Biol Trace Elem Res (2017) 176:225–238
DOI 10.1007/s12011-016-0832-y

Trace Mineral Micronutrients and Chronic Periodontitis—a Review

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Received: 2 August 2016 / Accepted: 22 August 2016 / Published online: 31 August 2016

Abstract Trace mineral micronutrients are imperative for optimum host response. Populations worldwide are prone to their insufficiency owing to lifestyle changes or poor nutritional intake. Balanced levels of trace minerals like iron (Fe), zinc (Zn), selenium (Se) and copper (Cu) are essential to prevent progression of chronic conditions like periodontitis. Their excess as well as deficiency is detrimental to periodontal health. This is specifically true in relation to Fe. Furthermore, some trace elements, e.g. Se, Zn and Cu are integral components of antioxidant enzymes and prevent reactive oxygen species induced destruction of tissues. Their deficiency can worsen periodontitis associated with systemic conditions like diabetes mellitus. With this background, the present review first focusses on the role of four trace minerals, namely, Fe, Zn, Se and Cu in periodontal health followed by an appraisal of the data from case control studies related to their association with chronic periodontitis.

Keywords Chronic periodontitis · Copper · Iron · Selenium · Trace minerals · Zinc

Introduction

Chronic periodontitis is an immunoinflammatory condition initiated by the dental plaque biofilm. It results in inflammation of the supporting structures of the tooth, namely, the periodontal ligament and alveolar bone. It has been suggested that the periodontal lesion is essentially a "wound" that requires favourable host environment to heal completely [1]. If the latter is not available, the disease process continues ultimately resulting in tooth loss.

Diet plays an important role in the maintenance of tooth structure after eruption [1]. Nutritional deficiencies are an important risk factor for periodontitis but their direct effect on it is not well documented [2, 3]. They mainly influence the immune response in the body and subsequently the progression of periodontal disease.

Nutrition is derived from specific components of diet like proteins, fats, carbohydrates (macronutrients) and vitamins, minerals, trace elements, antioxidants, amino acids and polyunsaturated fatty acids (PUFA) (micronutrients). The World Health Organization (WHO) has rightly described the micronutrients as the "magic wands required in minuscule amounts (micrograms or milligrams per day) to enable the production of enzymes, hormones and other substances for proper growth and development of the body" [4]. It has been reported that micronutrient deficiencies affect around 2 billion people worldwide [5]. The problem is so grave that lately, it has been regarded as a "silent epidemic affecting the people of all ages and genders around the globe" [5–7].

Amidst the array of micronutrients, minerals make up about 4 % of the body weight and are mainly present in the skeleton, enzymes and hormones [1]. They help in regulating and maintaining the normal heart rhythm, muscle contraction, nerve conduction and the acid–base balance. Minerals have been classified as major minerals (>100 mg/day) or as trace minerals (<100 mg/day). The major minerals are sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and sulphur (S) while the trace minerals are iron (Fe), zinc (Zn), selenium (Se), copper (Cu), iodine (I), fluoride (F),

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cobalt (Co), chromium (Cr), manganese (Mn) and molybdenum (Mo) [1].

Overcoming micronutrient malnutrition is exigent due to a wide discrepancy in its causes which range from normal physiologic changes (e.g. altered taste sensations, swallowing, reduced appetite and metabolism) to medications and hospitalization in special cases, as well as social issues like diminished finances and literacy [8, 9]. These subsequently influence the intake of food leading to nutritional imbalances.

Even though there is enormous data supporting the direct and indirect role of vitamins and major minerals on periodontal and general health, there is little evidence on the functions of trace mineral micronutrients. This may be related to their low concentrations in body fluids and tissues, the irregularities between their levels in blood and target tissues, as well as the lack of functional tests and information on their optimal levels for laboratory investigations [10].

Literature reveals that trace mineral insufficiency has far reaching consequences both systemically and orally [11]. Currently, oral conditions like dental caries, periodontitis, oral cancer and precancerous conditions as well as mucosal disorders have been linked to their deficiencies but the exact mechanisms of their associations have not been established [12–15]. Their deficiency may promote periodontal disease either by acting synergistic to the infection initiated by periodontopathogens or by influencing the biological gradients and haemostasis of the host response.

With this background, the present review first focusses on the role of trace minerals like Fe, Zn, Se and Cu in periodontal health followed by an appraisal of the data from case control studies related to their association with chronic periodontitis.

Etiopathogenesis and Strategies in the Management of Chronic Periodontitis

Chronic periodontitis is a multifactorial condition precipitated by complex interactions of dental plaque biofilm with the host immunoinflammatory response. Besides, numerous environmental and host associated risk factors have been suggested to play an important role in its pathogenesis [16, 17]. These include genetics, smoking as well as nutritional factors which modify the host response and support the progression of the disease [16, 17].

It has been suggested that if the biofilm is allowed to accumulate, it creates a favourable environment for bacterial species that act as a bridge between the early and late colonizers, e.g. the *Fusobacterium nucleatum* [18, 19]. They stimulate a stronger host response resulting in gingival inflammation. This increases the supply of nutrients (e.g. heme) which favour proliferation of late colonizers like *Porphyromonas gingivalis* (*P. gingivalis*) [18]. This phenomenon was termed as "incipient dysbiosis" [18]. It produced a disproportionate host response in a susceptible individual wherein excessive cytokines, ROS and matrix-degrading metalloproteinases are produced [18]. They destroy periodontal tissues and generate mediators that create a state of chronic non-resolving inflammation [18]. They even enter into systemic circulation and worsen the already existing conditions like diabetes mellitus, rheumatoid arthritis and cardiovascular disorders [20].

Numerous strategies have been utilized to modify the progression of periodontitis. This includes removal of biofilm to promote colonization of health-promoting microbial species. This enhances resolution of inflammation and restores normal structure and function of the tissues [18]. It may be achieved by both non-surgical and surgical periodontal therapy. Additionally, various host-modulating agents have been applied as an adjunct to routine periodontal procedures in order to stimulate a positive host response. This includes application of agents like subantimicrobial dose doxycycline, non-steroidal anti-inflammatory drugs, nitrous oxide (NO) synthase inhibitors, mitogen-activated protein kinase (MAPK) inhibitors, nuclear factor-kappa B inhibitors, tumour necrosis factor antagonist as well as dietary components like omega-3 fatty acids and micronutrients (dietary minerals, e.g. Fe, Zn, Se, Cu and vitamins A, B complex, C, D and E) [21, 22]. All these supplements have an immunomodulatory property. They provide resistance to infection as well as control the inflammation [21]. The following section would detail the role of trace minerals in periodontal health.

