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The relationship between vitamin C and periodontal diseases: A systematic review

Running title: Relation of vitamin C to periodontitis

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Abstract: Vitamin C is important in preventing and slowing the progression of many diseases. There is significant evidence linking periodontal disease and vitamin C. We aimed to systematically review studies addressing the relationship between vitamin C and periodontal disease and the preventive ability of vitamin C against periodontal disease. Electric searches were performed using PubMed, EMBASE, Cochrane Library, and Web of Science. Studies addressing the relationships between periodontal disease and vitamin C in adults aged over 18 years were included. Quality assessment was done using Critical Appraisal Skills Program guideline and GRADE-CERQual. Seventy hundred and sixteen articles were retrieved and fifteen articles (7 cross-sectional studies, 2 case-control studies, 2 cohort studies, and 4 randomized controlled trial [RCT]) were selected by reviewing all articles. Vitamin C intake and blood level were negatively related to periodontal disease in all 7 cross-sectional studies. Subjects who suffer from periodontitis presented lower vitamin C intake and lower blood vitamin C level than subjects without periodontal disease in the two case-control studies. Patients with lower dietary intake or lower blood level of vitamin C showed greater progression of periodontal disease than did the controls. Intervention using vitamin C administration improved gingival bleeding in gingivitis but not in periodontitis. Alveolar bone absorption was also not improved. The present systematic review suggested that vitamin C contributes to reduced risk of periodontal disease.

Key words: Vitamin C, Periodontal disease, Periodontitis, Gingivitis

1 Introduction

Numerous people in the world suffer from periodontal diseases [1]. It is estimated that these diseases affect up to 90% of the world population [2]. Progressed periodontitis destroys the alveolar bone that supports teeth, finally leading to loss of teeth. Moreover, periodontitis is likely to increase the risk of various illnesses, including diabetes mellitus type 2, cardiovascular disease, and adverse pregnancy outcomes [3].

Periodontal disease, an inflammatory disease that is initiated by bacterial infection and subsequently progresses via an aberrant host response, mainly contributing to periodontal tissue destruction [4]. Polymorphonuclear leukocytes (PMNs) are critically involved in biophylaxis against periodontopathogens [5,6]. At the site of infection, PMNs induce an antimicrobial response by triggering various intracellular signaling pathways including reactive oxygen species (ROS) [7]. ROS have a cytotoxic effect on periodontal tissues at higher concentrations [8,9]. Oxidative stress by PMNs may be a crucial cause of periodontal tissue damage in periodontal disease [10]. Patients with periodontitis have higher levels of biomarkers that indicate ROS-induced-tissue damage compared with controls [11-13].

Vitamin C is an important nutrient that exerts reducing and anti-oxidant effect, scavenges free radical, and acts as an enzyme cofactor in cells [14, 15]. Since vitamin C scavenges excessive ROS, this nutrient is considered to be an important dietary oxidant for periodontal health [16]. Vitamin C also plays roles in preventing and slowing the progression of periodontal disease via the induction of differentiation of periodontal ligament progenitor cells [17].

To prevent periodontal disease, evidence addressing nutritionally effective approaches must be generated. In the last two decades, the relationship between vitamin C intake and periodontal disease has been evaluated in epidemiological studies. In addition, the ability of vitamin C to prevent periodontal disease has been analyzed in clinical studies. Vitamin C has been quantified using dietary intake or blood vitamin C concentrations in these studies. In addition, periodontal disease consists of gingivitis and periodontitis, which have different pathologies, with the possibility of different associations with vitamin C. However, no comprehensive systematic review of the literature has been conducted to date.

Therefore, this study aimed to systematically review the relationship between the dietary intake and blood concentration and periodontal disease, as well as the preventive ability that vitamin C exerts against periodontal disease.

2. Material and methods

A protocol addressing a priori research questions, a comprehensive literature search, inclusion criteria for studies, screening methods and reasons for exclusion, data abstraction, scientific study quality, data analysis, and synthesis was developed and used to minimize bias.

2.1. Literature search

In the present systematic review, inclusion criteria were defined according to the PRISMA guidelines and the PICO model: P (Population): “human adults”, I (Intervention or exposure): “impact of vitamin C on periodontal disease”, C (Comparison): “different intake of vitamin C, different concentration of blood vitamin C or no administration of vitamin C” and O (Outcome): “periodontal disease parameters” [18]. Two independent authors (AT and HM) assessed the

eligibility of studies by screening the titles and abstracts, according to PICO model.

