The antibacterial effects of vitamin D3 against mutans streptococci: an in vitro study

Purpose
This study aims to evaluate the antimicrobial effects of the cholecalciferol vitamin D3 against Streptococcus sobrinus (Strep. sobrinus) and Streptococcus mutans (Strep. mutans) bacteria in vitro that is considered the main causative bacteria in dental caries development.

Materials and Methods
The antimicrobial effects of vitamin D3 were evaluated against Strep. sobrinus and Strep mutans using the agar disc diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of vitamin D3 were determined using a microdilution method following the guidelines by the Clinical Laboratory Standards Institute (CLSI). Scanning electron microscope (SEM) was used to evaluate the morphological changes of bacterial cells following exposure to vitamin D3.

Results
Strep. sobrinus was more sensitive to vitamin D3 compared to Strep. mutans bacteria. The MIC values of vitamin D3 against Strep. sobrinus and Strep. mutans were 60 µg/mL and 250 µg/mL respectively whereas the MBC values were 120 µg/mL and 500 µg/mL, respectively. Moreover, significant changes in the bacterial morphology were observed in treated bacterial cells with vitamin D3 as compared to the untreated control bacteria using SEM.

Conclusion
These findings suggested that vitamin D3 has excellent antimicrobial effects against Strep. sobrinus and Strep. mutans and may be considered as a promising compound in the prevention of dental caries in the future. Further research is recommended to elucidate the mechanism of vitamin D3 on these bacteria.

Keywords: Vitamin D3, Cholecalciferol, Streptococcus sobrinus, Streptococcus mutans, antibacterial effect

Introduction
Vitamin D is an essential component in the growth, maturation, and physiology of tissues and organs. It regulates the calcium-phosphorus metabolism and mineralization of bone tissue, including teeth (1). The lack of vitamin D during the tooth development period may lead to tooth developmental defects which makes the tooth more susceptible to bacteria attachment and colonization, and then eventually initiation of dental caries (2). Enamel hypoplasia is one of these developmental defects and is considered a significant risk factor for dental caries in children (3).

Dental caries and vitamin D deficiency are common health issues worldwide that are prevalent through all age groups and affect health and wellbeing (3). Dental caries has a negative impact on normal growth and on the quality of life of the affected individuals (4, 5). Recent studies

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have indicated a significant association between vitamin D deficiency and higher dental caries prevalence among children and adults (3, 6).

The potential role of vitamin D in inducing the innate immunity and improving the body’s resistance against different pathogens is well documented (7). Theoretically, the role of vitamin D in combating diseases is conceptualized by modulating the immune response of the infected host by production of antimicrobial peptides and inducing cell-specific receptors related to pathogen clearance (8). Almost all human cells have a specific vitamin D receptor (VDR), including B and T lymphocytes, macrophages, dendritic cells, and monocytes (7). Vitamin D boosts the expression of powerful antimicrobial peptides, such as cathelicidin and β-defensin as well as cytokines response that exist in neutrophils, monocytes, and natural killer cells through its effects on the VDRs. Additionally, the level of vitamin D has a direct influence on macrophages, enhances oxidative burst of macrophages including maturation, production of cytokines and releases hydrogen peroxide. In addition, vitamin D assists neutrophil motility and phagocytic function (7).

Moreover, the efficacy of vitamin D against diseases is not only via modulation of the immune system but also via direct antimicrobial activities against different bacteria although little is known about the direct effects of vitamin D on bacteria such as Mycobacteria (8). The mechanism by which vitamin D inhibits Mycobacterial growth remains to be studied further. Vitamin D inhibits Helicobacter pylori growth (9) via the collapse and destabilization of the cell membrane structures and ultimately lysis of the bacterial cells (9). Vitamin D inhibits the growth of Porphyromonas gingivalis by decreasing the virulence factors of associated genes contributing in bacterial colonization, inactivation of host defence mechanisms, tissue destruction and nutrient acquisition (10). Besides that, it was indicated that vitamin D derivatives are bactericidal and possess lytic activity against Strep. mutans and target the bacitracin-associated efflux system (11).

