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Original Research Paper

Comparison of Vitamin D Levels in Serum and Severity of Periodontal Parameters in Post Menopausal Women with and Without Chronic Periodontitis A Comparative Study

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ABSTRACT:
Background and Objectives: The biologically active form of vitamin D, 1,25 dihydroxy vitamin D (1,25(OH)2D) and its receptor, the vitamin D receptor (VDR), play roles in maintaining oral immunity and the integrity of the periodontium. Owing to the immunomodulatory, anti-inflammatory, and antibacterial properties of 1,25(OH)2 D or vitamin D3, a sufficient serum level of vitamin D3 is necessary for the maintenance of periodontal health. Hence this study is aimed at evaluating the effect of serum vitamin D3 levels on the periodontal status of post- menopausal women. **Materials and Methods:** Twenty three post-menopausal women aged between 50 to 65 years satisfying the inclusion and exclusion criteria were selected and randomly divided into two groups: Test group (With chronic Periodontitis) and Control group (Without Chronic Periodontitis).Clinical parameters like Oral Hygiene Index, Modified Sulcular bleeding Index, Gingival Index, Probing Pocket Depth, and Clinical attachment level were recorded on the same day of obtaining blood samples from the individual for estimation of Vitamin D3 in serum. **Results:** In the present comparative study a statistically significant inverse association between bleeding index scores and serum vitamin D3 levels in case and control groups and an inverse but not statistically significant association was observed between pocket probing depth scores and serum vitamin D3 levels in the case group. The study proved that serum vitamin D3 levels in the optimal range played a pivotal role in improving the periodontal status of the individual. **Conclusion:** This present study with all its limitations, gives us a clearer perspective of the role of serum vitamin D3 levels in reducing the severity of periodontal disease though it does not have any influence in the prevention of periodontal disease as such. Nevertheless, the immnomodulatory and anti-inflammatory action of Vitamin D3 on the general and gingival health of every post-menopausal women is noteworthy and can be suggested to be used as an adjuvant tool in screening of periodontal status of every individual.

Keywords: Serum Vitamin D3,chronic periodontitis, post -menopausal women, bleeding index, probing pocket depth.

INTRODUCTION:

Vitamin D is a fat soluble vitamin has an essential role in the functions of the body. Post-menopausal females with osteoporosis are at an increased risk of periodontal attachment loss and tooth loss .Current studies indicate most asymptomatic postmenopausal women and even some premenopausal women in India are deficient in Vitamin D irrespective of exposure to sunlight and hence most postmenopausal women are in a condition of receiving Vitamin D supplementation. Studies across the world have found that women who are receiving osteoporosis treatment reported decreased periodontal probing depth and clinical attachment loss, the amount of space between teeth and surrounding tissue due to bone loss and lower gingival bleeding than those who were not receiving therapy. The World Workshop of Periodontology 2017 has defined Periodontitis is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus. Its primary features include the loss of periodontal tissue support, manifested through clinical attachment loss (CAL) and radiographically assessed alveolar bone loss, presence of periodontal pocketing and gingival bleeding.[2] The interplay of bacterial infection and host response to bacterial challenge is significant, and the disease is alterable by environmental, acquired risk factors and

genetic predisposition of the individual. Vitamin D is hypothesized to reduce risk for progressive periodontitis & tooth loss via its influence on bone health, inflammation and the immune response. Vitamin D plays a significant role in maintaining healthy periodontal and jaw bone tissues, alleviating inflammation processes, stimulating post-operative healing of periodontal tissues and the recovery of clinical parameters. It is known for its anti-inflammatory and anti-oxidant property as well. Thus this studyfocused to investigate the role of VitaminD on the oral health and periodontal status of postmenopausal women.

AIM:

To assess the Vitamin D levels in serum and severity of periodontal parameters among post-menopausal women with and without periodontitis.

OBJECTIVES:

To compare the vitamin D levels in serum among post-menopausal women with and without periodontitis.
To study the clinical parameters like plaque index, gingival index, probing pocket depth, clinical attachment loss in post-menopausal women with varying levels of vitamin D deficiency with and without periodontitis.

MATERIALS AND METHODS:

The present study was permitted to be conducted by the Scientific and Ethical Committee Review board of Best Dental Science College and Hospital, Madurai. The study was carried out during the period from January 2020 to November 2021.

STUDY POPULATION:

- The study population was recruited from patients attending outpatient clinics of Department of Periodontics, Best Dental Science College and Hospital, Madurai.
- A total of 23 subjects (post-menopausal women) were included in the study of which 12 patients were taken as test patients with chronic periodontitis and 11 patients taken as control patients without chronic periodontitis.
- All the participants in the study were verbally informed about the nature, risks and benefits of the study and also a written informed consent was obtained.

CRITERIA FOR SELECTION OF SUBJECTS:

Inclusion Criteria:

- Post-menopausal women with age between 50 and 65 years.
- Patient not under oestrogen related hormonal medication or Vitamin D supplementation.
- Patient with presence of main index teeth for assessing periodontal parameters.

Exclusion Criteria:

- Post-menopausal women below the age of 50 years and post-menopausal women below age of 55 years who had undergone hysterectomy.
- Patients with presence of less than 20 teeth.
- Patients with systemic complications like carcinoma and bleeding and clotting disorders.

