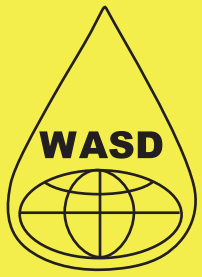# THE EFFECT OF VITAMIN D AND/OR CALCIUM SUPPLEMENTATION ON PERIODONTITIS

THE VITAM... SUPP... ON P...

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THE EFFECT OF VITAMIN D AND/OR CALCIUM SUPPLEMENTATION ON PERIODONTITIS

OBJECTIVE

To perform a systematic review and assess whether the use of vitamin D and calcium supplements can benefit the prevention of periodontitis and tooth loss among adults and elderly.

METHODS

Research of MEDLINE, LILACS, Cochrane Library, Scopus and Clin-icalTrials.gov and reference lists of relevant papers to identify exper-imental and observational studies on the effect of vitamin D and/or calcium supplementation on clinical parameters of periodontitis. Primary outcomes were clinical attachment loss (CAL), probing depth (PD) and tooth loss.
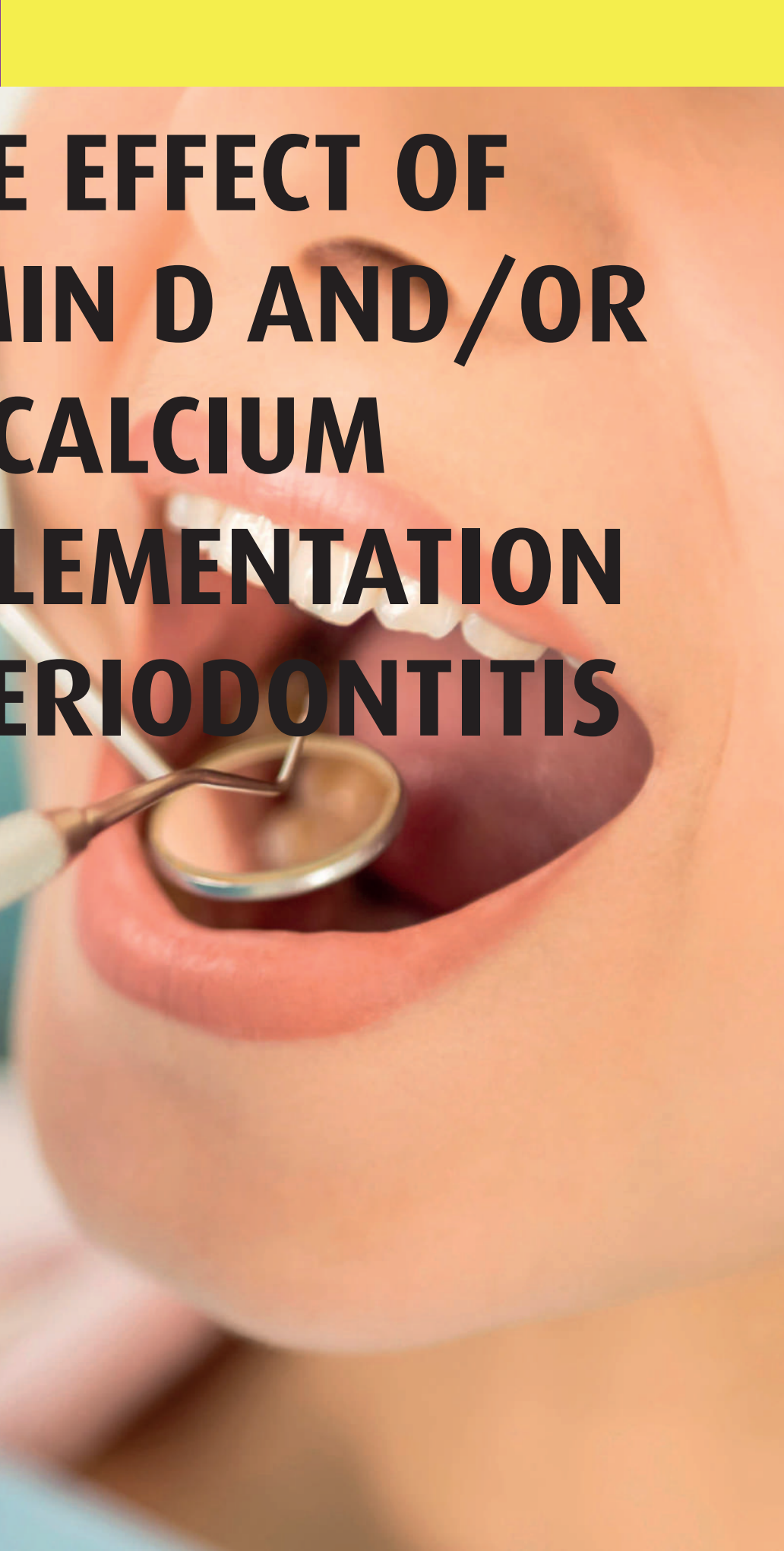
RESULTS

Four studies were included. Me-ta-analysis across studies showed a standardized mean difference (SMD) of −0.194 (95% CI: −0.777 to 0.389) and −0.168 (95% CI: −0.736 to 0.400) for CAL and PD, respectively.

