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Effect of Vitamin D Supplementatiton Against of Periodontal Pocket Depth Changes and Clinical Attachment Loss in Healing Process of Chronic Periodontitis Disease

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Abstract
Introduction: Vitamin D can be beneficial in the treatment of chronic periodontitis as an antibacterial and antiinflamation effect. Its act to elliminate periodontopatogen bacterial and inhibit the release of proinflammatory mediators by immunomodulator action of innate and adaptif immunity to resolve the inflamatory process of the periodontal disease. This study was aimed to evaluate the effect of vitamin D suplementation towards the levels of vitamin D in the blood, as well as to determine periodontal pocket depth (PPD), and clinical attachment loss (CAL) in healing process on chronic periodontitis disease. **Material & Methods**: A total of 45 individuals of chronic periodontitis were colected and randomized divided into three groups. vitamin D suplementation (placebo, 5,000 IU and 10,000 IU) was gave blindly one tablet per week within 6 weeks to all of the subject after received scaling and root planing (SRP) treatment. All the measurement like periodontal clinical parameters using a periodontal probe and serum vitamin D levels using the ELISA (Enzyme Linked Immunosorbent Assay) technique were noted before and after SRP treatment. **Results:** the results showed that there were significant differences of all clinical periodontal parameters in each treatment group compare to placebo group ($p < 0.05$) but between 5,000 IU and 10,000 IU group showed that there were no significant different ($p>0.05$). **Conclusion:** Vitamin D suplementation at 5,000 IU can supported in chronic periodontitis treatment which provide a new direction in management of periodontal treatment and healing process more rapidly.
Keywords: Chronic Periodontitis; Vitamin D Suplementation ; CAL ; PPD

1. Introduction

Periodontitis is an infectious disease caused by specific microorganism of periodonto patogen bacterial that causes inflammation and progressive destruction of periodontal tissue (Novak and Novak, 2012). Inflammation comes from untreated gingivitis, and if the process continues, it can invade tissues beneath the tooth structure and will form a periodontal pocket, periodontal ligament damage that leads to clinical attachment loss and alveolar bone resorption and finally make tooth loss (Banjar and Alshamman, 2014).

In Indonesia some of studies reported that measured levels of serum vitamin D on the Indonesian people have vitamin D deficiency (< 20 ng/ml), although Indonesia is a country which gets many sun exposure but the pattern of life Indonesia people is likely to avoid the sun, using sunblock and daily intake of vitamin D from diet is still lacking (Oemardi et al, 2007; Setiati, 2008; Yosephin et al, 2014).

Vitamin D may play a role as an immunomodulator by suppress function of immune overload (Bartley, 2010). This suggests that vitamin D may be helpful in the treatment of chronic periodontitis, because not only a direct effect of the bones metabolism and teeth but also because as an antibacterial effect against periodontopatogen bacterial and antiinflammatory effect by inhibiting the release of proinflammatory mediators that contribute to the periodontal destruction (Amano et al, 2009). Therefore, vitamin D may be considered as potential supplement that provides a beneficial effect in the wound healing process of periodontal tissues (Bashutski et al, 2011).

2. Material and Methods

The reseach which carried out was pre and post test randomized single blind controlled trials with the aim to determine the effect of vitamin D supplementation on serum levels of vitamin D, periodontal pocket dept (PPD) and clinical attachment loss of teeth (CAL) in the healing process of chronic periodontitis. The subject of this research is that adults (male and female) aged 31 to 55 years old who suffer from chronic periodontitis and visited the Periodonsia Installation of FKG USU in June-August 2016.

Inclusion criteria: adult 31 to 55 year old as new patients diagnosed with chronic periodontitis, who willing to do of periodontal tissues examination as well as a blood sample, that signed by informed concern. Exclusion criteria: still in surgical or non surgical periodontal therapy during last 6 months, still undergoing orthodontic treatment, had a history of systemic diseases (Diabetes, Hypertension, Heart, Kidney, Liver) or systemic conditions else that can affect the treatment and on compliance subject, direct sun exposure more than 3

hours a day, consuming nutritional supplements (Estrogen; biphosphonate, fat soluble vitamins) more than six months, taking antibiotics or antiinflammatory drugs, steroids, and anticonvulsant drugs, as well as have smoking habit.

