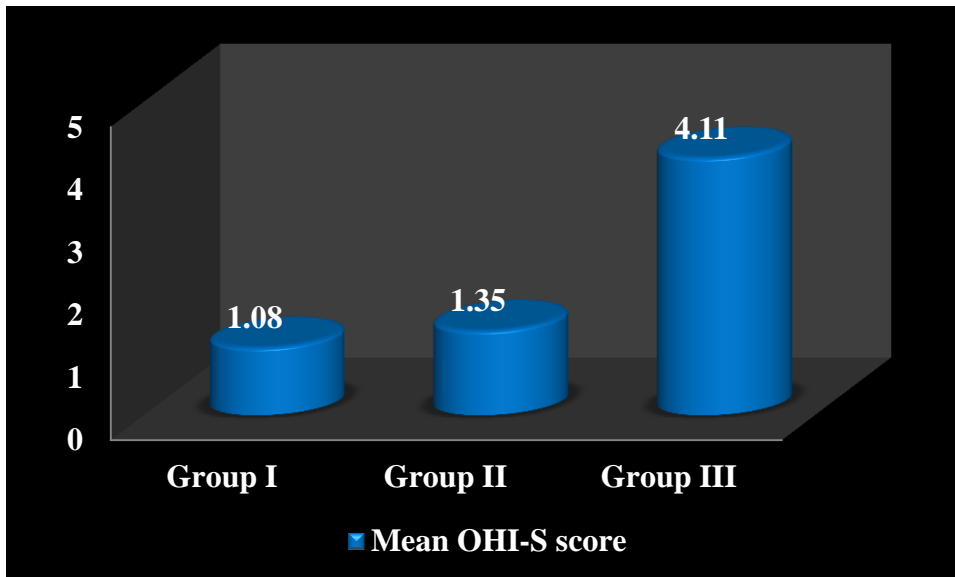
Graph 6: Mean OHI-S levels among 3 groups.

(I) Group	(J) Group	Mean Difference (I-J)	p value
Group I	Group II	-0.2715	**0.6820**
	Group III	-3.032	
Group II	Group I	-0.271	**0.0001**
	Group III	-2.761	
Group III	Group I	-3.032	**0.0001**
	Group II	-2.761	

Table 12: Post hoc test for multiple comparison of OHI-S score among 3 groups.

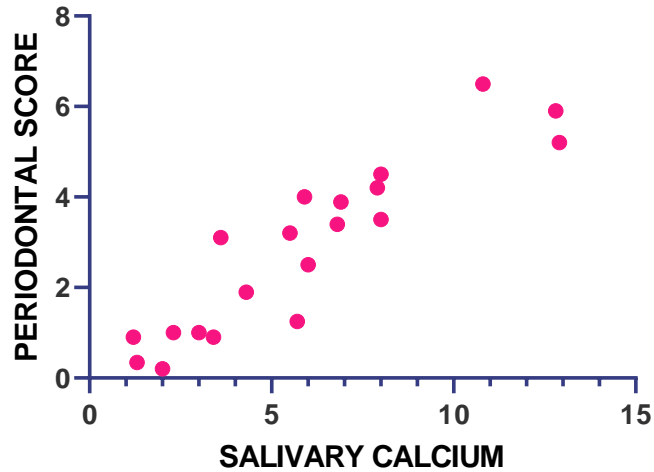| Pearson's r	-0.5974
95% confidence interval	-0.8223 to -0.2100
R square	0.3564
p value	**0.0054**

Table 13: Pearson's correlation of salivary estradiol level and xerostomia score
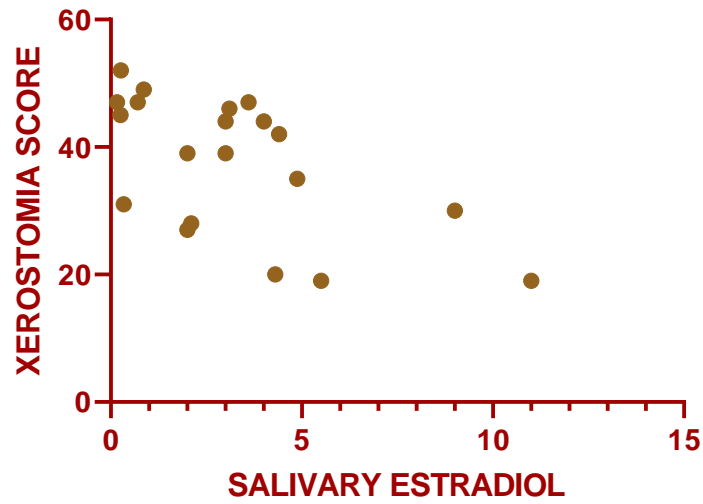
Graph 7: Pearson's correlation of salivary estradiol level and xerostomia score