Mutans streptococci mainly Streptococcus mutans (Strep. mutans) and Streptococcus sobrinus (Strep. sobrinus) are Gram-positive and facultative anaerobic bacteria and are mainly found in the oral cavity. They are the main causative bacteria responsible for initiating dental caries (12); these bacteria can easily produce extracellular polysaccharides in large quantities from fermented carbohydrates and are strongly bound to teeth surfaces. They are able to survive in an acidic environment (13, 14). Therefore, eradicating such cariogenic bacteria would be considered a basic and essential step in preventing dental caries.

Recently, searching for novel antimicrobial agents is of great interest where overuse or misuse of antibiotics and antibacterial agents have led to antimicrobial resistance (15). Several antimicrobial agents such as chlorhexidine, triclosan and cetylpyridinium chloride are widely used as effective antibacterial agents against oral pathogens to reduce dental plaque and oral diseases including dental caries (16). However, side effects such as tooth discoloration and bacterial resistance still hinder their use (17, 18). Antibiotics is still an expensive option and misuse of them results in significant antibiotic resistance and contributes to increased health care costs (18). Using other alternative therapeutic products such as vitamin D₃ which is considered an inexpensive prophylactic option could be an essential step to discover a novel antimicrobial agent since the search for novel antimicrobial agents has been of great interest in the last few decades.

To the best of our knowledge, two previous studies by Grenier et al. (10) and Saputo et al. (11) have determined the antibacterial activities of vitamin D against Strep. mutans. However, in these studies (10, 11), different study methods and different vitamin D compounds (alfacalcidol, doxercalciferol, and calcitriol) were used. The antimicrobial activity of vitamin D₃ against Strep. sobrinus was very much lacking in the literature. Hence, this study may extend our knowledge about the antibacterial activity of another vitamin D compound which is cholecalciferol vitamin D₃ against the two most cariogenic bacteria that causes dental caries, namely Strep. sobrinus and Strep. mutans bacteria. Therefore, we hypothesized that vitamin D₃ might inhibit the growth of these bacteria which in turn may help in preventing dental caries. The objective of this study is to assess the antibacterial effects of cholecalciferol vitamin D₃ against Strep. sobrinus and Strep. mutans in vitro.

**Materials and Methods**

**Preparation of vitamin D₃**

100 mg of analytical standard vitamin D₃ (Cholecalciferol) was obtained from Sigma Chemical (Sigma-Aldrich, Germany, Cat. No.: 47763) and was dissolved in 4mL of 95% ethanol to obtain 25mg/mL stock solution. This stock solution was then diluted in distilled water to obtain the working stocks and to reduce ethanol toxicity. The working stocks were aliquoted and kept at −80°C until used; once the working stocks were used, they were discarded.

**Bacterial strains and growth conditions**

Bacterial strain from the glycerol stock under −80°C was sub-cultured. The Strep. sobrinus DSM 20742 obtained from the German Collection of Microorganisms and Cell Cultures (Germany) and Strep. mutans (ATCC 25175 American Type Culture Collection, USA) were cultured on Brain heart infusion broth (BHI) and Brain heart infusion agar at 37°C under aerobic conditions for 18–24 hours. Microbiological media was obtained from Sigma-Aldrich (St. Louis, MO, USA and Oxoid Ltd, Basingstoke, UK) and prepared according to the manufacturer’s instructions.

**Antibacterial susceptibility assay**

The antibacterial susceptibility of vitamin D₃ was investigated using the disc diffusion method on Mueller-Hinton agar plates (Sigma-Aldrich, St. Louis, MO, USA). Agar plates were inoculated with bacterial suspensions at a concentration of 1×10⁸ CFU/mL. Then sterile blank discs (6-mm diameter) which were impregnated with 20 µL of (500, 1000, 2000, and 4000 µg/mL) cholecalciferol vitamin D₃ solutions were applied to give a final concentration of 10, 20, 40 and 80 µg/disc respectively, together with a positive (0.12% chlorhexidine) and negative control (2% ethanol). Preliminary experiments were carried out to test the effects of the sol-
vent (ethanol) on the tested bacteria which showed that at the dilution used, ethanol had no effect on bacterial growth. After 24 hours incubation at 37°C, the inhibition zones were observed and measured in millimetres.

