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Effect of menopausal estrogen deficiency on dentin formation: A comparative analysis of mineralization front and predentin thickness in pre and postmenopausal women

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SUMMARY

Objective. The effects of menopausal estrogen deficiency on dentin and its uncalcified organic matrix, termed predentin, in humans have not been documented so far in the literature. Thus, the present study was conducted to study the effects of menopause on dentin by analyzing the mineralization front in predentin and measuring the predentin thickness in teeth of pre and post-menopausal women.

Material and methods. 80 freshly extracted teeth, collected from pre and postmenopausal women (40 from each group), between 30-60 years of age group, were decalcified and predentin thickness was measured and compared at the cuspal tip, middle third and apical third. Age-wise inter and intragroup comparison was done to know the influence of aging on predentin thickness. Wider metadentin zone, indicative of delay in mineralization process was observed and analyzed by grading them. Unpaired 't' test was employed for the statistical analysis.

Results. There was no difference in predentin thickness between pre and post-menopausal women at all three sites. With advancing age, the predentin thickness decreased in both the study groups. Within the same age range (41-50 years), the predentin thickness slightly increased in postmenopausal women than premenopausal women. The presence of metadentin zone was more frequent ($p<0.001$) in the postmenopausal women.

Conclusions. Estrogen deficiency during menopause affects the dentin by causing delay in mineralization process and manifests as wider metadentin zone. Further studies on a larger scale including pre and postmenopausal women of 41-50 years are suggested for knowing the effect of menopause on predentin thickness.

Keywords: dentinogenesis, estrogen, menopause, metadentin, predentin thickness.

INTRODUCTION

Dentin is the hard tissue that constitutes the major proportion of body of each tooth and serves as both a protective covering for the pulp and as a support for the overlying enamel (1, 2). Formation of dentin or dentinogenesis is similar to the formation of bone (3). Calcium is responsible for the hardness of both bone and dentin and normal function of their formative cells namely, osteoblasts and odontoblasts respectively. Thus, disturbances of calcium metabolism and homeostasis adversely affect bone and dentin (4). Parathyroid hormone, calcitonin and vitamin D are the primary hormones involved in calcium homeostasis (5). The sex hormone estrogen plays an osteoprotective role by decreasing the deportation of calcium from the bones (6, 7) and decreasing urinary excretion of calcium by stimulation of active vitamin D metabolites (5).

Dentin forms throughout life without the simultaneous resorption phase seen in bone. This makes dentin an ideal model for the study of variations in the formation of mineralized tissue (2). Dentinogen-

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esis involves two steps, namely, the formation of the organic matrix, termed predentin, and its subsequent mineralization (3).

A routinely uniform area of uncalcified dentin known as predentin exists between the mineralized dentin and the pulpal surface. Maturation of the organic matrix, secreted by odontoblasts takes place by incremental mineralization in this zone (3). Increased predentin thickness is seen when matrix production exceeds mineralization, the condition being caused by either reduced rate of mineralization or increased rate of organic matrix formation (2).

Estrogen deficiency during menopause causes calcium imbalance resulting in osteoporosis of the bone. This osteoporosis is brought about by an altered turnover with the osteolytic component surpassing the osteoblastic component. Dentin, however, does not show turnover. Instead, it continues to form throughout the life. Thus, the effect of estrogen deficiency in teeth of postmenopausal women can only be established through the rate of mineralization at the predentin front or the predentin thickness. This effect has not been studied in humans so far. Thus, the present study was conducted to evaluate the same by analyzing and comparing the predentin thickness in teeth of pre and postmenopausal women.

MATERIALS AND METHODS

Study samples

This cross-sectional study was approved by the institutional ethical clearance committee. The study was conducted on 80 freshly extracted teeth (N=80) of pre (n=40) and postmenopausal women (n=40) within the age group of 30-60 years. We considered convenient sampling and selected 40 subjects in each group. The specimens were sourced from patients visiting teaching dental hospitals. The patients were selected based on a pre-study questionnaire and clinical examination. To ascertain their menopausal status, the subjects were questioned on their last menstrual cycle. The subjects with self-report of minimum of five years since their last menstrual cycle were included in the post–menopausal group. Healthy, non-carious, non-restored, erupted teeth extracted for various periodontal and prosthetic reasons were used for the study.