Essential Trace Mineral Micronutrients for Periodontal Health

As already stated, the trace minerals influence periodontal health due to their ability to affect the hard and soft tissues locally and the immunoinflammatory mechanisms systemically. Their optimum concentration of <100 mg/day is a prerequisite for the maintenance of oral tissues in a healthy state [1]. The role of some important trace mineral micronutrients essential for periodontal health would be discussed in the following section (Fig. 1)

Iron

Fe is indispensable for human life. It is crucial for erythropoiesis and haemoglobin (Hb) formation in the bone marrow [1]. The Recommended Daily Allowance (RDA) for Fe varies with age. Its requirement is highest in the women of reproductive age group (18 mg/day), while in the middle-aged adults, it is about 8 mg/day [23]. Its reduced absorption results in iron deficiency anaemia (IDA) where the Hb levels are <12 g/dl for adult non-pregnant women and <13 g/dl for adult men [24]. Furthermore, there is decreased serum Fe and ferritin levels and increased total iron binding capacity (TIBC).

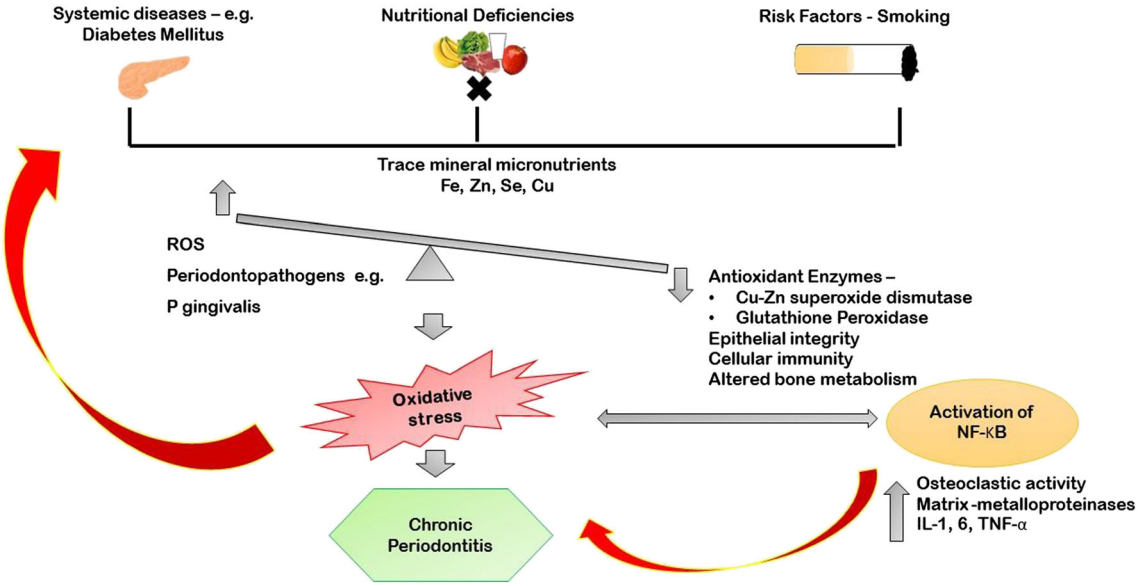

Fig. 1 Role of trace mineral micronutrients (Fe, Zn, Se, Cu) in chronic periodontitis

It is important to distinguish IDA from anaemia of chronic disease (ACD) which is more prevalent in individuals with chronic inflammatory conditions [25]. In ACD, there is decreased release of Fe from the stored forms. Laboratory investigations generally reveal decreased levels of Fe with normal or increased ferritin and normal or reduced TIBC. Although serum ferritin levels are the most reliable diagnostic marker for identifying anaemia, care should be observed when interpreting these levels, as chronic infections or inflammation may also result in its increased levels [26]. The causes for anaemia may vary from reduced dietary intake to occult gastrointestinal bleeding, chronic inflammation, gastric polyps, non-steroidal anti-inflammatory drugs, carcinoma of the gastrointestinal tract and inflammatory bowel disease [27].

Optimal levels of Fe are a prerequisite for periodontal health and a shift in either direction may be detrimental. Although the evidence for Fe deficiency directly causing chronic periodontitis is weak, the reverse association is comparatively strong [28–32]. It has been suggested that inflammation due to chronic periodontitis results in increased levels of pro-inflammatory cytokines which suppresses erythropoiesis in bone marrow [31, 33, 34]. Subsequently, the number of circulating erythrocytes is reduced, resulting in ACD [34]. Imbalances in Fe levels affect the periodontium by influencing the bone metabolism, periodontopathogens and host immunoinflammatory reactions. These may be explained as follows:

Influence on Bone Metabolism

The role of Fe in periodontal ligament (PDL) and alveolar bone homeostasis and function was recently verified in an animal model [35]. The PDL cells have the ability to regulate their Fe uptake by expressing the light and heavy chain subunits of heteromeric ferritin [35, 36]. This further controls the cytodifferentiation of these cells into osteoblasts and mineralization, thereby affecting the bone density [35, 36]. Observations from animal studies have revealed that IDA results in aberrant bone morphology and microarchitecture, decreased density and strength of femoral and vertebral bones [37, 38]. This may be seen in alveolar bone as well [39].

Besides the deficiency, Fe overload also produces negative effects on alveolar bone remodelling [40]. It suppresses the differentiation, function and apoptosis of osteoblasts resulting in osteoporosis and osteopenia [41, 42]. This is significant in postmenopausal females where reduced oestrogen levels combined with Fe excess (due to menopause) may further reduce the bone density [43]. This was revealed in both animal and human studies where altered Fe levels compromised the ability of the pleuripotent PDL cells to differentiate into osteoblasts and subsequently affected the repair and remodelling of the inner wall of alveolar bone [35, 43].