A total of 45 subjects were collected and divided into three groups consisting of 15 people. Group A were given Placebo, group B were given of 5.000 IU vitamin D, while Group C was given 10.000 IU vitamin D. Before intervention (scaling and root planning/SRP treatment and vitamin D suplementation) the blood of subjects was taken as much as 2 cc. After SRP the PPD and CAL were measured as the initial measurement, and then given vitamin D supplements as much as 1 tablet per week for six weeks in blinded. After six weeks suplementation, vitamin D levels, PPD and CAL were measured again. All patients were informed how the maintenance of oral hygiene at home, including how to brush teeth properly.

2.1 Material

Questionnaires, stationary, periodontal probe, Periodontal status assessment sheet, diagnostic set (mirror, explorer, pinset, nierbeiken), Disclosing agent, Ultrasonic scaler, polishing set, Disposable Syringes for taking blood, Torniquet, alcohol swab, plester, ELISA kit Vitamin D (Immunotek; merk DBC, Canada) with 96 wells, Sentrifuge, Vortex, Micropipet single (1000 µl, 100 µl, 20 µl, 200 µl) and multiple, Eppendorf tube, Freezer -80 ^{o}C, Plate shaker incubator, Automatic strip washer, Parafilm sheet, and Microwell plate absorbance reader.

2.2 Methods of Collecting Data

Scaling and root planing/SRP treatment was done before periodontal measurement to eliminate supra and subgingiva calculus so the measurement could be easy to do and to avoid bias. The periodontal measurement technique by probing around the gingival sulcus using periodontal Probe University Of North Carolina/UNC) # 15 on 6 (six) sides per tooth is mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, distolingual) and selected the teeth that represent the worst surface from the sixth sextant. The way to probe for pocket depth is by inserting the probe with light pressure into the pocket in parallel with the long axis of the tooth, "move" in the circumferensial around the surface of each tooth to detect the deepest penetration areas and keep the probe to contact with the surface teeth until any resistance is felt. Probing conducted from interproximal distal and mesial teeth of vestibular surface, then continued on interproximal distal and mesial of oral surface, once that is done in the middle of the teeth on the the vestibular and oral surface (Jacob, 2011). Pocket depth/PPD can be read on the calibration prob. Pocket criteria: Mild = 3 to 4 mm, Moderate = 5 mm, severe = > 6 mm. CAL measurement is calculated from the distance between the cemento enamel junction to the base of periodontal pocket. CAL criteria: Mild = 1 to 2 mm, Moderate = 3 to 4 mm, and Severe = > 5 mm. PPD and CAL measured by 3 (three) examiner (Periodontist) which had been calibrated before the study begin (Holtfreter et al, 2015)..

Serum levels of vitamin D 25(OH)D was measured before vitamin D supplementation and after six (6) weeks intervention. As much as 2 cc of blood taken and centrifuged at 3000 rpm for 15 minutes to obtain serum samples and then stored in a freezer at -80 °C. Serum level of vitamin D was measured by using ELISA (Enzyme Linked Immunosorbent Assay) technique. A total of 25 mL of solution calibrators, controls, and serum samples were inserted into the well using a micropipette tips yellow. After that a total of 150 mL of incubation buffer inserted into each well using a micropipette multi chanel. All wells closed with notepad, dark microplate coated aluminum foil lid and then incubation above the plate shaker incubation for 60 min at 37 ° C and speed of 400 rpm. Wells washed with 300 mL of wash buffer for 3 times using automatic flowed strip washer. A total of 150 mL of conjugate working solution was added to the wells and cap back all the wells with a notepad, dark microplate lids and coated aluminum foil and incubated again on plate shaker incubation for 30 min at 37 °C and a speed of 400 rpm after that wells were wash again. A total of 150 mL of TMB substrate was added to the wells. Incubation back over the plate shaker incubation for 15 min at 37 °C with a speed of 400 rpm. Add 50 mL solution of stopping solution in each well and knocked the well plate so that no bubbles. Measuring of each absorbance wells using a microplate reader tool at wavelength of 450 nm.