Patients suffering from systemic diseases, that, affect calcium homeostasis such as poorly controlled diabetes mellitus, hypertension, renal failure, nephrotic syndrome, endocrine disorders affecting calcium level (thyroid, parathyroid disorders), gastrointestinal surgical interventions (jejunoileal bypass and subtotal gastrectomy) and patients undergoing cortisol medication and treatment for chronic systemic conditions and infections were excluded from the study. Microscopically, teeth with internal resorption were excluded.

Study method

Freshly extracted teeth were rinsed in normal saline and immediately transferred to 10% neutral buffered formalin to allow fixation of pulp. Fixation was enhanced by drilling a hole at the cementoenamel junction of single-rooted teeth and cutting one of the roots of multi-rooted teeth at the apex; followed by slow injection of 10% formalin into the pulp through the hole or apical cut at a rate of 1 ml (millilitre)/min within ten minutes of extraction. Thus, in multirooted teeth, as one of the roots was cut at the apical end, the predentin thickness was measured in the remaining root. After 24-48 hours of fixation in around 50-100 ml of fixative at room temperature (ranging between 25-34 degree Celsius), the teeth were placed in five percent nitric acid for decalcification that routinely took eight to ten days for completion. The end point of decalcification was confirmed physically by slight bending of the teeth. The time for decalcification varied by the size of the teeth (less for anterior and more days for posterior teeth). The decalcified teeth were then water washed, processed manually and embedded longitudinally in paraffin. The embedded paraffin blocks were trimmed and the central pulpal exposure with maximum pulpal volume is taken for further histological staining. Two longitudinal serial sections of 5 μ thickness were stained with Haematoxylin and Eosin (H&E) to determine the predentin thickness.

The sections were viewed, using Trinocular research microscope (Olympus BX 51, Japan) at 20x magnification on the computer using 3 chip CCD camera (0.5 magnification) and stored on the hard disk for further measurements. All the measurements were in microns (μ). The examination and final assessment of the slides and images were blinded.

Measurement of predentin thickness

Predentin thickness was measured at five sites for each tooth, including the cusp tip (C), two on each wall at the middle third (M1,M2) and two on each wall in the apical third of the root (A1, A2). Three random measurements were done at each site using Image Proplus Software ver. 4.1.0.0 (Media Cybernetics, USA). The mean of the three values was considered as the predentin thickness of the site. The predentin thickness at the cusp tip (C), middle

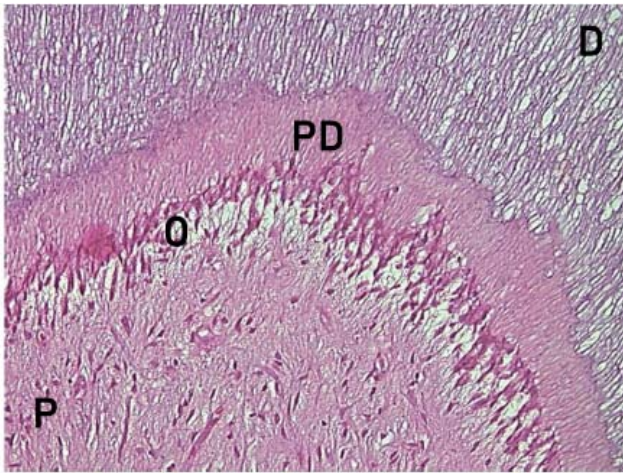

Fig. 1. Grade 0 metadentin – no metadentin (D – dentin, PD – predentin, O – odontoblastic layer, P – pulp)

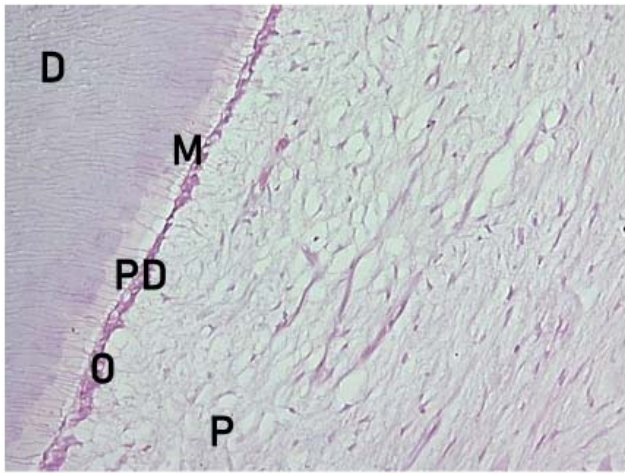

Fig. 2. Grade 1 metadentin – extending upto <1/3rd of predentin thickness (D – dentin, M – metadentin, PD – predentin, O – odontoblastic layer, P – pulp)

third (M=M1+M2/2) and apical third (A=A1+A2/2) was calculated for each tooth. The data was then transferred to Microsoft Excel worksheet for further statistical analysis. The data was checked for normal distribution and appropriate tests of significance were used.