ARMAMENTARIUM:

- Armamentarium for clinical evaluation:
- Mouth mirror
- Explorer
- Williams periodontal probe
- UNC 15 periodontal probe
- Armamentarium for collection of blood sample:
- Tourniquet
- Butterfly shaped scalpel and vein set of 22 gauges.
- 5 ml syringes
- Vaccutainers
- Test tubes containing clot activator(red coloured)
- Armamentarium for transport of collected blood sample:
- Ice packs within thermocol box

CLINICAL PARAMETERS MEASURED:

- Gingival Index (GI)
- Oral Hygiene Index (OHI)
- Probing pocket depth (PPD)
- Clinical attachment loss (CAL)
- Modified Sulcular Bleeding Index (SBI)

Clinical periodontal parameters such as Gingival Index by Loe and Silness(1963),Oral Hygiene Index by by Greene and Vermillion (1964) , Modified Sulcular bleeding index by Mombelli(1987) and, Pocket probing depth (PPD) by CPI index put forth by Jukka Ainama (1982) and clinical attachment level (CAL) by means of Staging & grading periodontitis put forth in 2017 World workshop on classification of periodontal and peri implant diseases by AAP and EFP were recorded in the test and control group on the day of sample collection.
The study participants of post-menopausal women aged 50 to 65 years with and without chronic periodontitis would be asked to report on the day of sample collection. Prior to sample collection periodontal parameters were assessed by measuring the

Gingival Index, Modified Sulcular Bleeding Index, Probing Pocket depth and Clinical attachment loss. Periodontitis was defined as two or more interproximal sites with CAL> 4 mm that were not on the same tooth[3] . Gingival bleeding was assessed by inserting the same manual type periodontal probe approximately 2 mm into the gingival sulcus/pocket at three gingival sites per tooth, except third molars. Each site was scored either 0 (absence) or 1 (isolated bleeding spots),2 (confluent bleeding line),3(profuse bleeding) scores. The mean of all gingival bleeding scores was computed, representing the proportion of all sites assessed that bled in the mouth (when multiplied by 100, this represents the percentage of bleeding sites in a mouth). Serum sample of 5 ml would be withdrawn from the patient after taking informed consent. The collected serum sample would be allowed to clot in clot activator containing vials and transported to the laboratory within 4 hours for Vitamin D estimation. Withdrawal of blood for serum vitamin D3 estimation was conducted on the same day as the clinical periodontal examinations. Assays were conducted using a Competitive Electro chemiluminescence immunoassay.

GROUPING OF THE PATIENTS:

The samples were divided into two groups

Group Name	Group Type	Details
A	Test	Patients with chronic periodontitis
B	Control	Patients without chronic periodontitis.

STATISTICAL DATA ANALYSIS:

Data were analysed using the Statistical Package for the Social Sciences (SPSS) software (IBM, Armonk, NY).Frequencies corresponding percentages were used for the descriptive analysis. The chi-square test was used to compare the three levels of 25(OH) D (deficient, insufficient and sufficient) between different groups. A multivariate logistic regression model was performed to examine the association between periodontitis and 25(OH) D serum levels. Statistical significance was set at 0.05

RESULTS:

The present study was conducted in the Department of Periodontology and Oral Implant ology, Best Dental Science College, Madurai.

A total of 12 cases and 11 controls matched for age (50 ± 6 years) and sex (only females) participated in the present study. In the present study among the 12 postmenopausal women in the case group minimum age was 50 and maximum age was 62 years and a mean age of 53.5 years. Among the control group of 11 post-menopausal women, minimum age of 50 and maximum age of 63 and a mean age of 53.5 years participated which has been depicted in tables 1 and 2.Among the participants in the case group only 8.3% participants had a good oral hygiene (0-1.2), while 66.7% had fair oral hygiene(1.3-3) and 25% had poor oral hygiene(3.1-6). Among the control group 45.5% had good oral hygiene (0-1.2)and 54.5% had fair oral hygiene(1.3-3) based on the OHI index given by Loe and Silness and depicted in tables 3 and 4.

The gingival index reflecting the inflammatory status of the gingiva was found to have a valid 16.7% of mild score(0.1-1), 33.3% of moderate score(1.1-2)and 50% severe scores (2.1-3)among the case groups and found to have a valid 54.5% of mild score(0.1-1),36.4% of moderate score(1.1-2) and 9.1% of severe score(2.1-3)among the control groups and enlisted in tables 5 and 6.

Table 1: Descriptive data based on Age distribution in case group

Descriptive Statistics-Case

	N	Minimum	Maximum	Mean	Std. Deviation
AGE	12	50	62	55.00	4.671
Valid N (list wise)	12				

GROUP = CASE

Table 2: Descriptive data based on Age distribution in control group

Descriptive Statistics-Control

	N	Minimum	Maximum	Mean	Std. Deviation
AGE	11	50	63	53.55	3.778
Valid N (list wise)	11				

GROUP = CONTROL

Table 3: Distribution of Oral Hygiene Index values among the case group

ORAL HYGIENE INDEX-CASE GROUP

	Frequency	Percent	Valid Percent	Cumulative Percent
GOOD	1	8.3	8.3	8.3
FAIR	8	66.7	66.7	75.0
POOR	3	25.0	25.0	100.0
Total	12	100.0	100.0	

GROUP = CASE

Table 4: Distribution of Oral hygiene index values among the control group

ORAL HYGIENE INDEX –CONTROL GROUP

	Frequency	Percent	Valid Percent	Cumulative Percent
GOOD	5	45.5	45.5	45.5
FAIR	6	54.5	54.5	100.0
Total	11	100.0	100.0	

