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Melatonin as a Treatment Option for Periodontitis: A Systematic Review

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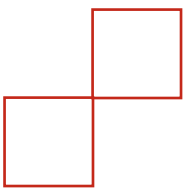

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ABBREVIATIONS

OS = Oxidative Stress

FR = Free Radicals

ROS = Reactive Oxygen Species

AO = Antioxidant

SOD-1 = Superoxide Dismutase

Trx = Thioredoxin

Ref-1 = Redox factor-1

OGG = 8-oxoguanine glycosylase

UA = Uric Acid

TNF-α = Tumor Necrosis Factor-α

HbA1c = Hemoglobin A1c (amount of glucose attached to hemoglobin)

H_2S = Hydrogen Sulfide

NH_3 = Ammonia

CAL = Clinical Attachment Loss

CEJ = Cemento-enamel junction

PD = Probing Depth/ Pocket Depth

NSPT = Nonsurgical periodontal therapy

IL-1β = Interleukin-1β

IL-6 = Interleukin 6

PGE2 = Prostaglandin E2

ABSTRACT

Objectives: Provide an overview of the applications of melatonin as an adjunctive treatment of periodontitis and compare the results between topical and systemic melatonin by evaluating Probing Depth (PD) and Clinical Attachment Level (CAL) changes and determine possible adverse effects.

Materials and Methods: Following the recommended methods for systematic reviews (PRISMA), an electronic search was conducted in the databases of Cochrane, MEDLINE, PubMed and Scopus on melatonin and periodontitis. The following PICO question was applied to guide the systematic review: Among patients suffering from periodontal disease with or without diabetes (P) does melatonin treatment with or without conventional non-surgical periodontal therapy (NSPT) (I) compared to non-melatonin treatment (C) improve PD and CAL levels more? (O)

Results: Among 84 potentially eligible articles, 7 complied with the inclusion criteria. Study characteristics including patient data as well as form, dosage, frequency, and duration of melatonin administration were noted. The clinical parameters PD and CAL were compared at Baseline (T0) and post-intervention (T1). Each study presented higher PD and CAL gain with adjuvant melatonin use.

Discussion: Although the results indicate success of each form of melatonin use, several limitations must be in considered, such as that seven articles are not enough to draw clear conclusions and the variables of each experiment varied greatly. Nonetheless this gives also rise to hope for future research.

Conclusions: Melatonin administration promotes periodontal health in terms of PD and CAL and systemical as well as topical use present no significant differences. Other than rare and minor instances of sleepinesss and headaches, no adverse effects have been observed.

KEYWORDS

I. Periodontal Disease
II. Melatonin
III. N-acetyl-5-methoxytryptamine
IV. Non-surgical periodontal therapy

1. INTRODUCTION

1.1 Oxidative Stress

The human body encompasses a multitude of chemical processes to function. Redox reactions, meaning oxidation-reduction reactions where electrons are transferred between two chemical species and resulting in the gain or loss of an electron provide metabolic regulations. This is fighting off any stimulus perceived by the body as threatening or also called stress (1). Oxidative Stress (OS) is defined as an altered biochemical homeostasis produced by environmental factors such as foods, air pollution, sunlight or other ionizing radiation, psychological pressure, chemicals such as pesticides or some drugs and many more (2, 3). This leads to the production of highly reactive molecules with a short lifetime containing one or more unpaired electrons in their last orbit and the need to have a reaction to become their stable form. In a healthy state the body can restrain by binding to them. However, if the rate of production of free radicals (FR) surpasses the bodies' ability to eliminate them, they find molecules of other cells, proteins, or genes to react with, which results in irreversible chemical disruption of healthy cell function and oxidative damage. This is favoring mutations and carcinogenesis and as a domino effect this can give rise to more FR and destruction (1).

FR can occur as a natural result of normal metabolic reactions, as well as under the influence of various external factors. The most significant FR recognized in biological systems are reactive oxygen species (ROS), which are chemically reactive oxygen-derived compounds which contain oxygen that easily react with other molecules in a cell. ROS are neutralized under physiological circumstances, hence preventing ROS-mediated tissue damage. When inflammation occurs, ROS generation rises, mostly by innate immune cells, such as neutrophils and macrophages, during the phagocytosis process (4).

The main detrimental effect of ROS on cell survival is the degradation of biological macromolecules, particularly DNA, RNA, proteins, and oxidation of

important enzymes. Accordingly, their high reactivity is often damaging in areas where it is produced in large quantities (5). However, not all ROS are free radicals, despite their reactive nature. One example is hydrogen peroxide (H2O2), which is a ROS but not a free radical (2). They play a role in the regulation of molecule's biological functions.

Cells have an antioxidant (AO) defense system that prevents the development of ROS and the resulting damage, as well as a method for removing OS. In healthy physiological conditions there is a dynamic balance between the ROS and the AO defense mechanism. OS occurs when the equilibrium shifts in favor of ROS, resulting in a reduction in AO defense and/or an increase in ROS activity. Thus, OS is defined as a potentially hazardous state caused by a shift in the ROS/AO balance toward ROS. Related health problems to OS include Parkinson's disease, Alzheimer's disease, myocardial infarction, cancers, diabetes, different inflammations, renal failure, hormonal imbalances, atherosclerosis, neurological disorders, pulmonary diseases and many more (7, 8). It is also related with a variety of oral health concerns. It is largely regarded as a factor in the start and progression of most oral illnesses. Mostly reported these include oral mucosal diseases such as recurrent aphthous stomatitis, oral lichen planus, oral leukoplakia, oral cancer, and oral squamous cell carcinoma (8,9).

Specific endogenous enzymes contributing to counteract destruction by oxidative stress include superoxide dismutase (SOD-1), thioredoxin (Trx), purinix/apyrimidinic endonuclease/redox factor-1(Ref-1) and 8-oxoguanine glycosylase (OGG), the latter two repairing DNA (10).

Saliva also contains antioxidants that are non-enzymatic, such as uric acid (UA), albumin, and ascorbic acid. The primary antioxidant in saliva is UA, which neutralizes radicals while it is transformed to allantoin, and it has been identified as a significant salivary biomarker. Therefore, it is not only significant for monitoring oxidative stress but also by counteracting it (8). Another newly discovered salivary antioxidant which will be explained more in the following is melatonin.

Furthermore, recent research approached the role of oxidative stress in caries and periodontal disease. Microbial biofilm (plaque) persistence on the dental surface and migration of this plaque into the surrounding periodontal pockets

induces the recruitment of leukocytes from the bloodstream to the infection site. 50-70% of the leukocytes are polymorphonuclear neutrophils and their infiltration serves as the first line of defense against the bacteria found in dental plaque in the periodontal pockets where they are utilizing a variety of defense mechanisms, including degranulation, chemotaxis, phagocytosis, and the release of ROS. The interaction between the subgingival biofilm and the host immune response including ROS are critical in the pathogenesis of periodontitis (4,11).

1.2 Antioxidants

AO are defined as substances that retard, inhibit, or eliminate oxidative damage to a target molecule (12). They donate one of their electrons to stop the electron-stealing of FR and thereby neutrale them. Although they lose an electron the antioxidant nutrients do not become FR because they are stable in any state. Antioxidants of note include Vitamin E (alpha tocopherol), Vitamin C (ascorbic acid), or Vitamin A (beta carotene), which all are examples of chain-breaking or scavenging vitamins. Antioxidant preventatives that primarily function by sequestering transition metal ions and inhibiting Fenton reactions and are predominantly proteins include albumin, transferring or lactoferrin (13).

Various studies analyze different antioxidants and their impacts on health. One of these found out that a dentifrice to which green tea catechins were added reduced periodontal inflammation my minimizing OS and inflammatory cytokine expression (14). The non-oxidized and non-fermented green tea contains catechins such as epigallocatechin-3 gallate, epicatechin-3 gallate, epicatechin, and epigallocatechin. The expression of Tumor necrosis factor-α (TNF-α), an inflammatory cytokine that appears through macrophages during inflammation and increasing ROS production (15,16) was more than 70% suppressed by topical green tea catechin supplementation (14).

1.3 Melatonin

As previously mentioned, another AO that is majorly on the popularity rise is melatonin. This is an indoleamine, a kind of neurotransmitter released following

the circadian rhythm by the pineal gland. Mostly it is known as the “sleeping hormone” that regulates the body’s sleep-wake cycle, and many people administer exogeneous melatonin as a pill or sublingual spray to promote sleep. Endogenous melatonin is created not only in the pineal gland but also in a variety of organs by melatonin-forming enzymes in multiple tissues, including the retina, ovaries, gastrointestinal tract, immune system cells and more creation sites are discovered. For example, it was proven that melatonin is not only secreted but also created by the salivary glands (17,18).

Melatonin has been shown to be a multifunctional pleiotropic molecule (19). It acts as an antioxidant by scavenging free radicals and as an immunomodulator, modulator of the circadian rhythm, oncostatic agent and more functions are still being found. Its anti-inflammatory properties limit the overexpression of pro-inflammatory mediators and cytokines (20). Recent studies compared the levels of present melatonin in saliva and gingival crevicular fluid and determined that it has an inverse correlation with the severance of periodontal disease. That is, it is present in lower amounts in patients with periodontal deteriorations, as well as in other dental diseases. Healthy control groups presented higher melatonin levels, suggesting it to have a protective role (21,22).

For this reason, high expectations are emerging, that it may be able to protect the oral cavity from free radicals produced by inflammatory illnesses (23). Since the past decade many animal studies are being conducted, experimenting with endogenous as well as artificially made melatonin that was applied topically as gel formulas and systemically through a pill or sublingual spray to assess its effects on dental and periodontal tissues (24,25).

Melatonin is presumably present in the mitochondria of all animals and plants, where it is also found in chloroplasts, and has been traced back to origin of life, where it seemingly evolved in bacteria which were phagocytosed by eukaryotes. It is synthesized by the amino acid tryptophan. In animals, tryptophan is hydroxylated to 5-hydroxytryptophan which with the generation of serotonin is then decarboxylated. Serotonin is acetylated to N-acetylserotonin or it is methylated to create 5-methoxytryptamine. Eventually these are methylated or acetylated to produce melatonin (26). To obtain supplements, the first melatonin commercially available was made by the extraction of pineal glands of animals,

such as cows. Along with this come many disadvantages, most importantly the risk of viral contamination by the animal tissue. Nowadays melatonin is artificially synthesized in laboratories by complex chemical procedures (27).

1.4 Periodontal Disease

Periodontal disease is an inflammatory, non-contagious, chronic condition affecting the surrounding tissues of the teeth. Periodontally healthy conditions preside a symbiosis between the oral microbiota and the immune response of the host. Various influences can disturb this symbiosis where the increase of most often opportunistic gram-negative bacteria cause a proinflammatory dysbiosis. The first stage of periodontal disease is gingivitis, which is the inflammation of the gums accompanied by bleeding, swelling and erythema due to bacterial biofilm and plaque formation. The compounds including toxins, enzymes, H_2S and NH_3 released by plaque bacteria cause an inflammatory host response which potentially leads to gingival pocket formation along with retaining more plaque and bacteria. A gingival pocket is the pathologic loss of tissue between tooth and gingiva that creates a space between them. The pathologic mechanism can progress to Periodontitis, causing the deepening of the pockets and involving the bone, generating its resorption around the teeth and with that irreversible damage, such as tooth mobility and tooth loss (28,29).

About more than 50% of the world population are thought to be affected moderately by the disease and between 5% to 20% suffer from severe generalized periodontitis (30). Next to the already stated main groups of periodontal disease (Gingivitis and Periodontitis), periodontal disease is further classified according to chronicity, severity, etiology, and extent. Its severity is determined based on the amount of clinical attachment loss (CAL). This is the distance between the cemento-enamel-junction (CEJ) to the periodontal sulcus. Pocket depth (PD) is the distance between the gingival margin to the apical portion of the gingival sulcus. While in healthy conditions it is 1 to 3 mm deep with progress of periodontal pathology it can become deeper. Some conditions, such as the use of medications can cause gingival overgrowth and with that result in a pocket depth of greater than 3 mm, even though there is no gum recession. This

is called a pseudo pocket. In other situations, the gingival recession can be great enough to be apical from the CEJ and the PD is less than 1 mm, falsely masking the severity of the recession. For this reason, measuring both PD and CAL is more exact (31).

To classify the severity and progress of periodontal disease there has been established the 2017 world workshop classification as an update to the International Classification of Periodontal Diseases in 1999. It divides the stages of periodontitis in four: Stage I is defined as ‘early’ or ‘mild’ periodontitis with less than 15% of bone loss, Stage II is termed as ‘moderate’ periodontitis with bone loss in the coronal third of the root, stage III is ‘severe’ periodontitis with bone loss until the middle third of the root and stage IV is defined as ‘very severe’ with bone loss in the apical third of the root. Furthermore, periodontitis is classified into either ‘localized’, when less than 30% of the teeth are affected and generalized when there are more affection sites. The velocity is categorized in grade A (slow) with less than 0.5% of bone loss divided by age, grade B (moderate) with 0.5% to 1% of bone loss divided by the age and grade C (rapid) with more than 1% of bone loss divided by the age (32,33).