Analysis Of Data

Data were presented as means ± standard deviation (SD) for each investigated parameter. Kruskal Wallis test for multiple comparisons was used to compare differences between groups while to compare before and after supplementation of vitamin D each intragroup using the Wilcoxon test. The significance level was set at $P < 0,05$. All statistical analyses were performed employing the SPSS software version 16.0 for Windows software system.

3. Result

The demographic data in table 1 showed that concerning gender distribution from 45 subjects resulted there were no significant differences between group A, B, and C regarding gender. The recruitment age categories showed the following percentages of subjects: 30 to 40 : 24,44%, 41 to 50 about 60,00%, and age > 50 : 15,56%. It means that the mean ages of subjects did not differ between the three groups.

Table 1. Demographic data

Variable	Intervention							
	Group A Placebo (n = 15)		Group B Vit D 5.000 IU (n = 15)		Group C Vit D 10.000 IU (n = 15)		Total	
	n	%	n	%	n	%	N	%
Gender								
Male	2	13.30	1	6.7	1	6.7	4	8.89
Female	13	86.7	14	93.3	14	93.3	41	91.11
Age								
30-40	1	6.7	8	53.3	2	13.3	11	24.44
41-50	11	73.3	6	40.0	10	66.7	27	60.00
>50	3	20.0	1	6.7	3	20.0	7	15.56

The average distribution of parameters PPD, CAL, and 25(OH)D at baseline from three group before intervention were presented on Table 2.

Table 2. Baseline comparison of parameters in three groups

Variable	Group A (Placebo)	Group B Vitamin D 5.000 IU	Group C Vitamin D 10.000IU	*P-value*
	Mean ± SD	Mean ± SD	Mean ± SD	
PPD (mm)	4,26 ± 0,35	4,38 ± 0,29	4,47 ± 0,19	0,093
CAL (mm)	5,91 ± 0,63	5,70 ± 0,53	5,78 ± 0,42	0,566
25(OH)D(ng/ml)	35.94 ± 18.22	42.58 ± 21.58	41.83 ± 18.75	0.585

There was no statistically significant differences in all parameter of periodontal measurment (PPD, CAL) and serum level of vitamin D 25(OH)D (P value > 0,05). The average distribution of PPD including moderate criteria (respectively > 4mm) while the CAL including severe criteria (respectively > 5 mm). Beside of that The average distribution of 25(OH)D in three group including insufficient vitamin D criteria (< 50 ng/ml).

Analysis of the differences vitamin D levels before and after the intervention are shown in Table 3 (*: P-value Significant).

Table 3. Average Levels of Vitamin D (ng/ml) Intragroup Comparison Before and After Intervention

Levels of Vitamin D (ng/ml)	n	Mean ± SD	P-value
Group A. Placebo			
Before	15	35,95 ± 18,22	
After	15	21,36 ± 12,96	0,004*
Group B. 5.000 IU			
Before	15	42,58 ± 21,57	
After	15	55,49 ± 23,67	0,001*
Group C. 10.000 IU			
Before	15	41,83 ± 18,76	
After	15	63,29 ± 16,32	0,001*

The intragroup comparison of level 25(OH)D parameter in Group A, group B, and group C (Table 3) showed that there was statistically significant difference after six weeks (P < 0,05). Levels of Vitamin D in the placebo group had a significant reduction (deficiency), whereas in the group of 5,000 IU and 10,000 IU group had an increasing of levels vitamin D that significantly be optimal (51 to 100 ng / ml).

Figure 1 represent the intergroup comparison of 25 (OH)D before and after six weeks.