GROUP = CONTROL

Table 5: Distribution of Gingival index values among the case group

GINGIVAL INDEX –CASE GROUP

	Frequency	Percent	Valid Percent	Cumulative Percent
MILD	2	16.7	16.7	16.7
MODERATE	4	33.3	33.3	50.0
SEVERE	6	50.0	50.0	100.0
Total	12	100.0	100.0	

GROUP = CASE

Table 6: Distribution of Gingival index values among the control group

GINGIVAL INDEX-CONTROL

	Frequency	Percent	Valid Percent	Cumulative Percent
MILD	6	54.5	54.5	54.5
MODERATE	4	36.4	36.4	90.9
SEVERE	1	9.1	9.1	100.0
Total	11	100.0	100.0	

GROUP = CONTROL

Table 7: Distribution of Clinical Attachment Level (CAL) values among the case group

CLINICAL ATTACHMENT LEVEL_CASE

	Frequency	Percent	Valid Percent	Cumulative Percent
MILD	2	16.7	16.7	16.7
MODERATE	9	75.0	75.0	91.7
SEVERE	1	8.3	8.3	100.0
Total	12	100.0	100.0	

a. GROUP = CASE

Table 8: Distribution of Clinical Attachment Level (CAL) values among the control group

CLINICAL ATTACHMENT LEVEL_CONTROL

	Frequency	Percent	Valid Percent	Cumulative Percent
MILD	7	63.6	63.6	63.6
MODERATE	4	36.4	36.4	100.0
Total	11	100.0	100.0	

a. GROUP = CONTROL

The serum vitamin D values according to The US Preventive services Task force (USPSTF) recommendations are

Category	**Limits**
Deficient	**<20 ng/ml**
Insufficient	**20 to 30 ng/ml**
Desirable	**30 to 100 ng/ml**
Toxic	**>100 ng/ml**

The vitamin D3 levels among the study group were found to be insufficient (20 to 30 ng/ml)in 16.7% of test group,36.4% of control group accounting for a mean of 26.1% with insufficient levels of serum Vitamin D3. Deficient Vitamin D3 levels (<20 ng/ml) were found in 50% of study group and 63.6% of control group accounting for a mean of 48.1% for both case and control group. Desirable levels of Vitamin D3 (30 to 100 ng/ml) was found in 33.3% of the case group accounting for mean valid percent of 17.4%.The above data has been enumerated in tables 9 and 10 respectively.

Table 9: Distribution of Vitamin D3 among the cases and the controls

VITD3_INDEX

GROUP			**Frequency**	**Percent**	**Valid Percent**	**Cumulative Percent**
CASE		**INSUFFICIENT**	**2**	**16.7**	**16.7**	**16.7**
		DEFICIENT	**6**	**50.0**	**50.0**	**66.7**
		DESIRABLE	**4**	**33.3**	**33.3**	**100.0**

		Total	**12**	**100.0**	**100.0**	
CONTROL		**INSUFFICIENT**	**4**	**36.4**	**36.4**	**36.4**
		DEFICIENT	**7**	**63.6**	**63.6**	**100.0**
		Total	**11**	**100.0**	**100.0**	

Table 10: Distribution of Vitamin D3 for both the cases and control group

VITD3_INDEX

		Frequency	Percent	Valid Percent	Cumulative Percent
	INSUFFICIENT	6	22.2	26.1	26.1
	DEFICIENT	13	48.1	56.5	82.6
	DESIRABLE	4	14.8	17.4	100.0
	Total	23	85.2	100.0	
Total		27	100.0		

A normal distribution of Vitamin D levels was observed in both the cases and control groups. Additionally the majority of participants had either deficient or insufficient levels of vitamin D (n=12), mean of 24.17 among case group and (n=11), mean of 17.27 among control group. If bleeding on probing and gingival index were decreased, the participant was considered to have a good oral hygiene and assuming the hypothesis that increased Vitamin D levels in serum would be beneficiary to the individuals periodontal status irrespective of the oral hygiene the present study focused to assess the vitamin D3 status in serum with bleeding index as a primary outcome and gingival index and clinical attachment level as secondary parameters.

Analysis of the distribution of characteristics pertinent to this study viz. bleeding index, oral hygiene index and risk factor (ie decreased levels of vitamin D3) within the study population by exposure category or outcome status was performed using x2 tests for categorical variables and unpaired t-test for analysis of variances (ANOVAs) for continuous variables. Two sided p-values <0.05 were considered statistically significant. Table 11 shows the level of clinical significance of clinical parameters among the study group namely oral hygiene index (p<0.002),bleeding index (p<0.001),probing depth (p<0.004) and clinical attachment level (p<0.001) respectively compared to the control group, inferring that there is statistically significant difference between groups in terms of Oral hygiene index, bleeding index, probing depth, clinical attachment and not for vitamin D3 level.