A literature search was performed using the PubMed, EMBASE, Cochrane Library, and Web of Science databases using the following search items: ("periodontal disease" AND "vitamin C" OR "periodontal disease" AND "ascorbic acid").

2.2. Quality Assessments

The studies, including cohort studies, case-control studies and randomized controlled trials (RCTs) that met the inclusion criteria were assessed using the Critical Appraisal Skills Programme (CASP) in terms of the quality of methodology [19-21]. The checklist for cohort studies was partly modified and applied to cross-sectional studies (e.g questions concerning the follow-up of participants were excluded). Two independent authors (AT and HM) calculated the strength and weaknesses of each study using the CASP checklist items and assigned them a grade of "High", "Moderate," or "Low" ; Two independent authors (AT and HM) resolved disagreements with consensus.

The GTADE CERQual was used to assess the confidence of key findings of this systematic review. Two reviewers independently reviewed findings according to the guidance for GTADE CERQual [22]. CERQual bases this evaluation on four criteria: (a) methodological limitations of included studies supporting a review finding, (b) the relevance of included studies to the review question, (c) the coherence of the review finding, and (d) the adequacy of the data contributing to a review finding.

2.3. Data extraction

Data were extracted from selected literature by two independent authors (AT and HM) using a data extraction sheet specifically developed. Disagreements were resolved by consensus after discussion. Relevant data extracted from each study that were rated as eligible included first author, publication year, setting, type of study, number of subjects, confounding factors, and main findings including both adjusted odds ratio and 95% confidence intervals (CIs).

3. Results

3.1. Literature searches and study characteristics

The initial database search yielded 716 relevant studies, and records were retrieved through a manual search. After the removal of duplicate records, 240 records were screened. Of these studies, 194 were not relevant based on the criteria. The remaining 46 articles were screened by further analysis.

From these 46 potential relevant articles, 31 did not meet the criteria. Finally, 15 publications (7 cross-sectional studies [23-29], 2 case-control studies [30,31], 2 cohort studies [32,33]), and 4 RCTs [34-37]) were selected for systematic review, as shown in the flow chart (Figure 1).

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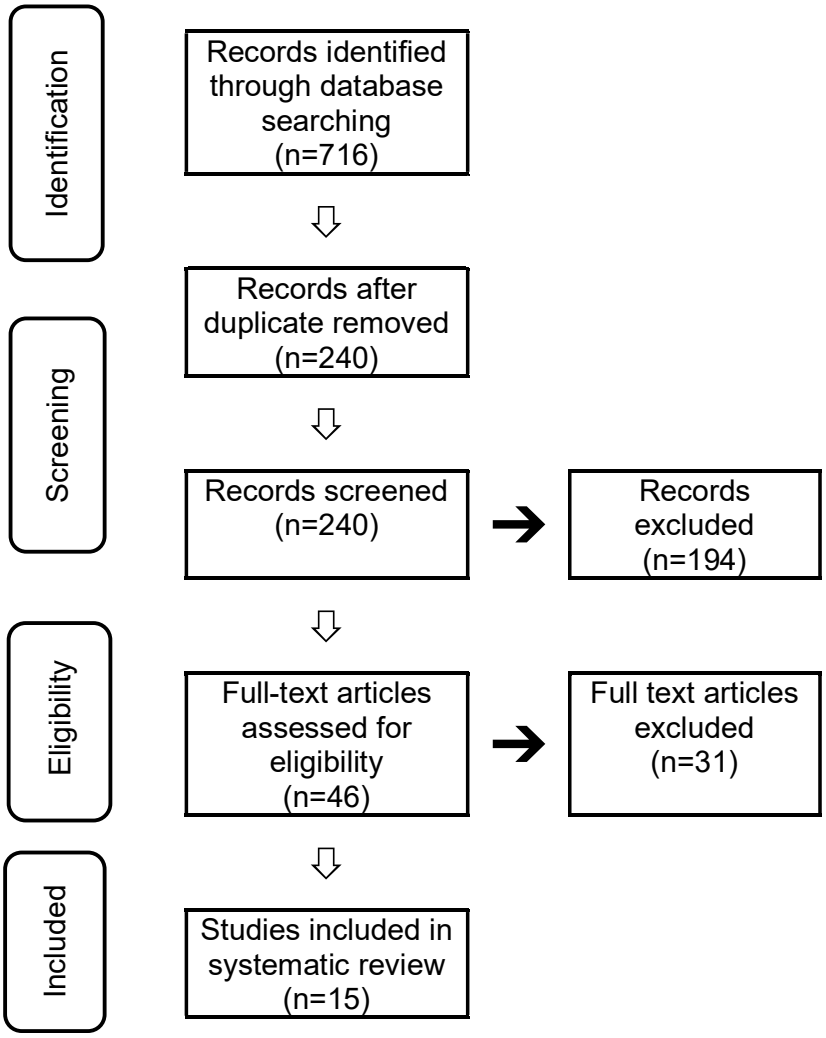

Figure 1 Flow diagram of literature search

Excluding the RCTs, 6 of the remaining 11 studies estimated vitamin C intake from dietary investigation and 6 studies measured blood vitamin C concentration. One study evaluated vitamin C based on both dietary intake and blood concentrations. Intervention studies compared the periodontal status before and after vitamin C administration.

