

Investigate the Relationship between Vitamin D3 and Cathelicidin with Tooth Decay Caused by Streptococcus Mutans

Teba Saddam Abdulhadi¹, Osama Nadhom Nijris²

^{1,2}Department of pathological analysis, College of applied sciences, University of Samarra/Iraq

Email: usama.n@uosamarra.edu.iq

Abstract

The study included the collection of 20 blood samples from people with teeth decay that was diagnosed and the causing was the bacterial type *Streptococcus mutans* and 20 blood samples of healthy people, the level of vitamin D3 and the Cathelicidin that called LL37 peptide was measured by using the ELISA technology and compared it with healthy people, and it turns out that those with tooth decay had to have teeth, the level of vitamin D3 and the LL37 is less than the normal level and there was a moral difference between them and the healthy people, and it turns out that there is a relationship between the levels of vitamin D3 and the LL37 in the body that has an important role in maintaining the oral cavity and thus reducing the occurrence of tooth decay.

Keywords: Cathelicidine, LL37, Vitamin D3, Streptococcus mutans.

1. Introduction

Vitamin D3 is a steriods hormone that is mainly obtained from sun exposure and also from food and nutritional supplements, as foods that contain vitamin D3 naturally rarely and can be found in oily fish (such as salmon, Mackerel, Herring) and fish oils (Like COD Live Oil), vitamin D3 mainly works as a hormone, and its glandular activity enhances the balance of phosphate and calcium in the blood by regulating intestinal absorption (Botelho et al., 2020; Mustafa, et al.,2020).

Also, vitamin D3 plays a major role in bone and teeth minerals, and when levels are not organized, it can lead to Rachitic Tooth, which is a term released from the incomplete mineral teeth that are severely vulnerable to breakage and decay as the teeth are organs that contain a large mineral percentage surrounded by abone, and it consists of three distinctive solid tissue, which is enamel, dentin and Cementum, and the process of removing the teeth occurs in parallel with the skeleton mineralizer (D'Ortenzio et al., 2018; Foster et al., 2014; Abdulwahed,et al.,2020).

It is worth noting what the wang (2004) has indicated, where he discovered another relationship with vitamin D3, which is its association with an immune peptide called Cathelicidin (LL37), where vitamin D3 stimulates the production of this peptide and is a member of the large ketogenic family of antimicrobial peptides called Cathelicidin that has an activity Wide -range plays an important role in the balance of the oral cavity by adjusting the content of inflammatory cytokin, and thus its cellular effects on the tissues of the tooth, which provides an appropriate environment for the formation of blood vessels and enhancing the distinction of intermediate stem cells and their migration and

limit One of the effects of inflammatory factors derived from bacteria is due to its deadly activities of other microorganisms (tokajuk et al., 2022).

The aim of our current study is to measure the concentrations of vitamin D3 and Cathelicidin LL37 of blood samples and find the relationship between the levels of Cathelicidin and Vitamin D3 and the infection of the tooth decay caused by Steptococcus mutans.

2. Materials and Methods

Sampling collection

20 samples of blood samples were taken to those with teeth decay, who were diagnosed that they have Streptococcus mutans using all traditional methods of implantation, microscopic and biochemistry diagnosis, and 20 samples of healthy people, as this was done by withdrawing 20 ml of venous blood by a medical syringe used for one time and blood placement with pipetes especially to collect blood and left the room temperature for ten minutes to be coagulated, then placed in the centrifugation device for 10 minutes and at a speed of 3000 rpm, then withdraw the serum with a microscopic and placed in clean sterile tubes, and the concentration of vitamin D3 and measuring the level of Cathelicidin concentration using the ELISA technique.