Table 11: Comparison of two groups for the values of serum Vitamin D, Bleeding Index (BI) in %, Oral Hygiene Index (OHI) score, Probing Depth (PD) and Clinical Attachment Level (CAL) in mm

Group Statistics

PARAMETERS	GROUP	N	Mean	Std. Deviation	Std. Error Mean	P VALUE
OHI	CASE	12	2.6667	1.07309	0.30977	0.002
	CONTROL	11	1.1818	0.87386	0.26348	
VITD3	CASE	12	24.17	9.447	2.727	0.051
	CONTROL	11	17.27	6.002	1.810	

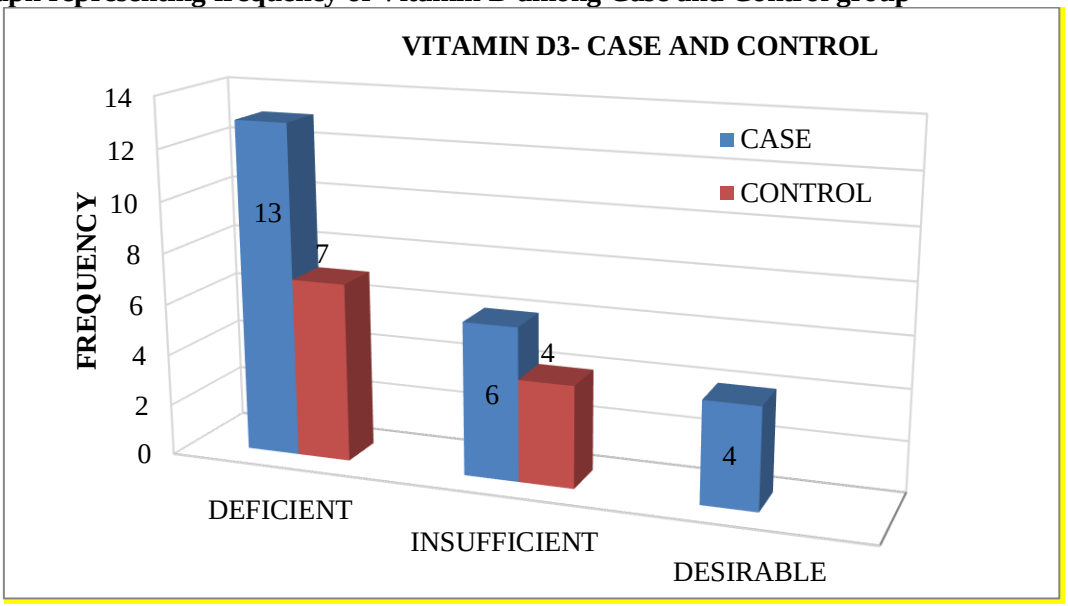
BI	CASE	12	43.3%	18.5%	5.3%	0.001
	CONTROL	11	19.9%	5.5%	1.6%	
PD	CASE	12	3.17	0.718	0.207	0.004
	CONTROL	11	2.18	0.751	0.226	
CAL	CASE	12	3.7500	0.86603	0.25000	0.001
	CONTROL	11	2.6364	0.50452	0.15212	

P value obtained from Independent sample T-test for ANOVAs p value < 0.05 is significant

Graph 1 is a representation showing the frequency of distribution of Vitamin D3 level among the control group with a greater frequency of deficient levels and Vitamin D3 level among the case group with a greater frequency of deficient levels followed by insufficient and least of all desirable levels of Vitamin D3.

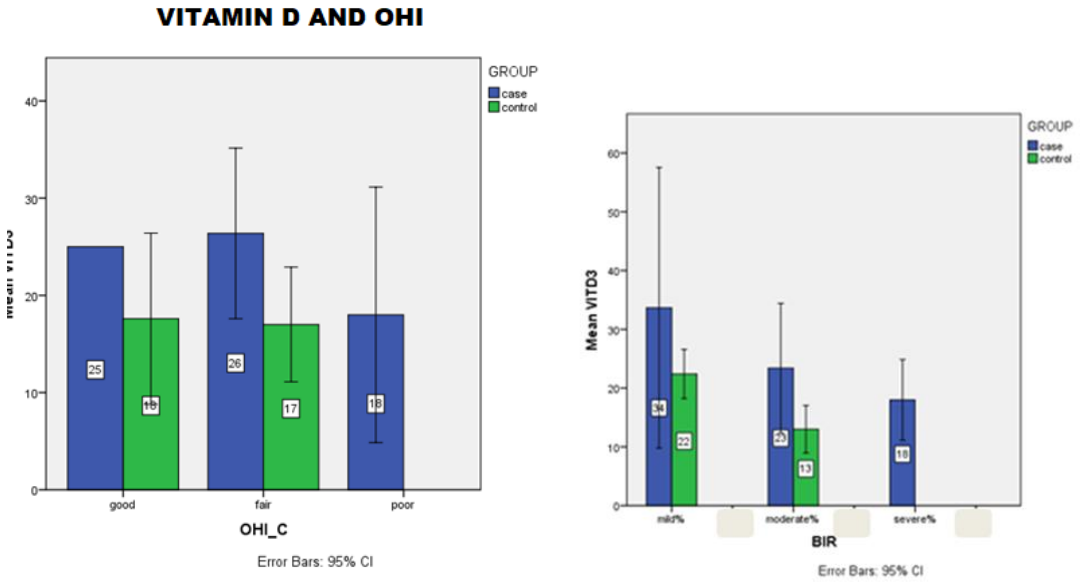
Graph 2 represents comparison of serum Vitamin D levels and Bleeding index values between case and control groups depicting mild bleeding scores among the case and control groups for whom vitamin D levels were greater than 25 ng/ml and severe bleeding scores among the case group alone as the serum vitamin D levels fall below 20 ng/ml.