Influence on Periodontopathogens

Fe is an essential micronutrient required at various stages of cellular metabolism in microorganisms [44]. Its overload from either genetic predispositions, therapeutic interventions or nutritional status has often been regarded as a risk factor for chronic periodontitis [45]. This could be related to the high dependency of periodontopathogens like *P. gingivalis*, *Prevotella intermedia* (*P. intermedia*) and *Treponema denticola* (*T. denticola*) on host Fe reserves [46–48]. The latter may be in the form of heme (Fe complexed with protoporphyrin IX), enzymes and proteins like haemoglobin, myoglobin, transferrin and lactoferrin [49].

As the levels of free Fe are extremely low in the body fluids, these pathogens have evolved mechanisms to fulfil their requirements [45, 49]. The main source of Fe in gingival crevicular fluid is haemin [49]. It is released during the active disease process due to bleeding. However, this haemin is not readily available as it is rapidly and irreversibly bound by haptoglobin in crevicular fluid. To overcome the scavenging effects of haptoglobin, *P. gingivalis* releases haemaglutinins like gingipains [45]. These are cysteine proteases which bind the red blood cells, lyse them and release haemoglobin. This increases the availability of haemin. Besides, *P. gingivalis* utilizes inorganic Fe, transferrin and lactoferrin for its growth [45]. This was also evident from the results of a study which showed that periodontopathogens like *P. gingivalis*, *Tannerella forsythia*, *T. denticola*, *P. intermedia*, *Parvimonas micra*, *Fusobacterium* species, *Campylobacter* species, *Capnocytophaga* species and *Selenomonas* species were more often present at the diseased sites and the degree of periodontal destruction was directly correlated to their haemolytic activity [50].

In order to counteract these pathogens, the body has also developed processes to prevent the degradation of bound Fe [51]. These include secretion of molecules like siderocalin, lactoferrin, natural resistance-associated macrophage protein-1 (Nramp-1), transferrin receptor-1 (TfR1), hepcidin and ferroportin [51].

Siderocalin binds to siderophores (released by bacteria for the uptake of free and transferrin bound Fe), lactoferrin binds to free Fe, Nramp1 and TfR1 act by depleting Fe^{2+}, Co^{2+} and Mn^{2+} from the phagosome and decreasing the intracellular Fe content. Hepcidin is an antimicrobial peptide which reduces free Fe levels and its sequestration within the host cells [51]. Ferroportin is a receptor for hepcidin. It binds to hepcidin to reduce the export of cellular Fe to plasma. All these mechanisms prevent the utilization of Fe by the intracellular pathogens [50]. Impairment of any of these processes may result in ACD [29, 52, 53].

Here the importance of periodontal therapy in improving the haematological indices should be highlighted. Reports have shown that non-surgical periodontal reduced the microbial load which further improved the anaemic status and ferritin levels in patients with chronic periodontitis [28, 54].

Influence on Host Response

Fe is essential for both innate and adaptive immune responses [55]. Its deficiency weakens the cell-mediated immunity as revealed by reduced lymphocyte count, interferon-γ (IFN-γ) and IL-2 levels and the functions of natural killer (NK) cells. The delayed type of hypersensitivity response mediated through CD4+ lymphocytes is also disrupted [56, 57].

Besides, Fe overload also has a negative impact on immunity. It results in reduced immunoglogulin E (IgE) production, high CD4+ to CD8+ T cell ratios, low numbers of CD28+ cells and impaired CD8+ T-cell function [58].

Furthermore, Fe plays an important role in oxidative burst, i.e. the release of reactive oxygen species (ROS) from macrophages and neutrophils. A shift in the levels may cause oxidative stress leading to periodontal destruction. This is mainly related to the conversion of hydrogen peroxide to ROS through Fenton reactions catalysed by free Fe. Subsequently, there is activation of matrix metalloproteinases (MMPs) which degrade the extracellular matrix components. This further activates the nuclear factor-kappa B (NF-κB) pathway stimulating the release of pro-inflammatory cytokines like IL-1β, IL-6, IL-8 and TNF-α which destroy the periodontal tissues and alveolar bone.

Fe is required for the activation of enzymes like inducible nitric oxide synthase, myeloperoxidase and NADPH oxidoreductase during bacterial phagocytosis [55, 59, 60]. Its deficiency may inhibit the activity of protective enzymes like myeloperoxidase, which are essential for the bactericidal activity of macrophages [28]. Additionally, it may reduce the levels of oxygen in the gingival tissues which further activates the inflammatory cascade.

Thus Fe is essential for immunological homeostasis. The general consensus is that both elevated and reduced levels are detrimental to the host immune responses, as well as susceptibility to infections. As it is critical for the proliferation and survival of pathogens, the mechanisms to control its levels need to be tightly regulated in order to guarantee a protective response with a simultaneous restricted access for pathogens.

Zinc

Zn is an integral component of a large number of macromolecules and a cofactor for over 50 enzymes including carbonic anhydrase, alkaline phosphatase, alcohol dehydrogenase and superoxide dismutase (SOD) [1]. Optimal levels of Zn are imperative for growth and development, protein and DNA synthesis, neuro-sensory functions, cell-mediated immunity and thyroid and bone metabolism.

Zn deficiency is more prevalent in children, elderly and patients with immunosuppressive disorders due to dietary deficiencies or poor absorption [61]. Its deficiency increases the frequency for infections and degenerative pathologies. It may be regarded as a food for brain as it plays an important role in cognition and psychological functioning. Zn is crucial for maintaining a balance among micronutrients [62]. For example high intakes of Zn depresses the Cu absorption. It is even required for the absorption of food folate, vitamins A and E. It is an essential element involved in growth and development of periodontal tissues. Its role in periodontitis is mainly related to its influence on oral mucosa, bone metabolism and host response. These may be explained as follows:

Influence on Oral Mucosa

Animal studies have revealed that dietary deficiency of Zn leads to poorer periodontal health. It alters the thickness and keratinization of oral mucosa which becomes more susceptible to infections. Furthermore, deeper periodontal pockets and thicker palatal tissues have been reported in Zn deficient rats [63, 64].

Influence on Host Response

Zn deficiency affects the functioning of immune cells like monocytes (all functions), natural killer cells (reduced cytotoxicity), neutrophils (reduced phagocytosis), T cells and lymphocytes (decreased apoptosis) [62]. Furthermore, it increases the secretion of pro-inflammatory cytokines.