Statistical analysis

Unpaired t-test was employed to compare the predentin thickness in both the study groups and p-value of 0.05 or less was considered to be statistically significant. Also, age-wise inter and intragroup comparison was done using unpaired t-test to look for changes in predentin thickness.

Assessment of metadentin

Literature indicates the presence of 1-2 μ wide intermediary zone termed as metadentin between the non-mineralized predentin and completely mineralized dentin (8). In completely mineralized dentin, collagen fibers and intercollagen spaces appear denser and more homogenous, and completely mineralized whereas, in metadentin only the collagen fibers are mineralized. Thus, metadentin is a distinct anatomical and physiological transitional zone wherein mineralization occurs (8) and the volume of unfused calcospherites present here can be used to establish the severity of delay in mineralization. In this study, the presence of metadentin and its thickness was examined and graded by a system devised by authors based on grading of globular dentin in vitamin D resistant hypophosphatemic rickets (9).

Grading of metadentin zone

Grade 0 – Dentin-predentin junction is linear or globular with few calcospherites that may/may not be attached to the dentin-predentin border. No metadentin is observed (Fig. 1).

Grade 1 – Metadentin is seen extending for <1/3rd of distance between dentin-metadentin border and pulpal end of dentin (Fig. 2).

Grade 2 – Metadentin is seen extending for >1/3rd to <2/3rd of distance between dentin-metadentin border and pulpal end of dentin (Fig. 3).

Grade 3 – Metadentin is seen extending for >2/3rd of distance between dentin-metadentin border and pulpal end of dentin (Fig. 4).

Two examiners did the grading and the values were statistically analyzed between pre and postmenopausal women by employing unpaired t-test.

Table 1. Sample distribution: Group I – premenopausal women within the age group of 30 – 45 years (Pre-M); Group II – postmenopausal women within the age group of 45 – 60 years (Post-M)

		Menopausal status	
No of subjects		**Pre-M**	**Post-M**
n=80		40	40
Age (in years)	Range	30-50	45-60
	Mean±SD	39.2±5.9	54.2±4.8
Root in teeth	Single	14	22
	Multiple	26	18
Unfused calcospherites		4/40=10%	17/40=42.5%
		Z=3.55 P<0.001, highly significant	

RESULTS

A total of 80 freshly extracted teeth (N=80) were collected for the study with equal numbers (n=40) from premenopausal and postmenopausal women. Age range was from 30-50 years (mean age of 39.2±5.9 years) among the premenopausal women,

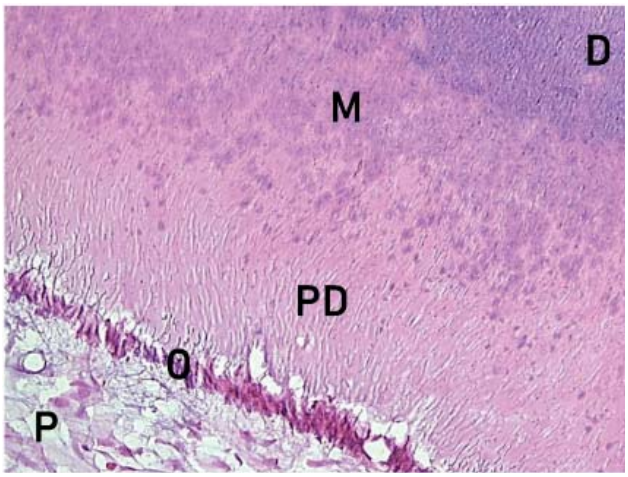

Fig. 3. Grade 2 metadentin – extending upto >1/3rd but <2/3rd of predentin thickness (D – dentin, M – metadentin, PD – predentin, O – odontoblastic layer, P – pulp)