| Pearson's r	0.9129
95% confidence interval	0.7892 to 0.9654
R squared	0.8334
p value	**0.0001**

Table 14: Pearson's correlation of salivary calcium levels and periodontal score

Graph 8: Pearson's correlation of salivary calcium levels and periodontal score

Pearson's r	-0.2126
95% confidence interval	-0.5988 to 0.2538
R squared	0.04521
p value	**0.3681**

Table 15: Pearson's correlation of salivary calcium levels and salivary estradiol levels
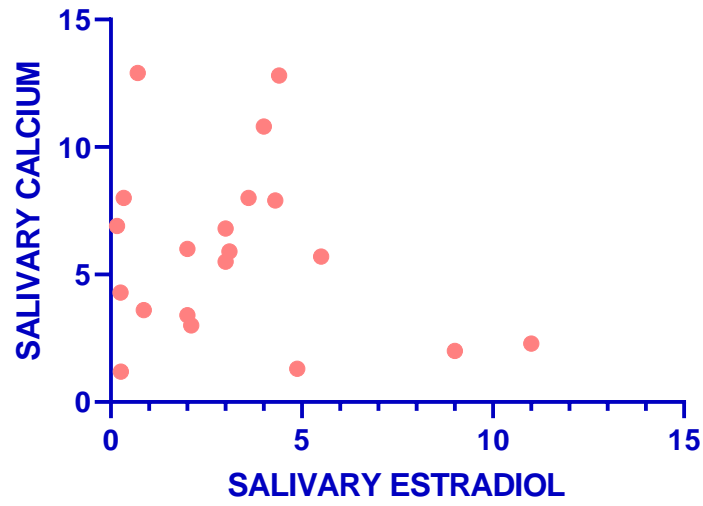
Graph 9: Pearson's correlation of salivary calcium levels and salivary estradiol levels

Discussion

DISCUSSION

Menopause is a permanent cessation of menstruation due to loss of ovarian follicular activity. It occurs between the fourth and fifth decade of life[1].The WHO has classified midlife of women into 3 categories, Menopause as the year of the final physiological menstrual period retrospectively designated as one year without flow (unrelated to pregnancy or therapy) in women aged $\geq$ 40 years. In Premenopausal (35 to 39 years) there is decreased fertility and fecundity which appear as the foremost manifestations of ovarian follicle depletion and dysfunction, despite the absence of menstrual changes. Perimenopause stage is the period of years immediately before menopause and the first year after menopause[50].In Indian population the average menopausal age is 46.2±4.9 years[3]

According to staging of reproductive aging in women (STRAW +10) criteria the midlife of women is classified into three phases are reproductive, menopausal transition and menopausal phase. The menopausal transition phase is divided into early and late phases. In this phase there is irregular menstrual cycle. The estradiol (E2) levels are fluctuating, FSH levels tend to increase and the progesterone levels may be normal or decreased. During menopause there is a decline in estradiol levels, progesterone is not produced after menopause and the FSH levels stabilize. Anti-mullerian hormone, Inhibin-B are other hormones

which are low in both menopause transition and menopausal phase. The antral follicular count is also low in both the phases[51]

Saliva is considered as an ultra filtrate of plasma and is used as a medium to measure endocrine hormones. Estradiol is strongly bound to steroid hormone binding globulin and weakly to albumin in blood. Only 1% estradiol exists as free form or biologically active form in blood. This hormone is transferred from blood stream into salivary gland by the process of passive diffusion through lipophilic layer of cells and glandular epithelial cells. This mechanism allows only biologically active form of estradiol to be secreted in saliva. Hence; it can be used as an indicator to measure biologically active estradiol levels. In our study we measured salivary estradiol levels in saliva. Based on the review of literature several studies were conducted by various authors by categorizing midlife of women into various age groups. Agna Hosseini et al[32],estimated stimulated and unstimulated salivary estradiol levels in post menopausal women with or without oral dryness to find out if there exists any relation between salivary estradiol and xerostomia among postmenopausal women. Sema dural et al[38],estimated salivary flow rate and salivary pH and compared it with DMFT index scores and and oral hygiene status in menopause and healthy women. Rajashri et al[43],had two study groups premenopausal and post-menopausal women and estimated periodontal status and oral hygiene status and compared between the groups.