It is an integral component of antioxidant enzymes and its altered levels cause oxidative stress. While the deficiency leads to reduced protection of sulpha-hydryl groups and increased production of ROS, excessive levels may act as prooxidant by eliciting a decline in erythrocyte Cu–Zn SOD [65]. This enzyme has been localized in the human periodontal ligament where it prevents free radical induced damage [66]. Therefore, its optimum levels are a prerequisite to maintain the tissues in a healthy state.

Influence on Bone Metabolism

Zn plays an important role in bone metabolism and its deficiency causes osteoporosis [67, 68]. This has been attributed to reduced activity of enzymes involved in bone matrix synthesis, altered coupling mechanism of bone remodelling, reduced osteoblastic activity and increased formation of osteoclast like cells. It promotes bone mineralization by acting as a cofactor for alkaline phosphatase [69]. Some workers have reported an inverse association between serum Zn levels and marginal alveolar bone loss [70]. This could be related to altered bone collagen metabolism with a significant reduction in collagen synthesis and turnover as well as reduced alkaline phosphatase activity [71].

Thus, adequate levels of Zn are imperative for both protective immune and bone regenerative processes. This would further prevent the progression of periodontitis.

Selenium

Se is a mineral antioxidant essential for the proper functioning of various organ systems. Its biological functions are mainly exerted through selenoproteins which are a group of antioxidants involved in activation, proliferation and differentiation of cells of innate and adaptive immunity [72]. They prevent exacerbation of immune responses in chronic inflammation. Various selenoproteins are glutathione peroxidase (GPX), thioredoxin reductases, deiodinases and selenoproteins P, K and S [72]. Among them, the GPX system is most widely investigated.

The GPX is a group of eight isoenzymes of which GPX 1 and 4 are most abundant and are found on all immune cells and tissues. The GPX3 is present in plasma. The GPX enzymes utilize Se at their active sites to detoxify ROS including hydrogen peroxide and phospholipid hydroperoxide. The redox balance at the cellular level regulates the selenoproteins which indirectly modulate immune cell signalling and function [72]. Likewise, Se regulates the activity of transcription factors (i.e. NF-κB and activator protein-1) and associated gene expression. It even reduces the levels of TNF-α and cyclooxygenase-2 produced by macrophages in response to lipopolysaccharides and downregulates the expression of adhesion molecules (i.e. intercellular adhesion molecule-1 and vascular cell adhesion molecule-1). Besides, it helps in metabolism of arachidonic acid and eicosanoids. It reduces monocyte adhesion and migration through endothelial cells. This is mainly related to Se induced shedding of L-selectin. At cellular level, dietary Se influences the leukocytic functions like adherence, migration, phagocytosis and cytokine secretion.

The RDA for Se is 40 μg for adults and its extreme levels, i.e. both depletion (Keshan disease) and toxicity (selenosis) are detrimental to overall health. Its optimal levels were calculated by an expert committee involving representatives from WHO, the food and drug administration (FAO) and International Atomic Energy Agency (IAEA). They suggested that the normal levels of Se were equivalent to those necessary to achieve two thirds of maximal GPX activity. It was observed that Se concentration of 1.1 mmol/l was associated with maximum GPX activity while a level of 1.2 mmol/l or less resulted in reduced immune function. Furthermore, a serum concentration of 1.5 mmol/l was proposed to be essential for cancer protection [73–76].

The deficiency of Se may be related to poor dietary intake, presence of chronic diseases and ingestion of drugs that reduce its absorption and proper utilization. Some studies even suggest that alcohol intake may result in poor concentrations of Se [77]. However, a positive correlation associated with high meat consumption in alcoholics was reported in another study [78].

The beneficial effects of Se on periodontium are mainly due to its antioxidant effects. In vitro and animal studies showed that addition of Se to α-tocopherol accelerated the proliferation rate and wound healing process. This was related to increased synthesis of basic fibroblastic growth factor and type I collagen from both gingival and periodontal ligament fibroblasts in the presence of Se [79, 80]. Studies have reported progression of chronic periodontitis in diabetic subjects with reduced levels of Se [81].

Thus, Se is a protective trace mineral micronutrient for the periodontium and its inclusion in the diet may be vital in

"dietary regulation of inflammatory cascade" [61]. This would further prevent the progression of destructive periodontal disease.

Copper

Cu is essential for immunity and combating the oxidative stress induced by reactive oxygen and nitrogen species. It acts as a co-enzyme for cytochrome-C and SOD and is involved in electron transport of proteins. The RDA for Cu is 900 μg [82]. It is required in association with Fe for the formation of haemoglobin and is stored bound to ceruloplasmin, a Cu-dependent ferroxidase. Ceruloplasmin helps in oxidising Fe so that ferritin is utilized. Therefore, its deficiency may cause anaemia [83].

Cu deficiency has a negative influence on neutrophils, macrophages, T cells and NK cells. There is impaired production of IL-2 and excessive production of pro-inflammatory cytokines, such as TNF-α, MMP-2 and -9 which degrade the collagen and extracellular matrix components in PDL. As it serves as a cofactor for metalloenzyme like SOD, an essential antioxidant for chronic periodontitis, optimal levels of Cu are essential for preventing exacerbation of inflammatory pathways [13].

Evidence on the Role of Trace Mineral Micronutrients in Chronic Periodontitis

The role of trace mineral micronutrients in periodontal health has been investigated in numerous studies [13, 81, 84–95]. As stated earlier, the trace mineral micronutrients influence host response which indirectly affects the progression of periodontitis. In order to elucidate this effect, literature search was made through the databases like Pubmed Central-Medline, Scopus, Web of science and Google Scholar to include all the observational, experimental and clinical trials from 2012 to 2016 related to the role of Fe, Zn, Se and Cu in chronic periodontitis.

The inclusion criteria included observational, experimental and clinical trials in human subjects aged ≥18 years. Only the free full text articles in English language were included. A combination of keywords like iron, zinc, selenium, copper and periodontitis were used to search these databases. Any study that involved direct measurement of above micronutrients in body fluids, i.e. serum, saliva or gingival crevicular fluid (GCF) of chronic periodontitis subjects were included.