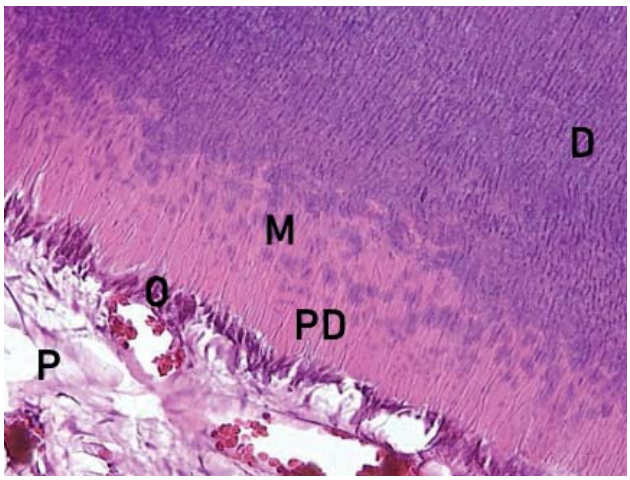

Fig. 4. Grade 3 metadentin – extending upto >2/3rd of predentin thickness (D – dentin, M – metadentin, PD – predentin, O – odontoblastic layer, P – pulp)

and the postmenopausal women age range was 45 to 60 years (mean age of 54.2±4.8 years) (Table 1).

The mean predentin thickness (MPDT) was compared site-wise and age-wise in the study groups.

Site-wise measurement and intergroup comparison of MPDT in the study groups

The MPDT in teeth of the premenopausal women at the cusp tip, middle third and apical third was 23.95µ (9.32µ to 83.41µ), 19.48µ (8.55µ to 58.55µ) and 18.68µ (6.55µ to 43.05µ) respectively. In the postmenopausal group, the MPDT was 22.66µ (11.20 to 46.59µ) at the cusp tip, 20.27µ (8.58µ to 48.02µ) at the middle third and 17.95µ (8.26 µ to 48.22µ) at the apical third.

Site-wise intergroup comparison at all three sites showed no statistically significant difference in the MPDT between the two groups at all the three sites (p value=0.61, 0.70, 0.68 respectively at the cusp tip, middle third & apical third) (Table 2).

Age-wise measurement and comparison of MPDT in study groups

Intragroup age-wise measurement and comparison of MPDT

I) In premenopausal women

The MPDT among teeth of 30-40 years old premenopausal women and 41-50 years old premenopausal women were compared and the older group showed a lower MPDT at all three sites (Table 3).

II) In postmenopausal women

Similarly, on comparison of MPDT between 41-50 years and 51-60 years age groups of the postmenopausal group, results showed that MPDT decreased in the elderly group except at the apical third (Table 3)

Intergroup age-wise measurement and comparison of MPDT

Both the study groups included women in the 41-50 years age group and were compared and within the similar age group (41-50 years), there was a statistically insignificant (p-value= 0.42, 0.41, 0.76 respectively at the three sites) increase in the MPDT in the teeth of postmenopausal women compared to premenopausal women (Table 4)

Grading of metadentin

Wider zone of unfused calcospherites termed metadentin was observed in 4 (10%) premenopausal and 17 (42.5%) post-menopausal women with statistically highly significant p-value of < 0.001 (Table 5)

DISCUSSION

In the present study, the predentin thickness was measured at five different sites in each tooth and the av-

Table 2. Comparison of predentin thickness in premenopausal (Pre-M) and postmenopausal (Post-M) women at cuspal tip, middle third and apical third (NS – not significant)

Sites of measurement of predentin thickness	Particulars	Menopausal status		Mean difference	Pre-M vs Post-M Unpaired t-test	
		Pre-M	Post-M		t value	p value
Cuspal tip (in µ)	Mean ± SD	23.95±9.79	22.66±9.04	0.99	0.26	0.80, NS
	Range	9.32-83.41	11.20-46.59			
Middle third (in µ)	Mean±SD	19.48±9.71	20.27±8.41	0.79	0.30	0.70, NS
	Range	8.55-58.55	8.58-48.02			
Apical third (in µ)	Mean±SD	18.68±8.06	17.95±7.68	0.73	0.42	0.68, NS
	Range	6.55–43.05	8.26–48.22			

erage of three different measurements was considered at each site. This strengthened the study by increasing the sample size. The measured predentin thickness at various sites was ranging from 6.55µ to 58.5µ, with the mean value of 20.23µ ±10.03µ. These values are on par with the physiologically normal predentin thickness mentioned in standard textbooks (1, 10). A very high predentin thickness (83.41µ) was seen at the cusp tip of a 40 years old premenopausal subject. This extreme value could be attributed to severe attrition found at the incisal edge of the tooth and was excluded from further statistical analysis. The predentin thickness measured at all three sites showed reduction that was widest at the cusp tip, followed by the middle third and narrowest at the apical tip in both the pre and postmenopausal study groups. This pattern corresponds with the expected predentin thickness within physiological and functional requirements.