Measuring the level of vitamin D3 in the blood serum

Then take the serum samples collected and measure the level of vitamin D3 using ELISA technology, according to KIT instructions through the following steps:

ELISA technology based on the principle of competitive-ELISA has been used, where the small

ELISA plate available in this group was used in advance with vitamin D3, where during the reaction vitamin D3 is competing in the sample or standard with a fixed amount of vitamin D3 to support the solid phase of the sites in the detector the vital anti-vitamin D3 body is washed, the unrelated sample is washed, and the Avidin-Horseradish Peroxidase (HRP) is added to each small plate pits and is incubated, and then the Substrate TMB solution is added to each hole, and then the enzymatic reaction is finished by adding the stop and converting solution. The color from the blue to yellow, then the optical density density (OD) is measured by a spectrum of 450 nm ± 2 nm.

Measuring the level of LL37 concentration in the blood serum:

The blood serum samples and the use of the ELISA technology were taken with the LL37 peptide and this was done according to the instructions on the kit as follows:

ELISA technology based on the Sandwich-ELISA principle has been used, where the small Elisa plate available in this collection is painted with an anti-Human LL-37 anti-37 body, HUMAN LL-37 and Avidin-Horseradish Perxidase (HRP) respectively to each plate pits and embraced, then the rest of the

ingredients are removed by washing and then add the substrate solution to each hole, as only those pits containing Human LL-37, Anti-biological and Avidin-HRP body will appear in blue, then the enzymatic reaction is ended by adding a stop solution and the color turns yellow, and the optical density (OD) is measured by a spectrum length of 450 nm ± 2 nm. The OD value is suitable for the Human LL-37 concentration, after which the Human LL-37 concentration is calculated in the samples by comparing OD for samples with a standard curve.

3. Results and Discussion

The level of vitamin D3 and the LL37 was measured for twenty samples for people who suffer from tooth decay and twenty samples for healthy people using the ELISA technology, It was divided into two groups, the first group A which includes samples for people with tooth decay and the second group B which includes samples for healthy people, was the level of vitamin D3 and the LL37 peptide for people with tooth decay in the first group less than the normal level, while the second group was the levels within the normal rate and after the statistical analysis was conducted, the results were shown in Table 1.

Groups Parameters	Mean ± SD		F- value	P - value
	A	B		
VD3	9.15±7.125	27.00±4.091	94.403	0.0001*
LL37	5.20±2.042	14.00±2.317	162.402	0.0001*

* p ≤ 0.01

Table 1 above shows a moral height at the VD3 level in Group B compared to Group A and as in Figure 1:

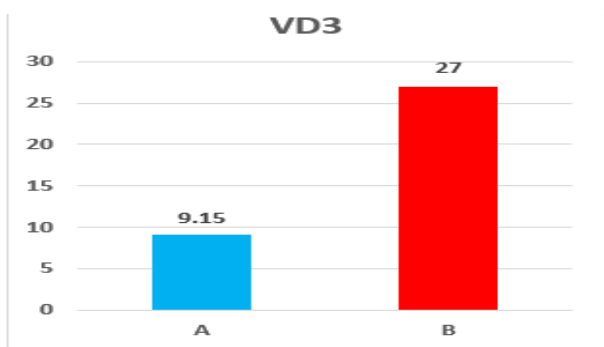


Figure 1:- Comparing the level of vitamin D3 between Group A and Group B

Also, Table 1 above shows the presence of a moral height at the LL37 level in Group B compared to Group A and as in Figure 2:

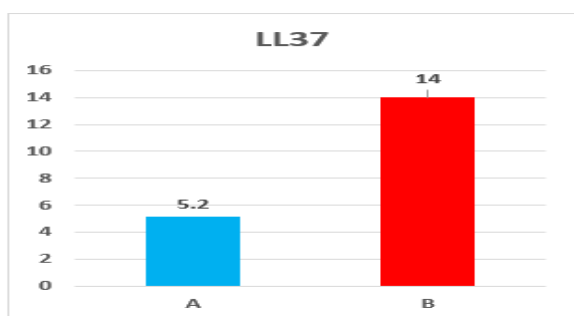


Figure 2: - Comparison of the level of the LL37 between Group A and Group B

Group A and Group B

Vitamin D3 plays a major role in regulating the level of calcium and phosphorus in the teeth, and therefore the deficiency of vitamin D3 leads to an abnormal mineralization of the teeth and affects the development of the teeth, and this is what was found in the results of our current study, as it was found that people who suffer from tooth decay have levels of vitamin D3, and this is what came in accordance with what Almoudi *et al.*, (2021) mentioned that It was found that young children and adults with vitamin D3 deficiency in the blood suffer from higher tooth decay compared to individuals who have adequate levels of vitamin D3 in the blood, Where vitamin D3 supplements have been associated with a 47 % reduction in cavities, in addition to that, the levels of vitamin D3 in the blood that exceed 40-30 ng / ml., And Gyll *et al.*, (2018) confirmed in its results, the importance of vitamin D3 to the teeth, as it concluded that the relationship between vitamin D3 and caries is an inverse relationship. The higher the vitamin D3 in the body, the more tooth decay.

And Al-Samarraie, *et al.*,2021 ; Saputo *et al.*, (2018) made a test of Alfacalcidol or Calcitriol or Doxercalciferol (It is vitamin D3 analogues) has the ability to prevent the formation of the biofilm, as these threes have shown the ability to prevent the formation of the biofilm of *Strep.mutans* and thus

reduce the occurrence of tooth decay, and Confirm this Almoudi *et al.*, (2021) has proven that the treatment of *Strep. mutans* with vitamin D3 led to major changes in the bacterial cell by making damage to the biofilm, and Swapna and Abdulsalam (2021) has found vitamin D3 plays a major role in oral health, so individuals need to ensure a balanced diet of vitamin D3 in their food, where reviews indicate that vitamin D3 deficiency was greatly involved with oral diseases and was associated with the main issue of dental defects, and the failure of oral treatment, and tooth decay and thus doctors need to confirm the level of vitamin D3 before treating any oral condition to achieve successful results for treatment.

Vitamin D3 in addition to its functions related to the bone and the regulation of calcium and phosphorus in the teeth, stimulates antimicrobial peptide (AMP), the most important of which is Cathelicidin known as LL37, which has a large -range activity against the esteemed bacteria, which is valued and honored, including *Strep. mutans* (Dürr *et al.*, 2006), as the results of our current study were found when examining the LL37 Peptide level in people with a deficiency in vitamin D3, they have low levels of LL37, This is also in conformity with a study by Jeng *et al.*, (2009) that was also found that the LL37 level is low in people with a low level of 1,25 (OH) 2D3 (which is the effective form of vitamin D3), as Liu *et al.*, (2006) found that Vitamin D3 therapy has improved the production of LL37 and developed from the possibility of killing microorganisms.

The presence of LL37 in saliva is considered one of the ways of defense in it because it is a natural barrier to the oral cavity, as the anti-inflammatory effect on the site of the injury was linked to an increase in the LL37 in the ventricular cells and the epithelial cells, which prevents increased permeability of the cells surrounding the cell and thus this effect is related to inflammation that can lead to topical accumulation of tissue fluids, and the LL37 is also interacting with the bacterial membrane, which leads to disrupting the safety of this membrane and leakage of the ingredients inside the cell of the bacteria as well as inhibition of the biofilm (Chen *et al.*, 2019).

From the above results and what we have reached, one of the important points in our current study is to find a strong relationship between the level of vitamin D3 and the LL37, which has an important role in preserving the oral cavity and thus reducing the occurrence of tooth decay, as we found that vitamin D3 is directly responsible for stimulating production LL37 These results supported a study of their conducted Al-Jubori *et al.*, (2022) which has proven that the LL37 level is linked to the level of vitamin D3 in the blood, and that the presence of a sufficient level of vitamin D3 and the LL37 will discharge the biofilm of bacteria, which is one of the most important factors of the virulence of *Strep. mutans* and thus their existence at a sufficient level will reduce the occurrence of tooth decay.

4. Conclusions

Our current study showed the effect of the level of vitamin D3 on the occurrence of tooth decay, as the presence of a sufficient level of vitamin D3 protects against *strep. mutans* through its effect on the formation of its biofilm, which is the most important virulence factor of bacteria, and there is a direct relationship between the level of vitamin D3 and LL37 peptide as vitamin D3 is the main catalyst for the production of LL37.

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