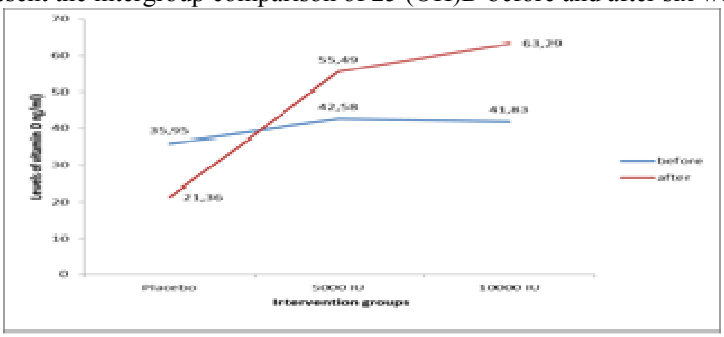

Figure 1. Graph of Comparison of Level 25(OH)D intragroups Before and After Intervention

Based on figure 1 above, levels of group 10,000 IU vitamin D increased of 21.46 ng/ml, higher than the group of 5,000 IU which increased by 12.91 ng/ml. However, vitamin D levels in the placebo group was

decreased of 14.59 ng/ml.

Table 4. Average of PPD (mm) before and after intervention. (*: P-value Significant)

PPD	n	Mean ± SD	P-value
Group A. (Placebo)			
Before	15	4,26 ± 0,35	0,001*
After	15	2,96 ± 0,36	
Group B. (5.000 IU)			
Before	15	4,38 ± 0,29	0,001*
After	15	2,64 ± 0,44	
Group C. (10.000 IU)			
Before	15	4,47 ± 0,19	0,001*
After	15	2,54 ± 0,81	

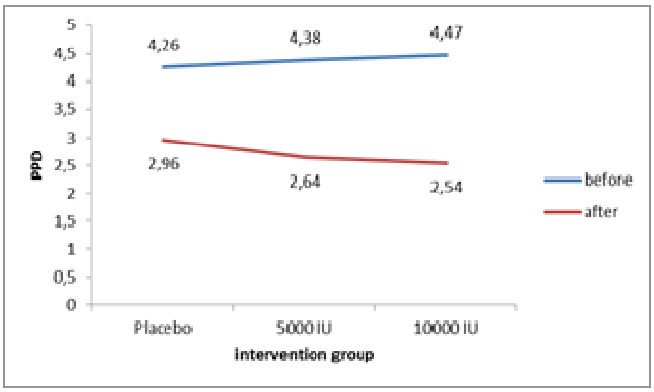

Figure 2. Graph of Comparison of PPD intragroups Before and After Intervention

Based on table 4 above there was statistically significant difference in the average value of PPD each of the groups I, II, and III after six weeks (P<0.05). Based on the graph Figure 2 above show pocket depth (PPD) was highest decline in the group of 10,000 IU of 1.93 mm, then 5,000 IU group reached 1.74 mm, while the PPD in the placebo group has smallest decline of 1.3 mm.

Table 5. Average of CAL (mm) before and after intervention (*: P-value Significant)

CAL	n	Mean ± SD	P-value
Group A. (Placebo)			
Before	15	5,91 ± 0,63	0,001*
After	15	4,27 ± 0,81	
Group B. (5.000 IU)			
Before	15	5,70 ± 0,53	0,001*
After	15	3,25 ± 0,83	
Group C. (10.000 IU)			
Before	15	5,78 ± 0,42	0,001*
After	15	2,93 ± 0,68	

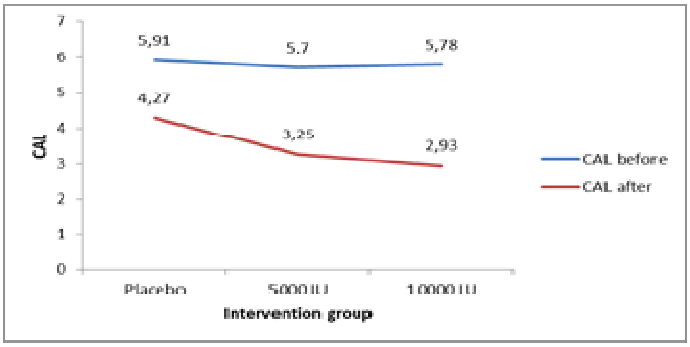

Figure 3. Graph of Comparison of CAL intragroups Before and After Intervention

Based on the table 5 above there was statistically significant difference in the average value of CAL each of the groups I, II, and III after six weeks (P<0.05). The largest decline CAL occurred in the group's 10,000 IU was 2,85 mm, in group of 5,000 IU of 2.45 mm while the lowest decline occurred in the placebo group of 1.64 mm.