Site-wise comparison of mean predentin thickness (MPDT) at the cusp tip, middle third and the apical third in both the study groups did not show statistical significant differences (p value=0.61, 0.70, 0.68 respectively). While there are no reported human studies on the subject, the other published animal studies (4, 11), have shown controversial results.

Devlin and Ferguson (11) studied the rate of incisor dentin calcification in the ovariectomized rats and found no changes in the dentin apposition rate due to ovariectomy. Conversely, Hietala and Larmas found ovariectomy caused activated dentin formation in adult rat molars. They postulated that lack of estrogen leads to increased production resulting in increased predentin thickness (4). Hietala and Larmas stated that this difference in results was the outcome of sample selection. Their study had analyzed molars exhibiting secondary dentinogenesis (4) whereas Devlin et al. (11) had used incisors that undergo primary dentinogenesis throughout life for his analysis.

Table 3. Age wise intergroup comparison of predentin thickness at cuspal tip, middle third and apical third between the premenopausal (Pre-M) and postmenopausal (Post-M) women

Age (in years)	Particulars	Cuspal tip (in µ)			Middle third (in µ)			Apical third (in µ)		
		Pre-M	Post-M	Difference	Pre-M	Post-M	Difference	Pre-M	Post-M	Difference
41-50	Mean±SD	20.70±6.60	23.40±9.83	2.7	18.25±7.65	20.69±7.13	2.44	16.14±4.88	16.78±5.41	0.64
	T	_	_	0.82	_	_	0.84	_	_	0.32
	P	_	_	0.42, NS	_	_	0.41, NS	_	_	0.76, NS

Table 4. Age wise intragroup comparison of predentin thickness in pre and post menopausal women at the cuspal tip, middle third and apical third

Premenopausal group				Postmenopausal group			
Age group (in years)	Cuspal tip (in µ)	Middle third (in µ)	Apical third (in µ)	Age group (in years)	Cuspal tip (in µ)	Middle third (in µ)	Apical third (in µ)
30-40	25.53±10.90	20.08±10.64	19.90±9.04	41-50	23.40 ±9.83	20.69±7.13	16.78±5.41
41-50	20.70±6.60	18.25±7.65	16.14±4.88	51-60	22.31±8.80	20.07 ±9.08	18.51±8.60
Mean difference	4.83	1.83	3.76	Mean difference	1.09	0.62	1.73
t-value	1.33	0.63	1.71	t value	0.34	0.24	0.78
p-value	0.19, NS	0.54, NS	0.10, NS	p value	0.74, NS	0.82, NS	0.44, NS

Unpaired t-test; NS – not significant.

Table 3. Comparison of predentin thickness in the single rooted and multi rooted teeth of premenopausal women (Pre-M) and postmenopausal (Post-M) women

Root	Particulars	Cuspal tip (in µ)			Middle third (in µ)			Apical third (in µ)		
		Pre-M	Post-M	Difference	Pre-M	Post-M	Difference	Pre-M	Post-M	Difference
SR	Mean±SD	30.51±12.23	25.26±9.31	5.25	24.32±11.60	22.53±9.58	1.79	22.80±9.46	20.21±9.10	2.59
	t	-	-	0.81	-	-	0.48	-	-	0.81
	p	-	-	0.42	-	-	0.63	-	-	0.43
MR	Mean±SD	20.43±6.97	19.50±7.81	0.93	16.88±7.54	17.52±5.85	0.64	16.46±6.34	15.18±4.29	1.28
	t	-	-	0.41	-	-	0.32	-	-	0.80
	p	-	-	0.69, NS	-	-	0.75, NS	-	-	0.43, NS

Unpaired t-test; NS – not significant; SR – single rooted teeth; MR – multi rooted teeth.