Assessment indications for periodontal disease included the community periodontal index (CPI), attachment loss, pocket depth, clinical attachment, bone loss, pocket depth (PD), and sulcus bleeding index (SBI).

3.2. Quality evaluation

The quality evaluation of studies was performed using CASP and is presented in Table 1. The strengths and limitations of each study are discussed therein. All included studies clearly focused on an issue. Subjects were recruited in an acceptable manner in all observational studies (n=11; [100%]). All the

studies measured outcomes to minimize bias (n=15; [100%]). With regard to exposure, 4 cross-sectional studies [23-26], one case-control study [31] and one cohort study [32] assessed dietary intake. Of these studies, five used dietary recall [23-26, 31], specifically, two studies used multiple dietary recalls [24, 31], and three used a single dietary recall [23, 25, 26]. Since a single 24-h dietary recall could not be considered to reflect one's habitual diet [38], multiple 24-h recalls are desirable and this technique was rated as "satisfactory". Two studies using multiple dietary recalls were rated as "satisfactory". One study used food frequency questions (FFQs), that were previously validated [32] and was rated as "satisfactory". Six studies used serum/plasma vitamin C levels for assessment [30-34, 36] and were rated as "satisfactory".

The incidence and development of periodontal disease is influenced by various factors, including demographic factors, socioeconomic factors, smoking systemic diseases (e.g. diabetes and obesity), and oral health habits [39-43]. When analyzing the relationship between relevant factor(s) and periodontal disease, these potential confounders must be controlled for in cross-sectional studies and cohort studies. Five studies covered all of these confounders [23-25, 32, 33] and were rated as "satisfactory".

The quality of studies was assessed according the number of "satisfied" scores as follows: cohort High-9, Moderate-8-10, Low-7 or less; others High-9, Moderate-7 or 8, Low-6 or less. Six studies were rated "High" and 9 were rated as "Moderate".

Table 1 The results of the critical appraisal assessment

(1) Cross-sectional study (for vitamin C intake)

	1	2	3	4	5a	5b	6	7	Quality evaluation
Lee (2017)	*1*	*1*	*0*	*1*	*1*	*1*	*1*	*1*	*moderate*
Luo (2018)	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*High*
Park (2017)	*1*	*1*	*0*	*1*	*1*	*1*	*1*	*1*	*moderate*
Nishida (2000)	*1*	*1*	*0*	*1*	*0*	*0*	*1*	*1*	*moderate*

(2) Cross-sectional study (for blood concentration)

	1	2	3	4	5a	5b	6	7	Quality evaluation
Chapple (2007)	*1*	*1*	*1*	*1*	*0*	*0*	*1*	*1*	*moderate*
Amarasena (2004)	*1*	*1*	*1*	*1*	*0*	*0*	*1*	*1*	*moderate*
Amaliya (2007)	*1*	*1*	*1*	*1*	*0*	*0*	*1*	*1*	*moderate*

1, satisfied; 0 not satisfied.

1 Clearly focused issue
2 Adequate recruitment method
3 Adequate measurement of exposure
4 Adequate measurement of outcome
5a Identification of all important confounding factors
5b Adequate study design accounting for the confounding factors
6 Application of the results to the population of a local area
7 Agreement with other available evidence

Table 1 The results of the critical appraisal assessment

(3) Case-control study

	1	2	3	4	5a	5b	6	7	Quality evaluation
Kuzmanova (2017)	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*High*
Staudte (2012)	*1*	*1*	*1*	*1*	*1*	*0*	*1*	*1*	*moderate*

1, satisfied; 0, not satisfied.