For treatment of periodontitis non-surgical periodontal therapy (NSPT) is widely used nowadays and throughout history and accepted as the “golden rule”. It consists of plaque removal, which includes regular tooth brushing and dental flossing to clean interdental spaces and supra- and subgingival scaling and root surface debridement with the help of ultrasounds and manual hand instruments such as curettes (34).

Many studies showed that periodontal disease is linked to several other diseases, among the top to diabetes type 1 and type 2, mainly to the latter one. Research focuses on the bidirectional relationship between them, on how diabetes increases periodontal disease as well as the other way around: how periodontal disease favors diabetes (35). The exact mechanisms of action have not yet been defined; however, several factors are accepted to be the major players. Very briefly, involved are altered cell functioning due to elevated immunoinflammatory responses and proinflammatory cytokines and with that altered wound healing and immune response (35,36).

2. Justification, Hypothesis and Objectives

2.1 Justification

Melatonin is known as the sleeping hormone and exogenous melatonin has shown great health benefits with exceptionally few toxic effects. Recently it has been a great interest in research for the general health due to its multifaceted characteristics. It functions as an antioxidant due to its scavenging capability of ROS and has shown to be beneficial not only in sleeping disorders but also in many other health alterations (37).

Periodontitis is one of the most common oral inflammatory diseases which involves tissue destruction consequently by the immune system to an excessive biofilm dysbiosis. It comes along with multiple problems, such as a reduced quality of life, functional and aesthetic problems, possible systemic effects, and high costs for the health systems.

Striving for the best and most conservative treatment methods is a key objective in medicine. Therefore, the integration of melatonin in periodontal treatment research, as well as the other way around, the consideration of the oral cavity in the search of health rewards of melatonin seems legitimate.

Conventional treatment of periodontitis as well as other infections in the oral cavity very often include antibiotics and anti-inflammatory drugs. In today's world antibiotic resistance is becoming an increasingly serious problem. Antibiotics are widely utilized, while in opposition novel antibiotics are only slowly being researched and found (38). Consequently, other therapeutic options are urgently needed to limit the use of antibiotics and antibiotic resistance. Melatonin may eventually be used in dentistry as a substitute for antibiotics in certain cases and could cause fewer side effects with at the same time local and systemic health benefits (39).

Those facts jointly support and underscore the scientific relevance of melatonin as a strategy for oral disease prevention and therapy.

Although the exact disease mechanism of the relationship between periodontitis and diabetes is still under investigation, it has been proven that these two pathologies are closely related in various ways and people affected by periodontitis present elevated HbA1c values (26, 27). Therefore, studies about patients with not only periodontal disease but also diabetes were included in this systematic review.

2.2 Hypothesis

Adjunctive melatonin supply in the treatment of periodontal disease provides better results compared to treatment without melatonin administration in terms of reducing probing depth (PD) and clinical attachment loss (CAL).

2.3 General Objectives

The aim of this systematic review is to provide an overview of the applications of melatonin as an adjunctive treatment of periodontitis and to determine possible side effects.

2.4 Specific Objectives

i. Determine if administration of topical or systemic melatonin improves periodontitis.
ii. Compare the results between topical and systemic melatonin administration.
iii. Review possible adverse effects of melatonin administration for periodontitis.

3. Materials and Methods

This systematic review was conducted according to the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (42). The latest publications were investigated to bring forth an overview of endogenous or exogenous melatonin in relation to Periodontitis

3.1 Identification of the PICO question

The biomedical online databases PubMed, Scopus, Medline, and Cochrane were used to search for articles with scientific relevance indexed about the applications of melatonin systemically or topically in periodontitis treatment published until January 2021 to answer the following question: Among patients with periodontal disease (P) does adjunctive treatment with melatonin (I) compared to conventional non-surgical treatment without melatonin (C) superiorly improve periodontal health (O)?

The study question was formed according to the PICO question (Population, Intervention, Comparison, Outcomes). The format of the question was established as the following:

- P (Population): Patients suffering from periodontal disease with or without Diabetes Mellitus
- I (Intervention): Melatonin treatment adjunctively with or without conventional non-surgical therapy with or without adjunctive treatment.
- C (Comparison): Patients suffering from periodontal disease that undergo conventional non-surgical therapy without adjunctive melatonin treatment
- O (Outcome): Treatment with melatonin prevents or improves periodontal disease in a better way than treatment without melatonin

3.2 Source of information and database

An individual search on each selected platform mentioned above was performed to obtain the articles to answer the PICO question and the objectives. On all databases the filter for the following languages was applied: English, Spanish, German and Turkish. Furthermore, the filter of articles between January 2011 and 12th of January 2022 was applied. The Boolean operators 'AND' and 'OR' were used to combine the keywords. The keywords were: "Periodontal Disease", "Periodontitis", "Pyorrhea", "Periodontal Therapy", "Melatonin", "N-acetyl-5-methoxytryptamine", "Pineal hormone melatonin", "Conventional therapy", "Non-surgical periodontal therapy", "NSPT", "Scaling and Root Planing", "Clinical attachment level", "Bleeding on Probing", "Pocket Depth", "Side Effects", "Adverse Effects"

Table 1 Databases used in the Search

Database	Search	Filters	Date
Scopus	ALL (("Periodontal Disease" OR "Periodontitis" OR "Pyorrhoea" OR "C hronic Periodontitis" OR "Aggressive Periodontitis" OR "Periodontal Atrophy") AND ("Melatonin" OR "N-acetyl-5 methoxytryptamine" OR "Pineal hormone melatonin" OR "Receptor, Melatonin, MT1" OR "Receptor, Melatonin,MT2" OR "Receptors, Melatonin+") AND ("Conventional therapy" OR "Non-surgical periodontal therapy" OR "NSPT" OR "Scaling and Root Planing" OR "Dental Scaling") AND ("Clinical attachment level" OR "CAL" OR "Bleeding on Probing" OR "BOP" OR "Pocket Depth" OR "PD" OR "Periodontal Index" OR "Gingival Pocket" OR "Gingivitis, Necrotizing Ulcerative" OR "Periodontal Pocket"))	Date January 2012-January 2022 Languages: English OR Spanish OR German OR Turkish	10.02. 2022
Medline Complete	ALL (("Periodontal Disease" OR "Periodontitis" OR "Pyorrhoea" OR "Chronic Periodontitis" OR "Aggressive Periodontitis" OR "PeriodontaAtrophy") AND ("Melatonin" OR "N-acetyl-	Date January 2012-January 2022	10.02. 2022

	5 methoxytryptamine" OR "Pineal hormone melatonin" OR "Receptor, Melatonin, MT1" OR "Receptor, Melatonin, MT2" OR "Receptors, Melatonin+") AND ("Conventional therapy" OR "Non-surgical periodontal therapy" OR "NSPT" OR "Scaling and Root Planing" OR "Dental Scaling") AND ("Clinical attachment level" OR "CAL" OR "Bleeding on Probing" OR "BOP" OR "Pocket Depth" OR "PD" OR "Periodontal Index" OR "Gingival Pocket" OR "Gingivitis, Necrotizing Ulcerative" OR "Periodontal Pocket"))	Languages: English OR Spanish OR German OR Turkish	

3.3 Search on each database

The search on PubMed was ("Melatonin"[Mesh]) AND "Periodontitis"[Mesh]. Included were controlled clinical trials and randomized controlled trials.

On Scopus, MEDLINE and Cochrane it was ALL (("Periodontal Disease" OR "Periodontitis" OR "Pyorrhoea" OR "Chronic Periodontitis" OR „Aggressive Periodontitis" OR "PeriodontalAtrophy") AND ("Melatonin" OR "N-acetyl-5methoxytryptamine" OR "Pineal hormone melatonin" OR "Receptor, Melatonin,MT1" OR "Receptor, melatonin, MT2" OR "Receptors, Melatonin+") AND ("Conventional therapy" OR "Non-surgical periodontal therapy" OR "NSPT" OR "Scaling and Root Planing" OR "Dental Scaling") AND ("Clinical attachment level" OR "CAL" OR "Bleeding on Probing" OR "BOP" OR "Probing Depth" OR "PD" OR "Periodontal Index" OR "Gingival Pocket" OR "Gingivitis, Necrotizing Ulcerative" OR "Periodontal Pocket")).

3.3.4 Search in Journals

Furthermore, a manual search of the reference lists of the obtained articles and mentioned related articles lists was carried out to find additional relevant publications.

3.4 Inclusion and exclusion criteria

Upon choosing the articles, the following criteria were applied:

Inclusion Criteria

- Population: Adult (at least 18 years old) patients suffering from periodontitis. They are either systemically healthy or diagnosed with with type 2 diabetes mellitus
- Intervention: Test group: NSPT (Non-surgical periodontal treatment) protocol which includes ultrasounds and scaling and root planning with manual curettes combined with topical or systemic melatonin treatment with or without other host modulators (e.g., combined with Vitamin C administration)
- Control group: The same NSPT protocol without melatonin, with a placebo and with or without other host modulators
- Outcomes: Reduction in probing depth (PD) and/or clinical attachment loss (CAL) of all the teeth in the patients' mouth or only the teeth with periodontal pockets of 4 mm or more. Secondary outcomes are changes in the bleeding or plaque index.
- Types of studies: Randomized controlled trials, case-control studies, double-blind placebo-controlled trials, cohort studies

Exclusion Criteria

Excluded were those studies where endogenous melatonin was measured without administering exogenous melatonin and where the intervention was not compared to a placebo treatment. Furthermore, excluded were studies that focused on any other parameters than PD and/or CAL throughout and at the end of the treatment. Papers that reported duplicated data (data that had already been published elsewhere were also excluded.

3.5 Search Strategy

A three-stage selection process was carried out. The selection of studies was carried out by two reviewers (NRN & AP). The first stage reviewed the titles to eliminate irrelevant articles. In the second stage the title and abstracts were filtered according to the type of studies, language, number of patients, interventions, and outcome variables. In the third stage each article was read completely, and the data was taken according to the eligibility to be included in the systematic review.

3.6 Data Extraction

The following information was extracted from the studies about adjunctive melatonin treatment and organized in a table which included the following criteria: Author, Name of Study, Journal, Type of Study, Year, Language, Funding, Country, Ethical Approval, Objectives, Participant's Age and gender, Type of Periodontitis, Type of Melatonin Application, Number of Participants, Inclusion and Exclusion Criteria, Diagnosis tools of Periodontitis, Diabetes assessment (if applicable), Administration Protocol, Assessment Times, Biochemical and periodontal result measurement tools, Treatment duration, Outcomes, Study limitations, complications and special observations.

3.7 Risk of Bias in individual studies:

The Newcastle – Ottawa Quality Assessment scale cohort studies and the CASP Randomized Controlled Trial Standard Checklist Tools were used to assess the risk of bias. If a study had a low risk of bias in all domains, it was considered to have a low risk of bias. If it had at least one doubt in any domain, it was marked to have an unclear risk of bias. And if it showed at least one high risk of bias or doubtful specification it was considered a high risk of bias.

4. Results

4.1 Study Selection

The process of the article selection is summarized in **Error! Reference source not found.**, showing the flow diagram of study selection process with results of the literature search according to PRISMA 2020 guidelines. A total of 84 articles were obtained in the initial search process: Scopus (n = 74) and Medline (n = 10). After removing duplicates with the online tool ‘Endnote’, an individual cross-search in Journals and in the bibliographies of other articles was done but no new relevant articles were found. After screening the titles and abstracts, of these 76 articles 57 were excluded based on the exclusion criteria. In the second screening, 12 more articles were excluded based on not fitting in the inclusion criteria which is demonstrated in **Error! Reference source not found.**. Finally, 7 articles met the inclusion criteria and were included in this systematic review.