Naijlaa.S.Al.Obaidi et al[49],estimated salivary estradiol levels and compared it with oral hygiene status among premenopausal and post-menopausal women. In our study we had 3 groups, Group I was formed by normal menstruating women, Group II was formed by premenopausal women and group III was formed by postmenopausal women. This grouping was done based on criteria for classification of midlife of women by world health organization and stages of reproductive aging in women criteria mainly to estimate the endocrinological changes and their oral implications during each phase of midlife of women.

In our study the age of the subjects in group I was between 25- 35 years, in group II it is 35-45 years and in group III it is 45-60 years. However, subjects who fall under premenopausal category were excluded since there will be fluctuating hormone levels during this phase. WHO suggested >40 years as menopausal age but in our study menopausal women (group III) between 45-60 years were included because the average age of menopausal in Indian women is 46.2 ± 4.9 years.[51]

Vallimaa et al, have put forth that ER β receptors are present in the acinar and ductal cells of the salivary glands. During menopause low circulating estradiol level are present so sufficient level of hormones is not available to bind with the receptors. This leads to alteration in quantity as well as quality of salivary secretion. This is suggested as a probable cause for xerostomia which is a

common oral symptom in postmenopausal women. Agha Hosseini et al[32], conducted a study to evaluate the relationship between unstimulated and stimulated salivary estradiol level and xerostomia among post menopausal women he used xerostomia XI inventory questionnaire to evaluate the oral dryness in postmenopausal women. Minicucci et al[35], conducted a study to assess the impact of salivary flow and xerostomia in menopause and menarche and found that severe levels of xerostomia was found during menopause. He assessed xerostomia using xerostomia XI inventory questionnaire. Singh et al,[24] also assessed oral dryness in postmenopausal women using xerostomia XI inventory questionnaire. So in our study also we have used xerostomia XI inventory questionnaire by Thomas et al,[49] to evaluate xerostomia among the groups since it helps in quantification of symptoms of xerostomia. We also assessed the relation between xerostomia and salivary estradiol among postmenopausal women.

Gill et al[37], in his study evaluated unstimulated salivary flow rate with oral symptoms in premenopausal women and postmenopausal women and found out that xerostomia was significantly higher in postmenopusal women when compared to premenopausal women. Divya Parakh et al, in her study evaluated the effect of menopause on saliva and dental health. It was found that xerostomia levels were higher in postmenopausal women when compared with normal women. The results of this study was in accordance to the results of our study.

The xerostomia score were measured among the subjects of three groups using xerostomia XI inventory questionnaire. Xerostomia was absent in normally menstruating women, premenopausal women exhibited mild levels of xerostomia. While the post-menopausal women exhibited higher xerostomia scores indicating severe levels of oral dryness. One-way ANOVA test and post hoc turkey test for multiple comparison was performed. The results were significant with a p-value of 0.001. Pearson's correlation test between salivary estradiol levels and xerostomia score among post-menopausal women revealed that as the salivary estradiol levels decrease the xerostomia scores increased among the postmenopausal women.

Minicucci et al,in his study assesses salivary flow rate and xerostomia using xerostomia XI inventory questionnaire in postmenopausal women. It was found that salivary flow rate was less in postmenopausal women and they did not show any clinical symptoms of xerostomia. The results of the above study were contradictory to our study because in their study group out of 30 postmenopausal women 16 postmenopausal women were under hormone replacement therapy but in our study we have excluded postmenopausal women under hormone replacement therapy and 85% of postmenopausal women had severe levels of xerostomia.[35]

Tivis et al[21], in his study measured salivary estradiol levels and serum estradiol levels among postmenopausal women who are not under any estrogen substitutes. He put forth that there were low circulating levels of estardiol in both serum and saliva. The results of his study were in accordance to our study in which low salivary estradiol was appreciated in 95% of the postmenopausal women.