1 Clearly focused issue
2 Adequate recruitment method
3 Adequate selection of controls
4 Adequate measurement of exposure
5a Equal treatment of the groups
5b Adequate study design accounting for the confounding factors
6 Application of the results to the population of a local area
7 Agreement with other available evidence

(4) Cohort study

	1	2	3	4	5a	5b	6a	6b	7	8	Quality evaluation
Iwasaki (2012)	*1*	*1*	*1*	*1*	*1*	*1*	*0*	*1*	*1*	*1*	*moderate*
Iwasaki (2013)	*1*	*1*	*1*	*1*	*1*	*1*	*0*	*1*	*1*	*1*	*moderate*

1, satisfied; 0, not satisfied.

1-5b: Same as cross-sectional study
6a Completion of follow-up
6b Sufficient length of follow up
7 Application of the results to the population of a local area
8 Agreement with other available evidence

Table 1 The results of the critical appraisal assessment

(5) RCT

	1	2	3	4	5	6	7	8	9	Quality evaluation
Shimabukuro (2015)	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*High*
Gokhale (2013)	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*High*
Staudte (2005)	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*High*
Abou (2010)	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*High*

1, satisfied; 0, not satisfied.

1. Clearly focused issue
2. Randomized assignment of patients
3. Proper selection of patients
4. Blinded experiment
5. Similarity of the groups at the beginning of the trial
6. Equal treatment of the groups
7. Application of the results in the context
8. Consideration of clinically important outcomes
9. Benefits outweigh harms and costs

We analyzed the confidence of our five findings using CERQual. Three findings were assigned a moderate confidence rating, and two were assigned a low confidence rating. A detailed explanation of confidence ratings was shown in Table 2.

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Table 2 The results of CERQual grading

Key Finding	Studies Supporting Key Finding	Methodological limitations	Relevance	Coherence	Adequacy	Overall Assessment of Confidence	Explanation of Judgement
①Adults with lower dietary vitamin C intake have higher incidence and severity and more progression of periodontal disease than those with higher dietary vitamin C intake	23-26, 30, 32	Minor methodological concerns in 1/6 study with adjustment for confounding factors	No or very minor concerns about relevance	No or very minor concerns about coherence	Minor concerns about adequacy as information lacked richness (1/6)	High	Finding graded as high because of only minor concerns about methodological quality and adequacy of contributing papers
②Adults with lower blood vitamin C level have higher incidence and severity and more progression of periodontal	27-31, 33	Moderate methodological concerns in 3/6 studies with adjustment for confounding factors and in one studies with sampling.	No or very minor concerns about relevance	No or very minor concerns about coherence	Moderate concerns about adequacy as information lacked richness (2/5)	Moderate	Finding downgraded because of concerns about methodological quality and adequacy of contributing papers

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disease than those with higher dietary vitamin C level.							
③ Administration of vitamin C improve periodontal disease	34-37	No or very minor methodological concerns	Minor concerns about relevance on the specification of intervention (1/4)	Minor concerns about coherence given that the effect is limited on gingivitis	Moderate concerns about adequacy of data given the small number of studies	Moderate	Finding downgraded because of relevance, coherence and adequacy concerns of contributing papers

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3.3. Relation of vitamin C intake/level to periodontal disease in cross-sectional studies

Four studies analyzed the relation of vitamin C intake to periodontal disease. Two studies used the same subjects (participants in the fourth Korean National Health and Nutrition Examination Survey) [23, 25]. Both studies set 2/3 as the cut-off score for the CPI. Lee et al found that the highest quartile (>132.2mg/day) of vitamin C intake had a significantly lower CPI score than the lowest quartile (≤47.34 mg/day) among adults aged 19 years and older 23 (Table 3). Park et al showed that the lower intake group had a significantly greater proportion of CPI≥3 compared with the higher intake group using a cut-off point of the median value of vitamin C intake (81.3mg/day) among women or nonsmokers aged 19-39 years [25] (Table 3).

Two other large-scale studies have found that periodontal markers exhibit significant associations with dietary intake of vitamin C. Luo and colleagues showed that those with insufficient intake of vitamin C had more severe periodontal disease [24] (Table 3). Another study revealed an association between reduced dietary vitamin C and increased risk for periodontal disease [26] (Table 3).

Three studies have investigated the correlation between attachment loss and serum/plasma vitamin C levels [26-28]. Two studies have shown that the extent of attachment loss was negatively correlated to plasma vitamin C levels among subjects. Amarasena *et al* demonstrated that serum vitamin C concentrations were negatively correlated to clinical attachment loss [28] (Table 2). Chapple et al demonstrated that the prevalence of severe periodontitis was significantly higher in subjects with serum vitamin C levels <8.52 mmol/L compared with subjects with other vitamin C concentrations [27] (Table 3). One study demonstrated that the extent of attachment loss was negatively correlated to plasma vitamin C levels among subjects [29] (Table 3).