Graph 1: Graph representing frequency of Vitamin D among Case and Control group



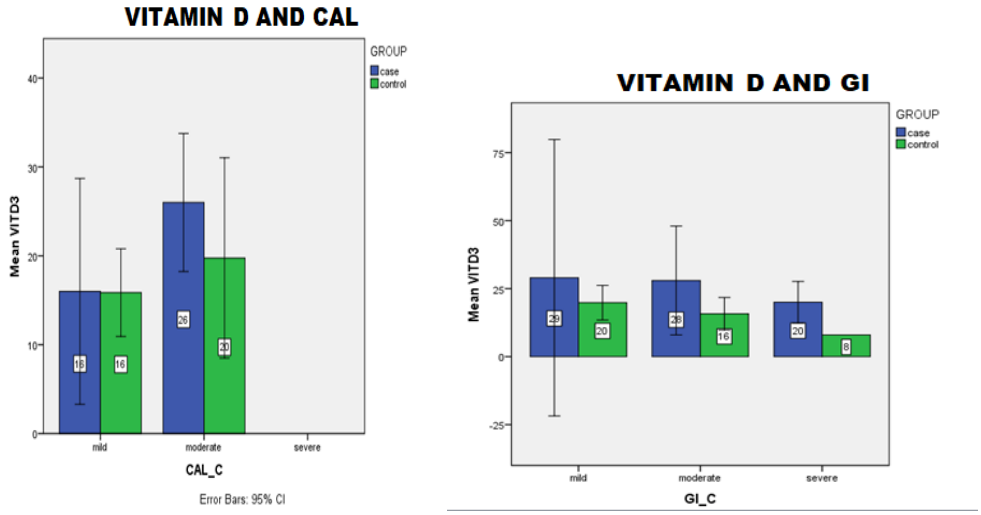
Graph 3 represents comparison of serum Vitamin D levels and Gingival index values between case and control groups depicting fair oral hygiene scores among the case and control groups for whom serum vitamin D levels were greater than 25 ng/ml and a general distribution of good,fair and poor oral hygiene scores among the case and control groups as the vitamin D levels fall below 20 ng/ml.

Graph 2& 3:Graphs representing comparison of serum Vitamin D levels and Oral hygiene index values and bleeding index values between case and control groups



Graph 4 represents comparison of serum Vitamin D levels and Gingival index values between case and control groups depicting mild and moderate gingival index scores among the case and control groups for whom vitamin D levels were greater than 25 ng/ml and severe gingival index scores among the case and control group alone as the serum vitamin D levels fall below 20 ng/ml.

Graph 4& 5: Graphs representing comparison of serum Vitamin D levels and Gingival index values and CALvalues between case and control groups



ODDS RATIO AND ASSOCIATION PATTERNS AMONG THE PERIODONTAL PARAMETERS AND SERUM VITAMIN D3 LEVELS:

Assuming the research question whether there is association between vitamin D levels and bleeding index, Oral hygiene index and probing depth levels the Odds ratio was used to interpret the results.The table 12 depicts an odds ratio of 0.015 with a 95% confidence interval of 0.527 in the lower bounds and 0.075 in the upper bounds for bleeding index values establishing a definitive protective role of Vitamin D in the periodontal tissues which is statistically significant.

Table 12: Odds Ratio

Model		Unstandardized Coefficients		Standard Coefficients	T	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	39.083	5.566		7.021	0.000	26.680	51.485
	BI	-0.344	0.119	-0.675	-2.896	0.016	-0.609	-0.079
2	(Constant)	17.857	10.087		1.770	0.110	-4.962	40.676
	BI	-0.301	0.100	-0.591	-3.009	0.015*	-0.527	-0.075
	PD	6.113	2.584	0.464	2.365	0.042	0.267	11.958

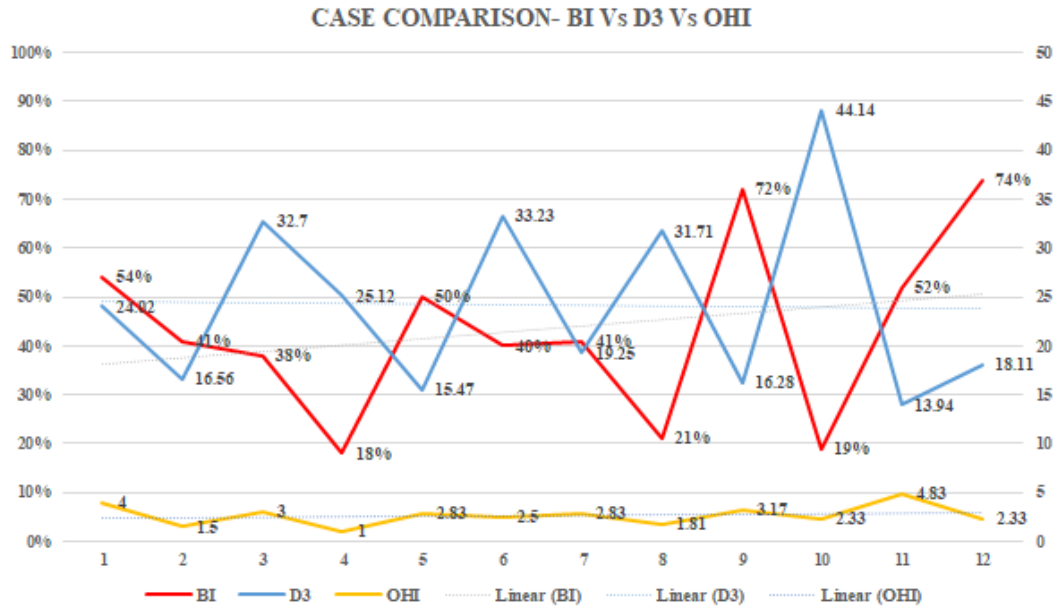
***p value 0.015 –statistically significant**

Thus it can be interpreted that for every single unit raise of Vitamin D there is a definitive decrease in bleeding index by 0.59 .Further more in the same table the odds ratio of 0.042 with a 95% confidence interval of 0.267 in the lower bounds and 11.95 in the upper bounds has been found in relation to probing depth signifying a probable protective role of Vitamin D levels over the probing depth levels though not statistically significant. Thus it can be interpreted that every single unit rise in vitamin D may decrease probing depth by 0.46.