**Minimum inhibitory concentration and minimum bactericidal concentration**

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth microdilution method following the National Committee for Clinical Laboratory Standards (19). In this study, MIC and MBC experiments of vitamin D₃ against Strep. sobrinus were carried out. Strep. sobrinus showed high sensitivity to vitamin D₃ at lower concentrations; however, these low concentrations did not work on Strep. mutans, hence higher concentrations of vitamin D₃ were used. The vitamin D₃ stock used for Strep. sobrinus was 240 µg/mL whereas 2000 µg/mL was used for Strep. mutans.

Serial dilutions of vitamin D₃ stocks were carried out in BHI broth in a sterile 96-well plate. Then, 100 µL of bacterial inoculum (a final concentration of 1×10⁶ CFU/mL) was added to each well. These assays were tested in triplicates along with positive and negative controls. The positive controls contained bacterial cells in BHI broth to determine the bacteria growth throughout the experiment. The negative controls contained two-fold serial dilutions of the tested vitamin D₃ in BHI broth without any bacteria and served as primary negative control to determine the changes in absorbance due to the different vitamin D₃ concentrations. In addition, another negative control contained uninoculated BHI broth without vitamin D₃ to evaluate the sterility of the BHI broth (20). Then the plates were incubated aerobically at 37°C for 24 hours. The growth of bacteria was determined at OD 600 nm using a microplate Spectrophotometer (Infinite M200 Pro, Tecan). The MIC was assessed by subtracting the mean OD 600 values of the incubated test medium from the mean OD 600 absorbance of the dilution used, ethanol had no effect on bacterial growth. The MBC was determined by taking 10 µL aliquot from the clear wells and were plated on BHI plates and incubated at 37°C for 24 hours. The MBC was considered as the lowest concentration of tested vitamin D₃ at which the OD 600 absorbance falls below 0.05 with respect to the primary negative control (21). Three triplicate experiments were completed at different time intervals.

The MBC was determined by taking 10 µL aliquot from the clear wells and were plated on BHI plates and incubated at 37°C for 24 hours. The MBC was defined as the lowest concentration of tested vitamin D₃ that did not show any bacterial growth on BHI plates.

**Scanning electron microscope** (SEM)

In this experiment, the morphological changes were assessed for the untreated and treated Strep. sobrinus and Strep. mutans with vitamin D₃ application using SEM.

Briefly, overnight cultures of Strep. sobrinus and Strep. mutans were treated with cholecalciferol vitamin D₃ at MIC values and incubated for 18–24 hours at 37°C along with untreated bacteria cultures that serve as growth controls. The treated bacteria were fixed in 2.5% glutaraldehyde for 4–6 hours then washed with 0.1 M sodium phosphate buffer (pH 7.2) and post-fixed in 1% osmium tetroxide for 2 hours at 4°C. After washing again with 0.1 M sodium phosphate buffer, the samples were dehydrated using a series of alcohols.

The specimens were coated with a thin layer of platinum and were observed under SEM.

**Statistical analysis**

The data was entered and analysed using Statistical Package for Social Sciences version 20.0 (SPSS Inc., Chicago, IL, USA). No data corrections were applied before the analysis. Bacterial measurement data under SEM were presented as the mean± standard deviations. The distribution of the data did not meet the requirements for normality and homogeneity of variance assumptions and therefore the length and width measurements between untreated and treated bacteria were determined by the nonparametric Mann–Whitney U test. The confidence interval was set to 95% and p < 0.05 was considered statistically significant.

**Results**

**Antibacterial activity of vitamin D₃**

In this experiment, vitamin D₃ was investigated to evaluate its antibacterial activity against Strep. sobrinus and Strep. mutans using the disc diffusion method. The results revealed no inhibition zones for both bacteria against all tested concentrations of vitamin D₃.

**Minimum inhibitory concentration and minimum bactericidal concentration**

The MIC is considered the lowest vitamin D₃ concentration that inhibited bacterial growth, as measured at OD 600. The MBC is defined as the lowest concentration of tested vitamin D₃ that did not show any bacterial growth on BHI plates. The MIC values of vitamin D₃ against Strep. sobrinus and Strep. mutans were 60 µg/mL and 250 µg/mL, respectively, as shown in Figure 1 and 2. The MBC of vitamin D₃ against Strep. sobrinus and Strep. mutans were 120 µg/mL and 500 µg/mL, respectively.