Table 3 Summary of Cross-sectional studies on the relationship between of vitamin C and periodontal disease

(1) For vitamin C intake

Reference	Study sample	Measurement of vitamin C	Measurement of periodontal status	Control of confounding factors[a]	Key results
Lee *et al* (2017)	10930 individuals (≥19 yrs, Korea)	A 24-h dietary record (adequate/in adequate vitamin C intake)	*CPI score* Periodontitis CPI= 3 or 4	1, 2, 3, 4, 5	Lowest intake (<47.3mg/day) vs highest intake (≥132.2mg/day) Adjusted odds ratio (aOR)= 1.28[95% confidence interval (CI)= 1.10, 1.50]
Park *et al.* (2017)	2049 individuals (19-39 yrs, Korea)	Complete one-day 24-h recall interviews	*CPI score* Periodontitis CPI= 3 or 4	1, 2, 3, 4,	Lower intake (<81.3mg/day) vs higher intake (≥81.3mg/day) aOR= 1.66 [95%

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					CI = 1.04–2.64] for women, aOR=1.49 [95% CI = 1.04-2.14] for nonsmokers
Luo *et al.* (2018)	6415 Individuals (≥30 yrs, US)	24-h recall interviews	*Pocket depth (PD) Attachment loss (AL)* Increased severity	1, 2, 3, 4, 5	Vitamin C intake ≤20.65mg/day vs ≥112.91/da7 aOR=1.401[95% CI = 1.121.74]
Nishida *et al.* (2000)	12419 individuals (20 yrs and over, US)	24-h dietary record	*Clinical attachment level* Periodontal disease ≥1.5	1, 3	Vitamin C intake (<0-29 mg/day) vs (>180mg/day) aOR = 1.30

[a] The following variables were adjusted in the analyses: 1, demographic factors; 2, socioeconomic factors; 3, smoking/alcohol; 4, flossing/brushing; 5, diabetes, hypercholesterolemia, hypertension, and obesity

(2) For blood concentration

Reference	Study sample	Measurement of vitamin C	Measurement of periodontal status	Control of confounding factors[a]	Key results
Chapple *et al.* (2007)	11895 individuals (≥ 20 yrs, US)	Serum vitamin C, anti-oxidant concentration	*AL, PD* Severe periodontitis: ≥2 mesiobuccal sites with AL≥5 mm and ≥1 mesiobuccal sites with PD≥4 mm	1, 2, 3, 5	Serum vitamin C concentration Highest (>70.41mmol/L) vs lowest (8.52mmol/L <) aOR=0.53[95%CI = 0.42-0.68]
Amarasena *et al.* (2004)	413 individuals (70 yrs and older, Japan)	Serum vitamin C	AL	1, 3, 4, 5	Serum vitamin C level-Attachment loss Coefficient=-0.04 [95%CI=-0.06--0.005]
Amaliya *et al.* (2007)	123 individuals (33-43 yrs, Indonesia)	Plasma vitamin C	AL	1, 2, 3, 4	Plasma vitaminC-Attachment loss Coefficient=-0.199

[a] The following variables were adjusted in the analyses: 1, demographic factors; 2, socioeconomic factors; 3, smoking/alcohol; 4, flossing/brushing; 5, diabetes, hypercholesterolemia, hypertension, and obesity

3.4. Relationship between vitamin C intake/level and periodontal disease in case-control studies

Serum vitamin C levels were compared between patients with periodontal disease and controls in two case-control studies [30, 31]. Both studies demonstrated that periodontitis patients have significantly lower serum vitamin C levels compared with controls (Table 4).

Table 4 Summary of Case-control studies on the relationship between of vitamin C and periodontal disease

Reference	Study sample	Measurement of vitamin C	Measurement of periodontal status	Control of confounding factors[a]	Key results
Kuzmanova *et al* (2017)	21 patients with periodontitis and 21 controls (≥19 yrs, Dutch)	Vitamin C plasma level	*Bone loss* periodontitis >1/3 of the root length		Plasma vitamin C level :periodontitis patients< controls (p=0.03)
Staudte *et al.* (2012)	42 patients with periodontitis (mean age 43.7 yrs) and 38 controls (mean age 40.5 yrs, Germany)	7-day food record Vitamin C plasma level	*PD* Chronic periodontitis: having≥5 teeth with periodontal sites exhibiting PDs≥3.5mm		Plasma vitamin C level: periodontitis patients< controls (p<0.05) Dietary intake of vitamin C: patients< controls (p<0.05)

[a] The following variables were adjusted in the analyses: 1, demographic factors; 2, socioeconomic factors; 3, smoking/alcohol; 4, flossing/brushing; 5, diabetes, hypercholesterolemia, hypertension, and obesity

3.5. Relationship between vitamin C intake/level and periodontal disease in cohort studies

Two cohort studies were included in the systematic review [32, 33]. The subjects of these studies were the same people living in Japan. Populations with higher dietary intake or serum levels of vitamin C exhibited less progression of periodontal disease progression (Table 5).