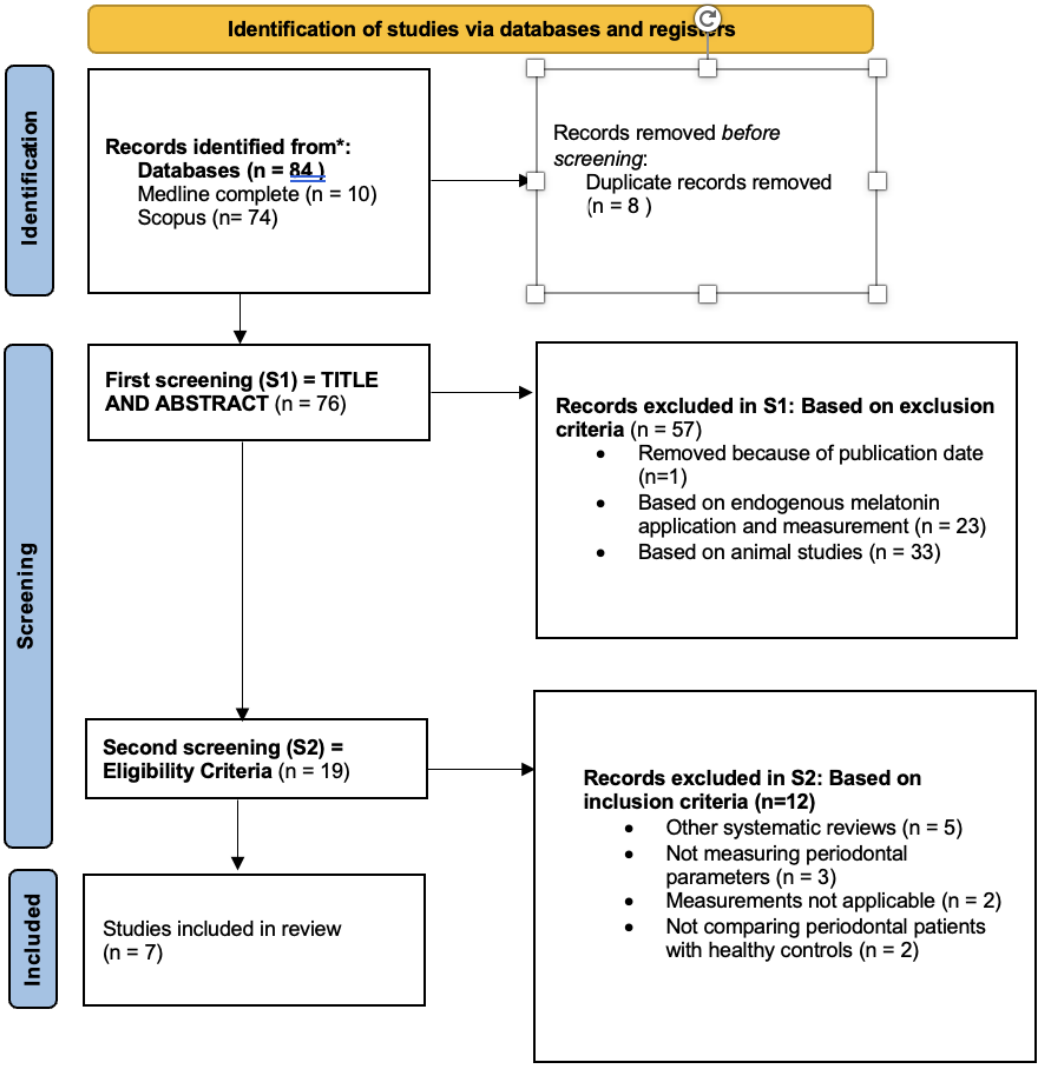


Figure 1. PRISMA 2020 flow diagram for searches of databases, registers and other sources

Table 2 Excluded Articles and reason for their exclusion

Autor and Year	Journal	Reason for exclusion
Liu, R. Y. et al, 2022 (43)	Inflammopharmacology	Systematic Review
Balaji, T, M. et al. 2022 (44)	Oral Diseases	Systematic Review
Chaves e Oliveira, P. et al. 2021 (45)	Oral Diseases	Systematic Review
Konecˇná, B. et al, 2021 (46)	International Journal of molecular sciences	Animal Study
Javid, A. et al, 2020 (47)	Inflammopharmacology	Not measuring periodontal parameters
Swarna Meenakshi, S. et al. 2020 (48)	Indian Journal of Dental Research	Systematic Review
Castro, M. M. L. et al., 2019 (49)	Oxidative Medicine and Cellular Longevity	Systematic Review
Virto, L. et al. , 2018 (50)	Journal of Periodontal Research	Animal Study
Cutando, A. et al, 2015 (51)	Journal Medicina Oral section of Periodontology	Not experimentally directly comparing periodontal patients with healthy controls
Bertl, K. et al, 2013 (22)	Springer	Not measuring periodontal parameters
Cutando, A. et al, 2013 (52)	Odontology(53)	Not experimentally directly comparing periodontal patients with healthy controls
Almughrabi, O.M. et al, 2012 (53)	Journal of periodontal research	Not measuring periodontal parameters

4.2 Study Characteristics

A total of 7 studies were analyzed in this systematic review (54–60). Six of these are randomized controlled clinical trials ((55–60) and one is a cohort study (54). Two studies were carried out in Iran (58,59), two in Egypt (55,57), one in Romania (54), one in Italy (56) and one in Spain (60).

All of these compared periodontal changes when adding melatonin administration versus non-melatonin application. Five studies (54,56–59) used systemic melatonin supplements in form of capsules and two studies (55,60) used topical gel or cream formulas.

A total of 362 subjects were analyzed, approximately half of which served as the control groups. The main characteristics are summarized in ***Error! Reference source not found.***. Briefly, the severity of evaluated periodontitis ranged from mild to severe chronic periodontitis. The age of the participants was mostly middle age between 40 to 50 years old and there were slightly more females than males. Three out of the seven studies included participants diagnosed with diabetes mellitus type 2 (54,58,60). The amount of administered melatonin varied from 1mg to 10mg of melatonin per day, with mostly 6mg per day as well as the duration of application which ranged from 20 days to 8 weeks, with mostly 4 to 8 weeks. All experiments included an additional treatment of NSPT, except one (60). One of the studies compared not only melatonin versus non-melatonin-application, but also melatonin in adjunct with vitamin C application (59).

Table 3 Main characteristics of the included articles.

Author / Year	**Country**	**Type of Study**	**Sample**					
			Sample Size	**Age (range/ mean) & Gender M/F**	**Type of Perio-dontitis**	**Melatonin Application**	**Additional Treatment**	**Diabetes**
Anton, M. et al/ 2021 (39)	Romania	Cohort study	50	49 – 56 N/A	Severe/ moderate/ superficial chronic periodontitis	6 mg melatonin net per day (2 tablets of 250mg with 3mg melatonin each) for 8 weeks	NSPT + oral hygiene, without mouthwash/antiseptic	yes
Ahmed, E. et al/ 2021 (55)	Egypt	Split mouth randomized controlled clinical trial	24	32-55 7/17	Stage II periodontitis	Topical Intrapocket application of 5% melatonin gel 1x per weeks for 4 weeks	NSPT + oral hygiene instructions	no

Tinto, M. et al/ 2019 (56)	Italy	Randomized controlled clinical trial	20	45.6 12/8	Untreated severe stage III periodontitis	1mg melatonin capusels per day for 1 month	NSPT + oral hygiene instructions + rinsing 2x per day 0.2% clorhexidine solution	no
El-Sharkawy, H. et al/ 2018 (57)	Egypt	Randomized Clinical Trial	74	38-55 41/33	Generalized chronic periodontitis	10 mg oral melatonin capsule daily for 2 months	NSPT	no
Bazyar, H. et al/ 2018 (58)	Iran	Double-blind Controlled trial	44	46-61 14/30	chronic periodontitis mild to moderate periodontitis (exclusion of severe periodontitis)	6 mg melatonin net per day (2 tablets of 250mg with 3mg melatonin each) for 8 weeks	NSPT	Yes (excluding insulin treatment)
Chitsazi, M. et al/ 2017 (59)	Iran	Randomozed clinical trial	60	41 29/31	Moderate to severe chronic periodontitis	2 mg melatonin per day for 4 weeks	NSPT	no
Montero, J. et al/ 2017 (60)	Spain	Randomized Clinical Trial	90	40-50 39/51	Chronic periodontitis	Topical (1% orabase cream) melatonin on attached gingiva surfaces for 20 days	Nothing, no brushing of those areas	yes

NSPT: Non-surgical periodontal treatment

4.3 Risk of Bias

One of the selected studies was a cohort study for which the Newcastle - Ottawa Quality Assessment Scale for Cohort Studies was used (61)(Table 4). Out of 9 possible points, it reached 6 and therefore was considered a high risk of bias. The assessment of the other six articles that were randomized controlled clinical trials was conducted with the CASP Randomized Controlled Trial Standard

Checklist was utilized (62) (Table 5 and annex for full questionnaire). The overall results were average, where the most important criteria were met, however methodologies were not always revealed in detail, which may have some concerns that should not be overlooked.

Table 4 Newcastle - Ottawa Quality Assessment Scale for Cohort Studies (61)

Quality Assessment Criteria	
Author/ Year	Anton, M. et al/ 2021
Representativeness of the exposed cohort	*
Selection of the non-exposed cohort	*
Ascertainment of exposure	*
Demonstration that outcome of interest was not present at start of study	No
Study control for age/gender and additional factor	*
Assessment of outcome	*
Was follow-up long enough for outcomes to occur	*
Adequacy of follow up of cohorts	*
Overall Quality Score (max=9)	6

** : Low risk of bias, - : High risk of bias*

Table 5 CASP Randomized Controlled Trial Standard Checklist (62)

Author/Year	**Ahmed, E. et al/ 2021** (55)	**Tinto, M. et al/ 2019** (56)	**El-Sharkawy, H. et al/ 2018** (57)	**Bazyar, H. et al/ 2018** (58)	**Chitsazi, M. et al/ 2017** (59)	**Montero, J. et al/ 2017** (60)
1.Did the study address a clearly focused research question?	*	*	*	*	*	*
2. Was the assignment of participants to interventions randomised?	*	*	*	*	*	*

3. Were all participants who entered the study accounted for at its conclusion?	*	*	*	*	-	*
4. Were the participants 'blind' to intervention they were given? -Were the investigators 'blind' to the intervention they were giving to participants? -Were the people assessing/analysing outcome/s 'blinded'?	* * -	* * -	* * -	* * -	- * -	* - *
5. Were the study groups similar at the start of the randomised controlled trial?	*	No	-	No	*	*
6. Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)?	*	*	-	-	*	*
7. Were the effects of intervention reported comprehensively?	*	*	No	*	*	*
8. Was the precision of the estimate of the intervention or treatment effect reported?	No	No	-	*	No	No
9. Do the benefits of the experimental intervention outweigh the harms	*	-	-	-	*	-
10. Can the results be applied to your local population/in your context?	*	No	-	-	*	-
11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions?	-	-	-	-	*	-

*: Yes, No: No, - : Cannot tell.

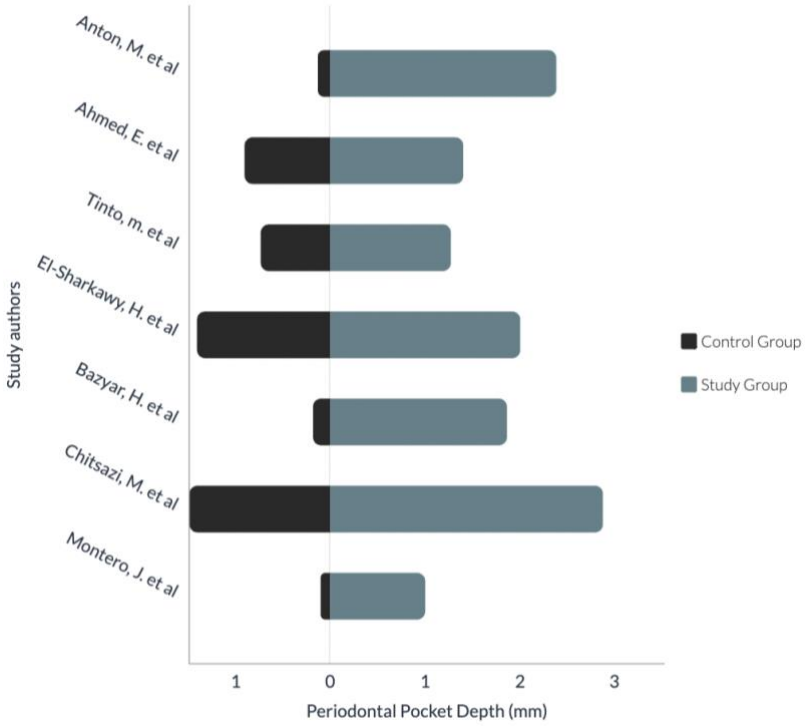
4.4 Results of individual studies

The main results of each article are presented in **Error! Reference source not found.**. The duration of melatonin application ranged from 20 days (60) to 2 months (57,58). Almost all studies measured the differences between the parameters PD and CAL in mm between the time before melatonin application and at the end of the treatment. Two studies solely analyzed PD (56,60). The study by *Chitsazi, M. et al* exclusively analyzed the addition of Vitamin C to melatonin in a second study group and reached more PD (3,35 mm) and CAL (3,3 mm) gain with Vitamin C compared to PD (2,87 mm) and CAL (3,07 mm) when only melatonin was applied (59). The results are illustrated in **Error! Reference source not found.** and **Error! Reference source not found.**. All studies started with nearly identical PD and CAL in their control and study groups, with the biggest deviation of 0,4 mm between the CAL at *T0* in the study of *Ahmed, E. et al* (55). Every article demonstrated improvement of the analyzed parameters in both the control and the study groups. Furthermore, every study obtained better results with the adjunctive use of melatonin than without it. The difference between control and study groups is especially notable for the parameter PD. Two studies showed the biggest difference for both PD and CAL. *Anton et al.* (54) obtained PD shrinkage of 2,38 mm in the melatonin group compared to 0,13 mm in the placebo group and decreased CAL values of 1,81 mm in the melatonin group and 0,04 mm in the control group. *Bazyar, H. et a*l (58) obtained PD shrinkage of 1,86 mm in the melatonin and in comparison, 0,18 mm in the placebo group. The same study obtained decreased CAL values of 1,45 mm in the study group and 0,23 mm in the control group. The least difference of both PD and CAL between the melatonin- and non-melatonin group has been noted in the trial by *El-Sharkawy, H. et al* (57), with a difference of 0,6 mm in the PD loss of the study and control group and 1,3 mm decreased CAL value between study and control group. Furthermore, the same study reported that 20% of their subjects had sleepiness and 10% mild headaches that resolved spontaneously, which was the only study that documented side effects. Besides that, none of the other included studies revealed any side effects by the use of melatonin.