Initial search resulted in 348 articles, of which 36 studies were selected after reading the title and abstracts (Fig. 2). Further discussion among the researchers resulted in 14 studies which were included in this review [13, 81, 84–95]. Their salient features, results and conclusions were recorded (Table 1). There was considerable heterogeneity among the studies with regard to study design, study period, study population, number, gender and age of participants.

All the studies included were case–control studies. The subjects in these studies had mild to moderate chronic periodontitis but were otherwise systemically healthy. However, seven studies involved subjects with type 2 diabetes mellitus, one study included subjects with rheumatoid arthritis and one another was done on smokers [13, 84–88, 90, 91]. Chronic periodontitis was defined as more than 30 % of the sites with clinical attachment level (CAL) ≥3 mm and pocket depth (PD)

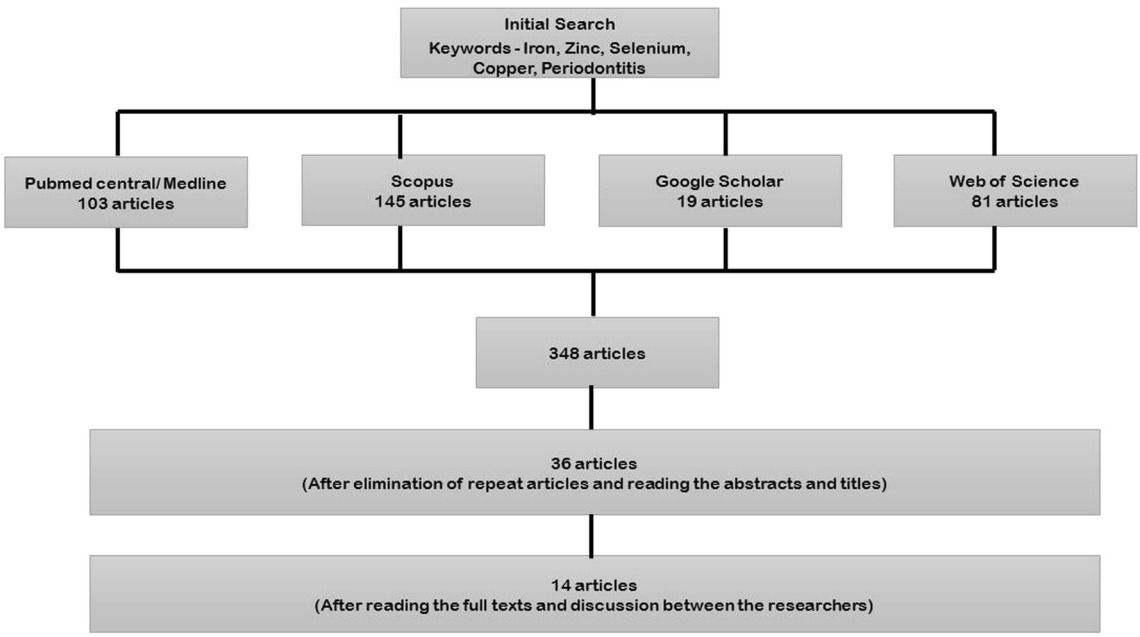

Fig. 2 Evidence search for the role of iron, zinc, selenium and copper in chronic periodontitis

Table 1 Studies reporting the role of iron, zinc, selenium and copper in chronic periodontitis

Authors	Aim of the study	Salient features	Results	Conclusion
Manea et al. [84]	• Determined the effect of chronic periodontitis on the levels of Ca, Mg, Zn and Cu in saliva and blood of smokers and non-smokers	• Cases: *n* = 30; 2 groups—smokers and non-smokers with periodontitis • Controls: *n* = 30; 2 groups—smokers and non-smoker without periodontitis • Salivary and blood levels of Ca, Mg, Zn and only salivary levels of Cu were measured in all the groups	• Increased salivary Cu and Zn levels in periodontitis group with highest concentration being in the smokers with periodontitis	• Increased Cu–Zn ratio in periodontitis was indicative of oxidative stress
Mahmood et al. [85]	• Determined periodontal status in subjects with rheumatoid arthritis (RA) and periodontitis and compared them with RA subjects without periodontitis and those with chronic periodontitis but no RA • Salivary levels of Cu, Zn and Mg were evaluated and compared to controls	• *n* = 75 female patients; 25 with RA and chronic periodontitis; 25 with chronic periodontitis but no RA and 25 healthy controls • Age: 40–50 years • Periodontal parameters and salivary elements, i.e. Cu, Zn and Mg were measured	• RA subjects had poor periodontal condition with higher levels of Cu but low levels of salivary Zn and Mg when compared to healthy controls	• Weak correlation between salivary levels of elements and periodontal parameters
Pushparani et al. [86]	• Assessed the serum levels of vitamin C and Zn in type 2 diabetes mellitus with and without periodontitis and their relation to the development of oxidative stress in type 2 diabetes mellitus with periodontitis	• *n* = 450 subjects; age: 25 to 56 years, 3 groups, consisting of 150 participants in each as follows: Group I—control healthy subjects Group II—type 2 diabetes mellitus without periodontitis Group III—type 2 diabetes mellitus with periodontitis • Clinical periodontal parameters, diabetic profile and serum levels of vitamin C and Zn were measured	• Low serum vitamin C and Zn levels in type 2 diabetics with periodontitis (group III) when compared to type 2 diabetics without periodontitis (group II), and healthy controls (group I)	• Low levels of serum Zn increase the probability for development of diabetes with periodontitis
Thomas et al. [81]	• Evaluated and compared the levels of glutathione, catalase, and Se in the serum of type 2 diabetic and healthy subjects with and without periodontal disease	• *n* = 150 subjects; 3 groups of 50 patients each; Group I: type 2 diabetes mellitus with chronic periodontitis Group II: systemically healthy with chronic periodontitis Group III: systemically healthy without periodontitis • Serum levels glutathione, catalase and Se were measured in all the groups	• Serum levels of all three biochemical parameters, i.e. glutathione, catalase and Se were significantly lower in diabetic patients with periodontitis when compared to healthy individuals with and without periodontitis	• The serum levels of biochemical markers including Se were inversely proportional to degree of periodontal inflammation and tissue destruction
Thomas et al. [13]	• Estimated and compared serum levels of Zn, Cu and Fe in type 2 diabetic patients and healthy individuals with and without periodontitis	• *n* = 150 subjects, 50 in each group; age: 35–60 years • Group 1—diabetes mellitus type 2 with periodontitis • Group 2—chronic periodontitis only • Group 3—healthy controls with no chronic periodontitis • Serum Zn, Cu and Fe were estimated	• Reduced levels of Zn and increased levels of Fe and Cu in subjects with diabetes and periodontitis when compared to healthy individuals with and without periodontitis	• Zn deficiency and elevated Cu and Fe are associated with an increased oxidative stress and altered immune response which promotes diabetic complications including periodontitis
Pushparani et al. [87]	• Evaluated the serum level of Ca and Fe in subjects with type 2 diabetes mellitus and chronic periodontitis	• *n* = 450 subjects, divided into 3 groups as follows: Group I—control healthy subjects	• Serum Ca and Fe levels were significantly increased in type 2 diabetes mellitus with periodontitis when compared to subjects with T2DM	• High concentration of Fe enhances growth and virulence of subgingival periodontopathogens that initiate active periodontitis