Agha Hosseini et al, in his study obtained stimulated salivary estradiol from postmenopausal women.He proposed that stimulated salivary estradiol levels were low in postmenopausal women. He also proposed that as stimulated salivary estradiol levels decreased there was an increase in xerostomia levels[32].He also conducted another study by measuring unstimulated salivary estradiol levels in and compared it with xerostomia status using xerostomia XI inventory questionnaire in post menopausal women. It was found that as unstimulated salivary estradiol levels decreased there was an increase in xerostomia scores[33]. The results of both these studies were in line with the results of our study.

In our study salivary estradiol levels were within normal limits in healthy menstruating women. In case of premenopausal women it was normal in 90% of the patients while there was a decline in 10% of the cases. The estradiol levels were low in 95% of the postmenopausal women. One-way ANOVA test and post

hoc turkey test for multiple comparisons was performed. The results were significant with a p-value of 0.01. Pearson's correlation also revealed that as the salivary estradiol level decreased the xerostomia levels increased.

Saliva in addition to estrogen and progesterone also contains calcium,phosphate,IgA and mucin. Ravindher singh et al[24],in his study had proposed there exists a significant correlation between serum oestrogen levels and salivary calcium concentration. Siva Reddy et al[23],Rabei et al[25], in their respective studies have proposed that salivary calcium can be used as a reliable parameter to determine regulation of bone metabolism in postmenopausal women. Madhura Vijay Rane et al,[52] in their study stated that increased salivary calcium levels are associated with the risk of development of periodontal disease. This is because salivary calcium is essential for calcification of dental plaque to supragingival and subgingival calculus. Since periodontitis and osteoporosis are important presentation of menopause we consider using salivary calcium as a biomarker, which would serve as an adjunct to evaluate these conditions.

Balwinder singh et al, estimated salivary calcium in postmenopausal women. There was a significant difference among salivary calcium in postmenopausal women who had xerostomia and who did not have xerostomia. It was found salivary calcium levels were high in postmenopausal women with oral dryness. The results of this study was in accordance to our study in which 40% of

the postmenopausal women showed high calcium levels while 90% premenopausal women and all the healthy menstruating women had normal salivary calcium levels.

Agha Hosseini et al[26],in his study estimated salivary calcium in postmenopausal women with or without xerostomia. The results showed that salivary calcium was high in postmenopausal women when compare to postmenopausal women without oral dryness. The result of his study was similar to the results of our study in which 40% of the postmenopausal women had higher salivary calcium levels.

Ravindher Singh et al[24],in his study correlated serum oestrogen and salivary calcium levels among postmenopausal women. It was found that serum estrogen was low and salivary calcium levels were increased in postmenopausal women with oral dryness when compared to post menopausal women without oral dryness. It was found that as salivary estrogen level decreased the salivary calcium levels increased among postmenopausal women with oral dryness. The result of this study was similar to the results of our study.

In our study salivary calcium levels were normal in all healthy menstruating women. In premenopausal women 10% of them had normal levels while 90% had low circulating calcium levels. In post menopausal women, 45%

had higher calcium levels 25% had normal calcium levels and 35% had low levels of salivary calcium. One-way ANOVA test and post hoc turkey test for multiple comparisons were performed. The results were significant with a p-value of 0.01.Pearson's correlation test was done and it was found that as the salivary estradiol level decreased there was an increase in salivary calcium levels in postmenopausal women and also it was observed an increase in salivary calcium level was related to the increased occurrence of periodontal disease among postmenopausal women.

Minnucci et al,[35]in his study had stated that during menopause there is a significant decrease in salivary flow rate. This reduction is salivary volume is responsible for the development of xerostomia symptoms among postmenopausal women. It causes poor flushing of debris by saliva thereby giving rise to poor oral hygiene status and it also leads to loss of antibacterial properties of saliva which is causes increases the risk of development of dental caries, periodontal disease in postmenopausal women. So in our study we have evaluated oral hygiene status using OHI-S scores and periodontal status using Russell's periodontal index scores among the groups.