Table 5 Summary of Cohort studies on the relationship between of vitamin C and periodontal disease

Reference	Study sample	Measurement of vitamin C	Measurement of periodontal status	Control of confounding factors[a]	Key results
Iwasaki *et al.* (2012)	264 individuals (77 yrs, Japan)	Food frequency questions	Number of teeth having AL of 3mm or greater regression (8 yrs prospective)	1, 2, 3, 4, 5	Lowest vitamin C intake (reference) vs Middle:0.76 (0.60-0.97) vs Highest:0.72 (0.56-0.93)
Iwasaki *et al.* (2013)	264 individuals (72 yrs, Japan)	Serum vitamin C	Number of teeth having AL of 3mm or greater regression (2 yrs retrospective)	1, 2, 3, 4, 5	Highest vitamin C level (reference) vs Middle: 1.12 (1.01-1.26) vs Lowest:1.30 (1.16-1.47)

[a] The following variables were adjusted in the analyses: 1, demographic factors; 2, socioeconomic factors; 3, smoking/alcohol; 4, flossing/brushing; 5, diabetes, hypercholesterolemia, hypertension, and obesity

3.6. Improvement in periodontal status by vitamin C in RCTs

Four studies have analyzed if the intervention administration of vitamin C has a preventive effect on periodontal status. Two studies investigated the effect of local (dentifrice containing ascorbic acid) or systemic (grapefruit consumption) administration of vitamin C on periodontal disease [34, 36] (Table 6). Dentifrice presented improvement in reducing gingivitis [34]. Grapefruit consumption improved the sulcus bleed index in patients with chronic periodontitis [36]. Two additional studies involved clinical trials investigating the additional effect of vitamin C in combination with non-surgical treatment of periodontal disease. Gokhale et al. presented that, non-surgical treatment combined with vitamin C supplementation exhibited a significant reduction in the SBI of patients with gingivitis [35] (Table 6). In contrast, vitamin C does not exhibit an additional effect on the improvement in clinical measures of periodontitis [35, 37] (Table 6).

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(5) Summary of RCT studies in the improvement of periodontal status by vitamin C

References	Study sample	Intervention	Measurement of periodontal status	Key results
Abou *et al*., (2010)	30 individuals with chronic periodontitis (Syria)	Non-surgical periodontal therapy+ vitamin C administration	PD, CAL, BPO. Plaque index (PI), Gingival index (GI)	Vitamin C did not offer additional effect to non-surgical periodontal therapy on improvement in clinical measures.
Shimabukuro *et al*.,(2015)	300 individuals with gingivitis (Japan)	Dentifrice containing L-ascorbic acid 2-phosphate magnesium salt	GI, Gingivitis severity index (GSI)	GI Test group: from 1.22 ±0.03 to 0.73±0.03 Control: from 1.16± 0.03 to 0.84±0.03 GSI Test group: from 1.09 ±0.04 to 0.69±0.03 Control: from 1.13± 0.04 to 0.78±0.03
Gokhale *et al*.,(2013)	120 individuals (30-60 yrs, India)	Non-surgical periodontal therapy+ vitamin C administration	PI Sulcus bleeding index (SBI) PD	SBI Mean of differences (Scores at baseline-Scores after 2 weeks) SRP + Vitamin C: 0.56±0.26 SRP: 0.28±0.12 PI and PD were not unaffected.
Staudte *et al*., (2005)	58 individuals with periodontitis (22-75 yrs, Germany)	Two weeks of grapefruit consumption	PI, SBI, probing pocket depths (PPD) Plasma vitamin C level	SBI Test group (non-smokers): from 1.68±0.6 to 1.05± 0.6, p<0.05) Diseased controls (from 1.64±0.5 to 1.67 ±0.5) PI and PPD were unaffected. Plasma vitamin C level increased significantly after grapefruit consumption

4. Discussion

Literature review articles addressing the association of periodontitis with various nutrients, including vitamin C have been published [44-46]. To the best of

our knowledge, this is the first systematic review that overviews findings and critically appraise the quality of studies addressing the relationship between vitamin C and periodontal disease.