Table 1 (continued)

Authors	Aim of the study	Salient features	Results	Conclusion
		Group II—type 2 diabetes mellitus without periodontitis Group III—type 2 diabetes mellitus with periodontitis • Serum concentrations of glucose, glycated haemoglobin (HbA1c), Ca and Fe were measured	without periodontitis and controls	• Elevated serum Ca and Fe were directly associated with alveolar bone loss and oxidative stress which predisposed diabetic subjects to periodontitis
Pushparani et al. [88]	• Evaluated serum levels of Zn and Mg in subjects with type 2 diabetes mellitus with periodontitis and correlated them with the lipid profile, i.e. serum cholesterol, high density lipoprotein, low density lipoprotein and triglycerides	• n = 600; 4 groups of 150 participants each as follows: ◦ Group II—type 2 diabetes without periodontitis ◦ Group III—type 2 diabetes with periodontitis ◦ Group IV—periodontitis without type 2 diabetes • Age range: 25 to 55 year • Serum levels of Zn and Mg measured in all subjects	• Significant reduction in the levels of serum Zn and Mg in type 2 diabetics with periodontitis when compared to controls	• Zn and Mg deficiency may play a role in the development of DM
Medikeri et al. [89]	• Evaluated the effects of quantification of *C. rectus* on serum Fe, total binding capacity (TIBC) and transferrin levels in chronic periodontitis and healthy sites.	• n = 120 subjects, 74 male and 46 female; age: ≥18 years; 2 groups—60 subjects with chronic periodontitis and 60 without chronic periodontitis • Serum levels of Fe, TIBC and transferrin were measured • The quantified bacterial count was compared with blood samples • *C. rectus* was detected in both groups; total frequency and prevalence of *C. rectus* in both groups: 26 in males and 16 in females	• Significant increase in *C. rectus* count in chronic periodontitis • Reduced Fe and elevated and TIBC and transferrin levels in periodontitis • Regression analysis showed a significant linear relationship between *C. rectus* counts and decreasing Fe levels and consequently increasing serum transferrin and TIBC in chronic periodontitis	• Periodontopathogens like *C. rectus* reduce serum Fe levels in chronic periodontitis thereby predisposing to anaemia of chronic disease
Pushparani [90]	• Reported the influence of low serum Zn and increased level of lysosomal enzyme, acid phosphatase in subjects with both type 2 diabetes mellitus and periodontitis	• n = 600 subjects; age: 25 to 56 years, divided into four groups, consisting of 150 participants in each group as: Group I: control healthy subjects Group II: type 2 diabetes without periodontitis Group III: type 2 diabetes with periodontitis Group IV: periodontitis without diabetes • Clinical periodontal, diabetic parameters and serum levels of Zn and acid phosphatase were measured	• Subjects with type 2 diabetes with periodontitis had lower Zn than those without them • Serum acid phosphatase levels in T2DM without periodontitis and only periodontitis subjects were significantly higher as compared to healthy controls	• Decreased Zn and elevated level of acid phosphatase may be a contributing factor in the progression of type 2 diabetes with periodontitis
Pushparani [91]	• Investigated the association between serum Zn and Fe levels among the type 2 diabetes mellitus (T2DM) with and without periodontitis	• n = 450 subjects; age group: 25 to 56 years divided into three groups: Healthy individuals (group I, n = 150) T2DM with periodontitis (group II, n = 150), T2DM without periodontitis (group III, n = 150) • Serum Zn and Fe levels were determined	• Serum Zn level in T2DM without periodontitis (group II) were significantly higher when compared to other groups. Their Fe levels were greater than group III but lesser than group I subjects.	• Increased level of serum Fe in T2DM with periodontitis can act as a strong pro-oxidant, which may catalyse Fenton reactions leading to the formation of reactive oxygen species • Furthermore, it depletes the Zn levels leading to aggravation of oxidative stress, increased cytokine production, insulin resistance and decreased

Table 1 (continued)