**Scanning electron microscope**

SEM examination was conducted to investigate the possible changes in the morphology of Strep. sobrinus and Strep. sobrinus.
many chronic diseases such as respiratory infections (22), or this is based on the findings that vitamin D regulates calcium and phosphate homeostasis that is essential for calcification, mineralization and maintenance of hard tissue, oral bone and teeth (2), or the fact that vitamin D regulates the expression of endogenous antimicrobial peptides which are human cathelicidin (LL-37) and defensins that have broad spectrum antimicrobial activities against many bacteria (8, 26). The results of this study showed that cholecalciferol vitamin D3 was able to inhibit the normal growth of Strep. sobrinus and Strep. mutans and altered their normal cell morphology. Therefore, it suggests that cholecalciferol vitamin D3 has a direct antibacterial action against these bacteria, which is totally different from its hormonal effects.

The antibacterial susceptibility of vitamin D3 was investigated using the disc agar diffusion method. This method is one of the popular methods used to determine the antimicrobial effects of an agent (27). However, this test can be considered for materials which are soluble and capable of diffusing into the surrounding environment (28). This may explain why there was no zone of inhibition (ZOI) in the present study. It appears that the insolubility of the cholecalciferol vitamin D3 may have hindered its diffusion to the surrounding agar surface and the inhibition zone.

In recent years, there was increasing attention towards the sunshine vitamin. Few studies had reported the antibacterial activities of vitamin D analogues including vitamin D3 products against different bacteria including Mycobacteria (8), Helicobacter pylori (9) and Streptococcus mutans (10, 11). Varied MIC values were reported from previous studies depending on the applied methods, vitamin D compounds used and bacteria species. Hosoda and colleagues (9) have found that vitamin D3 species (vitamin D3; 25-hydroxyvitamin D3; 1α,25-Dihydroxyvitamin D3) has a direct antibacterial action against these bacteria. A recent study found that the MIC for 1,25(OH)2D3 ranging from 3.125 to 6.25 µg/mL inhibited the growth of oral Porphyromonas gingivalis (10). Another study indicated that 1,25(OH)2D3 showed inhibition activities against S. mutans ATCC 35668 at MIC of 200 µg/mL, while MBC was > 400 µg/mL (10). In addition, a study by Saputo et al. (11) has determined the antibacterial activities of three vitamin D compounds, namely alfalcacidol, doxercalciferol, and calcitriol against Strep. mutans. They have concluded that vitamin D derivatives possess lytic activity against Strep. mutans at MIC of 16 µg/mL (11). In addition, the minimum biofilm inhibitory concentration of doxercalciferol and alfalcacidol was 64 µg/mL and 128 µg/mL, respectively; however, no biofilm formation inhibition was detected using calcitriol at any of these concentrations (11).

Both Strep. sobrinus and Strep. mutans are considered the most cariogenic bacteria causing dental caries; they are equally virulent in causing dental caries (12). Currently, chlorhexidine is considered the most effective oral antimicrobial agent due to its broad-spectrum action against Gram positive and Gram negative bacteria (29). Research has found that Strep. sobrinus has a higher resistance to chlorhexidine compared to Strep. mutans, and it may reappear earlier in saliva and plaque at higher levels than Strep. mutans after the application of chlorhexidine (30). However, in this study, we found that Strep. sobrinus is more sensitive to vitamin D3 compared to Strep. mutans, as the MIC and MBC values of vitamin D3 against Strep. sobrinus were lower than Strep.

**Discussion**

Vitamin D deficiency has been linked to the aetiology of many chronic diseases such as respiratory infections (22), asthma, allergic diseases (23), rheumatoid arthritis (24). Vitamin D supplements in asthmatic patients is associated with reduction of bacterial respiratory infections including *H. influenzae*, *S. pneumoniae*, *beta-haemolytic Streptococcus spp.*, *S. aureus*, and *Chlamydia pneumoniae* (22).