The average distribution of parameters PPD, CAL, and 25(OH)D post intervention from three group were presented on Table 6 below.

Table 6. Intergroup comparison of parameters after 6 weeks post intervention (*: P-value Significant)

Variabel	Placebo	5.000 IU	10.000 IU	P-value
	Mean ± SD	Mean ± SD	Mean ± SD	
PPD (mm)	2,96 ± 0,36	2,64 ± 0,44	2,54 ± 0,81	0,052*
CAL (mm)	4,27 ± 0,81	3,25 ± 0,83	2,93 ± 0,68	0,000*
25(OH)D(ng/ml)	21,36 ± 12,96	55,49 ± 23,66	63,28 ± 16,31	0,000*

Table 6 showed that there was statistically significant difference after six week post intervention of parameters PPD, CAL, and level of vitamin D (P<0,05). Statistical test results using *mann whitney test* showed that there was a significant difference of PPD, CAL, and the levels of vitamin D among the placebo group against group of 5.000 IU and 10.000 IU (P<0.05) but there was no significant differences between groups of 5,000 IU and 10,000 IU (P>0.05). The result of the research showed that both vitamin D 5.000 IU and 10000 IU equally contribute the same effect.

4. Discussion

According to the Dietary Reference Intakes (DRIs) and the Food and Nutrition Board (FNB) dose of 800 IU per day or 5000 IU per week is a safe dose for maintenance while the dose of 1200-2000 IU per day or 10,000 IU per week was a dose initial therapy in imflamation diseases (Ross et al, 2011). The result of this research showed between vitamin D is 5.000 IU and 10.000 IU giving the effect significantly did not differ to the improvement changes of pocket depth (PPD) and clinical attachment loss (CAL).These results indicate that lower dose of 5.000 IU is recommended in order to avoid side effects when given for the long term. This results was differ with Garcia study (2011) which gives a 400 IU vitamin D supplement combination of calcium 500 mg in patients with chronic periodontitis. It showed that there was no significant difference despite has swallow reduction of pocket depth, less gingival bleeding, as well as improvements of attachment loss but statistically showed not different.14 Anand et al, (2013) suggests that the intake of vitamin D 10,000 IU per week for 6 months is recommended and does not cause toksic.

The average of PPD and CAL both intra and inter intervention group showed a significant difference. Reduction of pocket depth (PPD) in the placebo group was 1.3 mm, smaller than the group of 5.000 IU of 1.74 mm and a the largest decline of PPD in the group 10.000 IU of 1.93 mm. Reduction of clinical attachment loss (CAL) in the placebo group is only 1.64 mm in, compared with group of 5.000 IU of vitamin D of 2.45 mm and a the largest decrease of CAL is group of 10,000 IU of vitamin D by 2,85 mm. It showed that the average improvement of PPD and CAL in the intervention group Vitamin D 5.000 IU and 10.000 IU higher than placebo group. In contrast to the meta-analysis study of Smiley et al, (2015) reported that maintenance therapy of SRP without additional therapy produce improvements of CAL only 0.5 mm while with the additional therapy (antibiotics, chlorhexidine, minocycline) resulting improved CAL slightly better 0.2 to 0 , 6 mm.16 it's means that adjuvant therapy with vitamin D is better than the other additional therapies because resulting in greater improvement of CAL and PPD.

The research of Bashutski et al (2011) reported that levels of vitamin D associated with the success of post surgery. Patients who severe chronic periodontitis with high levels of serum 25(OH)D > 50 ng/ml before having periodontal surgery reported had an improvement of clinical attachment and reduction in pocket depth within 12 months after surgery, whereas subjects who have deficiency of vitamin D did not show better benefits. According to Webb (2006) that vitamin D supplements was a source of vitamin D is that more effective and safer than sun exposure because of the detrimental effects caused by ultraviolet B radiation. It is in line with the statement of Kauffman (2009) that vitamin D supplementation is able to raise the level of serum 25-hydroxyvitamin D more adequate.