CONCLUSION

Vitamin D and calcium supplemen-tation seem to have some beneficial effect on clinical parameters of perio-dontitis as a complementary therapy. However, these findings need to be confirmed before being used in clin-ical practice.

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BIOGRAPHY

Mariana Tomaz Holds an MSc degree in Clinical Nutrition from the leading University of Portugal, the University of Porto. Mariana has previously conducted internships at the University of Westminster, University of Auckland and Netherlands Cancer Institute. She has worked in different fields ranging from Public Health to Oncogenomics and aims to work on a PhD regarding Oncology Nutrition research.

Melahat Macit Graduated from Ankara University, Faculty of Health Sciences, Department of Nutrition and Dietetics in 2012. She worked as a dietician at Kocaeli Public Health Directorate and Eskisehir Osmangazi University. She received her master degree at Nutrition and Dietetics. Currently, she is a student of Ph.D. at Gazi University. She also works as a research assistant at Ondokuz Mayıs University.

Maria Niza is a student pursuing medical in her third year. Earlier to this, she has finished her Bachelors degree in Nutritional Sciences at Egas Moniz Health Institute. During this time she did two internships at the University of Westminster and Nossa Senhora da Arrabida Hospital, where she became passionate about Public Health Nutrition and Clinical Practice.

The deficiency of vitamin D is considered a public health problem worldwide (Mithal et al., 2009). The American Institute of Medicine in 2010 recommended a dietary intake of vitamin D of 600 IU/d for children and adults (1–70 years) and 800 IU/d for elderly population (>70 years) (Institute of Medicine, 2010). Although there is still a hot debate on whether such daily targets are adequate or realistic, it was estimated that 1 billion people worldwide have vitamin D deficiency (<30 nmol/L or 12 ng/mL) or insufficiency (30–50 nmol/L or 12–20 ng/mL) (Holick, 2006, Miley et al., 2009). Vitamin D is obtained both through diet intake (10–20%) and by the cutaneous synthesis under the action of ultraviolet B (UVB) light (80–90%) from sunshine (Mithal et al., 2009).

Dietary Reference Intakes (DRI) recommends a daily calcium intake of 1000 mg/d for adults (19–50 years) and older males (51–70 years) and 1200 mg/day for older females (51–70 years) (Ross et al., 2011). However, many people are unable to reach their dietary targets. In 2011, a study showed that global calcium supply was of 684 mg (±211) capita/day and calcium deficiency risk was 32% worldwide (3.5 billion people) (Tulchinsky, 2013).

Periodontitis is a chronic problem characterised as a bacterial condition involving the gingivae and surrounding structures, which leads to soft tissue breakdown and loss of tooth-supporting bone if (Graves et al., 2011); untreated, tooth loss can follow. Tooth loss is associated with low self-esteem, impaired speech and chewing functions, which may result in a reduced ability to eat healthy and nutritious food (Moynihan and Bradbury, 2001).

Conventional periodontal therapy (PT) modalities includes both non-surgical and surgical treatment, often accompanied by local or systemic antibiotics (Stein et al., 2014). Conventional non-surgical PT consist of prophylactic dental polishing and removal of dental plaque, the sticky bacterial film that forms on the teeth over time, as well as scaling and root planning to remove calculus. In more severe cases of periodontal disease, when there is less adequate response to non-surgical therapy, surgical treatment might be needed (Grant and Boucher, 2010). Periodontitis is largely preventable by optimal oral hygiene and dental care; however it continues to be a common condition in adult and elderly populations (Boggess et al., 2011).

Calcium is the mineral that structures bones and teeth (Tanaka et al., 2014). Although the beneficial effects of calcium and vitamin D supplementation on bone health are well established in the literature their potential effect in periodontal disease and tooth loss has not been fully investigated (Heaney, 2002; Stein et al., 2014).

It is likely that a long-term insufficient intake of vitamin D and calcium could lead to a negative imbalance of calcium, which therefore could result in a secondary increase in calcium resumption from bone, including the alveolar bone; thus contributing to weakening of the tooth-attachment apparatus (Bion, 1939). It has been observed that individuals with osteoporosis or low bone mass also have higher alveolar bone loss (Hildebolt, 2005; Hildebolt et al., 2004). Vitamin D has been considered as a powerful immunomodulator as a result of its anti-inflammatory effect over the in-

BIOGRAPHY CONT.

Charlotte Jeavons is a Senior Lecturer for Public Health in the School of Human Sciences, Faculty of Education, Health and Human Sciences, University of Greenwich. Charlotte has previously worked in private clinical practices both in the UK and overseas and has developed a teaching resource for oral health that was issued to all primary schools in England. Apart from the projects with the Health Department, Charlotte also worked closely with the UK Public Health Register; she aims to develop a framework of competencies for public health practitioners leading to registration. Prior to this Charlotte hold a part-time teaching position for over 10 years at Queen Mary's University, Bart's and The London Dental Institute.