Authors	Aim of the study	Salient features	Results	Conclusion
				insulin secretion in T2DM with periodontitis
Herman et al. [92]	• Examined influence of periodontal disease on concentration of metals (Ca, Cu, Fe, Mg, Mn, Zn, Cd and Pb) in oral fluid and blood • Analysis of selected elements in oral fluid for identifying people with periodontitis	• Cases: n = 31 non-smoking patients with periodontitis (17 females, 14 males); mean age: 34.5 ± 10.4 years and controls: n = 29 non-smoking subjects (19 females and 10 males); mean age: 31.8 ± 16.8 years • Concentrations of 8 elements were measured in two groups The elements were divided into two groups: essential elements, Ca, Cu, Fe, Mg, Mn and Zn and toxic metals, Cd and Pb	• Most of the examined elements were elevated in the oral fluid of periodontal patients • The elevated levels of three metals: Cu, Mg and Mn were statistically significant • Levels of Cu were 5 times, Mn 3 times while Mg 2 times in oral fluids of periodontitis subjects when compared to controls.	• Elevated levels of minerals like Cu, Mn and Mg in oral fluids may be utilized to identify subjects with periodontitis
Boras et al. [93]	• Determined the salivary levels of phosphates ($PO4^{-}$), Cu, potassium (K), chloride (Cl^{-}), and sodium (Na), in subjects with and without periodontitis in order to explore its potential diagnostic implications	• n = 35 patients with periodontitis (23 females and 12 males) and n = 41 controls (32 females and 9 males); age range: 46.4 ± 17.2 years • Salivary levels of $PO4^{-}$, Cu, K, Cl^{-}, and Na, in subjects with and without periodontitis (either chronic or aggressive) were measured	• No significant difference in any of the clinical parameters between chronic and aggressive periodontitis patients • No significant difference in salivary $PO4^{-}$, Na, K, and Cl^{-} between cases and controls • Patients with periodontitis had significantly higher salivary Cu concentration compared to control group	• Increased salivary Cu levels indicated that either protective enzymes like superoxide dismutase and/or histatins were not working well, leading to progression of periodontitis • Host could also produce elevated levels Cu to combat bacterial infection
Carvalho et al. [94]	• Investigated the possible association between periodontitis and serum hepcidin and haemoglobin in systemically healthy individuals, as well as the association between IL-6 and hepcidin with complete blood count variables, inflammatory markers and Fe stores in individuals with chronic periodontitis	• Two groups—cases (n = 36) with periodontitis (at least two teeth with clinical attachment level ≥6 mm and probing depth ≥5 mm in one or more sites and controls (n = 31), without periodontal infection • Laboratory tests: complete blood count, total cholesterol, fasting glucose, triglycerides, albumin, oxaloacetic transaminase, creatinine, uric acid, erythrocyte sedimentation rate (ESR), CRP, IL-6, hepcidin, serum Fe, ferritin, Transferrin Saturation Index, and urine test for abnormal elements and sediment analysis	• The complete blood count, as well as Fe, hepcidin levels and haematocrit were lower in the group with periodontitis • Higher ESR and mean levels of in inflammatory markers in groups with periodontitis although not statistically significant	• Lowest haematocrit levels in periodontitis due to less number of red blood cells • Reduced serum Fe levels in periodontitis due to anaemia of chronic disease blocking intracellular Fe release. • In systemically healthy individuals with periodontitis the immunoinflammatory cells produce pro-inflammatory cytokines and acute phase reactants like C-reactive protein and hepcidin • Hepicidin is imperative for Fe haemostasis and metabolism
Kasuma et al. [95]	• Investigated the relationship between Zn consumption and matrix metalloproteinases-8 levels in GCF of subjects from Minangkabuenese, West Sumatra, Indonesia	• n = 60 patients; aged 17–30 years, were categorized into three groups—mild gingivitis, mild periodontitis and healthy groups • MMP-8 levels and Zn consumption were estimated	• Increased levels of MMP-8 with reduced Zn intake • Zn levels reduced from Healthy to mild gingivitis and were least in periodontitis group	• Zn supplementation is advisable to reduce periodontal inflammation

≥5 mm and at least two teeth in each quadrant with the condition. A minimum of 20 teeth were present in all the subjects. In most of these studies, the body fluids used for estimation of Fe, Zn, Se and Cu were serum or saliva.

All the studies suggested that optimal levels of micronutrients Fe, Zn, Se and Cu were imperative for periodontal health. There was a positive association between their reduced serum levels and chronic periodontitis. However,

elevated levels of Fe showed a negative impact on periodontal health [13, 87, 89, 90]. It was suggested that Fe chelation increased expression of lipopolysaccharide related genes which supported dental plaque biofilm formation. Furthermore, its reduced levels upregulated the genes involved in its uptake and downregulated the genes associated with its storage as well as the oxidative stress response to periodontopathogens [87]. High concentration of Fe enhanced the growth and virulence of subgingival plaque bacteria like *P. gingvalis*, *T. denticola*, *Aggregatibacter actinomyce temcomitans* and initiated active periodontitis. Besides serum, its elevated levels have been reported in oral fluids, i.e. GCF and saliva of subjects with periodontitis [96]. This further supports the view that elevated Fe in localized periodontal environment could promote destructive periodontal disease.

Elevated levels of Cu were also related to periodontal destruction in few studies [13, 73, 84, 85, 93]. Cu and Zn metabolisms are closely associated. It has been suggested that elevated levels of Cu reduces Zn absorption. Its levels may be increased in Zn deficiency in gingiva leading to increased permeability of gingival epithelium to periodontopathogens [97]. Furthermore, it stimulates inflammatory infiltrate to produce more IL-1, resulting in a vicious cycle of periodontal destruction [98]. Besides, Cu–Zn and Se are important constituents of antioxidant enzymes like Cu–Zn SOD and GPX system respectively [81, 93]. Therefore, as already explained, their deficiency results in excessive oxidative stress and destruction of tissues.

Another important observation in few studies was that existing chronic conditions like type 2 diabetes mellitus and rheumatoid arthritis could aggravate deficiency of micronutrients like Zn [13, 85, 86]. In diabetics, there is excessive urinary excretion of Zn resulting in its deficiency while Fe is elevated. As diabetes mellitus has a two way relationship with periodontitis and the latter is its complication, it may be suggested that imbalances in the levels of Zn and Fe may further promote progression of periodontitis in diabetics.

Smoking is another important risk factor for periodontitis. One study reported reduced levels of Zn and elevated levels of Cu in saliva of smokers with periodontitis [84]. Therefore, smoking could aggravate periodontitis by causing imbalances in the levels of these micronutrients. Similar results have been reported in previous studies where serum Se and erythrocyte GPX activity were reduced in smokers [98]. Furthermore, their erythrocyte Cu-Zn–SOD activity and serum ceruloplasmin concentrations were elevated, while serum Zn concentrations were depressed. Smoking even affected Fe homeostasis and increased the concentration of haemoglobin [98].

The major challenges in the interpretation of these studies were lack of uniformity, differences in socio-demographics, as well as the lifestyle factors. There was only limited data on intervention trials of trace mineral micronutrients. Only one study suggested that Zn supplementation may be beneficial in promoting periodontal health [95]. Furthermore, most of the studies utilized serum levels of trace minerals with only a few reporting levels in saliva. Since periodontal destruction occurs in a localized environment, measurement of these micronutrients in oral fluids like saliva and GCF would be more reflective of the changes occurring in periodontal environment. Therefore, prospective studies with larger sample sizes, investigating their levels in saliva and GCF are needed. Strategies for their supplementation in order to prevent the progression of chronic periodontitis should be developed after randomized controlled trials.