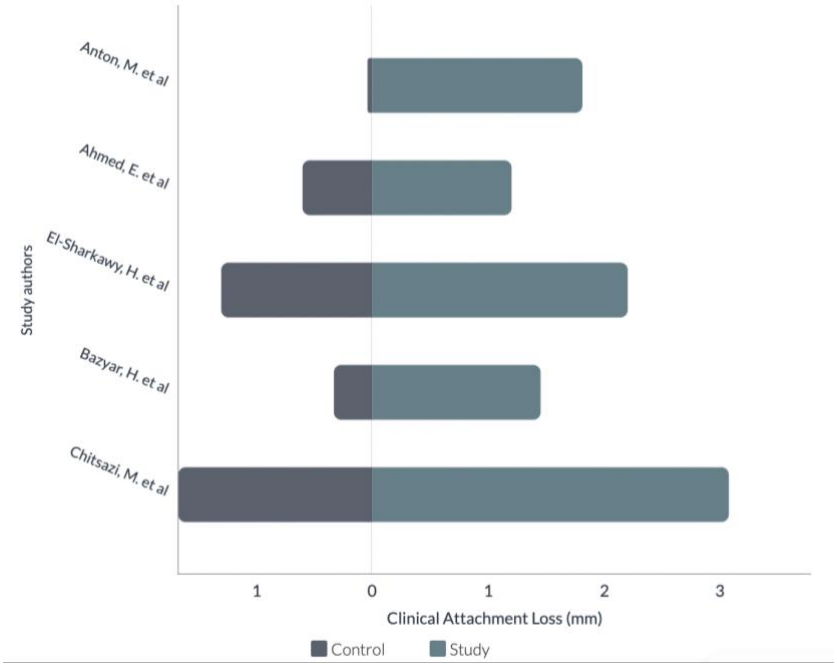
Table 6 Changes of PD and CAL in mm in control and study groups before and after intervention

Author/ Year	T1	Variables	Control Group		Study Group		2. Study Group (Mel + Vit C)	
			T0	T1	T0	T1	T0	T1
Anton, M. et al/ 2021 (39)	8 weeks	PD (mm):	4.53	4.40	4.65	2.27		
		CAL (mm):	3.02	2.98	3.05	1.24		
Ahmed, E. et al/ 2021 (55)	3 months	PD (mm):	4.0	3.1	4.3	2.9		
		CAL (mm):	4.3	3.7	4.7	3.5		
Tinto, M. et al/ 2019 (56)	6 months	PD (mm):	3.40	2.67	3,72	2.45		
		CAL (mm):	N/A	N/A	N/A	N/A		
El-Sharkawy, H. et al/ 2018 (57)	6 months	PD (mm):	4.4	3.0	4.3	2.3		
		CAL (mm):	4.7	3.4	4.8	2.6		
Bazyar, H. et al/ 2018 (58)	8 weeks	PD (mm):	4.54	4.36	4.45	2.59		
		CAL (mm):	3	2.77	3.04	1.59		
Chitsazi, M. et al/ 2017 (59)	6 months	PD (mm):	6.40	4.92	6.41	3.54	6.43	3.08
		CAL (mm):	6.23	4.56	6.29	3.22	6.30	3.00
Montero, J. et al/ 2017 (60)	20 days	PD (mm):	2.7	2.6	2.8	1.8		
		CAL (mm):	N/A	N/A	N/A	N/A		

T0: Baseline, T1: Assessment immediately or later after the intervention, MEL: Melatonin, Vit C: Vitamin C, PD: Probing depth, CAL: Clinical attachment loss, N/A: Not applicable.



Graph 1 PD (mm) Difference T0-T1 comparison between control and study group. T0: Baseline, T1: Assessment immediately or later after the intervention



Graph 2 CAL (mm) Difference T0-T1 comparison between control and study group. T0: Baseline, T1: Assessment immediately or later after the intervention

5. Discussion

This systematic review informs about the outcome of melatonin application topically as well as systematically in periodontal patients. The objective was to evaluate if the administration of melatonin additionally to conventional treatment improves periodontal parameters and furthermore to compare the results between topical and systemic application. PD and CAL were analyzed, and side effects reported.

5.1 Description of Methodology

The form of melatonin, its concentration as well as its technique of administration differed significantly between the different studies. All but one studies conducted NSPT and in addition intervened with melatonin application. Five of the seven studies used systemic melatonin application in the form of tablets and capsules (54,56–59), and two applied it topically as a cream (55,60). Almost all application forms showed heterogeneity in its dosage, additives, application frequency as well as in the duration of the intervention. Two studies used 6mg of melatonin per day in form of two capsules with 3mg each, two times a day for 8 weeks (54,58). Another study applied 1mg melatonin capsules per day for 4 weeks (56) and in contrast a different one applied 10mg melatonin per day for 8 weeks (57). Apart from the doses, frequency and time span, the sample populations also showed variations among the articles. This systematic review includes articles about systemically healthy as well as diabetes patients. Three studies included patients with diabetes, of which one examined only diabetes type II (54), one with diabetes type II but excluding those who underwent insulin treatment (58), or including diabetes type I and type II patients (60). Furthermore, the sample populations did not coincide in terms of their state of periodontal disease. The types of periodontitis in the present articles not only varied among each other from generalized, chronic and mild (54,57–59) to severe periodontitis (54,56,59), but some also included a broad range of the disease within their own samples. Namely the studies conducted by *Anton et al.* contained subjects with severe, as well as with superficial chronic periodontitis (54) and *Chitsazi et al.*

encompassed moderate and severe periodontitis (59). Lastly, the sample sizes among the analyzed articles varied from 24 subjects (55) to 90 (60), the average size being 52.

5.2 Summary of main results.

Recent research has shown that administering melatonin therapy to individuals with periodontal disease results in an improvement in the status of the illness. The purpose of this study was to get closer to making a decision about whether or not there is an improvement and to compare the results that were achieved by systemic applications with the results that were achieved by topical applications in regard to the most important clinical periodontal parameters, which were PD and CAL. This review included a total of seven publications, each of which compared melatonin application plus NSPT with merely traditional NSPT, with the exception of one study by *Montero et al.,* that compared melatonin application to having no therapy at all (60).

The findings of the current investigation provide evidence to support the idea that the presence of additional melatonin supplementation is more effective in lowering PD and CAL than its absence. However, the fact that all studies showed very similar results even though their variables differed significantly from one another, such as mode of application, dosage, frequency, duration, and sample variables including sample population size, age, general health, and periodontal state make it impossible to ascertain how specific factors influence the outcome. Nonetheless, the ubiquitous use of melatonin was associated with an improvement in periodontal markers. In order to better understand the actual process, additional tests need be undertaken under specified, consistent conditions.

5.3 Probing Depth and Clinical Attachment Level

The clinical measurement of periodontal disease is based on PD, which is the distance from the gingival margin to the apical portion of the gingiva, and CAL,

which describes the distance between the cemento-enamel-junction and the periodontal sulcus. Both are measured in millimeters with a periodontal probe. The studies that were considered for this systematic review took measurements of PD and CAL before the beginning of the experimental intervention at baseline (T0) and after it had been completed (T1). Some studies measured T1 immediately at the end of their intervention (54,58,60), while the rest assessed T1 later, the latest being after 6 months in the studies by *Tinto et al.* (56), *El-Sharkawy et al.* (57) and *Chitsazi et al.* (59).

Although PD and CAL do not always coincide due to factors such as gingival hyperplasia or severe recession, all the articles included in this current review showed consistent correlations between their measured PD and CAL. Notably, all of the articles without exception came to the same conclusion, indicating that the study groups given melatonin obtained a greater reduction of PD and CAL than the control groups did. The biggest contrast of results between melatonin and non-melatonin application have been gathered by the three studies that used diabetic patients as their population sample (54,58,60), illustrated in Graph 1 and Graph 2. Causal to this can be that melatonin improves diabetic parameters, such as lowering fasting glucose, glycated hemoglobin, and insulin resistance, as is supported by the systematic reviews carried out by *Delpino et al.* (63) and *Lauritzen et al.* (64). Among the three studies included in the present systematic review, *Anton et al.* additionally demonstrated lower glycated hemoglobin levels in the melatonin group compared to the control group (54). *Bazyar et al.* obtained lower Interleukin-6 (IL-6) and C-reactive protein (CRP) levels in the melatonin groups, which are both predicting biomarkers of diabetes (58).

Finally, the results after melatonin intervention in the study by *Montero et al.* revealed lower levels of interleukin-1β (IL-1β), interleukin-6, and prostaglandin E2 (PGE2) values in addition to a reduction in PD and CAL (60). Therefore, the results of correlation between improved periodontal and diabetic parameters after melatonin intervention coincide with the general assumption, that periodontitis and diabetes are linked to each other.

All population samples of the non-intervention groups, albeit less than in the melatonin intervention groups showed reduced PD and CAL results. The potential reason lies in the fact that NSPT has been conducted in all groups regardless of melatonin intervention. The one study that makes the exception is

by *Montero et al.* which at the same time shows the least changes of PD and CAL in study and control groups. Nonetheless, it should be noted that concurrently this study had the shortest duration of intervention and assessment (T1) of 20 days (60). Therefore, the results of the here included studies do not clarify whether the increased PD and CAL improvement is due to NSPT, treatment duration or assessment time. Analyzing the intervention periods of the other included studies does not indicate a clear correlation between experiment duration and increased PD and CAL success. As a contrast to *Montero et al.* the study conducted by *Chitsazi et al*. had the highest success rate with up to 3,07 mm CAL reduction in the melatonin group and 1,67 mm CAL reduction in the control group with similar PD reduction results, while its intervention duration was 4 weeks, but the periodontal parameters were measured after 6 months (59). This gives rise to the question whether a longer recovery period is required to reach PD and CAL gain. Furthermore, the experiment by *Chitsazi et al.* had a sample size of 60 subjects and with those three times as large as *Tinto et al.* with 20 subjects. It also used a double as high melatonin dosage with 2mg net systemic melatonin per day while *Tinto et al.* used 1mg per day (56), and showed lower risk of bias. Nevertheless, that does not explain the improved results among the non-melatonin groups. The increased changes of PD and CAL in each included study that conducted NSPT compared to the study by *Montero et al.* that left NSPT out, indicate, that NSPT improves periodontal parameters regardless of melatonin intervention. This is supported by the generally accepted “golden rule” of periodontal treatment and suggested by numerous studies and systematic reviews, such as the ones conducted by *Khan et al.* (65) and *Botelho et al.* (66).

5.4 Variations Melatonin Administration

This systematic study had several aims, one of which was to evaluate the efficacy of systemic melatonin against topical melatonin. Two of the seven studies conducted experiments using topical melatonin, whereas the others used systemic formulations in the form of capsules. The randomized controlled trial by *Ahmed et al.* used a topical cream once every week for four weeks and obtained fewer contrasting results between the melatonin and non-melatonin group (55).

Montero et al. (60). used a cream every day for twenty days and achieved a very high difference between the intervention and study groups, suggesting that higher frequency increases success more than higher duration. Considering the findings of all randomized controlled trials and the cohort study together, there is no discernible difference between topical and systemic administration. Since only two of the seven included trials used topical administration and the other five utilized systemic administration, it is hard to draw a definitive conclusion.

Chitsazi et al. (59) observed the greatest PD and CAL increase at assessment after administering 2mg of systemic melatonin per day for four weeks. However, the non-melatonin group also had successful results, which may be attributable to applied NSPT and the fact that the population sample had at least mild to severe periodontal disease, resulting in a greater range of improvement. Out of the included studies the highest used dose of melatonin per day was reported by *El-Sharkawy et al.* (57), where 10mg melatonin was administered daily over a period of 2 months and PD and CAL were measured after 6 months. The results showed a significant difference between the melatonin and non-melatonin groups, however, while the findings were satisfactory, they were not exceptional. As a result, increasing the dosage and duration may result in greater improvement, but the key is finding out which dose and time is most effective. The experiment by *El-Sharkawy et al*. used five times the melatonin dosage for two times as long (2 months) than the one by *Chisatzi et al.* (4 weeks), but received slightly lower PD and CAL gain, even though both studies equally measured the values after 6 months. Although no clear conclusion can be drawn due to the small number of studies, this indicated that rather than high dosage, the frequency and recovery time of several months determine PD and CAL gain.