Berit Heitmann is a Clinical Professor for Public Health in the University of Copenhagen where she focuses on nutritional epidemiology (diet assessment methods, reporting issues and bias consequences) and interventions linking dietary changes to obesity and disease development. Berit also works at the Paker Institute and is the leader of the Research Unit for Dietary Studies where she manages several observation and intervention settings. Berit Heitmann was also a professor in nutritional epidemiology at University of Southern Denmark (between 1999 to 2017) and is a visiting professor at University of Sydney, Australia, the Boden Institute of Obesity, Nutrition, and Exercise & Eating Disorders (since 2011).

hibition of the production of cytokine by the immune cells that which enhances the secretion of peptides (e.g. cathelicidin and β-defensin) by macrophages with potent antibiotic activity and increasing phagocytosis activity (White, 2008). Therefore, it might play a role in fighting inflammation caused by periodontitis.

Unravelling and testing calcium and vitamin D prophylactic and therapeutic values and promoting their consumption might lead to new frontiers to tackle dental disease. Some studies (Dietrich et al., 2004; Garcia, 2011; Hildebolt et al., 2004; Krall et al., 2001) have suggested that vitamin D and/or calcium supplementation results in reduced alveolar bone loss and attachment loss. However, findings from these primary studies need to be selected and summarised in a systematic fashion before drawing any valid inference on the potential effect of vitamin D and/or calcium supplementation on tooth loss and periodontitis.

The aim of this systematic review is to assess whether calcium and vitamin D supplementation will improve the response to periodontal therapy on clinical parameters of periodontitis, and to review the evidence on vitamin D and/or calcium supplementation on preventing tooth loss in adult and elderly people with chronic periodontitis.

METHODS

This systematic review was written according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009). The protocol for this systematic review with meta-analysis was registered in the PROSPERO database (42016040034).

ELIGIBILITY CRITERIA

The studies included adults ($\geq$ 18 years) or elderly ($\geq$ 65 years) population with chronic periodontitis (according to authors' definition) currently undergoing conventional PT. Studies of participants who had undergone specialist PT prior to the study and the maintenance phase were also included. No restrictions of the conventional PT (e.g. dentists, dental hygienists or dental therapists) and health status of participants (e.g. hypertension, diabetes, obesity, etc.) were applied. Individuals aged less than 18 years or with aggressive periodontitis and pregnant women were excluded. No geographic restriction was applied, as the region where the studies were conducted may assist understanding the possible relationship between vitamin D, season and latitude.

Supplemental calcium (citrate, carbonate, gluconate or lactate) and/or vitamin D (vitamin D3 (cholecalciferol) or vitamin D2 (ergocalciferol)) or active forms of vitamin D (1a-hydroxyvitamin D (alfacalcidol) or 1.25-dihydroxyvitamin D (calcitriol)) were the interventions required. Studies proved that using any dose of calcium and vitamin D, for certain period or, administered as monotherapy or in combination with

BIOGRAPHY CONT.

Dr Amanda Rodrigues Amorim Adegboye is an Associate Professor in Public Health in the School of Human Sciences, Faculty of Education, Health and Human Sciences, University of Greenwich. Prior to that, Amanda has worked as a Senior Lecturer in International Public Health & Nutrition at the University of Westminster where she was a leader for the Food, Nutrition and Public Health research group, course leader on postgraduate research methods and project, an elected member of the faculty research committee and supervisor of several undergraduate and postgraduate students. Amanda has completed her first degree in Human Nutrition at Rio de Janeiro State University in Brazil and holds an MSc and PhD degree in Epidemiology. Amanda has a strong background in quantitative methods, systematic reviews, design of public health interventions and service evaluation.

other therapies were included. Studies offering vitamin D and/or calcium in addition to multi-supplementation were not considered.

Regarding the study design, randomised controlled trials (RCT), cluster RCT, quasi-RCT, controlled before and after the observational studies with available data on two groups (one taking the supplement and the other one not taking such supplement) were considered eligible for inclusion. The non-exposed group were defined as the regular group that followed diet without supplementation, identical placebo or non-intervention.

OUTCOMES

The primary outcomes of this review were probing depth (PD) in mm; clinical attachment level (CAL) in mm; tooth loss and adverse events. It was pertinent to look closely at this last outcome once higher doses of calcium or vitamin D supplementation could cause advert gastrointestinal events or toxicity. The secondary outcomes were calculus or plaque indices; bleeding on probing (BOP); and alveolar bone loss.

SEARCH STRATEGY

A literary methodology was carried out by two reviewers (M.R.T. and M.S.M.) and a third reviewer (A.R.A.) was involved when consensus was not reached regarding the eligibility. The research was conducted, using the following databases from inception to present: MEDLINE through PubMed; Cochrane Controlled Trials Database; Cochrane Database of Systematic Reviews; LILACS through BIREME, ClinicalTrials.gov and Scopus.

Methodological filters for study design were not applied in the electronic searches, as these reduce the sensitivity of searches. Relevant studies were included regardless of status of publication (published, unpublished or ongoing), date of publication or the date when the study was conducted.

