Original Article

Estimation of Vitamin D Levels Using a Chairside Diagnostic Test Kit in Patients with Gingivitis and Periodontitis: A Cross-Sectional Study

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Abstract

Aim: Periodontitis is an inflammatory condition of the periodontium that is instigated by microbial biofilms developed on the teeth. The purpose of the study was to ascertain the vitamin D status of gingivitis and periodontitis patients while maintaining a healthy group as the control using simple low-cost chairside pre-coated with 25-OH Vitamin D antigen rapid test kits. **Materials and Methods:** From outpatients visiting the college's periodontics clinics, a total of 101 patients were screened. The study sample includes 38 patients in the periodontitis group, 32 in the gingivitis group, and 31 in the healthy group. A middle-digit needle was used to collect blood samples, which were put into a test cassette with membrane that had been coated with 25-OH Vitamin D antigen on the test line area of the strip. Vitamin D Quick Test (Natejah) Semi-quantitatively detects 25-hydroxyvitamin D (25 (OH) D) in human finger-stick complete blood at a cutoff convergence of 30 ± 4 ng/mL. Vitamin D blood levels below 80 nmol/L are considered to have deficient vitamin 25(OH)D levels. Clinical parameters between healthy, gingivitis, and different stages of periodontitis subjects were compared using one-way ANOVA and Tukey's multiple comparison. Fisher's exact test was done to compare vitamin D levels in the three groups. **Results:** Fisher's exact test revealed that there was a statistically significant increase in the number of subjects with stages 4 and stage 3 periodontitis who lacked vitamin D levels (less than 80 nmol/L). Whereas least deficient in Vit D were noticed among Healthy and Gingivitis subjects. When compared to healthy individuals, gingivitis, and various stages of periodontitis had significantly higher mean PI values in a Tukey's multiple comparison. Vitamin D deficiency was found to be 27.5% in subjects with gingivitis and 71.5% in subjects with periodontitis. **Conclusion:** Periodontitis is associated with vitamin D deficiency, in contrast to gingivitis and healthy subjects. The severity of periodontitis was likewise linked to the amount of vitamin D in the individual's blood. When compared to expensive, time-consuming, and laborious laboratory methods, the findings of this study suggest that a simple inexpensive chairside pre-coated with 25-OH Vitamin D antigen rapid test kits can be considered a viable alternative for determining vitamin D levels.

Keywords: *Gingivitis, periodontitis, vitamin D deficiency*

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Introduction

Chronic periodontitis is none other than an inflammatory condition of periodontium that is

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initiated due to microbial biofilms and the bacterial products instigates the immune response of the host leading to the tissue destruction of structures that support the teeth, and alveolar bone.[1] Due to this pathological phenomenon, chronic periodontitis is reckoned as the major reason for tooth loss.[2,3] This disease has a remarkable caustic effect on the nutritional status and quality of life.[4,5] Chronic periodontitis is interconnected to other systemic conditions like cardiovascular disease and type 2 diabetes mellitus.[6,7] Vitamin D is a fat-soluble vitamin associated with the regulation of calcium absorption in the body, gets processed in the liver region up to 25-hydroxyvitamin D (25(OH)D), and after that kidneys convert it to its active form, that is, 1,25-dihydroxyvitamin D (1,25-(OH)2D).[8] Since the vivacious circulating metabolite 25(OH)D in the blood is employed for defining the vitamin D status of the patient.[9] However, no consensus has been established for optimal levels of 25 (OH) D, and the majority of experts assert <50 nmol/L (20 ng/mL) of being an inadequacy of vitamin D and 25 (OH) D levels should remain as high as 75 nmol/L (30 ng/mL) to achieve optimal vitamin D context.[8,10]