5.5 Limitations

Even though the present systematic review's findings point to the same direction, it is important to emphasize certain limitations. Due to the limited scientific proof of melatonin's antioxidative and health-promoting properties up to date, relatively few investigations on its effect on periodontitis have been

undertaken and published until now. Seven studies do not collect sufficient data to make a definitive declaration; nonetheless, given the results are all similar, the present systematic review indicates that additional testing will likely yield encouraging outcomes. Variables such as dosage, technique, additives in the manufacturing process, frequency, and duration of melatonin administration varied greatly between the included studies. These factors make it impossible to determine which criteria must be met for the optimal outcome. In addition, the procedure for NSPT, which plays a crucial role in periodontal improvement, was inconsistent. Some studies did not describe their methodology in detail, while those that did utilized different session lengths and numbers. Furthermore, the operators that performed the NSPT in research environments most likely have varied skill levels and procedures, and it is unknown how these differences translate to general dentists. In addition, the success of NSPT depends on characteristics such as tooth type, furcation involvement, and smoking. All included articles in this systematic review excluded patients who smoked. According to a study by *Van der Weijden et al.,* treatment success was frequently higher for single-rooted teeth than for molars, particularly for those with furcation involvement. The here included studies did not disclose the type of teeth on which NSPT was performed, hence introducing an added significant variable that demonstrates inconsistency. Besides, the success rate was correlated with the severity of periodontal disease, similar to the results of the present systematic review (67).

Some included studies recommended further mouth rinsing with chlorhexidine solution (56), while others did not do the NSPT or brush their teeth to solely analyze the effect of melatonin (60). Each study included in this systematic review aggregated data to provide a summary by a statistical approach based on mean values for PD and CAL changes. While this provides for an overall assessment of the influence of melatonin on a patient's periodontal health, it should be noted that in relation to the beforementioned, periodontitis is often a site-specific condition, and by combining specific site-level assessments, crucial information may be lost. The sample populations included a wide range of ages (32-61 years), variances in the presence and type of diabetes as well as periodontal disease. The gender was also uneven; however, it may have a minor role in PD and CAL gain. Matter of fact, the range of circumstances with a

consistent good outcome may also be a strength for demonstrating the efficacy of melatonin.

This systematic review, which found no serious side effects or complications, suggests that its use as an adjunct in periodontal therapy is safe. However, it should be noted that melatonin research is still in its early stages, and thus negative effects cannot be ruled out until more extensive and long-term research is conducted. The same is true for the effects of synthetic melatonin over the endogenous melatonin release and how the body's negative feedback response operates. Conclusively much more research with various variables is required to make a clear indication. It should be noted that according to the risk of bias assessment in this present review, biases cannot be discarded completely although the most important criteria have been met. In the clinical practice adjuvant melatonin use may potentially reduce the excessive use of antibiotics and other conventional treatments for periodontal disease. More research with consistent variables and larger population samples appears promising for achieving the greatest success to increase periodontal health.

6. CONCLUSIONS

The purpose of this systematic review was to provide an overview of melatonin's use as an adjuvant therapy for periodontitis. Based on the primary objective melatonin may be delivered systemically or topically to promote periodontal health.

In relation to the secondary aims, both administration methods enhance periodontal parameters. Comparing systemic vs topical treatment for pocket depth and clinical attachment level gain both methods were successful and revealed no significant differences.

Other than rare and minor instances of sleepiness and headaches, no adverse effects have been observed.

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ANNEX

TITLE: “Melatonin as a Treatment Option for Periodontitis: A Systematic Review”

RUNNING TITLE: Melatonin as a Treatment Option for Periodontitis

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ABSTRACT

- Objectives: Provide an overview of the applications of melatonin as an adjunctive treatment of periodontitis and compare the results between topical and systemic melatonin by evaluating Probing Depth (PD) and Clinical Attachment Level (CAL) changes and determine possible adverse effects.
- Materials and Methods: Following the recommended methods for systematic reviews (PRISMA), an electronic search was conducted in the databases of Cochrane, MEDLINE, PubMed and Scopus on melatonin and periodontitis. The following PICO question was applied to guide the systematic review: Among patients suffering from periodontal disease with or without diabetes (P) does melatonin treatment with or without conventional non-surgical periodontal therapy (NSPT) (I) compared to non-melatonin treatment (C) improve PD and CAL levels more? (O)
- Results: Among 84 potentially eligible articles, 7 complied with the inclusion criteria. Study characteristics including patient data as well as form, dosage, frequency, and duration of melatonin administration were noted. The clinical parameters PD and CAL were compared at Baseline (T0) and post-intervention (T1). Each study presented higher PD and CAL gain with adjuvant melatonin use.
- Discussion: Although the results indicate success of each form of melatonin use, several limitations must be in considered, such as that seven articles are not enough to draw clear conclusions and the variables of each experiment varied greatly. Nonetheless this gives also rise to hope for future research.
- Conclusions: Melatonin administration promotes periodontal health in terms of PD and CAL and systemical as well as topical use present no significant differences. Other than rare and minor instances of sleepinesss and headaches, no adverse effects have been observed.

***Keywords**: Periodontal Disease, Periodontal Disease, Melatonin, N-acetyl-5-methoxytryptamine, Non-surgical periodontal therapy*

1. INTRODUCTION

Oxidative Stress (OS) is an an altered biochemical homeostasis that is favoring several diseases. Recent research approached the role of oxidative stress in caries and periodontal disease (1,2). Antioxidants (AO) are defined as substances that retard, inhibit, or eliminate oxidative damage to a target molecule (2). One AO that is majorly on the popularity rise is melatonin, which is an indoleamine released by the pineal gland following the circadian rhythm (3). Mostly it is known as the "sleeping hormone" that regulates the body's sleep-wake cycle, and many people administer exogeneous melatonin as a pill or sublingual spray to promote sleep. Melatonin has been shown to be a multifunctional pleiotropic molecule, that acts as an antioxidant by scavenging free radicals (FR), as an immunomodulator, oncostatic agent and more functions are still being found. Its anti-inflammatory properties limit the overexpression of pro-inflammatory mediators and cytokines (3). Recent studies compared the levels of present melatonin in saliva and gingival crevicular fluid and determined that it has an inverse correlation with the severance of periodontal disease (4). Since the past decade many animal studies suggested that melatonin applied topically as gel formulas and systemically through a pill or sublingual spray showed positive effects on dental and periodontal tissues (5). Periodontal disease is a highly prevalent inflammatory, chronic condition affecting the surrounding tissues of the teeth. Its severity is determined based on the amount of clinical attachment loss (CAL), which describes the distance between the cemento-enamel-junction to the periodontal sulcus. Pocket depth (PD) is the distance between the gingival margin to the apical portion of the gingival sulcus, that in healthy conditions is 1 to 3 mm deep (4,6). For treatment of periodontitis non-surgical periodontal therapy (NSPT) is widely used and accepted as the "golden rule" (7). Methods other than antibiotics and anti-inflammatories are urgently researched for periodontal treatment. The present systematic review aimed to determine if melatonin application may be a viable solution and improve PD and CAL levels, as well as compare systemic with topical administration and reveal possible adverse effects.

2. MATERIALS AND METHODS

Protocol and focused question: The present systematic review was carried out according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). The following focus question was employed according to the PICO question: Among patients suffering from periodontal disease with or without diabetes (P) does melatonin treatment with or without conventional non-surgical periodontal therapy (NSPT) (I) compared to non-melatonin treatment (C) improve PD and CAL levels more? (O)

Selection criteria: Studies were excluded based on the following criteria: studies older than 10 years, animal studies, only measuring endogenous melatonin. The inclusion criteria were studies that analysed adults older than 18 years old suffering from periodontitis, either systemically healthy or diagnosed with type 2 DM and executing the intervention by applying melatonin systemically or topically to compare PD and CAL at baseline and after the intervention.

Search strategy: The search was executed in the databases of Cochrane, MEDLINE, PubMed and Scopus on melatonin and periodontitis to find articles published between January 2012 and January 2022. The algorithm used for the search was the following: ("Periodontal Disease" OR "Periodontitis" OR "Pyrorrhoea") AND ("Melatonin" OR "N-acetyl-5methoxytryptamine") AND ("Conventional Therapy" OR Non-surgical periodontal therapy" OR "NSPT" OR "Scaling and Root Planing") AND ("Clinical Attachment Level" OR "CAL" OR "Probing Depth" OR "PD"). Screening methods and data extraction: A three-stage selection process was carried out. The selection of studies was carried out by two reviewers (NRN & APC). The first stage reviewed the titles to eliminate irrelevant articles. In the second stage the title and abstracts were filtered according to the type of studies, language, number of patients, interventions, and outcome variables. In the third stage each article was read completely, and the data was taken according to the eligibility to be included in the systematic review.

Risk of bias in individual studies: The Newcastle - Ottawa Quality Assessment scale for cohort studies and the CASP Randomized Controlled Trial Standard Checklist

tools were used to assess the risk of bias. If a study had a low risk of bias in all domains, it was considered to have a low risk of bias. If it had at least one doubt in any domain, it was marked to have an unclear risk of bias. And if it showed at least one high risk of bias or doubtful specification it was considered a high risk of bias. **Data extraction:** The following information was extracted from the studies about adjunctive melatonin treatment and organized in a table which included the following criteria: Author, Name of Study, Journal, Type of Study, Year, Language, Funding, Country, Ethical Approval, Objectives, Participant's Age and gender, Type of Periodontitis, Type of Melatonin Application, Number of Participants, Inclusion and Exclusion Criteria, Diagnosis tools of Periodontitis, Diabetes assessment (if applicable), Administration Protocol, Assessment Times, Biochemical and periodontal result measurement tools, Treatment duration, Outcomes, Study limitations, complications and special observations.

3. RESULTS

Study selection: The process of the article selection is summarized in **Error! Reference source not found.**. A total of 84 articles were obtained in the initial search process: Scopus (n = 74) and Medline (n = 10). After removing duplicates with the online tool 'Endnote', an individual cross-search in Journals and in the bibliographies of other articles was done but no new relevant articles were found. After screening the titles and abstracts, of these 76 articles 57 were excluded based on the exclusion criteria. In the second screening, 12 more articles were excluded based on not fitting in the inclusion criteria. Finally, 7 articles met the inclusion criteria and were included in this systematic review. Characteristics of included studies: The main characteristics of the analyzed study groups and controls are summarized in Table 1. Briefly, the severity of evaluated periodontitis ranged from mild to severe chronic periodontitis. The age of the participants was mostly middle age between 40 to 50 years old and there were slightly more females than males. Three out of the seven studies included participants diagnosed with diabetes mellitus type 2 (8–10). Melatonin administration varied from 1mg to 10mg of

melatonin per day, with mostly 6mg per day as well as the duration of application which ranged from 20 days to 8 weeks, with mostly 4 to 8 weeks. All experiments included an additional treatment of NSPT, except one (10). One of the studies compared not only melatonin versus non-melatonin-application, but also melatonin in adjunct with vitamin C application (11). Risk of bias: One of the studies used the Newcastle-Ottawa Quality Assessment Scale for Cohort Studies(12) as seen in TABLE. It scored 6 out of 9 and was considered a high risk of bias. The CASP Randomized Controlled Trial Standard Checklist (13) was used to evaluate the other six RCTs (TABLE). The overall results were average, meeting the most important criteria, but methodologies were not always detailed, which raises some concerns. Results of individual studies: The main results of each article are presented in Table 2. The duration of melatonin application ranged from 20 days (10) to 2 months (9,14). Almost all studies measured the differences between the parameters PD and CAL in mm between the time before melatonin application and at the end of the treatment. Two studies solely analyzed PD (10,15). The study by Chitsazi, M. et al exclusively analyzed the addition of Vitamin C to melatonin in a second study group and reached more PD (3,35 mm) and CAL (3,3 mm) gain with Vitamin C compared to PD (2,87 mm) and CAL (3,07 mm) when only melatonin was applied (11). The results are illustrated in **Error! Reference source not found.** and **Error! Reference source not found.**. All studies started with nearly identical PD and CAL in their control and study groups, with the biggest deviation of 0,4 mm between the CAL at T0 in the study of Ahmed, E. et al. (16). Every article demonstrated improvement of the analyzed parameters in both the control and the study groups. Furthermore, every study obtained better results with the adjunctive use of melatonin than without it. The difference between control and study groups is especially notable for the parameter PD. Two studies showed the biggest difference for both PD and CAL. Anton et al. (8) obtained PD shrinkage of 2,38 mm in the melatonin group compared to 0,13 mm in the placebo group and decreased CAL values of 1,81 mm in the melatonin group and 0,04 mm in the control group. Bazyar, H. et al (9) obtained PD shrinkage of 1,86 mm in the melatonin and in

comparison, 0,18 mm in the placebo group. The same study obtained decreased CAL values of 1,45 mm in the study group and 0,23 mm in the control group. The least difference of both PD and CAL between the melatonin- and non-melatonin group has been noted in the trial by El-Sharkawy, H. et al (14), with a difference of 0,6 mm in the PD loss of the study and control group and 1,3 mm decreased CAL value between study and control group. Furthermore, the same study reported that 20% of their subjects had sleepiness and 10% mild headaches which resolved spontaneously. Besides that, none of the other included studies revealed any side effects using melatonin.