In addition to SRP therapy, systemic conditions like the body's immune response affecting in the successful of periodontal treatment (Eaton and Ower, 2015). Vitamin D deficiency thought to be associated with decreased innate and adaptive immune system so the body vulnerable againts to the inflamation (Antonoglou, 2015). Inflammation in chronic periodontitis triggered by proinflammatory cytokines IL-1β, IL-17, IL-6 and TNF-α that enhance the development of alveolar bone resorption through osteoclastogenesis process that characterized by increased expression of RANKL. Effect of vitamin D [1,25 $(OH)_2D_3$] in the adaptive immune system is characterized by the turn of the proinflammatory effects Th1 / Th17 to be antiinflammatory effect of Th2 / Treg which lowers proinflammatory cytokines and increase the amount of IL-10, IL-4 and IL-13 so

Porphorymonas gingivalis bacteria could be eliminated or called intraceluler clearance bacteri (Hewison, 2012). In the innate immunity vitamin D [25(OH)D] will reduce inflammation because it induces the release of LL-37 molecule Cathelicidin and Defensin (Cannel et al, 2014). Cathelicidin can repair to the damage in chronic periodontitis through the vitamin D signaling (Schauber et al, 2007).

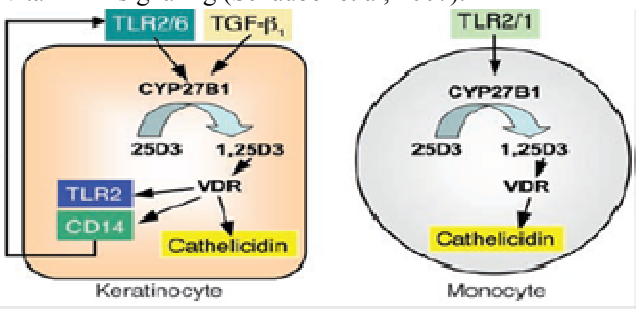

Figure 3. *Vitamin D Signaling* in repair fuction of healing process
(Schauber et al, 2007)

Active vitamin D [1,25$(OH)_2D_3$] that bind to vitamin D receptors, especially in keratinocytes and monocytes cells will initiate an antibacterial response. TLR2 receptors on the surface of specific cells to recognize negative and gram positive bacteria such as Peptidoglycan and Lipoteichoid acid molecules whereas TLR1 and TLR6 will recognize Triacetylated and Diacetylated Lipopeptide. This condition will lead to an increase the number of CYP27B1 receptor as forming vitamin D inside the cell, and add to increase the signal amount of 1,25 $(OH)_2D_3$ in the cell. Bonding of vitamin D and vitamin D receptor (VDR) in the keratinocyte cell would trigger TLR2 and CD14 to produced more to increase the recognition of the bacteria, so that the repair process happens faster. But in monocytes cell addition of 1,25$(OH)_2D_3$ does not trigger receptors TLR2/1, but vitamin D inhibits the expression of TLR2/1 more and trigger hiporesponsif of pathogenic bacteria (Schauber et al, 2007).

In addition to keratinocytes cells and monocytes, another cells involved in the healing process in periodontal tissues include fibroblast cells, platelets, macrophages, neutrophils, endothelial cells, B cells and T cells. Inside the fibroblast cells, platelets, macrophages, lymphocytes, and keratinocytes there are Transforming Growth Factor (TGF-β1), which triggers the proliferation, migration, and the synthesis of extracellular molecules TGF-β. It also trigger the enzyme CYP27B1 to convert 25(OH)D into the active form 1,25$(OH)_2D_3$ which binds to vitamin D receptor (VDR) in cells thereby increasing the production of Cathelicidin as antimicrobial protein that acts as an innate immunity (Hakkinen et al, 2012).

5. Conclusion

Finally, the conclusions from this study was 5,000 IU of vitamin D may have an effect and are very helpful in reducing pocket depth and clinical attachment loss of teeth so could support the healing process after SRP treatment for chronic periodontitis patients because of its function as an antibacterial and antiinflammatory.