Researchers have discovered the crucial positive relation between 25 (OH) D levels as well as bone mineral density along with the association between lesser risks of fractures and vitamin D supplementation.[11,12] Studies showed that an increased level of production potential of generating active vitamin D tends to enhance the bactericidal activity like beta-defensins and cathelicidin.[13] Few *in vitro* researches have proved that 1,25-(OH) 2D has potential to inhibit the maturation, proliferation, and variation of dendritic cells right from the monocytes and the potential to suppress the proliferation as well as the T-lymphocytes' cytokine production.[14,15] It is a known fact that a positive relation is observed exhibiting low levels of vitamin D, development of gingivitis and periodontal disease attributing it to the bone loss reaction towards bacterial plaque.[16,17] Though gingivitis is a mild form of periodontal illness which if not treated initial stages can lead to serious conditions or even tooth loss in the case of susceptible patients.[18] Various risk factors hold the accountability of such kind of disorder which even includes diabetes mellitus, smoking, and some sorts of medications like genetic factors, oral contraceptives, vitamin D deficiency, and genetic factors.[19,20] So many studies recommend that vitamin D levels be evaluated prior to treatment to prevent vitamin D deficiency and improve post-surgery outcomes.[21] For periodontal pathoses diagnosis, numerous oral biomarkers like saliva, blood, or gingival crevicular fluid form a diagnostic tool. In comparison to this vitamin D chairside diagnostic tests enable prompt, noninvasive, and precise disease prediction along with early detection of disease.[22] It goes without saying that more research is needed to find out how vitamin D status affects the development of gingivitis and periodontal disease. These results are still not enough, and there is no evidence that have any conclusive research on this subject using chairside Vit D kit to determine its levels, which is where the proper research is required. The aim of the study was to determine the vitamin D status of gingivitis and periodontitis patients, keeping healthy group as control using simple low-cost chairside pre-coated with 25-OH Vitamin D antigen rapid test kits. We hypothesize that vitamin D antigen rapid test kits can be considered as a reliable alternative to determine vitamin D levels.

Materials and Methods

This cross-sectional study was conducted from March to June 2021 and aimed at the establishment of the vitamin D status of patients with gingivitis and periodontitis with healthy group as control. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of College of Dentistry, Dar Al Uloom University, Riyadh, KSA (COD/IRB/2020/29) on February 22n 2021. After obtaining the informed consent a total of 101 patients visiting the periodontics clinics of the College of Dentistry, Dar Al Uloom University participated in the study, out of them 55 were female patients and 46 were male patients. The sample was collected after computer-generated randomization, and the estimation of the sample size was performed using the formula, $n = P(1 - P) Z^2/d^2$, $Z = 1.96$, $d = 0.05$, confidence level (5% margin of error) = 95%, and power = 80%. The calculated sample size for this study was hence 101. Thirty-one individuals fit into the healthy category, whereas 32 patients have gingivitis and 38 patients have periodontitis. The Periodontitis group is evidenced by ≥4 sites with pocket depth (PD) ≥ 4 mm and clinical attachment level (CAL) ≥ 1 mm, staging of the disease is based on the 2017 classification[7] Based on the new classification, the patients were grouped into stages 1, 2, 3, and 4.[7] PD and CAL were recorded to the near millimeter (at the deepest position of the certain interproximal site) by means of a UNC-15 probe. A single examiner performed clinical evaluations on each parameter. Intra-inspector adjustment was performed in 6 subjects with clinical status like concentrating on subjects. Calibration was declared reproducible when the variation between

measurements made at baseline and 48 h later was less than 1 mm in 95% of the instances. The criteria for including participants in the gingivitis group are as follows: gingival inflammation (Loe and Silness Gingival Index greater than 0.5), erythema, or edema, and clinically visible PD smaller than 3 mm. Healthy controls included the subjects who had absence of gingival inflammation as measured by the Loe and Silness Gingival Index 0.1, absence of erythema or edema, clinically evident PD 3 mm, and loss of attachment. A penned informed consent was acquired from all the patients ahead of the commencement of the study. Vitamin D measurement is done by collecting blood samples from the middle-digit needle stick into a test cassette containing membrane that is pre-coated with 25-OH Vitamin D antigen on the test line region of the strip which is the primary outcome. Vitamin D Rapid Test (Natejah) semi-quantitatively detects 25-hydroxyvitamin D (25 (OH) D) in human finger-stick [Figure 1]. Whole blood at a cutoff concentration of 30 ± 4 ng/mL. Vitamin D deficiency has blood 25(OH)D levels below 80 nmol/L were considered insufficient.

Exclusion criteria for study groups

1) Pregnancy or lactation; 2) systemic diseases that have the potential to alter the clinical status of the periodontal system and the metabolism of bone, or that require premedication prior to monitoring or treatment procedures, 3) treatment for periodontal disease in the previous six months.