4. DISCUSSION:

The objective was to evaluate if the administration of melatonin additionally to conventional treatment improves periodontal parameters and to compare the results between topical and systemic application. PD and CAL were analyzed, and side effects reported. Methodology: The form of melatonin, its concentration as well as its technique of administration differed significantly between the different studies. All but one studies conducted NSPT and in addition intervened with melatonin application. Five of the seven studies used systemic melatonin application in the form of tablets and capsules (8,9,11,14,15), and two applied it topically as a cream (10,16). Almost all application forms showed heterogeneity in its dosage, additives, application frequency as well as in the duration of the intervention. Two studies used 6mg of melatonin per day in form of two capsules with 3mg each, two times a day for 8 weeks (8,9). Another study applied 1mg melatonin capsules per day for 4 weeks (15) and in contrast a different one applied 10mg melatonin per day for 8 weeks (14). Apart from the doses, frequency and duration, the sample populations also showed variations. The types of periodontitis in the present articles not only varied among each other from generalized, chronic and mild (8,9,11,14) to severe periodontitis (8,11,15), but some also included a broad range of the disease within their own samples. Namely the studies conducted by *Anton et al.* contained subjects with severe, as well as with superficial

chronic periodontitis (8) and *Chitsazi et al.* encompassed moderate and severe periodontitis (11).

Summary of main results: Recent research has shown that administering melatonin therapy to individuals with periodontal disease results in an improvement in the status of the illness. The purpose of this study was to get closer to deciding about whether there is an improvement and to compare the results that were achieved by systemic applications with the results that were achieved by topical applications regarding the most important clinical periodontal parameters, which were PD and CAL. The findings of the current investigation provide evidence to support the idea that the presence of additional melatonin supplementation is more effective in lowering PD and CAL than its absence. However, the fact that all studies showed very similar results even though their variables differed significantly from one another, such as their mode of application, dosage, frequency, duration, and sample characteristics including sample population size, age, general health, and periodontal state make it impossible to ascertain how specific factors influence the outcome. Nonetheless, the ubiquitous use of melatonin was associated with an improvement in periodontal markers.

Probing Depth and Clinical Attachment Level: The clinical measurement of periodontal disease is based on PD, which is the distance from the gingival margin to the apical portion of the gingiva, and CAL, which describes the distance between the cemento-enamel-junction and the periodontal sulcus, measured in mm with a periodontal probe. The studies that were considered for this systematic review took measurements of PD and CAL both before the beginning of the experimental intervention at baseline (T0) and after it had been completed (T1). Although PD and CAL do not always coincide due to factors such as gingival hyperplasia or severe recession, all the articles included in this current review showed consistent correlations between their measured PD and CAL. This was the case even though PD and CAL naturally do not always coincide. Notably, all the articles without exception came to the same conclusion, indicating that the study groups given melatonin obtained a greater reduction of PD and CAL than the control groups did.

The biggest contrast of results between melatonin and non-melatonin application have been gathered by the three studies that used diabetic patients as their population sample (8–10), illustrated in Graph 1 and Graph 2. Causal to this may be that melatonin improves diabetic parameters, such as lowering fasting glucose, glycated hemoglobin, and insulin resistance, as is supported by the systematic reviews carried out by Delpino et al. (17) and Lauritzen et al. (18). All population samples of the non-intervention groups, albeit less than in the melatonin intervention groups showed reduced PD and CAL results. The potential reason lies in the fact that NSPT has been conducted in all groups regardless of melatonin intervention. The one study that makes the exception is by Montero et al. which at the same time shows the least changes of PD and CAL in study and control groups. Nonetheless, it should be noted that concurrently this study had the shortest duration of intervention and assessment (T1) of 20 days (10). Therefore, the results of the here included studies do not clarify elucidate whether the increased PD and CAL improvement is due to NSPT, treatment duration or assessment time. Analyzing the intervention periods of the other included studies does not indicate a clear correlation between experiment duration and increased PD and CAL success. As a contrast to Montero et al. the study conducted by Chitsazi et al. had the highest success rate with up to 3,07 mm CAL reduction in the melatonin group and 1,67 mm CAL reduction in the control group with similar PD reduction results, while its intervention duration was 4 weeks, but the periodontal parameters were measured after 6 months (11). This gives rise to the question whether a longer time period is required to reach PD and CAL gain Furthermore, the experiment by Chitsazi et al. had a sample size of 60 subjects and with those three times as large as Tinto et al. with 20 subjects. It also used a double as high melatonin dosage with 2mg net systemic melatonin per day while Tinto et al. used 1mg per day (15) and showed lower risk of bias. Nevertheless, that does not explain the improved results among the non-melatonin groups. The increased changes of PD and CAL in each included study that conducted NSPT compared to the study by Montero et al. that left NSPT out, indicate, that NSPT improves periodontal parameters regardless

of melatonin intervention. This is supported by the generally accepted “golden rule” of periodontal treatment and suggested by numerous studies and systematic reviews, such as the ones conducted by Khan et al. (19) and Botelho et al. (20) **Variations Melatonin Administration:** Two of the seven studies conducted experiments using topical melatonin, whereas the others used systemic formulations in the form of capsules. The randomized controlled trial by Ahmed et al. used a topical cream once every week for four weeks and obtained fewer contrasting results between the melatonin and non-melatonin group (16). Montero et al. (10) used a cream every day for twenty days and achieved a very high difference between the intervention and study groups, suggesting that higher frequency increases success more than higher duration. Considering the findings of all randomized controlled trials and the cohort study together, there is no discernible difference between topical and systemic administration. Chitsazi et al. (11) observed the greatest PD and CAL increase at assessment after administering 2mg of systemic melatonin per day for four weeks. However, the non-melatonin group also had successful results, which may be attributable to applied NSPT. Out of the included studies the highest used dose of melatonin per day was reported by El-Sharkawy et al. (14), where 10mg melatonin was administered daily over a period of 2 months and PD and CAL were measured after 6 months. The results showed a significant difference between the melatonin and non-melatonin groups, however, while the findings were satisfactory, they were not exceptional. **Limitations:** Few studies don't collect enough data to make a definitive statement, but the similar results suggest that additional testing will likely yield positive results. Dosage, technique, manufacturing additives, frequency, and duration of melatonin administration varied greatly between studies, making it impossible to determine optimal criteria. Also, the NSPT procedure was inconsistent, and the operators likely differed; it's unknown how these translate to general dentists. Each study in this systematic review aggregated data to provide a statistical summary of PD and CAL changes, which provides an overall assessment of melatonin's effect on a patient's periodontal health, but periodontitis is often site-specific, so combining

specific site-level assessments may lose crucial information. The variety of good outcomes may also be a strength in proving melatonin's efficacy. No serious side effects were reported, suggesting it is safe for periodontal therapy. However, negative effects cannot be ruled out until more extensive and long-term research is conducted. The same is true for the effects of synthetic melatonin on endogenous melatonin release and how the body's negative feedback response operates. In the clinical practice adjuvant melatonin use might reduce the excessive use of antibiotics and other conventional treatments for periodontal disease. More research with consistent variables and larger population samples appears promising for achieving the greatest success to increase periodontal health.

5. CONCLUSIONS

Melatonin can be systemically or topically delivered to promote periodontal health, where both methods improve periodontal parameters. Comparing systemic with topical treatment for pocket depth and clinical attachment level gain, neither showed significant differences. Other than rare and minor instances of sleepiness and headaches, no adverse effects have been observed.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest in this study.

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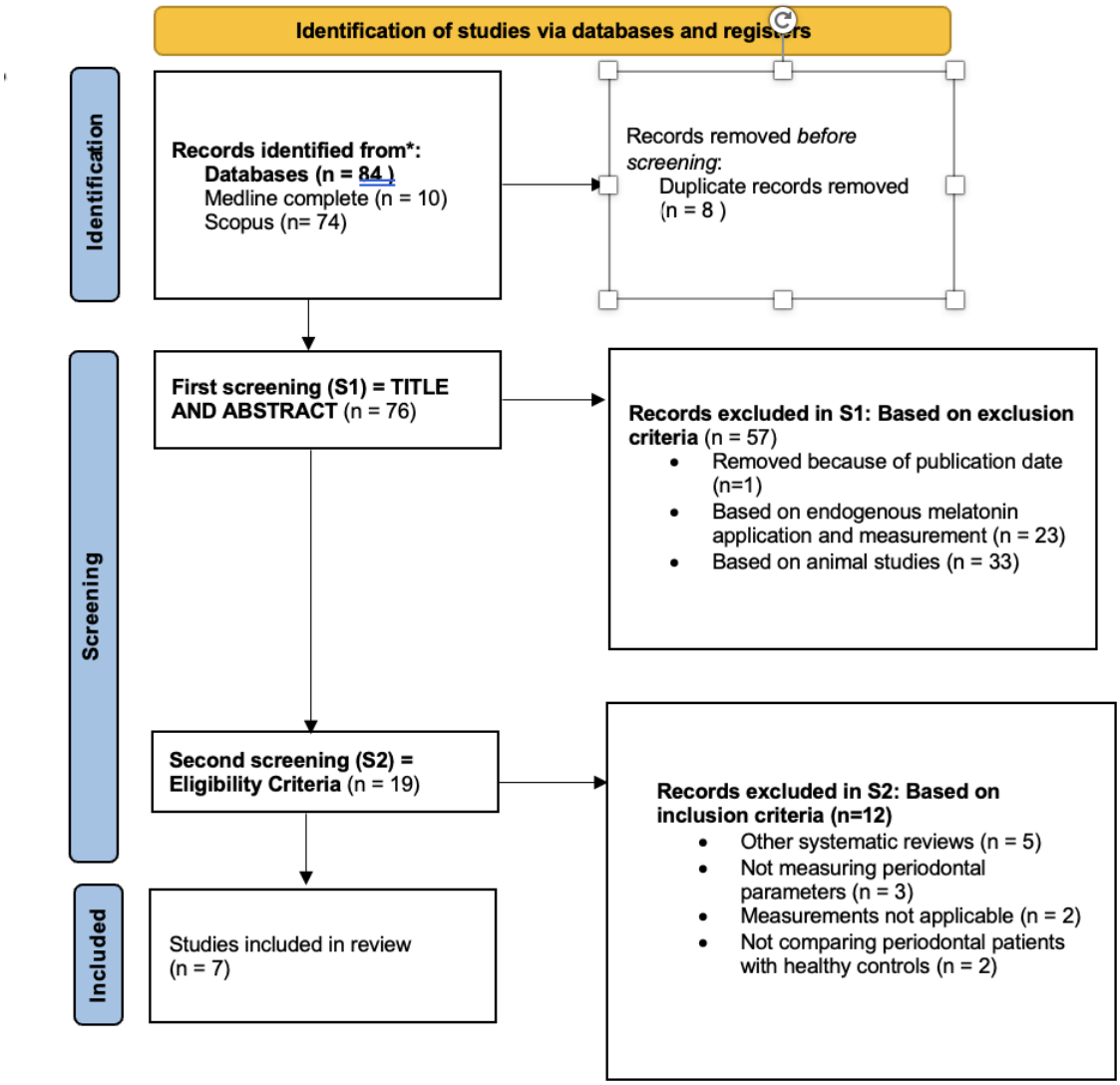


Figure 2 PRISMA 2020 flow diagram of the identification process and results of the literature search through databases, registers and other sources

Table 7 Main characteristics of the included articles

Author / Year	Country	Type of Study	Sample					
			Sample Size	Age (range/ mean) & Gender M/F	Type of Perio-dontitis	Melatonin Application	Additional Treatment	Diabetes
Anton, M. et al/ 2021	Romania	Cohort study	50	49 – 56 N/A	Severe/ moderate/ superficial chronic periodontitis	6 mg melatonin net per day (2 tablets of 250mg with 3mg melatonin each) for 8 weeks	NSPT + oral hygiene, without mouthwash/antiseptic	yes
Ahmed, E. et al/ 2021	Egypt	Split mouth randomized controlled clinical trial	24	32-55 7/17	Stage II periodontitis	Topical Intrapocket application of 5% melatonin gel 1x per weeks for 4 weeks	NSPT + oral hygiene instructions	no
Tinto, M. et al/ 2019	Italy	Randomized controlled clinical trial	20	45.6 12/8	Untreated severe stage III periodontitis	1mg melatonin capusels per day for 1 month	NSPT + oral hygiene instructions + rinsing 2x per day 0.2% clorhexidine solution	no
El-Sharkawy, H. et al/ 2018	Egypt	Randomized Clinical Trial	74	38-55 41/33	Generalized chronic periodontitis	10 mg oral melatonin capsule for 2 months	NSPT	no
Bazyar, H. et al/ 2018	Iran	Double-blind Controlled trial	44	46-61 14/30	chronic periodontitis mild to moderate periodontitis (exclusion of severe periodontitis)	6 mg melatonin net per day (2 tablets of 250mg with 3mg melatonin each) for 8 weeks	NSPT	Yes (excluding insulin treatment)

