Original Article

Vitamin D status and periodontal disease among pregnant and non-pregnant women in an underdeveloped district of Pakistan

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Abstract

Aim: To compare pregnant and non-pregnant females for vitamin D level and periodontal status and to determine if there is any association between the periodontal health and hypovitaminosis D in pregnant women. **Materials and Methods:** A cross-sectional study was conducted in Jhelum, Pakistan. Participants were pregnant females at ~ 12 weeks of gestation (n = 36) and non-pregnant (n = 35) females selected from the same locality. Periodontal parameters such as probing depth, bleeding on probing, and attachment loss were recorded. Serum samples were taken to measure blood indices and vitamin D levels. Chi-square test and Odds ratio were applied to determine the association between hypovitaminosis D and periodontal status. **Results:** Vitamin D deficiency was common in the pregnant group compared to non-pregnant (P < 0.001). Blood indices (hemoglobin, hematocrit, mean corpuscular volume) were significantly lower among the pregnant compared to the non-pregnant group (P < 0.001). However, there was no significant difference between the two groups for probing depth and attachment loss. **Conclusions:** Pregnant women were more deficient in Vitamin D than non-pregnant women. However, no association between low vitamin D levels and periodontal disease was seen in the studied population.

Key words: *Nutrition, periodontal disease, pregnant women, vitamin D*

INTRODUCTION

Studies have shown that gingival tissue can be affected in pregnancy.[1] Although pregnancy itself does not result in gingivitis, it may exacerbate the preexisting periodontal disease. Typical feature of pregnancy gingivitis is that the gums appear red, swollen with smooth texture and increased bleeding tendency.[2] The pregnancy-related gingival changes usually resolve within few weeks or months of delivery. The inflammatory changes are usually restricted to the gingiva and probably do not cause any permanent changes in the periodontal tissues.[3]

Vitamin D deficiency and insufficiency is a global problem.[4] Pregnancy puts an additional burden on mothers because they have to satisfy the demand of the developing fetus.[5] The fetal vitamin D levels are related with the maternal vitamin D levels,[6] and children born to vitamin D-deficient mothers show a higher incidence of rickets.[7] Therefore, it is important for pregnant women to maintain their plasma level of this hormone.

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Pregnant women are known to be highly vulnerable to vitamin D deficiency.[8-11]

Periodontitis is an infection of the gums and the associated alveolar bone characterized by a chronic inflammatory response in the affected tissues. Most of the destruction in the tissues in periodontal disease is a result of inflammatory cascade initiated by the host defense.[12] Vitamin D metabolites have been shown to modify the inflammatory response[13] and possess antimicrobial effects.[14] Thus, adequate vitamin D levels are likely to affect periodontal status. Grant and Boucher[15] investigated the relationship between hypovitaminosis D and periodontal disease using Bradford Hill's criteria. They concluded that hypovitaminosis D can act as a risk factor for periodontal disease. However, this association is yet to be studied in pregnant women.

Miley *et al.*[16] reported that the participants who consumed vitamin D and calcium supplements had shallower probing depths, lesser attachment loss, fewer bleeding sites, and lesser alveolar bone loss compared to controls. However, the study participants were healthy individuals whose vitamin D status was not assessed. Similarly, a clinical trial by Krall *et al*.[17] showed that the supplementation of vitamin D can reduce tooth loss due to periodontal reasons. The generalizability of their study is limited because their study population comprised elderly individuals only. In this context, it is imperative to study the periodontal disease in individuals who already are at a high risk of hypovitaminosis D. Therefore, we aimed to compare the vitamin D level and periodontal status of pregnant with non-pregnant women and planned to determine an association between periodontal disease and hypovitaminosis D.