Figure 1: Vitamin D rapid test (Natejah)

Statistical analysis

The data was organized and systematized in MS-Excel. Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS) version 20 software (IBM, Chicago, IL, USA). GI, PI, PD, and CAL between Healthy, Gingivitis, and different stages of periodontitis subjects were compared using one-way ANOVA and Tukey's multiple comparison. Fisher's exact test was done to compare vitamin D levels in the three groups.

Results

There were 47.4% of males with periodontitis whereas 52.6% of females had the same. Gingivitis was endured by 43.8% of males and 56.2% of females. A total of 45.2% of males were regarded to be healthy whereas 54.8% of females were deemed healthy. Descriptive statistics and gender details are mentioned in Table 1. Fisher's exact test displays a statistically significant higher number of subjects with deficient (less than 80 nmol/L) of Vit D were noticed among stage 4 and stage 3 Periodontitis. Whereas least deficient in Vit D were noticed among Healthy and Gingivitis subjects. (Fisher's exact value (5) = 12.23; P = 0.028) [Tables 2 and 3]. The mean difference of PI in the one-way ANOVA is statistically significant (F = 70.55; P = 0.001), GI (F = 107.23; P = 0.001) and pocket profundity (F = 33.85; P = 0.001), between healthy, gingivitis, and periodontitis subjects at various stages [Graph 1] in Table 4 and Graph 2. When compared to a healthy individual, gingivitis and various stages of periodontitis had significantly higher mean PI values in Tukey's multiple comparison. Likewise, statistically significant mean PI estimates were noted among Gingivitis groups when compared to different stages of periodontitis. (P < 0.001). When compared to healthy individuals, statistics revealed that gingivitis and various stages of periodontitis had significantly higher mean GI values. In a similar vein, the Gingivitis groups had significantly higher mean GI values than the various stages of periodontitis. When compared to stage 2 periodontitis, the mean GI values of stage 3 periodontitis were statistically significantly higher (P = 0.008).

Table 1: Gender details of the participants

Gender	Periodontitis		Gingivitis		Healthy	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
Male	18	47.4	14	43.8	14	45.2
Female	20	52.6	18	56.2	17	54.8
Total	38	100.0	32	100.0	31	100.0

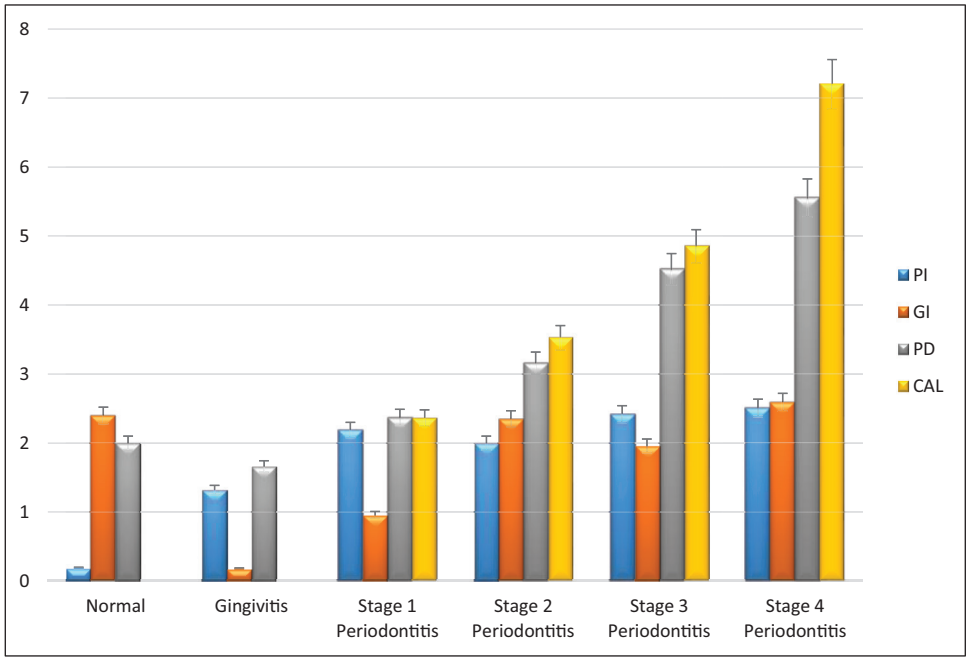
Table 2: Association of vitamin D level among healthy, gingivitis and different stages of periodontitis