Chitsazi, M. et al/ 2017	Iran	Randomozed clinical trial	60	41 29/31	Moderate to severe chronic periodontitis	2 mg melatonin per day for 4 weeks	NSPT	no
Montero, J. et al/ 2017	Spain	Randomized Clinical Trial	90	40-50 39/51	Chronic periodontitis	Topical (1% orabase cream) melatonin on attached gingiva surfaces for 20 days	Nothing, no brushing of those areas	yes

NSPT: Non-surgical periodontal treatment

Table 8 Newcastle - Ottawa Quality Assessment Scale for Cohort Studies (12)

Quality Assessment Criteria	
Author/ Year	Anton, M. et al/ 2021
Representativeness of the exposed cohort	*
Selection of the non-exposed cohort	*
Ascertainment of exposure	*
Demonstration that outcome of interest was not present at start of study	No
Study control for age/gender and additional factor	*
Assessment of outcome	*
Was follow-up long enough for outcomes to occur	*
Adequacy of follow up of cohorts	*
Overall Quality Score (max=9)	6

** : Low risk of bias, - : High risk of bias*

Table 9 CASP Randomized Controlled Trial Standard Checklist (13)

Author/Year	Ahmed, E. et al/ 2021	Tinto, M. et al/ 2019	El-Sharkawy, H. et al/ 2018	Bazyar, H. et al/ 2018	Chitsazi, M. et al/ 2017	Montero, J. et al/ 2017
1.Did the study address a clearly focused research question?	*	*	*	*	*	*
2. Was the assignment of participants to interventions randomised?	*	*	*	*	*	*
3. Were all participants who entered the study accounted for at its conclusion?	*	*	*	*	-	*
4. Were the participants 'blind' to intervention they were given? -Were the investigators 'blind' to the intervention they were giving to participants? -Were the people assessing/analysing outcome/s 'blinded'?	* * -	* * -	* * -	* * -	- * -	* - *
5. Were the study groups similar at the start of the randomised controlled trial?	*	No	-	No	*	*
6. Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)?	*	*	-	-	*	*
7. Were the effects of intervention reported comprehensively?	*	*	No	*	*	*

8. Was the precision of the estimate of the intervention or treatment effect reported?	No	No	-	*	No	No
9. Do the benefits of the experimental intervention outweigh the harms	*	-	-	-	*	-
10. Can the results be applied to your local population/in your context?	*	No	-	-	*	-
11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions?	-	-	-	-	*	-

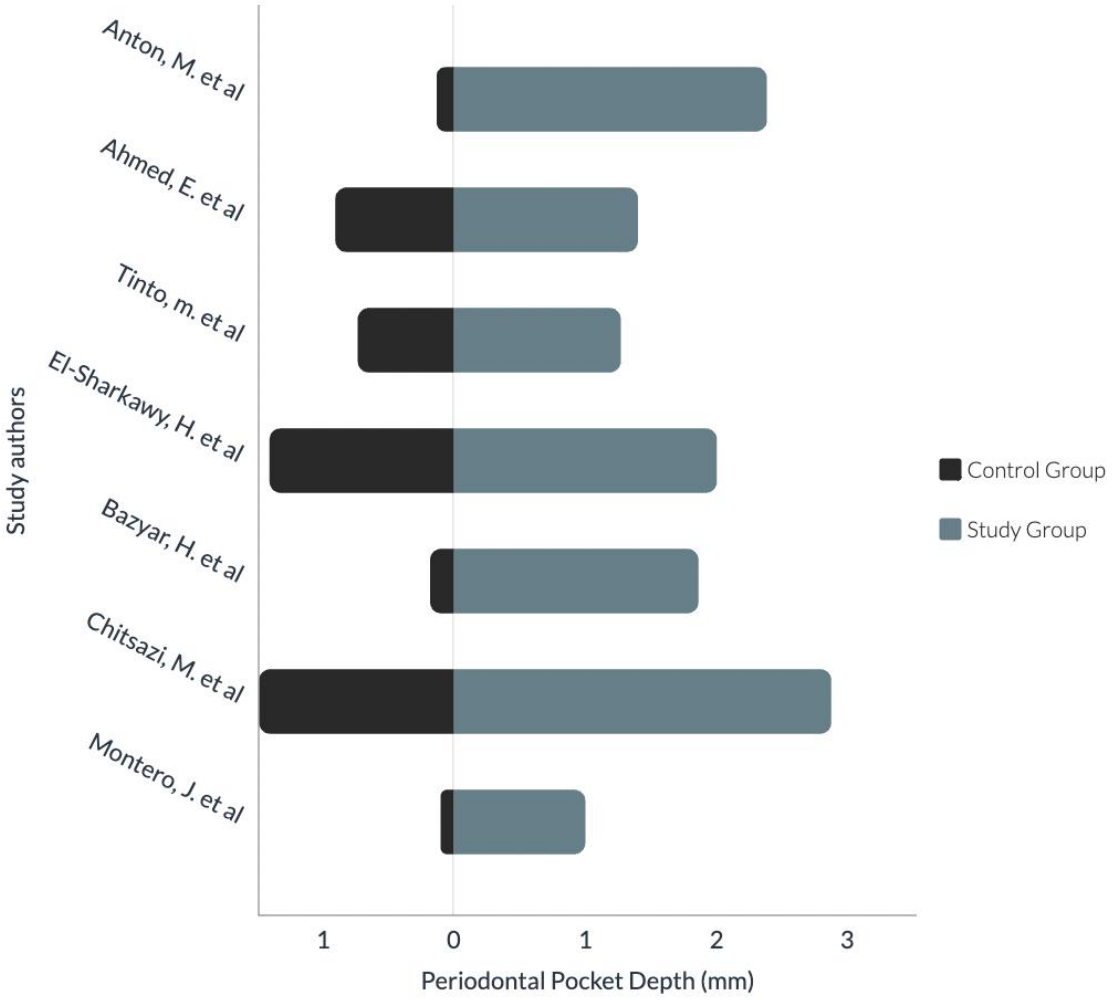
**: Yes, No: No, - : Cannot tell*

Table 10 Changes of PD and CAL in mm in control and study groups before and after intervention

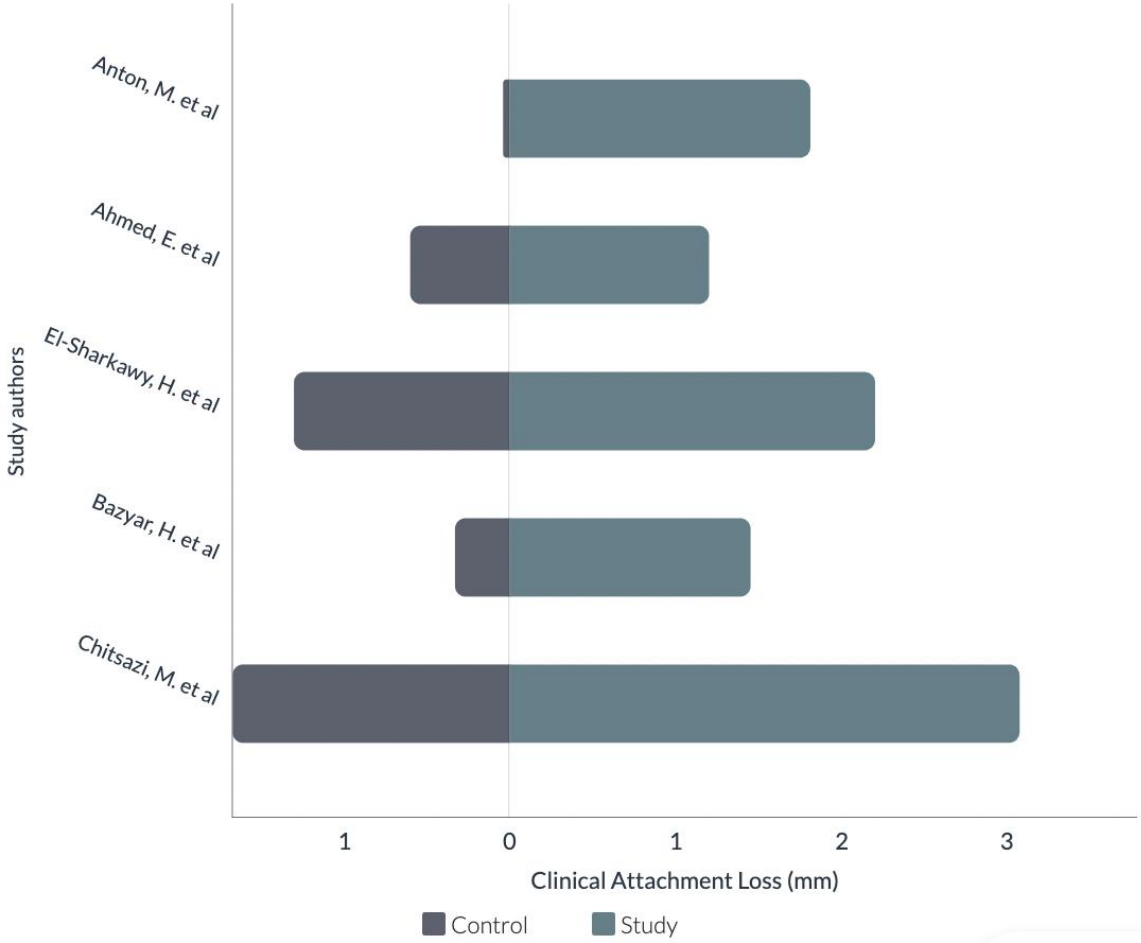
Author/ Year	**T1**	**Variables**	**Control Group**		**Study Group**		**2. Study Group (Mel + Vit C)**	
			T0	**T1**	**T0**	**T1**	**T0**	**T1**
Anton, M. et al/ 2021	8 weeks	PD (mm):	4.53	4.40	4.65	2.27		
		CAL (mm):	3.02	2.98	3.05	1.24		
Ahmed, E. et al/ 2021	3 months	PD (mm):	4.0	3.1	4.3	2.9		
		CAL (mm):	4.3	3.7	4.7	3.5		

Tinto, M. et al/ 2019	6 months	PD (mm):	3.40	2.67	3,72	2.45		
		CAL (mm):	N/A	N/A	N/A	N/A		
El-Sharkawy, H. et al/ 2018	6 months	PD (mm):	4.4	3.0	4.3	2.3		
		CAL (mm):	4.7	3.4	4.8	2.6		
Bazyar, H. et al/ 2018	8 weeks	PD (mm):	4.54	4.36	4.45	2.59		
		CAL (mm):	3	2.77	3.04	1.59		
Chitsazi, M. et al/ 2017	6 months	PD (mm):	6.40	4.92	6.41	3.54	6.43	3.08
		CAL (mm):	6.23	4.56	6.29	3.22	6.30	3.00
Montero, J. et al/ 2017	20 days	PD (mm):	2.7	2.6	2.8	1.8		
		CAL (mm):	N/A	N/A	N/A	N/A		

T0: Baseline, T1: Assessment immediately or later after the intervention, MEL: Melatonin, Vit C: Vitamin C, PD: Probing depth, CAL: Clinical attachment loss, N/A: Not applicable.



Graph 3 PD (mm) Difference T0-T1 comparison between control and study group.
T0: Baseline, T1: Assessment immediately or later after the intervention



Graph 4: CAL (mm) Difference T0-T1 comparison between control and study group. T0: Baseline, T1: Assessment immediately or later after the intervention

Annex 2. Prisma checklist

Section and Topic	Item #	Checklist item	Location
TITLE			
Title	1	Identify the report as a systematic review.	Title
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	10
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	11
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	15
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	13-14
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	13-14
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	16
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	16
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	16
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	N/A
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	16
Effect measures	12	Specify for each outcome the effect measure(s) (e.g., risk ratio, mean difference) used in the synthesis or presentation of results.	N/A
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	16
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	N/A
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	N/A
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	N/A
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	N/A
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	N/A
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	16

Section and Topic	Item #	Checklist item	Location
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	N/A
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	17-18
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	19
Study characteristics	17	Cite each included study and present its characteristics.	19-21
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	21-23
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	23
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	20-23
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	N/A
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	N/A
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	N/A
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	N/A
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	N/A
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	27-30
	23b	Discuss any limitations of the evidence included in the review.	31-33
	23c	Discuss any limitations of the review processes used.	31-33
	23d	Discuss implications of the results for practice, policy, and future research.	32-33
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	N/A
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	N/A
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	N/A
Competing interests	26	Declare any competing interests of review authors.	N/A
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	N/A

Annex 3. Risk of bias CASPE

CASP Randomised Controlled Trial Standard Checklist:
11 questions to help you make sense of a randomised controlled trial (RCT)

Main issues for consideration: Several aspects need to be considered when appraising a randomised controlled trial:

- Is the basic study design valid for a randomised controlled trial? (Section A)
- Was the study methodologically sound? (Section B)
- What are the results? (Section C)
- Will the results help locally? (Section D)

The 11 questions in the checklist are designed to help you think about these aspects systematically.