MATERIALS AND METHODS

Approval from Aga Khan University ethics committee (Ref: 147/Ped/ERC/2010) was taken to conduct a community-based, cross-sectional study at Jhelum, Pakistan. A list of the pregnant women was generated. These pregnant females were selected among 400 women already enrolled in a separate trial on birth outcomes. Pregnant females with gestational age of 12–16 weeks were enrolled during September 2011 to February 2012. Using the convenience sampling method, non-pregnant females were also selected (matched for area of residence and age). Participants provided written informed consent. Women with any known metabolic disease or missing >8 teeth were excluded. The interview took place at data collection centers and at women's residence. All study subjects underwent dental examination followed by the collection of venous blood. Laboratory analysis of blood samples was conducted at the Aga Khan University Nutrition Laboratory.

Sample size

The known prevalence of hypovitaminosis D among pregnant women is 69.6%. We assumed a conservative prevalence of periodontal disease among non-pregnant women as 50%. Keeping the level of significance at 5% and power of study at 80%, we required 36 participants in each group. Thus, a total of 72 women were needed for the study.

Periodontal probing depth (PD), clinical attachment loss (AL), and bleeding on probing (BoP) were assessed by a single trained dentist (FRK). Number of decayed, missed, and filled teeth (DMFT index) was also recorded. Vitamin D levels were measured using a Food and Drug Association approved method (DiaSorin, LIASON Inc, kit). The method is based on a direct competitive chemiluminescence immunoassay (CLIA) of vitamin D levels in serum.

Data analysis

Data was analyzed using the Statistical Package for the Social Sciences version 19.0, (IBM Inc. Chicago, IL, USA). Independent sample *t*-test was applied to compare continuous variables such as AL, PD, blood indices, and vitamin D levels between pregnant and non-pregnant women. Chi-square test (or Fisher exact test) was applied to determine the difference in the proportion of periodontitis (PD > 3 mm) and hypovitaminosis D (<20 ng/mL) participants in the two groups. Odds ratio with 95% CI was also computed. $P \leq 0.05$ was considered to be statistically significant.

RESULTS

A total of 36 pregnant and an equal number of non-pregnant participants were recruited in the study. The serum specimen of one of the participant in the non-pregnant group was of insufficient quantity to measure the 25(OH) D levels; therefore, 71 women (36 pregnant and 35 non-pregnant) constituted the final study group. There were no smokers or diabetics in the sample. None of the participants had received any implant or visited a dentist in the last 6 months. The participants in the two groups were of similar ages. Major differences were observed in the weight and

blood indices of the subjects. This can be attributed to the fact that one of groups comprised pregnant women. It was alarming to note that the vitamin D levels in both groups were low [Table 1]. Approximately 89% pregnant women were found to be vitamin D deficient compared to 54% non-pregnant ($P < 0.001$). One-third of pregnant women had severe deficiency (vitamin D ≤10 ng/mL) compared to only 3% non-pregnant ($P < 0.001$).The periodontal parameters, i.e. PD, AL, and BoP, were slightly better in the non-pregnant group [Table 2]. There was no statistically significant association between periodontal disease and vitamin D status in the two study groups [Table 3].

DISCUSSION

Pregnancy is a state where the likelihood of hypovitaminosis D is high. The low concentration of vitamin D among pregnant women is explained by increased physiological demand for the skeletal growth of fetus. Conventionally, poor periodontal health is considered to be a result of inadequate oral hygiene practices that result in poor mechanical removal of

Table 1: Characteristics of study participants

	Pregnant (*n*=36)				Non-pregnant (*n*=35)				*P*
	Mean	SD	*n*	%	Mean	SD	*n*	%	
Age (years)	26.6	4.5			26.1	5.0			0.93
Weight (Kg)	58.4	11.3			53.9	11.8			<0.01
Hb (gm/dL)	9.26	2.9			11.9	2.5			<0.001
HCT (%)	28.69	8.6			39.27	3.2			<0.001
MCV (fL)	74.20	11.4			82.87	9.3			0.04
Vitamin D level (ng/mL)	12.20	5.9			20.6	6.6			<0.001
Vitamin D status									
Severely deficient: 10.0 ng/mL			12	33.4			1	2.8	<0.001
Deficient: 10.1-20.0 ng/mL			20	55.5			18	51.5	
Insufficient: 20.01-30.0 ng/mL			4	11.1			13	37.1	
Sufficient: >30.01 ng/mL			0	0			3	8.6	
Parity status									
0			9	25.0					NA
1-2			16	44.4					
3-4			9	25.0					
5-8			2	5.6					