The research was conducted in English. The articles that were published in English, Portuguese, Spanish and Danish were considered. Titles and abstracts resulting from the research were screened. Retrieval of studies were performed when the titles or abstracts fulfilled the eligibility criteria described below regarding the type of participant, type of study, type of outcome and type of exposure/intervention. Studies that did not present an abstract but the titles were relevant to the topic were selected, thus the entire-text was screened for eligibility. Reference list of included studies were screened for further studies.

DATA EXTRACTION

Data from the studies were identified as to meet the inclusion criteria were extracted into a standardised data extraction form where the data extraction was

further pilot-tested using two studies. Extracted data were independently checked by a second reviewer (M.S.M.), for accuracy and completeness. Disagreements were settled by a third reviewer (A.R.A.). Data extracted included: study aims; study characteristics (country, latitude, year of publication and year of recruitment); study design; sample size; sampling strategy; duration of study; participant characteristics (inclusion and exclusion criteria, age, gender, confounders and health status); intervention setting (clinical, community, etc.); intervention fidelity (if the intervention was conducted as programmed); type of supplementation (vitamin D, calcium or combination of both, dose and duration of intervention); type of comparison group applied; key results; and source of funding. In case there was an incomplete or insufficient data in the article, the corresponding author was contacted via email.

QUALITY APPRAISAL

Methodological quality of each study included in the systematic review was assessed by a modified Downs and Black checklist (Downs and Black, 1998) designed specifically to appraise both randomised and non-randomised studies. The checklist composed of 27 "yes-or-no" questions which are further divided into five sections: Reporting; External validity; Internal validity - bias: Internal validity - confounding; and Power. The checklist provides both an overall score for each study quality and a specific score for each section. The five sections include questions on:

1. Reporting – the overall quality of the study;
2. External validity – the ability to generalize findings of the study;
3. Internal validity – to assess bias in the intervention and outcome measure(s);
4. Internal validity – to determine bias from sampling or group assignment;
5. Power – to determine if findings are due to chance.

Studies were classified into three groups: high (22 points or 80% of the total), medium (≥14 and $<$ 22 points or $\geq50\%$ and $<80\%$) or low quality ($<$ 14 points or $<50\%$).

STATISTICAL ANALYSIS

Meta-analysis was performed in accordance with the recommendations of Cochrane Handbook for Systematic Reviews of Interventions (Higgins et al., 2011). Meta-analysis, was only conducted when there was a sufficient number of studies available with homogeneous data (participants characteristics, intervention and outcome). However, we acknowledged "a priori" the divergent spectrum type of the same intervention in terms of dose, duration and type of participants. Hence, statistical heterogeneity might be inevitable; thus random-effects models were used. An inverse variance and Mantel-Haenszel methods were applied for continuous and binary outcomes, respectively. The aggregated results for the studies were presented in forest plots.

For dichotomous data, results were presented as summary odd ratio (OR) with 95% confidence intervals (95% CI). For continuous outcomes, for which the baseline data were available, results were reported as mean difference (MD) between the change in the supplementation and non-supplementation when the outcomes were measured in the same scale by all studies. If the same continuous outcomes were measured in different ways by different studies, the standardized mean difference (SMD) between groups was used.

Heterogeneity was initially assessed by visual inspection of forest plots to look at the consistency of effects across included studies and overlapping of confidence intervals. In addition, the following statistical tests were applied: Cochran's Q-test (p-value <0.10) and I^2 test (Higgins et al., 2011). An I^2 value greater than 50% was considered as an indicator of high heterogeneity. Whenever data permitted, subgroup analyses were conducted to assess the effect of supplementation according to subgroup

characteristics regarding different types of participants and intervention intensity and duration. As expected the following characteristics would introduce clinical and methodological heterogeneity: dose (high vs low); duration (<6 months vs ≥6 months); risk of bias/methodological quality (low quality vs moderate and high quality); participants age (elderly only; yes/no) and study location (studies conducted at latitude <400 N vs ≥400 N).