When the values of bleeding index, gingival index and vitamin D values are overlapped among the case group ie participants with chronic periodontitis through the graph depicted in graph 6, a maximum of 72% of bleeding index is observed as against an oral hygiene index of 3.17 and a serum vitamin D level of 16.28 ng/ml. and a minimum bleeding indices of 18% and 19 % for whom serum vitamin D levels were 25.12 ng/ml and 44.14 ng/ml and oral hygiene index values of 1 and 2.33 respectively. On an average the bleeding index values were in the range of 18% to 21% for the participants in the case group who had serum vitamin D levels >30 ng/ml irrespective of the oral hygiene index values which was in the range of 2 to 3.Thus even though the oral hygiene index values were on the higher end it had minimal effects on the inflammatory status of the individual provided the participant's serum Vitamin D levels were in the optimal range.

When the values of bleeding index, gingival index and vitamin D values are overlapped among the control group ie participants without chronic periodontitis through the graph predicted in graph 7 a maximum of 25% and a minimum of 12% bleeding index can be observed as against the Vitamin D levels of 9.98 ng/ml and 23 ng/ml and OHI values of 1.1 and 0.49 respectively. Among the control group generally a good oral hygiene maintanence in the range of 0.2 to 1 and serum vitamin D3 levels in the range of 10 to 26 ng/ml can be observed putting most of them in the insufficient category. There was a definitive reduction in bleeding index by 25% compared to the case group. If the effect of oral hygiene alone has to be analysed ie the presence of plaque and calculus alone as a factor for the development of gingival inflammation and the outcome of bleeding there should have been a minimal ie below 12% bleeding index in majority of the participants in the control group but on the contrary there was an overall 25% of bleeding index .Though bleeding and inflammation can be attributed to the plaque and calculus levels the protective effect provided by vitamin D on the gingival tissues can be found to be reduced in the control group owing to the insufficient levels of vitamin D.

Graph 6:Line chart representing comparison of serum Vitamin D levels against bleeding index and OHI values within the case group



Graph 7: Line chart representing comparison of serum Vitamin D levels against bleeding index and OHI values within the control group

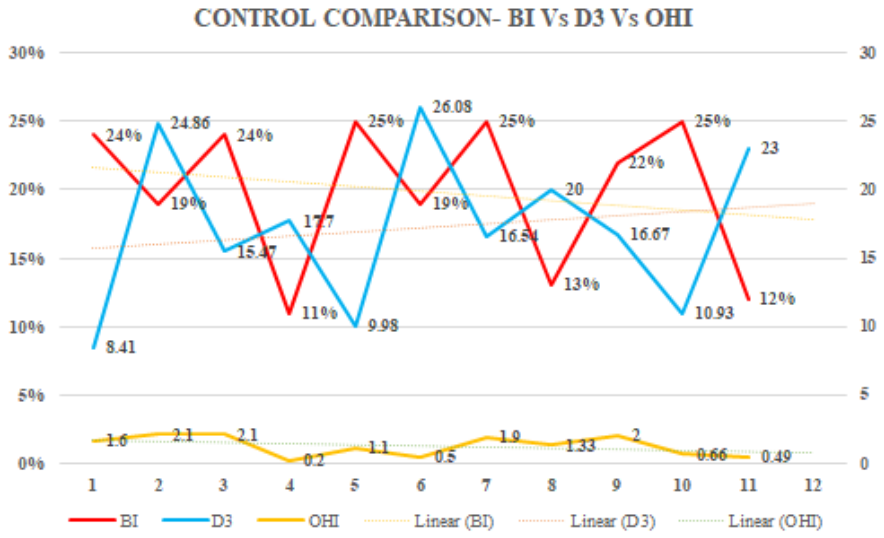


Table 13 Correlation between Vitamin D3 and clinical parameters of the case group

Case group	Correlation coefficient	Correlation
Vitamin D3 vs BI	-0.591	Negative. Good correlation
Vitamin D3 vs PPD	0.464	Very low correlation
Vitamin D3 vs CAL	0.446	Very low correlation/partial correlation
Vitamin D3 vs OHI	-0.148	Negative .Good correlation

Table 14 Correlation between Vitamin D3 and clinical parameters of the control group

Control group	Correlation coefficient	Correlation
Vitamin D3 vs BI	-0.695	Negative. Good correlation
Vitamin D3 vs PPD	0.619	Very low correlation
Vitamin D3 vs CAL	0.131	Very low correlation/partial correlation
Vitamin D3 vs OHI	-0.122	Negative .Good correlation

DISCUSSION:

There are scores of research papers in the literature reporting poor vitamin D status from all over India and some from other countries of the Indian subcontinent too.Among them significant with respect to our study are the studies done by Harinarayanan etal ,Paul etal and Farrant etal among south Indian post menopausal women[4,5,6,7,8]. In Farrants study 66% of women had hypovitaminosis D (25(OH)D concentrations <50 nmol /l and 31% were below 28 nmol /l.Harinarayanan and Paul etal too concluded that there was an overall higher prevalence of Vitamin D insufficiency and osteoporosis in the semiurban population of postmenopausal women.