4.1. Quality assessment of the studies

Several studies reviewed in this review article used intake data to assess vitamin C intake. Three studies used a single dietary recall [26, 28, 29]. Since these studies used large-scale samples from national surveys, obtaining data on multiple dietary recalls may be difficult. Nevertheless, many studies employed multiple dietary recalls, validated FFQ or objective evaluation by blood vitamin C level. The overall evaluation of vitamin C obtained from articles in this review is considered to be reliable.

Three of the eight cross-sectional studies and both cohort studies were also scored “satisfactory” in controlling for confounders. Moreover, although three studies did not control for all potential confounders, these studies controlled for almost all potential confounders when assessing the relationship between vitamin C level and periodontitis [27-29], thereby increasing the reliability of the current review’s findings.

The confidence of the findings ranges from moderate to high, based on the CERQual assessments. The main reasons for downgrading are methodological limitation and adequacy. Concerns about methodological limitation included sufficient adjustment for confounding factors. Downgrade in adequacy occurred when the concern about the richness of the data contributing to the review finding. Nonetheless, concerns found in coherence and relevance are limited and no study showed contradictory data in the relationship between vitamin C and periodontal disease, which provides higher confidence to the findings concerning this relationship.

4.2 Impact of vitamin C on periodontal status

All cross-sectional studies demonstrated significant associations of periodontal disease with dietary intake of vitamin C or blood vitamin C level. Subjects with periodontitis exhibited higher blood vitamin C levels than participants with healthy periodontal tissues in two case control studies. These findings suggest that vitamin C obtained from the diet is transferred to periodontal tissue via blood circulation, thus decreasing the risk of periodontal disease. However, a reverse causation could be deduced from the associations presented in cross-sectional studies. Thus it is possible that the associations may reflect the effect of periodontal disease on vitamin C intake due to decreased mastication rather that the effect of vitamin C intake on periodontitis. Longitudinal studies are needed to elucidate and verify this association.

A longitudinal relationship between vitamin C and periodontal disease was presented in two cohort studies in this review [32, 33]. The results obtained from the cohort studies support the mechanism that vitamin C obtained from diet reduces the inflammatory reaction in periodontal disease. However, given that these two cohort studies used the same population, further research using cohort studies is necessary to reinforce the reliability of the relationship.

Three of the four RCTs demonstrated improvements in periodontal indices (gingival index [GI], SBI or PD) [34-36], namely, an improvement in gingival condition, caused by vitamin C administration. Two of these studies used vitamin C intake from dentifrice or grapefruit [34, 36]. Grapefruit is rich in vitamin C, with levels that satisfy the recommended intake levels. On the other hand, grapefruit also contains the anti-oxidant vitamin, vitamin E in sufficient amounts, and its effect should not be overlooked. Administration of vitamin C alone or with non-surgical treatment has shown effects on improving gingival indices, SBI and GI. Vitamin C has a powerful anti-oxidative effect in living organisms, particularly at the intracellular level [47], and that is thought to decrease the oxidative stress generated in gingivitis. Additionally, vitamin C reduced the cytotoxic and apoptotic activity of *Porphyromonas gingivalis* C in human periodontal ligament cells and human gingival fibroblasts [48, 49], which may contribute to these effects. However, the reduction in the SBI score by vitamin C has not been seen in patients with periodontitis [35, 37]. Vitamin C also reduced gingival bleeding in gingivitis lesions but not in periodontitis lesions. It is speculated that when the inflammatory reaction extends from the gingiva to other periodontal tissues including the alveolar bone, some factors that inhibit the effect of vitamin C as anti-oxidant may be generated.

Vitamin C administration did not make an improvement in the pocket depth [35]. Another study also reported that Vitamin C administration has failed to improve the pocket depth and attachment level [50]. Reduction of the pocket depth requires regeneration of the alveolar bone. Although vitamin C induces the *in vitro* osteogenic differentiation of periodontal ligament progenitor cells [17], there has been no report that vitamin C triggers the bone regeneration *in vivo*, which is a likely explanation for the lack of pocket depth reduction after vitamin C administration.

Nevertheless, vitamin C administration has been observed to improve periodontal disease to some extent in the intervention studies reviewed. It could be considered that findings obtained from articles reviewed in this study are sufficient to demonstrate the preventive ability of vitamin C against periodontal disease.

There remains a possibility that periodontopathic pathogens lower blood vitamin C level by biodegradation. However, one study reported that significant change of serum ascorbic acid level could not found in patients with moderate of severe periodontitis after treatment with scaling and root planning [51]. This study suggests that the concentration of vitamin C in the blood influences the periodontal status, but not vice versa. This indicated that a change in periodontal health does not influence the blood vitamin C level.