Deepa et al[47],assessed periodontal health status among postmenopausal women and she found that destructive periodontitis were common in postmenopausal women and poor oral hygiene increased the risk of development

of periodontitis. The results of this study stood by our study in which 85% of the postmenopausal women had established destructive periodontitis and 15% had terminal periodontal disease.95% of the patients had poor oral hygiene.

Rukmini et al[12], in their study evaluated the effect of menopause on saliva and dental health. Oral hygiene status and periodontal status were recorded. The periodontal index scores as well as oral hygiene status were higher in postmenopausal women. The results of these study was in accordance to the results of our study in which normal periodontium was observed in healthy menstruating women. In group II 35% had gingivitis, 10% had beginning destructive periodontal disease, and 5% had established periodontal disease. In postmenopausal group 10% had beginning periodontal disease, 70% had established periodontal disease and 15% had terminal disease. One-way ANOVA test and post hoc turkey test for multiple comparisons were performed. The results were significant with a p-value of 0.01.With regard to the oral hygiene status; in our study 95% of postmenopausal women had poor oral hygiene status

Naijlaa.S.AL.Obaidi et al[48], in their study estimated salivary estradiol levels and oral hygiene status among postmenopausal women and found that the salivary estradiol levels were low and oral hygiene index scores were high indicating poor oral hygiene. The results of this study was in accordance to the results of our study were the oral hygiene status was appreciated among normal

controls it was good in 70%,fair in 25% and poor in 5% of the subjects. In pre-menopausal women the oral hygiene status was good in 60%, fair in 30% and poor in 10% of the subjects. In post-menopausal women oral hygiene was good in 5% and poor in 95% of the subjects. One-way ANOVA test and post hoc turkey test for multiple comparisons were performed. The results were significant with a p-value of 0.01

The result of our study suggests that saliva can be a preferred medium for measuring estradiol and calcium levels. Aging causes a considerable change in salivary flow rate and composition in addition to that menopausal phase also is responsible for the alteration in quantity and quality of saliva. This physiological change is the key factor behind poor oral hygiene, xerostomia, periodontitis which are the common oral manifestations of menopause. A strong association between salivary estradiol and calcium levels and oral health status put forth that the oral changes occurring during menopause are due to decline in estradiol levels produced by the ovaries. So, educating women about the impact of hormonal changes and its oral implications during menopausal phase is mandatory.

Summary and conclusion

SUMMARY AND CONCLUSION

The present study titled "Estimation of salivary estradiol and salivary calcium in postmenopausal women with or without xerostomia" was done to evaluate if saliva can serve as an alternative diagnostic aid to establish the relation between salivary estradiol and calcium levels with oral health status in postmenopausal women. Our study consisted of 20 healthy menstruating women, 20 premenopausal women and 20 menopausal women. Unstimulated saliva was collected from them and estimated for estradiol and calcium levels. Their oral health status was evaluated by determining Russell's periodontal score, Oral hygiene index score and xerostomia score.

The summary of the results are, salivary estradiol levels were low in postmenopausal women. High salivary calcium levels were appreciated among subjects of the study group. Destructive periodontal disease was present among postmenopausal women. Oral hygiene index was poor among post menopausal women. Severe levels of xerostomia were present in postmenopausal group. In postmenopausal women, as the salivary estradiol levels decreased the salivary calcium levels and severity of xerostomia also increased. Increased salivary calcium levels were related to the risk of development of periodontal disease. The overall impression was oral health status of menopausal women decline as the estradiol levels produced by the ovaries decline.

This study put forth that saliva can be used as an alternative to serum for estimation of estradiol and calcium levels. It also throws light on the fact that decline in estradiol level is responsible for various systemic and oral manifestations during menopause. During the study it was also observed that most of the menopausal women were not aware that the oral changes occurring in them were due to withdrawal of estradiol levels.

In order to conclude, we suggest saliva can be a preferred medium and an emerging alternative for serum to estimate estradiol and calcium levels. And it is also necessary to create awareness among women of all ages about the hormonal changes and their effects they will experience during various stages of their life. As a dentist, we have to educate them about the oral changes they will experience during menopause and emphasize its strong association between low estradiol levels. Oral hygiene instructions should be given for maintenance of healthy periodontiumMenopausal women who experience severe postmenopausal symptoms can be identified and the dentist and gynecologist can work hand in hand to treat the symptoms of these women.

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