Recommendations for Trace Mineral Micronutritional Therapy for a Healthy Periodontium

As wholesome nutrition is the mainstay of good health and immune system, micronutrient administration to overcome their deficiencies may be justified. There are two options to achieve this objective. First, dietary modification and second, the administration of supplements. It has been suggested that where dietary manipulation is difficult, fortified foods and supplements may be a more practical choice. However, the argument regarding the cost–benefit effects and use of multimicronutrient additives still prevails [99]. In a recent review, it was proposed that "there was no proof that boosting micronutrient intake above what can be achieved in a well-balanced diet would lead to a healthier life that can be extended beyond the genetic set point we inherit" [99]. Furthermore, scientists have concluded that, "The hypothesis that groups with low nutrient status may benefit from supplementation is yet to be formally tested" [100].

Even though, numerous studies have evaluated the relationship between micronutrients and periodontal disease, intervention studies utilizing trace minerals in humans are scarce [13, 28, 81, 101]. A randomized, parallel clinical trial evaluated the influence of multivitamin tablets as an adjunct to periodontal therapy over a 60-day period [102]. There was a significant improvement in probing depths of subjects with chronic periodontitis who received the experimental treatment. Similar results were reported in a recent study where fruit/vegetable or fruit/vegetable/berry juice powder concentrates were concomitantly administered with non-surgical periodontal therapy [103]. The subjects received six capsules daily over a period of 2 months. It was suggested that addition of berry juice powder increased the levels of antioxidant micronutrients which reduced extracellular oxidative stress. It even downregulated the intracellular redox-regulated pro-inflammatory gene transcription factors [103]. The results from these studies further strengthens

the view that nutritional supplements as an adjunct to periodontal therapy may have a positive impact on immunoinflammatory response.

The trace mineral micronutrients, Zn, Fe, Se and Cu, have been deeply investigated for their role in immune function and resistance to infection. However, their complex metabolism and interactions with each other, as well as their effect on some intracellular pathogens (e.g. Fe) make their administration even more perplexing [61]. Evidence from preclinical and clinical trials suggests that although Fe is essential for immune function, its supplementation does not necessarily decrease morbidity and mortality [61]. This could be related to the complexity of the mechanisms of Fe homeostasis, which leads to differences in the results of the epidemiological studies [61]. Alternatively, strong evidence exists for the use of Se and Zn as dietary modulators due to their antioxidant and antiinflammatory actions [104]. They prevent periodontal destruction by reducing the levels of plasma oxidative stress markers and pro-inflammatory cytokines. Furthermore, their administration in conjunction with periodontal therapy accelerated wound healing and improved the clinical periodontal parameters [79]. A study investigated the effect of antioxidant therapy on the progression of periodontal disease when used as a monotherapy and/or as an adjunct to non-surgical debridement [105]. About 6 mg of antioxidants was administered in three divided doses for 2 weeks in the form of soft gel containing lycopene 2000 μg, zinc 7.5 mg and selenium 35 μg [105]. It significantly improved the salivary antioxidant levels and response to periodontal therapy. Furthermore, administration of Zn may improve the levels of SOD and reduce the inflammation. This was revealed in a recent study on 43 postmenopausal females with chronic periodontitis. They were administered with about 27.5 mg of zinc sulphate monohydrate, once daily for 3 months in conjunction with non-surgical periodontal therapy. It significantly improved the SOD levels in subjects receiving combined therapies [101]. Besides, the beneficial effects of elemental Zn supplementation (15 mg/day, 5 days a week for 10 weeks) on gingival health and plaques scores have also been reported in children with Zn deficiency [106].

Even though some reliable evidence exists, caution should be practised when advising supplements, as risk for toxicity cannot be ruled out. However, despite these disagreements, following guidelines may be helpful in determining the micronutrient requirements for oral and general health [61, 107].

1. Dietary assessment of nutritional intake should be done by a dietician. This involves use of a questionnaire to explore the dietary intake over a period of time.
2. Clinical assessment of nutritional status through changes in skin, nails, eyes, hair, mouth, neck, abdomen and extremities should be done.
3. Screening tools like, the Malnutrition Universal Screening Tool (MUST), a five-step screening method, may be utilized to identify individuals who are malnourished or at risk of malnutrition [108]. This tool scores the malnutrition risk as low, medium or high which helps the workers to develop a strategic plan for improving the dietary intake of the subjects. Besides, the Mini Assessment (MNA) and Malnutrition Risk Scale (SCALES) have been utilized for vulnerable groups like elderly [109]. The MNA consists of 18 items and has greater ability to predict morbidity and mortality while the SCALES includes parameters like S—sadness, C—cholesterol, A—albumin, L—loss of weight, E—eating problem physical/cognitive and S—shopping problems [107]. The subjective global assessment relies on physical signs of under nutrition and patient's history and does not use laboratory findings [110].
4. Anthropometric assessment, including weight, body mass index and arm circumference, may be used to calculate muscular circumference of the arm, which indicates the lean body mass [107]. Additionally, biometric impedance analysis to estimate total body water, extracellular water, fat-free mass and body cell mass helps in identification of malnourished individuals [111]. These parameters may be utilized in building a formal diet plan for the subjects.
5. Biochemical tests should be done to confirm the levels of specific micronutrients; however, they should be correlated with clinical parameters [10]. The nutrients may be supplemented if specific indications exists.
6. Administration of supplements should be further verified through the results of randomized controlled clinical trials in periodontitis subjects.

Conclusion

The trace mineral micronutrients like Fe, Zn, Cu and Se are essential for regulating the immunoinflammatory pathways. Their levels in the body may be reflective of the ongoing destructive process in chronic periodontitis. Moreover, any imbalances in subjects with conditions like diabetes may indicate a plausibility of development and worsening of diabetic complications like periodontitis. However, this alone does not justify their massive supplementation. A formal assessment combined with minor dietary shifts may be sufficient to achieve optimal levels for periodontal health. Future controlled clinical trials are warranted to elucidate their exact role in chronic periodontitis.

Acknowledgments None.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Funding Source None.

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