Present study in human subjects differed from these forementioned animal studies in regards to the following:

a) Estrogen: in gonadectomized rats, the effect of estrogen on hard tissues is lost completely. However, in postmenopausal women, the effect of estrogen though reduced is not lost entirely and may exhibit individual variations, resulting in statistically insignificant differences in predentin thickness.
b) Diet: diet can be controlled and can be made uniform for all the experimental animals, while diet in human subjects from different regions is not uniform.
c) Age: pre and postmenopausal women differ in their age range (30-50 years for the premenopausal group, 45-60 years for the postmenopausal group); whereas gonadectomized rats in animal studies were of similar age.

Menopause occurs between the fourth and fifth decades, and thereby age acts as an important confounding factor in determining the differences in predentin thickness between the two study groups. Odontoblast activity diminishes on completion of tooth development, reducing the rate of dentin formation with age (10). Also, the reason for extraction in both the study groups differ by age and can affect the pulpal inflammatory state and thereby dentinogenesis. Thus to differentiate the influence of age on predentin thickness from that of menopausal status, age-wise intra and intergroup comparison of MPDT was done between the two study groups. On age-wise intragroup comparison, the older groups of both pre and postmenopausal women showed decreased predentin thickness mirroring physiology. However, a slight increase was noticeable at the apical third of teeth in the postmenopausal group. This increase could be a result of apical periodontitis since most teeth in this age group were extracted due to periodontal disease.

Intergroup comparison of MPDT between teeth of pre and postmenopausal women was possible only in the age group of 41-50 years. Limiting the comparison to the same age group showed a slight increase in the predentin thickness of postmenopausal women at all the three sites. Though the results were not statistically significant (p value=0.42, 0.41, 0.76 respectively at the three sites), this was a noteworthy finding as this slight increase has occurred beyond the influence of aging, which can be attributed to lag of mineralization process caused by the decreased estrogen receptors (12) at the pulp – predentin junction (13).

Metadentin-intermediate zone of mineralization

This zone of predentin in normal conditions is narrow and fairly even in its contour and the young fibers in the predentin are evenly distributed throughout and are of regular size, shape, and direction (10). During observation in this study, the dentin-predentin junction was not even in few premenopausal and many of the postmenopausal cases. It was characterized by the presence of a broad zone of ongoing mineralization showing unfused calcospherites termed metadentin (8).

Wider metadentin zone was appreciable in many teeth of postmenopausal women than premenopausal women. A self-devised grading system was used to quantify the severity of delay in mineralization (Table 5).

Grading of metadentin revealed that about 42.5% of postmenopausal women showed a wider metadentin zone with unfused calcospherites that were statistically significant ($p<0.001$). The examination was done by two different observers to minimize the interobserver variation, which was negligible (2.5%).

The finding of broader metadentin with unfused calcospherites was similar to that of an animal study conducted by Bernick (12) in gonadectomized rats where he found disturbed dentin mineralization with increased interglobular dentin. He proposed that a disturbance in the organic matrix, both collagen, and the protein mucopolysaccharide complex have led to the interruption in the calcifying process (12).

Estrogen receptors are located at the predentin – odontoblast junction and in the pulpal blood vessels of teeth (13) and gonadal hormones are necessary for the synthesis of protein and specific enzymatic activity (12). Loss or reduction in estrogen levels at menopause causes an alteration in the state of aggregation and/or synthesis, of the protein mucopolysaccharide complex in dentin. This might lead to lack of fixation of acid mucopolysaccharides in the dentin matrix. The resultant depolymerized state of the ground substance in the dentin would not be conducive for the proper calcification resulting in extensive interglobular spaces (12). Thus, dentin mineralization is delayed at this zone by the altered state of dentin matrix proteins, and this suggested altered state of proteins is supported by the results of the present study.

The delay in the mineralization process causing wider zone of metadentin with unfused calcospherites cannot be related to aging or deficiency of calcium since calcium level in pulpal blood vessels increases with age (14).

Literature and recent studies speak about only bone changes in menopausal women and the changes in dentin are not addressed.

Further studies with inclusion of larger sample size, particularly within the same age group of 41- 50 years are required. Additionally, studies to correlate calcium and estrogen levels with varying grades of metadentin can shed further light on this topic. Age-matched males can be included in future studies as negative controls. Longitudinal studies in the same individuals before and after menopause will provide further information on the mechanism involved.

CONCLUSIONS

Postmenopausal women in this study population revealed a higher frequency and grade of metadentin than premenopausal women; this association is consistent with delayed mineralization and warrants further controlled, age-matched and longitudinal studies with biochemical validation.

STATEMENT OF CONFLICT OF INTEREST:

No conflict of interest

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