Independent samples t-test or Chi square (or Fisher's exact) test; Level of significance kept at 0.05

Table 2: Education, oral hygiene, DMFT, index and periodontal parameters

	Pregnant (*n*=36)				Non-pregnant (*n*=35)				*P*
	Mean	SD	*n*	%	Mean	SD	*n*	%	
Education status									
≤5 years of schooling			24	66.6			0	0	<0.001
≥6 years of schooling			12	33.4			35	100	
Oral hygiene practice									
Brush			26	72.2			32	91.5	<0.001
Traditional∞			10	27.8			3	8.5	
DMFT categories									
Low 0-2			23	63.8			19	54.3	0.556
Moderate 3-5			9	25.0			10	28.6	
High >5			4	11.2			6	17.2	
BoP									
Yes			18	50.0			13	37.2	0.04
No			18	50.0			22	62.8	
Dental parameters									
Number of teeth	29.7	3.7			30.23	1.7			0.473
PD (mm)	1.83	0.5			1.81	0.6			0.93
AL (mm)	1.2	0.9			0.74	0.85			0.16

Independent samples t-test; Chi square/Fisher's exact test was applied at P≤0.05; ∞, traditional methods refer to using Miswak (bark of the neemwood tree)

Table 3: Association between vitamin D status and periodontal disease in the study groups

Group	Vitamin D	PD >3mm		PD <3mm		Odds ratio (95% CI)	*P*
		n	%	*n*	%		
Pregnant	<20 ng/mL	4	11.1	28	77.8	1.42 (0.07-31.1)	0.99
n=36	>20.01 ng/mL	0	0	4	11.1		
Non-pregnant	<20 ng/mL	4	11.5	15	42.8	9.58 (0.47-193)	0.10
n=35	>20.01 ng/mL	0	0	16	45.7		

Chi square/Fisher exact test was applied at P≤0.05

plaque. However, recent literature indicates that periodontal disease is associated with a number of systemic health conditions.[18] Vitamin D can affect periodontal disease through its bone mineral density effect as well as its immunomodulatory effect through the stimulation of antimicrobial polypeptides such as cathelicidins and defensins. The protective effect of vitamin D against periodontal disease is weakened in the state of hypovitaminosis D. There is an emerging body of evidence that supports vitamin D as a determinant of periodontal health.[15]

Our study showed that there is no association between vitamin D status and poor periodontal health. This is contrary to Boggess *et al.*[19] who showed that hypovitaminosis D is independently associated with periodontal disease among black pregnant women. They recommended that improvement in vitamin D levels could be a potential intervention to improve the oral health of pregnant females. The data from the third National Health and Nutrition Examination (NHANES III) Survey indicated that low dietary intake of calcium and vitamin D is linked with periodontal disease.[20] Dietrich *et al.* used NHANES III dataset and reported that vitamin D levels were significantly and inversely correlated with AL in individuals aged >50 years, independent of bone mineral density.[21] In a subsequent analysis of the same dataset, Dietrich *et al.* also found that hypovitaminosis D was associated with high BoP.[22] It was inferred that vitamin D exerts its anti-inflammatory effect to reduce the gingival inflammation. Probably this is the reason why pregnant women with hypovitaminosis D in the present study showed an increased propensity toward BoP. However, we observed that BoP was not correlated with any increase in the PD or AL.