Some articles included in this review analyzed the effects of other vitamins that have anti-oxidative effect, such as vitamin A and vitamin E, on periodontal disease [27, 28, 34, 35]. Among anti-oxidant vitamins, only vitamin C showed a consistent association with periodontal disease in these studies. it is considered to be meaningful to analyze the association of vitamin C with periodontal disease

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4.3 Influence of other factors on the association between vitamin C and periodontal disease

A close link has been established between periodontitis and diabetes, and the associations among vitamin C, periodontitis and diabetes are of interest. The key articles in this review showed some evidence on the association between vitamin C and periodontal diseases in patients with diabetes. In Gokhale's study, vitamin C exhibited an additional effect on the improvement of gingival bleeding in periodontitis patients with diabetes [35]. Lee et al showed that patients with pre diabetes and diabetes exhibited a stronger association between vitamin C and periodontitis than did people who with normal blood sugar levels [25]. Patients with diabetes also showed low ascorbate levels [52]. This could because glucose inhibits the transportation of vitamin C to the cells and because of stimulation of the hexose monophospate shunt by vitamin C [53, 54]. In diabetic patients, vitamin C is considered to work less effectively. However, unlike in these studies, the key articles showed that vitamin C that is administered or taken in via food is likely to work effectively. Vitamin C also enhances immune function by supporting various cellular functions by of both the innate and adaptive immune systems [55], which may influence on the periodontal status of diabetic patients. It is speculated that there might be more complex interactions among vitamin C, diabetes and periodontal disease. Further intervention studies are warranted to elucidate this mechanism.

Park et al stated that the association between vitamin C and periodontitis was significant in non-smokers but not significant in smokers [25] In contrast, Nishida et al showed that vitamin C intake was significantly associated with periodontitis among current smokers and past smokers but not in non-smokers [26]. Although differences were found in periodontal markers, vitamin C standard, and adjusted confounding factors between these two studies, the differences in their results cannot be sufficiently explained by differences in these factors. Vitamin C is known to reduce oxidative stress caused by nicotine [56]. Some studies consistently demonstrated that smokers have lower vitamin C levels in the plasma and leukocytes than do non-smokers, probably because of the increased oxidative stress [57]. Complicated interactions may exist between the anti-oxidative effect of vitamin C and the oxidative stress effect induced by tobacco on the periodontal tissue. Further studies are needed to elucidate this perplexing mechanism(s).

4.4 Limitations

First, the indicators for periodontal diseases used in the reviewed studies were diverse, and this precluded us making comparisons between the results of studies. Evaluation for periodontal disease differs according to the indicator used, probably affecting the relationship between periodontal disease and vitamin C. For example, CPI has a risk of underestimation due to partial mouth recording [58]. The possibility of the fluctuation of results due to inconsistencies in the evaluation of indicators attenuates the reliability of this study.

Second, smoking and diabetes are factors that exacerbate periodontal disease and negatively influence the anti-inflammatory function of vitamin C. Some interactions may exist between vitamin C and the molecules released

from tobacco or those highly expressed in diabetes. However, evidence about this concern available from the articles included in this review is perplexing and the current study provides insufficient evidence addressing this concern.

4.5 Future direction

The use of different indicators for periodontal disease makes it impossible to compare the strength of associations between vitamin C and periodontal disease obtained from multiple studies. Quantitative assessment of periodontal status and the treatment effect of periodontal disease requires the unification of indicators

The possibility that smoking and diabetes pathology influences the preventive ability of vitamin C against periodontal disease is suggested by studies analyzing the association of these factors with vitamin C [52-54, 56, 57]. Further population-based research addressing this point may be of help in the comprehensive measures for preventing periodontal disease.

Vitamin C can be easily taken in from many types of foods. Collaboration between oral health professionals and dieticians in public health sectors is expected to be more effective when promoting oral health in community dwellings. Future studies should aim to identify the merits of collaboration between nutritionists and dental health professionals.

5. Conclusion

This review provided an overview and appraisal of studies analyzing the relation of vitamin C to periodontal disease. This review highlights the effect of vitamin C on the prevention of incidence and development of periodontal disease. Further studies should be performed to increase knowledge of the relationship between vitamin C and periodontal disease.

Authors' contributions

Authors AT, and HM contributed to the conception, design of the study, acquisition of data, interpretation of data, and manuscript revisions. AT contributed to the writing. HM contributed to the funding acquisition.

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Conflicts of Interest

The authors declare no conflict of interest.

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