Sensitivity analysis was performed omitting every single study, one by one, in successive steps, in order to evaluate the influence of each publication on the pooled effect size, in the context of significant heterogeneity (leave-one-out analysis). The impact of components of quality assessment included studies (for example, RCT versus observational studies, etc.) and the impact of studies at high risk of bias on the results of the meta-analyses was also explored. It was initially planned to the use a graphical test such as funnel plots to check for confounding (Egger et al., 1997). Howev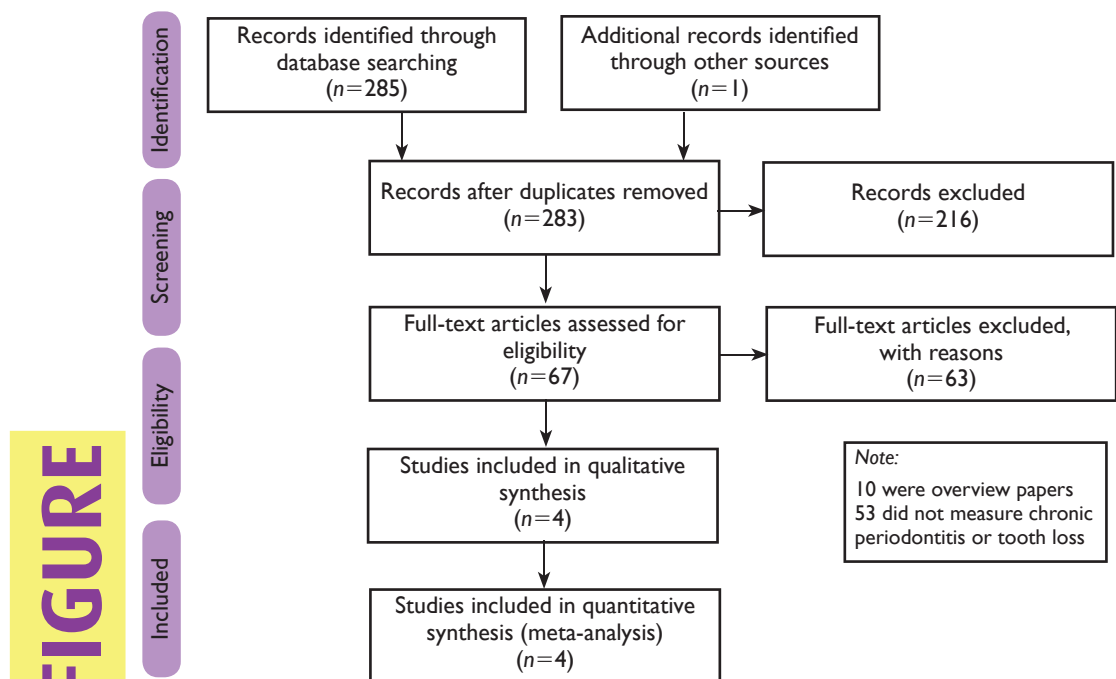er, the number of studies included was insufficient (less than 10 studies) to allow adequate assessment. All analyses were conducted in the statistical software package Open Meta-Analyst (DeCoster, 2004).

RESULTS

Out of the 283 unique articles identified in the search strategy (excluding duplicates from different databases) and one study identified via examining the reference list included studies, 216 abstracts did not meet the inclusion criteria and were therefore excluded from the review. The remaining 67 articles were assessed and, only four studies involving 339 participants that met the criteria for eligibility and were admitted in this systematic review. Figure 1 (PRISMA flow diagram) presents the number of papers identified, included, excluded and the reasons for exclusions (Moher et al., 2010).

Quality Appraisal

The quality assessment of each study is shown in Figure 2. No assessment scored above 21
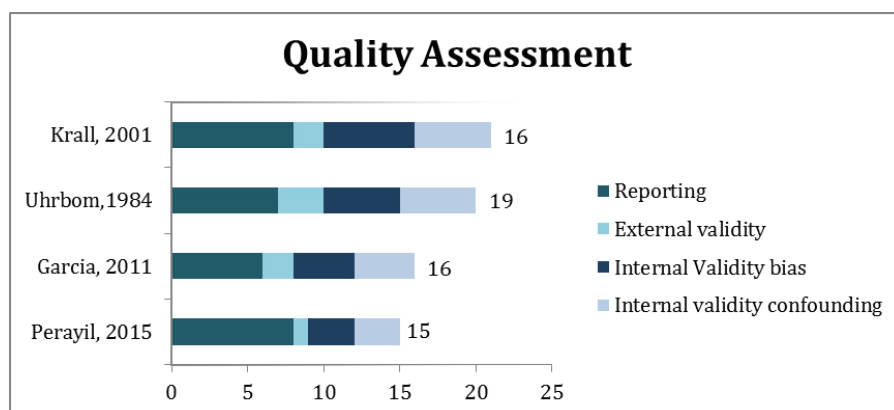

FIGURE 1

Flow Diagram Illustrating the Search and Selection Process of Studies

FIGURE

2

Quality Assessment

Krall, 2001	16
Uhrbom, 1984	19
Garcia, 2011	16
Perayil, 2015	15

■ Reporting
■ External validity
■ Internal Validity bias
■ Internal validity confounding

This Figure summarises methodological characteristics of all included studies

points, where the majority of the studies were of medium quality. Krall et al (2001) (Krall et al., 2001) and Uhrbom & Jacobson (1984) (Uhrbom and Jacobson, 1984) received the highest score (21 points) followed by Perayil et al (2015) (Perayil et al., 2015) with 18 points and Garcia et al (2011) (Garcia, 2011) with 16 points.

Perayil et al (2015) was the only study that scored one point in the Power section due to the performance of sensitivity analysis. Also Perayil et al (2015) presented the highest score for reporting.

Characteristics of Included Studies

The general characteristics and salient features of each study and details about intervention or exposure are presented in Table 1 and 2, respectively. The studies included diverse study designs. (n = 2 RCT; n = 1 non-randomized trial; and n = 1 longitudinal studies) With follow-ups ranging from 3 months to 24 months. All included studies focused on treating patients with chronic or severe periodontitis. Two studies included patients currently undergoing conventional PT (Perayil et al 2015; Uhrbom 1984) and two studies included participants who had undergone PT prior to the study (Garcia 2011; Krall 2001) and were in the maintenance phase. Both the studies were conducted in the United States of America

(Garcia, 2011; Krall et al., 2001), one study was conducted in India (Perayil et al., 2015) and one study in Sweden (Uhrbom and Jacobson, 1984).