How to use this appraisal tool: The first three questions (Section A) are screening questions about the validity of the basic study design and can be answered quickly. If, in light of your responses to Section A, you think the study design is valid, continue to Section B to assess whether the study was methodologically sound and if it is worth continuing with the appraisal by answering the remaining questions in Sections C and D.

Record 'Yes', 'No' or 'Can't tell' in response to the questions. Prompts below all but one of the questions highlight the issues it is important to consider. Record the reasons for your answers in the space provided. As CASP checklists were designed to be used as educational/teaching tools in a workshop setting, we do not recommend using a scoring system.

About CASP Checklists: The CASP RCT checklist was originally based on JAMA Users' guides to the medical literature 1994 (adapted from Guyatt GH, Sackett DL and Cook DJ), and piloted with healthcare practitioners. This version has been updated taking into account the CONSORT 2010 guideline (http://www.consort-statement.org/consort-2010, accessed 16 September 2020).

Citation: CASP recommends using the Harvard style, i.e., *Critical Appraisal Skills Programme (2021). CASP (insert name of checklist i.e. Randomised Controlled Trial) Checklist. [online] Available at: insert URL. Accessed: insert date accessed.*

Study and citation: Ahmed E, Shaker OG, Yussif N, Ghalwash DM. Effect of Locally Delivered Melatonin as an Adjunct to Nonsurgical Therapy on GCF Antioxidant Capacity and MMP-9 in Stage II Periodontitis Patients: A Randomized Controlled Clinical Trial. International Journal of Dentistry. 2021

Section A: Is the basic study design valid for a randomised controlled trial?			

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes
	☒
		No
		☐
			Can't tell
			☐

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes ☒	No ☐	Can't tell ☐
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes ☐	No ☐	Can't tell ☒

Section B: Was the study methodologically sound?			
4. • **Were the participants 'blind' to intervention they were given?** • **Were the investigators 'blind' to the intervention they were giving to participants?** • **Were the people assessing/analysing outcome/s 'blinded'?**	Yes ☒ ☒ ☒	No ☐ ☐ ☐	Can't tell ☐ ☐ ☐
5. Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?* • *Were there any differences between the study groups that could affect the outcome/s?*	Yes ☒	No ☐	Can't tell ☐

6. Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)? *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (e.g. tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes ☒	No ☐	Can't tell ☐

Section C: What are the results?

7. Were the effects of intervention reported comprehensively? *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	Yes ☒	No ☐	Can't tell ☐
8. Was the precision of the estimate of the intervention or treatment effect reported? *CONSIDER:* • *Were confidence intervals (CIs) reported?*	Yes x	No ☐	Can't tell ☐
9. Do the benefits of the experimental intervention outweigh the harms and costs? *CONSIDER:* • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	Yes x	No ☐	Can't tell ☐

Section D: Will the results help locally?			
10. **Can the results be applied to your local population/in your context?** *CONSIDER:* • *Are the study participants similar to the people in your care?* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes ☒	No ☒	Can't tell ☐
11. **Would the experimental intervention provide greater value to the people in your care than any of the existing interventions?** *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes ☐	No ☐	Can't tell ☒

Study and citation: Tinto M, Sartori M, Pizzi I, Verga A, Longoni S. Melatonin as host modulating agent supporting nonsurgical periodontal therapy in patients affected by untreated severe periodontitis: A preliminary randomized, triple-blind, placebo-controlled study. Journal of Periodontal Research. 2020 Jan 1;55(1):61–7

Section A: Is the basic study design valid for a randomised controlled trial?			

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	Yes
	☒
		No
		☐
			Can't tell
			☐

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes ☐	No ☐	Can't tell ☒
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes ☒	No ☐	Can't tell ☐

Section B: Was the study methodologically sound?			
4. • **Were the participants 'blind' to intervention they were given?** • **Were the investigators 'blind' to the intervention they were giving to participants?** • **Were the people assessing/analysing outcome/s 'blinded'?**	Yes ☒ ☒ ☐	No ☐ ☐ ☐	Can't tell ☐ ☐ ☒
5. Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?* • *Were there any differences between the study groups that could affect the outcome/s?*	Yes ☐	No ☒	Can't tell ☐

6. **Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)?** *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (co-tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes ☒	No ☐	Can't tell ☐

Section C: What are the results?			
7. **Were the effects of intervention reported comprehensively?** *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	Yes ☒	No ☐	Can't tell ☐
8. **Was the precision of the estimate of the intervention or treatment effect reported?** *CONSIDER:* • *Were confidence intervals (CIs) reported?*	Yes ☐	No ☒	Can't tell ☐
9. **Do the benefits of the experimental intervention outweigh the harms and costs?** *CONSIDER:* • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	Yes ☐	No ☐	Can't tell ☒

Section D: Will the results help locally?			
10. **Can the results be applied to your local population/in your context?** *CONSIDER:* • *Are the study participants similar to the people in your care?* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes ☐	No ☒	Can't tell ☐
11. **Would the experimental intervention provide greater value to the people in your care than any of the existing interventions?** *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes ☐	No ☐	Can't tell ☒

Study and citation: El-Sharkawy H, Elmeadawy S, Elshinnawi U, Anees M. Is dietary melatonin supplementation a viable adjunctive therapy for chronic periodontitis? -A randomized controlled clinical trial. Journal of Periodontal Research. 2019 Apr 1;54(2):190–7.

Section A: Is the basic study design valid for a randomised controlled trial?

	Yes	No	Can't tell

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of?*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	☒	☐	☐

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	☒	☐	☐
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	☒	☐	☐

Section B: Was the study methodologically sound?

	Yes	No	Can't tell
4. • **Were the participants 'blind' to intervention they were given?** • **Were the investigators 'blind' to the intervention they were giving to participants?** • **Were the people assessing/analysing outcome/s 'blinded'?**	☒ ☒ ☐	☐ ☐ ☐	☐ ☐ ☒
5. Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g., age, sex, socio-economic group) clearly set out?* • *Were there any differences between the study groups that could affect the outcome/s?*	☐	☐	☒

6. **Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)?** *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (co-tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes ☒	No ☐	Can't tell ☐

Section C: What are the results?

7. **Were the effects of intervention reported comprehensively?** *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	Yes ☒	No ☐	Can't tell ☐
8. **Was the precision of the estimate of the intervention or treatment effect reported?** *CONSIDER:* • *Were confidence intervals (CIs) reported?*	Yes ☐	No ☒	Can't tell ☐
9. **Do the benefits of the experimental intervention outweigh the harms and costs?** *CONSIDER:* • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	Yes ☐	No ☐	Can't tell ☒

Section D: Will the results help locally?			
10. **Can the results be applied to your local population/in your context?** *CONSIDER:* • *Are the study participants similar to the people in your care?* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes ☐	No ☒	Can't tell ☐
11. **Would the experimental intervention provide greater value to the people in your care than any of the existing interventions?** *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes ☐	No ☐	Can't tell ☒

Study and citation: Bazyar H, Gholinezhad H, Moradi L, Salehi P, Abadi F, Ravanbakhsh M, et al. The effects of melatonin supplementation in adjunct with non-surgical periodontal therapy on periodontal status, serum melatonin and inflammatory markers in type 2 diabetes mellitus patients with chronic periodontitis: a double-blind, placebo-controlled trial. Inflammopharmacology. 2019 Feb 1;27(1):67–76.

Section A: Is the basic study design valid for a randomised controlled trial?

1.	**Did the study address a clearly focused research question?**
	CONSIDER:
	• *Was the study designed to assess the outcomes of an intervention?*
	• *Is the research question 'focused' in terms of:*
	  • *Population studied*
	  • *Intervention given*
	  • *Comparator chosen*
	  • *Outcomes measured?*
		Yes ☒	No ☐ Can't tell ☐

2.	**Was the assignment of participants to interventions randomised?** *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	Yes ☒	No ☐ Can't tell ☐
3.	**Were all participants who entered the study accounted for at its conclusion?** *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	Yes ☒	No ☐ Can't tell ☐

Section B: Was the study methodologically sound?

4.	• **Were the participants 'blind' to intervention they were given?** • **Were the investigators 'blind' to the intervention they were giving to participants?** • **Were the people assessing/analysing outcome/s 'blinded'?**	Yes tell ☒ ☒ ☐	No ☐ ☐ ☐	Can't ☐ ☐ ☒
5.	**Were the study groups similar at the start of the randomised controlled trial?** *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?* • *Were there any differences between the study groups that could affect the outcome/s?*	Yes tell ☐	No ☒	Can't ☐

6. **Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)?** *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (co- tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes ☐	No ☐	Can't tell ☒

Section C: What are the results?

7. **Were the effects of intervention reported comprehensively?** *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	Yes ☒	No ☐	Can't tell ☐
8. **Was the precision of the estimate of the intervention or treatment effect reported?** *CONSIDER:* • *Were confidence intervals (CIs) reported?*	Yes x	No ☐	Can't tell ☐
9. **Do the benefits of the experimental intervention outweigh the harms and costs?** *CONSIDER:* • *What was the size of the intervention or treatment effect?* • *Were harms or unintended effects reported for each study group?* • *Was a cost-effectiveness analysis undertaken? (Cost-effectiveness analysis allows a comparison to be made between different interventions used in the care of the same condition or problem.)*	Yes ☐	No ☐	Can't tell ☒

Section D: Will the results help locally?			
10.	**Can the results be applied to your local population/in your context?** *CONSIDER:* • *Are the study participants similar to the people in your care?* • *Would any differences between your population and the study participants alter the outcomes reported in the study?* • *Are the outcomes important to your population?* • *Are there any outcomes you would have wanted information on that have not been studied or reported?* • *Are there any limitations of the study that would affect your decision?*	Yes ☐	No ☐ Can't tell ☒
11.	**Would the experimental intervention provide greater value to the people in your care than any of the existing interventions?** *CONSIDER:* • *What resources are needed to introduce this intervention taking into account time, finances, and skills development or training needs?* • *Are you able to disinvest resources in one or more existing interventions in order to be able to re-invest in the new intervention?*	Yes ☐	No ☐ Can't tell ☒

Study and citation: Chitsazi M, Faramarzie M, Sadighi M, Shirmohammadi A, Hashemzadeh A. Effects of adjective use of melatonin and vitamin C in the treatment of chronic periodontitis: A randomized clinical trial. J Dent Res Dent Clin Dent Prospects. 2017;11(4):236–40.

Section A: Is the basic study design valid for a randomised controlled trial?

	Yes	No	Can't tell

1. Did the study address a clearly focused research question?
CONSIDER:
• *Was the study designed to assess the outcomes of an intervention?*
• *Is the research question 'focused' in terms of:*
  • *Population studied*
  • *Intervention given*
  • *Comparator chosen*
  • *Outcomes measured?*
	☒	☐	☐

2. Was the assignment of participants to interventions randomised? *CONSIDER:* • *How was randomisation carried out? Was the method appropriate?* • *Was randomisation sufficient to eliminate systematic bias?* • *Was the allocation sequence concealed from investigators and participants?*	☒	☐	☐
3. Were all participants who entered the study accounted for at its conclusion? *CONSIDER:* • *Were losses to follow-up and exclusions after randomisation accounted for?* • *Were participants analysed in the study groups to which they were randomised (intention-to-treat analysis)?* • *Was the study stopped early? If so, what was the reason?*	☐	☐	☒

Section B: Was the study methodologically sound?

	Yes	No	Can't tell
4. • **Were the participants 'blind' to intervention they were given?** • **Were the investigators 'blind' to the intervention they were giving to participants?** • **Were the people assessing/analysing outcome/s 'blinded'?**	☐ ☒ ☐	☐ ☐ ☐	☒ ☐ ☒
5. Were the study groups similar at the start of the randomised controlled trial? *CONSIDER:* • *Were the baseline characteristics of each study group (e.g. age, sex, socio-economic group) clearly set out?* • *Were there any differences between the study groups that could affect the outcome/s?*	☐	☐	☒

6. **Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)?** *CONSIDER:* • *Was there a clearly defined study protocol?* • *If any additional interventions were given (co-tests or treatments), were they similar between the study groups?* • *Were the follow-up intervals the same for each study group?*	Yes ☒	No ☐	Can't tell ☐

Section C: What are the results?

7. **Were the effects of intervention reported comprehensively?** *CONSIDER:* • *Was a power calculation undertaken?* • *What outcomes were measured, and were they clearly specified?* • *How were the results expressed? For binary outcomes, were relative and absolute effects reported?* • *Were the results reported for each outcome in each study group at each follow-up interval?* • *Was there any missing or incomplete data?* • *Was there differential drop-out between the study groups that could affect the results?* • *Were potential sources of bias identified?* • *Which statistical tests were used?* • *Were p values reported?*	Yes ☒	No ☐	Can't tell ☐
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Study and citation: Montero J, López-Valverde N, Ferrera MJ, López-Valverde A. Changes in crevicular cytokines after application of melatonin in patients with periodontal disease. Journal of Clinical and Experimental Dentistry. 2017 Sep 1;9(9): 1081–7

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	☒
		No
		☐
			Can't tell
			☐

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