Earlier studies have shown that young children and adults who had low serum vitamin D had higher dental caries occurrence compared to individuals with adequate serum vitamin D levels (3, 6). Vitamin D supplementation was associated with a 47% reduced risk of caries (25). In addition, serum vitamin D levels above 30–40 ng/mL may significantly reduce the risk of dental caries (26). It is unclear whether the circulating hormone vitamin D has exerted a direct antibacterial activity against oral bacteria that causes dental caries, or this is based on the findings that vitamin D regulates calcium in response to cholecalciferol vitamin D3 application. The morphology of the tested bacteria was observed for the untreated and vitamin D3 treated bacterial cells. The untreated *Strep. sobrinus* and *Strep. mutans* exhibited the typical streptococcal appearance as ovoidal (elongated) cells with smooth uniform shape and intact cell membranes (Fig.3a, c and Fig.4a, c, e). However, the treated *Strep. sobrinus* significantly appeared shorter and swollen compared to untreated *Strep. sobrinus* bacteria (Fig.3b, d) with mean length of 0.96±1.95 µm, 0.78±0.11 µm p=0.021 and mean width of 0.47±0.04 µm, 0.51±0.06 µm p=0.048 for non-treated and treated bacteria, respectively. On the other hand, the treated *Strep. mutans* cells did not exhibit any clear changes in their size compared to the untreated cells. Additionally, both treated *Strep. sobrinus* and *Strep. mutans* bacterial cells showed distinct surface alternations of formation of cell membrane blebs (Fig.3b, d) and (Fig.4b), cell membrane damage/rupture (Fig.3f) and (Fig.4f), cell membrane clumping (Fig.3f), intracellular material leakage (Fig.4b), wrinkled and rough cell membrane (Fig.3f). Furthermore, the bacterium-to-bacterium contact area appeared flattened and wider in the treated *Strep. mutans* (Fig.4b). Thus, the observed morphological alternations in both bacteria appear to be related to the damage in cell wall and cell membrane.

**Figure 2. MIC value of vitamin D3 against Strep. mutans.**

**Vitamin D effect on mutans streptococci**
Figure 3. Scanning electron microscope of untreated Strep. sobrinus (3a,c,e). Strep. sobrinus treated with vitamin D$_3$ at MIC (3b,d,f) showing the formation of cell membrane blebs (red arrows) (3b,d), cell membrane damage/ruptured (green arrow) (3f), membrane clumping (blue arrows) (3f), wrinkled and rough cell membrane (yellow arrows) (3f).
Vitamin D effect on mutans streptococci

Figure 4. Scanning electron microscope of untreated Strep. mutans (4a,c,e). Strep. mutans treated with vitamin D3 at MIC (4b,d,f) showing the formation of cell membrane blebs (red arrow) (4b), intracellular materials leakage (blue arrows) (4b), and the bacterium-to-bacterium contact area appeared flattened and wider (orange arrow) (4b). Bacterial cell distortion (white arrows) (4d) and cell membrane damage/ruptured (green arrows) (4f).
mutans. Therefore, our findings indicate that vitamin D₃ has a potential promising antibacterial effect against cariogenic bacteria, mainly Strep. sobrinus.

Moreover, due to the absence of studies that evaluated the antibacterial activities of vitamin D₃ against oral bacteria, we were unable to compare our MIC and MBC values against the tested bacteria.

The microbial cell wall serves as a selective environmental barrier and contains determinants required for bacterial colonization and survival (31). The first barrier that an antimicrobial agent must overcome when interacting with its target is the bacterial cell wall (32). It was indicated that Gram positive bacteria were less sensitive to antibacterial agents compared to Gram negative bacteria because of the presence of a thicker peptidoglycan layer which acts as an additional barrier for the entry of antimicrobial agents inside the bacterial cells (33). From SEM results, it was demonstrated that treatment of Strep. sobrinus and Strep. mutans with cholecalciferol vitamin D₃ exhibited considerable morphological changes. Treated Strep. sobrinus cells appeared shorter compared to untreated cells (Fig.3b, d). It appeared that vitamin D₃ may impede the growth of Strep. sobrinus. Bacteria that grow in the presence of a compound which has antibacterial properties may experience environmental stress that could influence its ability to use nutrients efficiently and thereby slow down its normal growth (34). Morphological changes such as formation of blebs, wrinkled surfaces and cellular membrane damages were observed in the present study and the membrane damages are considered a key factor in the inactivation of bacteria (35). Such morphological changes in the surfaces of bacterial cells following the treatment with antimicrobial agent have been previously reported (35, 36, 37) and the results of the present study were consistent with them.