Holmes *et al*.[23] studied vitamin D status among pregnant women during 12th week of gestation and compared them with non-pregnant controls, both residing at >50 degree north latitude from the equator. Their sample mainly comprised Caucasian females in United Kingdom. They reported vitamin D deficiency and insufficiency among pregnant women were 96% and 99%, respectively. Our data suggests that, in Pakistani pregnant women (living at 33°N latitude from the equator), the vitamin D deficiency and insufficiency are 87 and 100%, respectively. This shows that, as far as pregnant women are concerned, the present study is in agreement with Holmes *et al.*[23] However, non-pregnant controls in Caucasian sample had 92% and 100% vitamin D deficiency and insufficiency, respectively, whereas Pakistani non-pregnant women showed 54% and 97% of vitamin D deficiency and insufficiency, respectively [Table 1]. In other words, the prevalence of vitamin D deficiency among non-pregnant Pakistani women is lower than that of their Caucasian counterpart. This difference can be attributed to more sunlight exposure, darker skin, and proximity to the equator. As a part of cultural and religious practice, a proportion of Pakistani women mostly keep their head, neck, and body covered. This could also restrict the amount of sunlight available to them. As a result, their underlying vitamin D deficiency is likely to get worse. This factor of covering body and face and acquiring vitamin D deficiency due to poor sunlight exposure is more significant among Pakistani females residing in European counties at higher latitude from equator.[24]

The strength of the present study is that the hypovitaminosis D and periodontal disease association was never addressed before among pregnant women in a developing country. Although Boggess *et al.*[19] did study this relationship by employing a case control design in USA, the present study is different as both pregnant and non-pregnant participants were compared. Because of poor dentist–population ratio, periodontal disease is more prevalent in the rural populations.[25]

Gursoy *et al*.[3] reported similar periodontal findings in the non-pregnant women in Finland, however, the present study is different as it has been conducted in the rural setting where non-pregnant women demonstrated better oral hygiene practices and less AL. This may be attributed to the biased selection of controls. Pakistani society is conservative and literacy levels are low, hence selection of non-pregnant participants was a challenge. Although the participants were matched by

age and residence, it was impossible to match them on educational status because only educated females could understand the purpose of research and consented to participate in the study [Table 2]. This has potentially introduced a bias caused by the effect of education on oral hygiene. The two groups were significantly different with respect to the oral hygiene practices. Thus, in this context, it is difficult to attribute the relatively poor periodontal health among pregnant women to low vitamin D alone. Education and oral hygiene practices might have operated as confounders.

With the progress of pregnancy, it may be speculated that the periodontal indices may change owing to physiological change in the hormones. The temporal relationship between the exposure and outcome cannot be studied in a cross-sectional study. We could not assess any association between vitamin D and periodontal disease in both the pregnant and the non-pregnant groups. One of the probable reasons may be a low prevalence of periodontal disease in the study sample. None of the participants in our sample were detected with severe periodontitis [Table 3]. This selection bias may have distorted the true relationship between hypovitaminosis D and periodontal disease (if there is one).

Another limitation was lack of an objective quantification of AL. Ideally, periapical dental radiographs should have been employed, however, because of the limited logistics in the rural community and pregnant participants, it was avoided.

Not all pregnant women have appropriate knowledge about oral health care.[26] In developed countries, where health systems are well-established, gynecologists routinely counsel the expecting mothers about the significance of periodontal disease in pregnancy and emphasize on maintaining appropriate oral health care.[27,28] However, in developing countries, like Pakistan and India, lack of a structured general and oral healthcare system (particularly in rural areas) keeps pregnant females vulnerable toward periodontal disease and the associated adverse health outcomes.[29,30]

There is a clear need for clinical trials to establish hypovitaminosis D as a risk factor for periodontal disease. This becomes more important for subsets of population that are already vulnerable to vitamin D deficiency.

CONCLUSIONS

Vitamin D deficiency was more prevalent among pregnant than the non-pregnant participants. There was no statistically significant association between hypovitaminosis D and periodontal disease among pregnant and non-pregnant women.

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Conflicts of interest

The authors confirm that there are no conflicts of interest regarding this publication.

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