There are 5 main factors affecting the status of gum bleeding in post menopausal women viz

1)Systemic status ie presence of any bleeding or clotting disorder or leukemia

2)Presence of systemic conditions like Diabetes Mellitus,drugs like anti coagulant therapy.

3)Hormone levels especially Oestrogen and use of Hormone Replacement Therapy.

4)Oral hygiene level(summation of plaque and calculus levels and the frequency of oral hygiene practices)

5) Presence of pathogenic bacteria

Among the 5 factors, the presence of bleeding or clotting disorder as a systemic condition is considered as an exclusion criteria in the present study.

Effect of sex Hormone Levels and HRT:

The physiological changes associated with menopause can cause some women to experience uncomfortable symptoms such as hot flashes and night sweats, vaginal dryness and dyspareunia, disturbed sleep and irritability/depression. Moreover, estrogen deficiency arising from menopause, in association with age-related factors, has been shown to increase the risk of developing cardiovascular disease, including coronary heart disease and stroke, colorectal cancer and osteoporosis [9]. Until recently, HRT was considered the single most effective treatment of menopausal symptoms and its use was recommended for the prevention of diseases associated with estrogen deficiency [10]. After the publication of the Women's Health Initiative (WHI) findings in 2002 and 2004 [11,12], the use of HRT at menopause has become a matter of debate and its utility has been questioned.

In past years, various studies have been conducted to evaluate the effect of HRT in modifying the periodontal conditions in postmenopausal women due to a possible connection between osteoporosis and the progression or severity of periodontitis [13]. HRT has also been associated with decreased levels of gingival bleeding [14]. It has been suggested that estrogen may have an inhibitory effect on gingival inflammation by inhibiting mediators (IL-1, TNF-α, IL-6, IL-1β, IL-8) and cellular mechanism of inflammation (PMN recruitment, lymphocyte activation) [15]. Similarly, estrogen supplementation may modulate the rate of breakdown of periodontal tissue through a mechanism involving down-regulation of matrix metalloproteinases (MMP-8 and MMP-13) and cytokines involved in bone resorption [16].

The findings of the study conducted by Pizzo etal indicate that, in postmenopausal women, long-term HRT was not associated with significant effects on periodontal status and clinical measures of periodontitis, thus suggesting that HRT may not confer protection against periodontitis. Periodontitis may be primarily related to the presence of plaque and to a lesser extent to hormonal changes such as estrogen deficiency. However, the possibility exists that the decreased estrogen levels associated with the postmenopausal period may contribute to the progression of periodontal disease by affecting the oral bone mass [17]. In the present study none of the participants were on Hormone Replacement Therapy potraying the minimal effect of Oestrogen Hormone on post menopausal womens periodontal status and the lesser importance of the same.

Effect of Diabetes:

Diabetes increases inflammation in the periodontal tissues. For example, gingival crevicular fluid (GCF; a fluid exudate that flows from the gingival margins) levels of PGE_2 and IL-1β are higher in type 1 diabetic patients with either gingivitis or periodontitis compared with those in non-diabetic individuals with the same level of periodontal disease. Preshaw etal indicated a clear inter-relationship between degree of hyperglycemia and severity of periodontitis [18].In the

present study 41% of the case group and 36% of the control group were found to be diabetic signifying that uncontrolled diabetics had greater severity of the disease and on the other hand when the diabetes was under control the inflammation levels too were markedly reduced.

Influence of Oral Hygiene practices:

Gingival bleeding occurs mainly due to inadequate plaque removal which results in the thinning, ulceration, necrosis of gingival epithelia coupled with engorgement of blood vessel. Gingival bleeding on tooth brushing is a form of provoked gingival bleeding, a vital feature and probably one of the most frequent complaints among patients with periodontal disease. Gingival bleeding occurs alongside other manifestations of periodontal diseases like gingival swelling, halitosis, food packing, pain, gingival recession, and tooth mobility.[19]

Maintenance of good oral hygiene helps to prevent dental problems mainly periodontal disease and dental caries. Poor oral hygiene and frequent consumption of sugars is known for many years as key behavioral risk factors for these two oral diseases.[20] Daily oral self-care to control the supragingival plaque may assist in slowing or reducing the shift to a pathogenic environment.[21]

In the present study 91% of the case group were found to brush once daily with toothbrush and only 8% were found to brush twice daily.On the other hand 45% of the control group brushed once daily and 54.5% of the same brushed twice daily signifying the profound influence of oral hygiene practices on inflammation and bleeding status.

Effect of outdoor activity:

Periodontal disease is directly caused by periodontal disease-causing bacteria, but indirect causes include host factors and environmental factors. These factors are thought to contribute significantly to the onset and progress of periodontal disease pathology. Environmental factors include oral cleaning conditions, smoking habits, and stress stimulation[22]. Exercise habits may be a new environmental factor or may simply exert an influence on known environmental factors. It is possible that the establishment of appropriate exercise habits improves periodontal disease through the activation of the immune system and a reduction in inflammatory cytokines, and it is presumed that this effect is similar to that of nonsurgical periodontal treatment on obese people. The available research suggests that exercise habits may have a synergistic effect in the improvement of periodontal disease with the usual periodontal treatment[23].

In the present study 66.6% of the case group and 41.6% of the control group were found to have participated in outdoor activities ie having exposure to sunlight partly as a result of their work and partly for exercise purpose such as walking thus signifying the remarkable influence made by outdoor exposure and physical activity among the case group that had significantly improved their bleeding scores.