The SEM analysis in the present study proposed a possible mechanism for the antibacterial action of vitamin D₃. Vitamin D₃ attaches to the treated bacterial cell wall through interactions with the peptidoglycan of this Gram positive strain. The adherence of cholecalciferol vitamin D₃ to the cell wall caused disruption to the bacterial cell wall and membrane, making them shrink, become rough and increase the internal cellular pressure causing bleb-like formation and eventually causing cell membrane rupture and bacteria damage. Based on SEM findings, it is evident that vitamin D₃ is considered a membrane-active agent and is toxic to these oral bacteria and therefore affecting its normal growth.

To our knowledge this is the first study assessing the antibacterial activity of cholecalciferol vitamin D₃ against Strep. sobrinus and Strep. mutans bacteria in vitro. Cholecalciferol vitamin D₃ exhibited MIC and MBC as well as clear morphological alternations on both bacteria even though the exact mechanism by which vitamin D₃ inhibited Strep. sobrinus and Strep. mutans growth remains to be discovered. More studies to evaluate its effects on the bacterial membrane ultrastructure need to be considered.

Conclusion

The findings of this study suggest that vitamin D₃ has a direct antimicrobial effect against mutants streptococci bacteria in vitro. It appears that vitamin D₃ is a membrane-active agent that affects bacterial cell wall and causes membrane disruption. It significantly altered the cellular structure of both the Strep. sobrinus and Strep. mutans cell walls and obviously hindered the normal growth of these bacteria. Therefore, vitamin D₃ could be considered as a promising compound that may be used in caries prevention. Further research is recommended to explicate the mechanism of antibacterial activity of vitamin D₃ on cariogenic oral bacteria.

Türkçe Özeti: Vitamin D₃’ün Mutans Streptokokklarına Karşı Antibakte- yel Etkileri: Bir in vitro çalışma. Amac: Bu çalışma, kolekalsferol vitamin D₃’in diş yüzüğü oluşumunda ana etken bakteri olarak kabul edilen Streptococcus sobrinus (Strep. Sobrinus) ve Streptococcus mutans (Strep. Mutans’a) karşı antimikrobiyel etkilerini in vitro olarak değerlendirerek amaçlamaktadır. Materyal ve Metod: Vitamin D₃’in Strep. sobrinus ve Strep. mutans’a karşı antimikrobiyel etkileri, agar disk difüzyon yöntemi kullanılarak değerlendirildi. Vitamin D₃’in minimum inhibitory konsantrasyonu (MIC) ve minimum bakterisit konsantrasyonu (MBC), Klinik Laboratuar Standartları Enstitüsü (CLSI) yönergelerine göre mikrodiffüzyon yöntemi kullanılarak belirlendi. Vitamin D₃ uygulanmasının takiben bakteri hücrelerinin morfolojik değişikliklerini de- ğerlendirmek için taramalı elektron mikroskobu (SEM) kullanıldı. Bulgular: Strep. sobrinus’un, Strep. mutans bakterilerine kyasala vitamin D₃’e daha duyarlı olduğu belirlendi. Vitamin D₃’in bakterileri üzerine MIC değerleri: Strep. sobrinus ve Strep. mutans için sırasıyla 60 µg/mL ve 250 µg/mL, MBC ise sırasıyla 120 µg/mL ve 500 µg/mL idi. Ayrıca vitamin D₃ ile tedavi edilmiş bakteri hücrelerinin bakteriyel morfolojisinde, tedavi edilmemiş kontrol grubu bakterilerine kıyasla, SEM kullanılarak önemli değişiklikler gözlemdi. Sonuç: Bu bulgular, vitamin D₃’in Strep. sobrinus ve Strep. mutans’a karşı üstün antimikrobiyel etkileri sahip olduğunu ve gecekteki diş yüzüklerinin önlenmesinde umut verici bir araç olarak düşünülürlebiliceğini ideri sürdü. Vitamin D₃’in, bu bakteri- ler üzerindeki mekanizmasını aydınlatmak için daha fazla araştırma yapılması önemlendi. Araştırmacı Kılavuzu: Vitamin D₃, kolekalsferol, Streptococcus sobrinus, Streptococcus mutans, antibakteriyel etki