Study groups		Vit D Levels		df	Fisher's exact value	*P* value
		deficient (less than 80 nmol/L)	sufficient (more than 80 nmol/L)			
Normal	*N*	9	22	5	12.23	**0.028***
	%	29.00	71.00			
Gingivitis	*N*	9	23			
	%	28.10	71.90			
Stage 1 Periodontitis	*N*	4	4			
	%	50.00	50.00			
Stage 2 Periodontitis	*N*	5	5			
	%	50.00	50.00			
Stage 3 Periodontitis	*N*	6	4			
	%	60.00	40.00			
Stage 4 Periodontitis	*N*	8	2			
	%	80.00	20.00			

*Bold values state that $P \leq 0.05$ is statistically significant; *N*: number of sample; df: degree of freedom

Table 3: Analysis of vitamin D levels and clinical parameters in the study groups

Parameters	Vitamin D levels	Periodontitis		Gingivitis		Healthy	
		N	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD
Age	Deficient (less than 80 nmol/L)	23	37.30 ± 6.88	9	35.67 ± 3.50	9	32.78 ± 3.35
	Sufficient (more than 80 nmol/L)	15	39.93 ± 8.55	23	33.17 ± 7.07	22	30.05 ± 6.02
Gingival Index	Deficient (less than 80 nmol/L)	23	2.51 ± 0.40	23	1.06 ± 0.50	22	0.18 ± 0.07
	Sufficient (more than 80 nmol/L)	15	2.23 ± 0.56	9	0.71 ± 0.36	9	0.21 ± 0.05
Clinical Attachment level	Deficient (less than 80 nmol/L)	23	4.78 ± 1.56				
	Sufficient (more than 80 nmol/L)	15	3.47 ± 1.39				



Graph 1: Distribution of mean PI, GI, PD, and CAL among normal, gingivitis and different stages of periodontitis. PI = Plaque Index, GI = Gingival Index, PD = Pocket depth, CAL = Clinical Attachment loss

Discussion

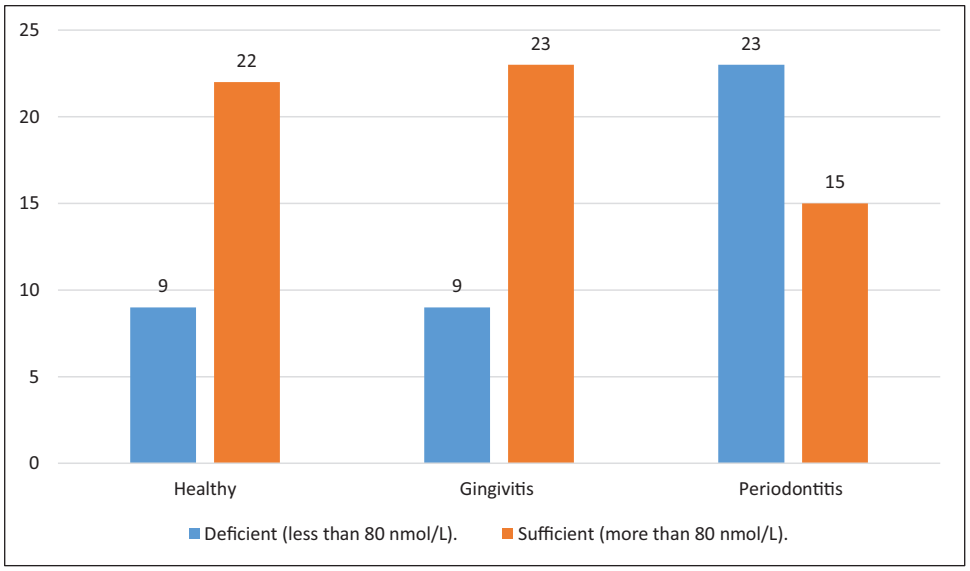
In the current study, out of 101 participants, 38 patients belong to periodontitis group, 32 patients belong to gingivitis group, and 31 patients fit in healthy group. Among these, the highest of 20 female and 18 male participants have periodontitis, whereas