Three studies examined vitamin D and calcium supplementation (Krall et al., 2001; Perayil et al., 2015; Garcia, 2011) and only one study focused on calcium supplementation alone (Uhrbom and Jacobson, 1984). The varied dosage of supplement taken across studies ranged from 250 IU/d to 1049 IU/d for vitamin D and from 500 mg/d to 1769 mg/d for calcium. The measured outcomes also varied across studies; however, all studies assessed at least one clinical parameter of periodontitis.

Adverse Effects

At least one out of four studies reported side effects (Uhrbom and Jacobson, 1984), such as gastro-intestinal disturbance in two of the supplemented subjects and gallbladder disturbance and itching in the placebo group. Additionally, two other subjects of the placebo group discontinued the supplementation due to lack of cooperation. No adverse effects have been reported by the authors as it was not mentioned in the text.

Compliance

Krall et al (2001) study, during its RCT, reported a compliance of 93% on the supplemented group

TABLE 1

General Characteristics of Included Studies

Author, year	Country	Location	Study Population	Sample Size	Age (y): mean, range	Season	Outcomes assessed	Definition of Periodontitis	Study Design	Type of Analysis
Garcia, 2011	USA	N: 38.49' 10.853" W: 90.27' 0.852'"	Post-menopausal women and men (50–80y) - moderate to severe period	51	*	Summer/Autumn	GI; PI, PD; AL; BOP; CI; FI	≥2 interproximal sites with ≥3mm of CAL and a minimum of two mandibular and one maxillary posterior teeth	LS	Student test
Krall, 2001	USA	N: 42.21' 36.297' W: 71.3' 31.968'	Elderly	145	65y; 71±5y	All year	TL; PPD	Scores of 3 or 4 indicate periodontal disease with pockets 3.5 mm or greater	RCT	Multivariable logistic regression/chi-squared test
Perayil, 2015	India	N: 9.55' 52.438" E: 76.16' 2.294"	Periodontal patients	77	35–55y	Summer	GI, PPD, CAL, BD, OHI-S	1 or more teeth chronic moderate periodontitis with >3–4 mm of CAL	CT non R	Independent two sample Test/paired sample test
Uhrbom, 1984	Sweden	N: 59.16' 30.945" E: 15.12' 48.277"	Periodontal patients	66	*	NA	PI; GI; PD; FI	Probing depths >3mm & >5mm	RCT	t-test & chi-square test

*Age appears on the table by study analysis group (confounders)

AL – Attachment Loss
BD – Bone Density
BOP – Bleeding on Probing
CAL – Clinical Attachment Loss
CI – Calculus Index
CT – Clinical Trial
FI – Functional Inflammation
GI – Gingival Inflammation
L – Latitude;
LS – Longitudinal Study
NA – Not Available
OHI-S – Oral Hygiene Index - Simplified
PD – Probing Depth
PPD – Probing Pocket Depth
PI – Plaque Index
RCT – Randomized Clinical Trial
Suppl – Supplementation/supplements
TL – Tooth Loss

TABLE 2 — Description of Intervention or Supplementation Intake

Author, year	25(OH)D Dose	Calcium Dose	Control or Non-Supplements Intake Group	Duration	Compliance Assessment	Confounders
Garcia, 2011	mean: 1049 IU/d	mean: 1769 mg/d	Non-users: usual dietary intake	12 months	Evaluation at baseline and 6, 12 months	Age, gender, race, smoking history and alcohol consumption
Krall, 2001	700 IU/d	500 mg/d	Placebo	2–3 years-measured every 6 month	Counted after 6 months	Baseline age, smoker, presence of diabetes, sex, education beyond high school, number of teeth loss
Perayil, 2015	250 IU/d	500 mg/d	Placebo	3 months	Evaluation at baseline and 3 months	Gender, age
Uhrbom, 1984	NA	1g/d	Placebo	6 months	Evaluation at baseline and 6 months	Gender, age

NA – not available
wks – weeks

The Tables 1 and 2 provide further details on Downs & Black (Downs and Black, 1998) final score on the quality of the 4 included studies ranged from 0 to 21 with a median score of 14 (maximum possible is 28). Scores for each of the five factors devised by Downs & Black (Downs and Black, 1998) varied from 0 to 9 for quality of reporting (maximum score = 11), o to 3 for external validity (maximum score = 3), 0 to 6 for internal validity bias (maximum score = 8), 0 to 5 for internal validity confounding (maximum score = 6) and all studies received a 0 to 1 for power calculation (maximum score = 1).

and 92% on the placebo group. During the follow-up period, only 63% (91 participants) of the subjects reported use of calcium supplementation and 54% (78 participants) reported the intake of vitamin D supplementation.