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Author contributions: MMM, ASH and HAT designed the study. MMM, ASH and HAT participated in generating the data for the study. MMM, SABN and NAEBE participated in gathering the data for the study. MMM, ASH, SABN and NAEBE participated in the analysis of the data. MMM, ASH and SABN wrote the majority of the original draft of the paper. MIAH, HAT and HBSGK participated in writing the paper. All authors approved the final version of this paper.

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References

1. Wójcik D, Krzewinska A, Szalewski L, Pietyrka-Michalowska E, Szalewski M, Krzewski S, et al. Dental caries and vitamin D3 in children with growth hormone deficiency: A STROBE compliant study. Medicine. Medicine 2018;97. [CrossRef]

2. Almoudi MM, Hussein AS, Abu Hassan MI, Schroth RJ. Dental caries and vitamin D status among children in Asia: A literature review. Pediatr Int 2019;61:327–38. [CrossRef]

3. Schroth RJ, Levi JA, Sellers EA, Friel J, Kliewer E, Moffatt ME. Vitamin D status of children with severe early childhood caries: A case-control study. Bmc Pediatr 2013;13:174. [CrossRef]
4. Sheiham A. Oral health, general health and quality of life. Bull World Health Organ 2005;83:644–5.

5. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet 2007;6:51–9. [CrossRef]

6. Song E-L, Song C-H, La S-A, Ock S-M, Ju S-Y. Associations of serum vitamin D level with dental caries in Korean adults. Korean J Fam Pract 2016;16:72–8. [CrossRef]

7. Youseff DA, Miller CWT, El-Abbassi AM, Cutchins DC, Cutchins C, Grant WB, et al. Antimicrobial implications of vitamin D. Dermatoendocrinol 2011;3:220–9. [CrossRef]

8. Greenstein RJ, Su L, Brown ST. Vitamins A & D inhibit the growth of mycobacteria in radiometric culture. Plos One 2012;7: P.E29631. [CrossRef]

9. Hosoda K, Shimomura H, Wanibuchi K, Masui H, Amgalanbaatar A, Hayashi S, et al. Identification and characterization of a vitamin D3 decomposition product bactericidal against Helicobacter pylori. Sci Rep 2015;5:8860. [CrossRef]

10. Conrads G, de Soet J, Song L, Henne K, Sztajer H, Wagner-Peerke J. Comparing the cariogenic species Streptococcus sobrinus and S. mutans on whole genome level. J Oral Microbiol 2014;6:26189. [CrossRef]

11. Forsten SD, Björklund M, Ouwehand AC. Streptococcus mutans. Saudi Dent J 2014;25:186–90. [CrossRef]

12. Almoudi MM, Hussein AS, Hassan MIA, Zain NM. A systematic review on antibacterial activity of zinc against Streptococcus mutans. Saudi Dent J 2018;30:283–91. [CrossRef]

13. Martínez de Tejada G, Sánchez-Gómez S, Rázquin-Olazaran I, Kowalski I, Koncis Y, Heinbockel L, et al. Antimicrobial activity of toothpastes containing natural extracts, chlorhexidine or triclosan. Braz Dent J 2014;25:186–90. [CrossRef]

14. Sinha R, Karan R, Khare SK. Interaction and nanotoxic effect of ZnO and Ag nanoparticles on mesophilic and halophilic bacterial cells. Bioresour Technol 2011;102:1516–20. [CrossRef]

15. Grant WB. A review of the role of solar ultraviolet-B irradiance and vitamin D in reducing risk of dental caries. Dermatoendocrinol 2011;3:193–8. [CrossRef]