Table 4: Mean comparison of PI, GI, PD among healthy, gingivitis, and different stages of periodontitis

Clinical parameters	*N*	Mean	SD	*F*	*P* value
PI					
Normal	31	0.19	0.09	70.555	**0.001***
Gingivitis	32	1.32	0.67		
Stage 1 periodontitis	8	2.19	0.60		
Stage 2 periodontitis	10	2.00	0.47		
Stage 3 periodontitis	10	2.42	0.23		
Stage 4 periodontitis	10	2.51	0.42		
GI					
Normal	31	0.18	0.06	107.238	**0.001***
Gingivitis	32	0.96	0.48		
Stage 1 periodontitis	8	2.35	0.50		
Stage 2 periodontitis	10	1.96	0.50		
Stage 3 periodontitis	10	2.59	0.23		
Stage 4 periodontitis	10	2.45	0.60		
PD					
Normal	31	1.66	0.65	33.858	**0.001***
Gingivitis	32	2.37	0.52		
Stage 1 periodontitis	8	3.16	0.45		
Stage 2 periodontitis	10	4.52	0.72		
Stage 3 periodontitis	10	6.55	1.65		
Stage 4 periodontitis	10	6.94	1.39		

Bold values state that $P \leq 0.05$ is statistically significant; *N*: number of sample; SD: standard deviation

PI = Plaque Index, GI = Gingival Index, PD = pocket depth, CAL = clinical attachment loss



Graph 2: Distribution of number of participants with deficient and sufficient vitamin D levels among all the groups

18 female and 14 male participants have gingivitis condition and 17 female and 14 male participants are healthy participants. The findings of this study analyzed the vitamin D levels among participants, vitamin D level is sufficient for gingivitis and healthy group than periodontitis in both males and females. However, when compared to male, the female has an equal level of sufficient and deficient level of vitamin D level among periodontitis group. However, the vitamin D level is sufficient for females in both gingivitis and healthy group. This evidenced that the vitamin D level is less among the periodontitis group of patients. This finding is consistent with the findings seen in periodontitis studies of Bhargava *et al*.[23] Their study observed a statistically significant relationship between 25(OH) level and periodontal parameters like GI, PPD, and CAL with patients showing overall low levels of serum vitamin D with chronic periodontitis suggesting that vitamin D supplementation may have a beneficial effect in periodontal pathology opening

new treatment approach for periopathology and Laky *et al*.[24] in their research revealed a strong relation between serum 1,25(OH)2D level and periodontal health status; in that subjects with a low 1,25(OH)2D were more likely to belong to the periodontitis group (OR = 0.97, 95% CI = 0.95–1.00).

The community health strategy's primary objectives include assisting with dental health examinations and disease prevention. Jagelavičienė *et al.*,[17] in their review, focused on vitamin D and periodontal pathology proposing that vitamin D brings about the suppression of the destructive effect of various microbes in periodontal tissue thus preventing chronic periodontists to the larger extent. It also maintains the homeostasis between the systemic and jaw bone density making it a vital treatment for option of patients with periodontitis. However, there is a dearth of research in the area of gingivitis, periodontitis, and low vitamin D levels affecting the community's health.[14-16] There has been no direct research in the literature that has looked at the link between periodontitis and vitamin D in the Indian population although there are investigations carried out on periodontal disorders in diabetes or obesity groups that are at high risk.[18,21,23] Similarly, most of the gingival research focused on exploring gingival diseases in general in worldwide and India. This emphasizes the significance of this research on the relationship between periodontitis, gingivitis, and vitamin D levels.[11,13,22]