According to Garcia et al. (2011) study, only two participants had dropped out, but one of them was replaced for another subject. The reason for withdrawal was not mentioned. The two other studies did not report any mishap regarding compliance neither was any information provided in the text.
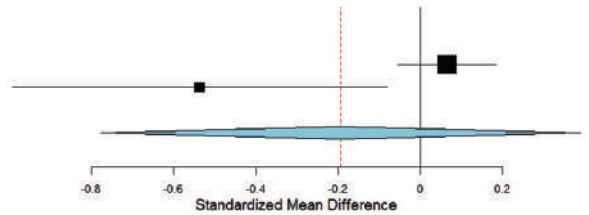
META-ANALYSIS RESULTS

Clinical Attachment Level (CAL)

Two studies have contributed with data for this outcome (Garcia, 2011; Perayil et al., 2015). The SMD obtained was –0.194 (95% CI: –0.777 to

FIGURE 3 Clinical Attachment Loss Meta-analysis: Standardized Mean Difference Forest Plot
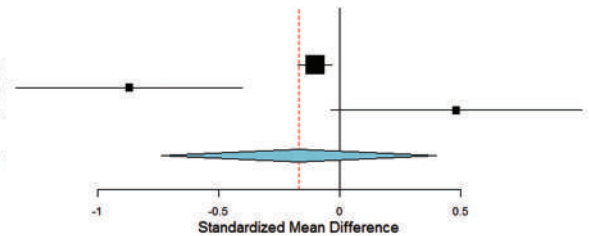
FIGURE 4 Probing depth Meta-analysis: Standardized Mean Difference Forest Plot

0.389). Overall, the I² was 83.98%, which provides statistical evidence of considerable heterogeneity of intervention effect (Figure 3). An explanation for this heterogeneity could be the due to difference in study design, since one was a longitudinal study (Garcia, 2011) and the other one was a non-randomised clinical trial (Perayil et al., 2015).

Tooth Loss

Meta-analysis was not performed for this outcome (Krall et al., 2001) because in this RCT, the results showed that only the group who received supplementation had a statistically significant correlation to a lower odds of tooth loss (OR=0.4; 95% CI: 0.2 to 0.9; p<0.05).

Probing Depth (PD)

Three studies contributed with data for the analysis of this outcome (Garcia, 2011; Perayil et al., 2015; Uhrbom and Jacobson, 1984). The polled effect (SMD) was of –0.17 with (95% CI: –0.74 to 0.40). The I² was 86.82, demonstrating the presence of considerable statistical heterogeneity among studies (Figure 4).

Subgroup analysis, by study design and type of supplementation was conducted in attempt to identify which study might be the source of heterogeneity (data not shown). It was found that heterogeneity was not eliminated after the exclusion of any of the three studies.

Calculus and Plaque Index

The data was received from the outcome of only one study (Garcia, 2011), therefore meta-analysis was not performed. The clinical measurements analysed in this longitudinal study, including calculus and plaque index, were worse in the "non-users" of supplementation (vitamin D and calcium) at baseline and throughout the study. In univariate analyses, the clinical and radiographic outcomes results (including calculus and plaque index) were not statistically significant.

Bleeding on Probing (BOP)

Only one of the studies reported data on this clinical parameter (Garcia, 2011), therefore meta-analysis was not performed. In the study, BOP was significantly lower at 6 months among supplement users compared to non-users, but with no significant results at 12 months of follow-up. When adjusting the results for variables such as race, smoking habits, alcohol and gender, it was observed that a marginally change was visible at 6 months follow-up. Therefore, at baseline, the effect of taking supplements was significant ($p=0.037$), borderline at 6 months ($p=0.058$) and not significant after 12 months ($p=0.142$).

Alveolar Bone Loss

This assessment was the outcome received from only one study (Uhrbom and Jacobson, 1984), therefore the meta-analysis was not performed. It was reported that calcium supplementation for 6 months had no effect on alveolar bone (results not shown).

DISCUSSION

This systematic review and meta-analysis summarises the essential methods for evaluating calcium and vitamin D supplementation effects on periodontitis and tooth loss among those under-going periodontal treatment. A rigorous methodology was followed to conduct this study and four articles were included to review according to the inclusion criteria.

Although our results were not conclusive, they indicate a potential effect of supplementation on CAL and PD. This is consistent with previous literature on the association of serum levels of vitamin D and periodontitis. Cohort studies, suggest an inverse association between the concentration of serum calcium and periodontal disease in young females (20–39 years), but not in males and elderly population (Nishida, 2003). In an another study, higher serum 25(OH)D levels were associated with less periodontal attachment loss in individuals with an age >50 years old, resulting therefore in significant inverse association between vitamin D concentrations and periodontal disease (Dietrich et al., 2004). Jabbar et al. (2011) also reported similar associations in women with postmenopausal osteoporosis (Jabbar et al., 2011). In the current systematic review only one study (Perayil et al., 2015) investigated the total concentration of vitamin D and calcium at baseline to assess whether the patients had a previous deficiency.

A vitamin D supplementation around 40 IU is necessary to raise the serum calcium levels for 0.70 nmol/l (Nishida, 2003). When participants had calcium or vitamin D deficiency, supplementation may have helped to maintain serum levels and therefore have impacted the periodontal status. Assessment of baseline serum levels might be important to demonstrate the effectiveness of supplementation. Hildebolt et al. (2004) reported that vitamin D dosage ranging from 500 IU to 2000 IU was essential to provide the anti-inflammatory effect (Hildebolt et al., 2004).