16. Balouiri M, Sadiki M, Ibsououda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal 2016;6:71–9. [CrossRef]

17. Weiss EI, Shalhav M, Fuss Z. Assessment of antibacterial activity of endodontic sealers by a direct contact test. Endod Dent Traumatol 1996;12:179–84. [CrossRef]

18. Rossi A De, Ferreira DCA, Silva RAB da, Queiroz AM de, Silva LAB da, Nelson-Filho P. Antimicrobial activity of toothpastes containing natural extracts, chlorhexidine or triclosan. Braz Dent J 2014;25:186–90. [CrossRef]

19. Grönroos L, Mättö J, Saarela M, Luoma AR, Luoma H, Jousimies-Somer H, et al. Chlorhexidine susceptibilities of mutants streptococcal serotypes and ribotypes. Antimicrob Agents Chemother 1995;39:894–8. [CrossRef]

20. Azari F, Nyland L, Yu C, Radermacher M, Mintz KP, Ruiz T. Ultrastructural analysis of the rugose cell envelope of a member of the Pasteurellaceae family. J Bacteriol 1993;157:1680–8. [CrossRef]

21. Kostoglou-Athanassiou I, Athanassiou P, Lyraki A, Raftakis I, Bener A, Ehlayel MS, Bener HZ, Hamid Q. The impact of vitamin D deficiency on asthma, allergic rhinitis and wheezing in children: An emerging public health problem. J Family Community Med 2014;21:154–61. [CrossRef]

22. Bener A, Ehlayel MS, Bener HZ, Hamid Q. Vitamin D and rheumatoid arthritis. Ther Adv Endocrinol Metab 2012;3:181–7. [CrossRef]

23. Hujoel PP. Vitamin D and dental caries in controlled clinical trials: systematic review and meta-analysis. Nutr Rev 2013;71:88–97. [CrossRef]

24. Wu H, Lu Y, Wang X, Xu S, et al. Vitamin D is associated with asthma, allergic rhinitis and wheeze in children. Int J Environ Res Public Health 2017;14:725. [CrossRef]

25. Grant WB. Vitamin D effect on mutans streptococci

26. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet 2007;6:51–9. [CrossRef]

27. Youseff DA, Miller CWT, El-Abbassi AM, Cutchins DC, Cutchins C, Grant WB, et al. Antimicrobial implications of vitamin D. Dermatoendocrinol 2011;3:220–9. [CrossRef]

28. Greenstein RJ, Su L, Brown ST. Vitamins A & D inhibit the growth of mycobacteria in radiometric culture. Plos One 2012;7: P.E29631. [CrossRef]

29. Hosoda K, Shimomura H, Wanibuchi K, Masui H, Amgalanbaatar A, Hayashi S, et al. Identification and characterization of a vitamin D3 decomposition product bactericidal against Helicobacter pylori. Sci Rep 2015;5:8860. [CrossRef]

30. Almoudi MM, Hussein AS, Hassan MIA, Zain NM. A systematic review on antibacterial activity of zinc against Streptococcus mutans. Saudi Dent J 2018;30:283–91. [CrossRef]

31. Zhu Y, Gasilova N, Jovic M, Qiao L, Liu B, Lovey LT, et al. Detection of antimicrobial resistance-associated proteins by titanium dioxide-facilitated intact bacteria mass spectrometry. Chem Sci 2018;9:2212–21. [CrossRef]

32. DePaola LG, Spolarich AE. Safety and efficacy of antimicrobial mouthrinses in clinical practice. J Dent Hyg. 2007;81:13-25.

33. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999;12:147–79. [CrossRef]

34. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspect Medicin Chem 2014;6:25-64. [CrossRef]

35. Orasmo EAC, Miyakawa W, Otani C, Khouri S. In vitro AFM imaging and AFM-PLLpPA effect of E. coli and Bacillus subtilis. J Appl Oral Sci 2011;3:193–8. [CrossRef]

36. Cho Y-S, Oh JJ, Oh K-H. Antimicrobial activity and biofilm formation inhibition of green tea polyphenols on human teeth. Biotechnol Bioprocess Eng 2010;15:359–64. [CrossRef]