Antonoglou *et al*.[25] conducted study among Finnish adults, observed no link between periodontitis severity and vitamin D level. However, the absence of correlation was primarily due to the Finnish population's overall lower vitamin D levels. Further, this study analyzes the vitamin D levels among the age of the participants, it is noted that both gingivitis and healthy group of people have deficient vitamin D levels across participants' age. This evidenced that vitamin D levels is not sufficient among periodontitis group of patients than other two group of aged patients. This shows that the periodontitis is significantly related to sufficient levels of vitamin D among periodontitis patients. This is consistent with our study findings suggesting that vitamin D insufficiency may play a role in the development and progression of periodontal disease. With respect to the Plaque Index across age groups, periodontitis, and healthy patients have more deficient scores than gingivitis group. On the pocket depth across age groups, all the groups have high deficient scores. In terms of gender, the score of Plaque Index was high in male among both gingivitis and healthy group of patients. The score of Gingival Index was high in females among periodontitis and healthy patients. The score of pocket depth was high in females among periodontitis and gingivitis patients. Increased vitamin D-binding protein levels in plasma have been linked to severe periodontitis. , which is confirmed by the research conducted by Hiremath *et al.*[26] Milan *et al*.[27] proposed that the gingivitis score changes in direct proportion to the dosage of vitamin D supplementation.[28] They observed that gingival score (baseline) every month comparing the baseline gingivitis score with later visits after the patient was given the oral vitamin D supplementation dosages from 500 to 2000 IU with results better with higher dosages ($P < 0.0001$). There is no clear evidence that vitamin D can prevent periodontitis or that this association is the cause. On the other hand, improving vitamin D deficiency with supplements and diets can have a significant positive impact on periodontal disease and the prevalence of periodontal disease. Vitamin D supplementation as a prophylactic approach may be an economical and safe technique to reduce the prevalence of periodontal disease. In addition, future long-term clinical studies using follow-up designs to investigate the effects of vitamin D supplementation on the prevention of inflammatory collapse of periodontal and gingival tissues may be needed. Researchers can investigate the feasibility and optimal dose of vitamin D supplementation and its impact on the prevention of periodontitis and gingivitis. Studies carried out by Jilani *et al*.,[29] Marian *et al*.,[30] Guo *et al*.[31] focused on the point that genetic background of the population can be a determinant of the discrepancy associated with vitamin D deficiency and periodontitis. Severe or persistent periodontitis in families of different generations indicates a genetic impact on the risk of periodontitis. The molecular mechanism that links vitamin D deficiency to periodontitis is based on the fact that vitamin D deficiency contributes to the progression of periodontitis, osteoporosis, decreased bone density, and the development of jawbone resorption. Vitamin D deficiency, on the other hand, not only reduces the risk of periodontitis, but also improves wound healing after periodontal surgery, increases the antibacterial resistance of the gingival epithelial cells, and reduces gingival inflammation. Our investigation highlighted the fact that optimal vitamin D levels can be established for a relatively low cost, this is one of the strengths of our study. To achieve optimal results for periodontal treatment, future studies can investigate the plasma vitamin D levels required before starting periodontal treatment. There were some limitations to this investigation. The sample size was limited due to the case-control design, and some parameters related to blood vitamin D levels, such as patient's physical

activity and socioeconomic status, were not included in the study. However, all individuals were recruited from the same outpatient facility to ensure sample homogeneity.

Conclusion

Vitamin D deficiency is linked to periodontitis, high levels of gingivitis, and overall good health in adults. This study's findings suggest that chairside pre-coated with 25-OH Vitamin D antigen rapid test kits can be considered as a viable alternative to check vitamin D levels when compared to expensive, time-consuming, and laborious lab methods. The severity of periodontitis was likewise linked to the amount of vitamin D in the individual's blood. A larger, nationwide long-term follow-up study is needed to screen for reduced vitamin D levels in patients with periodontitis and gingivitis to validate the findings of this study. Furthermore, further research into the factors of periodontal and gingival disease, as well as the possibility of recommending vitamin D supplementation, the appropriate dosage, and the effectiveness of preventing and reducing periodontitis and gingivitis may be useful.

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Conflicts of interest

There are no conflicts of interest.

Authors contributions

Pradeep Koppolu, Lingam Amara Swapna: Conceptualization, methodology, formal analysis, data interpretation, writing the original draft. Abdullah M. A. Alshahrani, Mohammad A. Y. Ghawas, Mohammed S. A. Almuqbil, Abdulmalik K. H. Almuhaydib: Data collection. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Ethical policy and Institutional Review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of College of Dentistry, Dar Al Uloom University, Riyadh, KSA (COD/IRB/2020/29) on February 22, 2021.

Patient declaration of consent

The author certify that she have obtained all appropriate patient consent forms. The patients have indicated in the form that they are comfortable with the publication of their clinical data in the journal. The patients are aware that their names and initials won't be published and that reasonable measures will be taken to keep their identities a secret.

Data availability statement

Data set available on request.

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