Vitamin D is related to a faster healing process, has anti-bacterial effects; strengthen the oral epithelium and promotes the immune response to microorganisms (Amarasena et al., 2008). Thus, the literature has been proposing vitamin D as a potent component in preventing periodontitis and

it is also beneficial in the treatment after surgery (Stein et al., 2014). Several papers have proposed that a high intake of calcium and vitamin D can strengthen remineralisation of the enamel, decrease demineralization and help to avoid alveolar bone loss by enhancing the condition and maintenance of a natural dentition (Krall et al., 2001). Furthermore, high levels of calcium and phosphate in saliva have been reported to inhibit bacterial biofilm formation (Danielsson et al., 2009), which may have prophylactic and therapeutic values in guarding against the development of oral pathologies (e.g. caries and periodontitis) (Signoretto et al., 2012). Finally, studies have shown that a sufficient intake of vitamin D may be important for the benefits of a calcium intake (Adegboye et al., 2013). These multiple pathways of vitamin D and calcium in the human body are potentially promising for an adjuvant treatment of patients with periodontitis, where the immune cells activated by the presence of bacteria expel inflammatory mediators that result in the destruction of supporting periodontal tissues, including connective tissue and alveolar bone (Garcia, 2011). Low serum 25(OH)-vitamin D concentration, might affect immune system function, reducing resistance to potentially harmful bacteria. In fact, vitamin D has anti-inflammatory properties and stimulates α- and β-defensins and cathelicidins and prevents bacterial growth (Miley et al., 2009; Schwalfenberg, 2011). These defences have several effects on bacteria and viruses such as Actinobacilus actinomycetemcomitans, Porphyromonas gingivalis, Fusobacterium nucleatum, Candida, and papilloma virus (Amarasena et al., 2008).

In this review, it was observed that there were some improvements in the clinical parameters of periodontitis, but the meta-analysis did not reach statistical significance. Additionally, analyses reported a high statistical heterogeneity therefore; the overall result should be interpreted with caution. This was expected since there are large varieties

of environmental, clinical, and methodological characteristics. Each study was conducted for different purposes and presents divergent latitudes, seasons, durations, doses of supplementation with or without added calcium and study populations with differing baseline calcium and 25(OH)D concentrations. Due to the limited number of studies, it was not possible to perform subgroup analysis according to dosage. The duration of exposure also varied considerably across studies. Perayil et al. (2015) conducted their study for 3 months and Krall et al. (2001) had the longest study duration with three years of RCT followed by 2-year of follow-up (Krall et al., 2001; Perayil et al., 2015). Period of supplementation is an important factor to see the effects of supplementation that may affect serum vitamin D status, if the patients have deficiency or are affected by the seasonal variation. However, these studies did not mention the effects of the seasonal changes during the year of the study.

Studies were conducted in countries with different exposure of the Sun, three studies were conducted at a latitude of >40, in the northern hemisphere, and only one study was conducted in a sub-tropical country (India) (Perayil et al., 2015). Perayil et al (2015) showed that Indian population had a deficiency of vitamin D, despite India being a country with high levels of exposure of the Sun, therefore assumptions cannot be made regarding the interactions among vitamin D supplementation, sun exposure and season (Perayil et al., 2015).

The current systematic review shows several strengths and limitations. The review was thoroughly performed, several searches of the literature were undertaken to identify all studies meeting the inclusion criteria for the review. The Five leading medical databases were examined to ensure that all published articles would be found. Also plans were to include studies in Portuguese, Spanish and Danish to avoid potential exclusion of some relevant studies; however, articles only in English language were found. One important

limitation is that papers lacked detailed information on the source of calcium supplementation (e.g. derived from diary). Previous research has shown that calcium intake, particularly dairy products, is implied to preserve teeth from falling among adults, but no association was found for non-dairy calcium intake (Adegboye et al., 2012).

It is important to note that some studies did not focus on our main outcomes, such as clinical attachment loss or probing depths, thus, for the purpose of this systematic review, it was a challenge to extract complete data.

The methodological quality was assessed by an adapted version of the Downs & Black check list (Downs and Black, 1998), in which question number 27 on statistical power - "Did the study have sufficient power to detect the difference due to chance being less than 5%?" was slightly modified to "Was a power analysis performed and described in the text?." where the majority of articles (3 out of 4) were of medium to low quality. The modification was made due to the fact that one of the aims of a meta-analysis is to take a number of inconclusive (and under-powered) studies and pool them so that pooled analysis has sufficient power to detect an effect if it exists. If the power is too low, the results will be imprecise. A small study will automatically be given less weight in the pooled analysis. Apart from that we have also included an extra question whether analysis was adjusted for confounders (applied for observational studies) or whether the main participants' characteristics between intervention and control group were balanced at baseline (applied for clinical trials).

CONCLUSION

Due to the limited number of studies and the lack of randomised controlled trials, more research is essential to reveal if there is any significant clinical benefit of calcium and vitamin D supplementation as a co-adjuvant therapy for chronic periodontitis.

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