Effect of Food habits:

In the present study on an average 92% of the case group and 91% of the control group were found to have a mixed diet including non vegetarian foods such as meat, fish, and poultry in their diet while only 8% of the case group and 9% of the control group were found to be pure vegeterians.

Effect of pathogenic bacteria:

In the study concerning relationships between selected bacterial species and clinical periodontal disease in the population-based setting of INVEST (Oral Infection and Vascular Disease Epidemiological Survey) by Demmer and Papapanau etal information was added to the existing evidence that the bacterial grouping *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* and *T. denticola* (or correlates of this grouping) are strong predictors of gingival inflammation and colonization by health-associated bacteria was inversely related to bleeding on probing suggesting that certain bacteria are potentially protective against manifest gingival inflammation[24].

Dietrich etal associated serum concentrations of 25(OH)D3 and gingival inflammation in a large sample of the civilian, non institutionalized US population [third National Health and Nutrition Examination Survey (NHANES III)] aged 13 to 90 years.Their study suggested that increased serum concentrations of vitamin D may be beneficial in regard to gingivitis susceptibility. This inverse association may be due to the antiinflammatory effect of vitamin D, which may be present in serum concentrations of 25(OH)D3 ≥90 –100 nmol/L[25,26].In the present study we have also a found a statistically significant inverse association between serum Vitamin D3 levels and gingival inflammation which is reflected as decreased score of bleeding index by 0.59 for every single unit rise in Vitamin D3 level above 20 ng/ml (50 nmol/L)given the fact that there is generally insufficiency in vitamin D levels among the Indian population as discussed in the previous section.

One of the largest prospective study to date on vitamin D status and progression of periodontal disease in postmenopausal women is the study by Millen etal in 2014 known as the Buffalo Osteoperio study[27]. The authors observed no associations between vitamin D status, assessed with baseline 25(OH)D concentrations and subsequent 5-year change in periodontal disease measures inclusive of changes in Alveolar Crestal Height(ACH) ,Clinical attachment level and probing depth.. These results did not vary greatly by baseline

periodontal disease status. There was some suggestion that among women with severe periodontal disease at baseline, higher baseline 25(OH)D concentrations may protect against periodontal disease progression, defined using ACH, CAL, and PD, but these results were not statistically significant. In our present study when we assessed the cross-sectional association between 25(OH)D3 and oral inflammation with probing depth and clinical attachment level in a cohort of 23 post menopausal women aged 50 to 65 years we observed an inverse but not statistically significant association with probing depth and clinical attachment level among the participants with chronic periodontitis. Every single unit rise in vitamin D may decrease probing depth by 0.46 and thus reduce the severity of progression of disease. Thus it can conferred that though Vitamin D has limited role in the prevention of periodontal disease the role played by the vitamin in arresting the progression and severity of the disease could not be ruled out.

In another ancillary study of the Women's Health Initiative (WHI) Observational Study (OS) conducted by Sahli etal[28] among females enrolled at the Buffalo, New York center which used information collected from the Buffalo Osteoporosis and Periodontal Disease Study (OsteoPerio Study) a lack of association was observed between five species of pathogenic oral bacteria and 25(OH)D concentrations in postmenopausal females in the OsteoPerio Study.

Limitations of the study:

1. The present study limits with minimum number of sample size when compared with other studies in addition to limited scope regarding the radiographic assessment of the hard tissue parameters such as Alveolar Crestal height.

CONCLUSION:

- The present study was conducted to evaluate the serum vitamin D3 levels among post menopausal women with and without periodontitis and to compare the severity of the periodontal parameters among the same. A total of 23 post menopausal women were included in the study of which 12 of them were participants with chronic periodontitis and 11 of them were without chronic periodontitis.
- The demographic characteristics of the participants were noted and the clinical parameters like Oral Hygiene Index, Bleeding Index, PPD,CAL were recorded on the same day of Serum Vitamin D3 estimation. From the results of the study, following conclusions could be arrived viz..
- An inverse association was observed between the serum Vitamin D3 levels and bleeding index which is statistically significant.
- An inverse association was observed between the serum Vitamin D3 levels and only probing pocket depth levels which is not statistically significant.
- There is no association present between serum Vitamin D3 levels and chronic periodontitis as such.
- The results of the present study indicate that serum Vitamin D3 levels make a profound influence on the soft tissue characteristics of the periodontium through the anti-inflammatory and anti-microbial effects that it exerts on the human periodontium. Our results support the hypothesis that Vitamin D plays a pivotal role in stabilising the periodontal health and thus conferring general health benefits to the patient on the whole. Hence Vitamin D3 screening can be used as an adjuvant tool in assessing the periodontal status of the individual. Vitamin D3 deficient patients with periodontitis should improve their serum vitamin D3 levels for improving their status through diet and lifestyle modification and on the other hand participants with sufficient levels of Vitamin D should improve their overall oral hygiene.
- To confirm and validate the findings of this study, a larger study with long term follow up and focussing on screening periodontitis patients for lower levels of 25(OH)D and interventional studies post Vitamin D supplementation seems to be needed. Moreover, exploring further determinants of periodontal disease such as use of advanced radiographic diagnostic aids in assessing ACH, and the use of interdisciplinary approach with help of medical personnel for optimal dose of Vitamin D supplementation may help a long way in recognizing the importance of this supplementation for the general and periodontal health of every post menopausal woman .

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