References

Amano, Y., Komiyama, K., dan Makishima M., 2009. Vitamin D and periodontal disease, *Journal of Oral science,* Vol.51(1) : 11 - 20

Anand, N., Chandrasekaran, C., Rajput, NS., 2013. Review article : Vitamin D and periodontal health:Current concepts, *Journal of Indian Society of Periodontology* - Vol 17 : 302 – 8

Antonoglou G, 2015. Vitamin D and Periodontal Infection, faculty of medicine, University of Oulu. Finlandia : 1 – 78

Banjar W. dan Alshamman, M H., 2014, Genetic factors in patogenesis of chronic periodontitis. *Journal of Taibah University Medical Science*.

Bartley J., 2010. Vitamin D: emerging roles in infection and immunity, Expert Rev. Anti Infect. Ther. Vol.8(12) : 1359 - 69

Bashutski JD., Eber, RM., Kinney, JS., Benavides, E., Maitra, S., 2011. The impact of vitamin D status on periodontal surgery outcomes. *J Dent Res* 90: 1007 – 1012

Cannel JJ, Grant WB, Holick MF. 2014. Vitamin D and inflammation. *Dermato-Endocrinology,* Vol.6:1

Eaton K dan Ower P, 2015. Textbook of Practical periodontics, Elseviers : 96 – 105

Garcia, MN., Hildebolt, CF., Miley, DD., Dixon, DA., Couture, RA., 2011. One-year effects of vitamin D and calcium supplementation on chronic periodontitis kronis. *Journal of Periodontol*, Vol.82, : 25 – 32

Hakkinen L, Larjava H, Koivisto L, 2012. Granulation tissue formation and remodeling, dalam Text book of Oral /Wound Healing : Cell biology and clinical management, Wiley Blackwell Pondicherry, India : 146-55

Heaney RP, Davies KM, Chen TC, Holick MF, dan Lux MJ. 2003. Human serum 25-hydroxyxholecalciferol response to extended oral dosing with cholecalciferol. *Am Journal Clin Nutr,* Vol.77,: 204 – 10

Hewison M, 2012. Review article : An update on vitamin D and human immunity. *Clinical endocrinology.* Vol. 76(3), : 315 - 325

Holtfreter B, Albandar JM, Dietrich T, dkk.,2015. Standards for reporting chronic periodontitis prevalence and severity in epidemiologic studies. *Journal of clinical periodontology*, Vol.42, : 407 - 412.

Jacob S, 2011. Measuring periodontitis in population studies : a literature review. *Rev.Odonto Cienc*. Vol.26(4) : 346 - 354

Novak KF. dan Novak MJ., 2012. Chronic periodontitis dalam Textbook of Carranza'Clinical Periodontology. 11^{ed}. : 160 - 4

Oemardi M, Horowitz M, Wishart JM, Morris HA, Need AG, Loughlin PD, Nordin EC. 2007. The effect menopause on bone mineral density and bone-related biochemical variables in Indonesian women. *Clinical Endocrinology*, Vol.67 : 93 - 100

Ross C, Taylor CL, Yaktine AL, Valle HBD, (2011). Dietary reference intakes : Calcium, Vitamin D, *Institute of Medicine of the national academies*, Washington : 243 - 50.

Schauber J, Dorschner RA, Coda AB, Buchau AS. dkk, 2007. Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism, Vol.117(3) : 803 – 11

Setiati, 2008. Pengaruh pajanan sinar ultraviolet B Bersumber dari sinar matahari terhadap Konsentrasi vitamin D (25(OH)D) dan Hormon paratiroid pada perempuan usia Lanjut Indonesia, Jurnal Kesehatan Masyarakat Nasional Vol. 2(4) : 147 – 53

Smiley CJ, dkk.,2015. Systematic review and meta-analysis of the nonsurgical treatment of chronic periodontitis by means os scaling and root planning with or without adjuncts. *Journal of American Dental Association*, Vol 146 (7)

Yosephin B, Khomsan A, Briawan D, dan Rimbawan. 2014. Peranan ultraviolet B sinar matahari terhadap status vitamin D dan tekanan darah pada perempuan usia subur. Jurnal Kesehatan Masyarakat Nasional Vol. 8 (6) : 256 – 60