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Assessment of Serum Magnesium and Nitric Oxide Levels in Periodontitis: A Cross-sectional comparative Study

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Abstract

Periodontitis is a chronic inflammatory disorder that causes severe irreversible destruction of the tissues and bone of the teeth. Nitric oxide (NO)**,** a free radical act as anti-inflammatory, proinflammatory and important inflammatory mediator, magnesium (Mg) is major macro mineral plays a critical role in regulating inflammatory and immune responses. Alterations in these biomarkers may contribute to the pathogenesis of periodontal disease. This research aims to evaluate serum nitric oxide and magnesium levels in patients with periodontitis and compare them with healthy controls. This cross-sectional comparative study included 72 participants, comprising 36 periodontitis patients and 36 healthy controls aged 25–40 years of both genders**.** Healthy controls were systemically and periodontally healthy individuals. Periodontitis was diagnosed by a qualified periodontist using clinical parameters including periodontal pocket depth, clinical attachment loss, and bleeding on probing. Serum nitric oxide and magnesium levels were measured using respective kits. Data was normally distributed and statistical analysis was performed using Student's *t*-test. Serum nitric oxide levels were significantly increased in periodontitis patients (78.92 ± 9.40 µmol/L) compared with healthy controls (48.75 ± 12.20 µmol/L) (p = 0.007), representing an increase of approximately 64%. Serum magnesium levels were significantly decreased in periodontitis patients (1.42 ± 0.24 mg/dL) compared with controls (1.99 ± 0.14 mg/dL) (p = 0.001), representing a reduction of approximately 29%.Periodontitis patients exhibited significantly elevated serum nitric oxide levels and significantly reduced serum magnesium levels. These findings suggest that nitric oxide-mediated inflammation and magnesium deficiency are associated with periodontal disease progression.

Keywords: Periodontitis, Nitric Oxide, Magnesium, Inflammation, Periodontal Disease.

Introduction

Periodontitis is a chronic inflammatory disease-causing destruction of tooth-supporting structures, including the periodontal ligament and alveolar bone. Although bacterial plaque initiates the disease process, the resulting host inflammatory response is primarily responsible for periodontal tissue destruction and eventual tooth loss (Meisel et al., 2005)

Nitric oxide (NO) is an important inflammatory mediator involved in periodontal disease. Excessive activation of inducible nitric oxide synthase (iNOS) leads to increased nitric oxide production in inflamed periodontal tissues. Elevated NO levels contribute to tissue injury, bone resorption, and periodontal attachment loss through the formation of reactive nitrogen species such as peroxynitrite (Batista et al.,

2002). Magnesium (Mg) is an essential mineral involved in numerous enzymatic and cellular functions. It acts as a natural calcium-channel blocker and suppresses activation of nuclear factor-kappa B (NF-κB), thereby reducing cytokine production and inflammatory responses (Meisel etal.,2005) Magnesium deficiency has been associated with increased oxidative stress, enhanced inflammatory activity, and periodontal tissue destruction (Wu et al.,2024). Several studies have reported reduced serum magnesium levels and elevated nitric oxide concentrations in patients with periodontitis compared with healthy controls (Khan et al., 2017 & Al-Rawi et al., 2025). Therefore, the present study was designed to evaluate serum magnesium and nitric oxide levels in patients with periodontitis and compare them with healthy controls.Increasing nitric oxide does not directly alter magnesium levels in the gums. Instead, they play complementary roles in managing periodontal disease: nitric oxide helps regulate the oral microbiome and modulate inflammation, while magnesium supports jawbone density and reduces systemic inflammatory markers. (Cruz-Velasquez and Rodríguez-Orozco, 2025). Objective of this study was to measure and compare serum nitric oxide (NO) and Magnesium (Mg) levels periodontitis patients & healthy controls.

Materials and Methods

This Cross-sectional comparative study.was conducted after the institutional ethical committee. A total of 72 participants were enrolled, comprising 36 clinically diagnosed periodontitis patients and 36 healthy controls. Participants were recruited from Fatima Memorial Hospital and Sir Ganga Ram Hospital, Lahore, using a non-probability convenience sampling technique. Inclusion criteria Individuals aged 25–40 years of either gender who provided informed consent were included in the study. Participants with a history of antibiotic or anti-inflammatory therapy within the previous two months, smokers, pregnant or lactating women, individuals receiving recent periodontal treatment, those taking vitamin or mineral supplements, and patients with chronic systemic diseases were excluded.

Diagnosis of Periodontitis Patients. Periodontitis patients were diagnosed by a qualified periodontist according to established clinical diagnostic criteria. (Graetz et al., 2019).

Sample preparation. 5 ml Venous blood samples were collected under aseptic conditions the serum was placed in Eppendorf tubes and centrifuged at 15,000 rpm for 15 mins at 4 °C. The supernatant was transferred to new sterile Eppendorf tubes and was stored at − 80 °C for biochemical analysis. Serum nitric oxide & magnesium was measured by using commercially available Kit on Micro lab 300.

Statistical analysis

The data was entered and analyzed using SPSS version. Results were expressed as mean ± standard deviation (SD), and comparisons between groups were performed using Student's *t*-test. A *p*-value < 0.05 was considered statistically significant.

Results

Comparison of Serum Nitric Oxide and Magnesium Levels between Periodontitis Patients and Healthy Controls

Patients with periodontitis exhibited significantly higher serum nitric oxide (NO) levels (78.92 ± 9.40 µmol/L) than healthy controls (48.75 ± 12.20 µmol/L), representing a 64% increase ($p < 0.001$). In contrast, serum magnesium (Mg) levels were significantly lower in periodontitis

patients (1.42 ± 0.24 mg/dL) compared with controls (1.99 ± 0.14 mg/dL), corresponding to a 29% decrease (p = 0.001). Student's t-test confirmed statistically significant differences between the groups for both biomarkers, indicating increased oxidative stress and reduced magnesium status in individuals with periodontitis (Figs. 1 and 2 respectively).

Discussion: More recently, Hajishengallis etal., (2021) emphasized that periodontal inflammation contributes to both local tissue destruction and systemic inflammatory disorders, while Lira-Junior and Figueredo (2016) highlighted the close relationship between periodontal inflammation and systemic disease mechanisms.

Our research demonstrates significantly higher level of serum nitric oxide (NO) in patients with periodontitis compared with healthy controls The approximately 64% increase in serum Nitric oxide (Figure 1) levels suggest a potential association between nitric oxide level and the inflammatory processes involved in pathogenesis and progression of periodontal disease. These findings are consistent with those reported by (Mani et al., 2013) that elevated salivary and plasma nitric oxide concentrations were positively associated with increased probing depth, plaque accumulation, and clinical attachment loss. Similarly, Reher et al. (2007) observed that salivary nitric oxide levels increased with the severity of chronic periodontitis, whereas Batista et al. (2002) reported enhanced inducible nitric oxide synthase (iNOS) expression in diseased periodontal tissues. Liu et al. (2025) reported that Low levels of nitric oxide (NO) play a protective role act as vital signalling molecule and assist immune system to destroy pathogen and maintaining oral homeostasis. While in chronic bacterial infection nitric oxide synthesis increase, act as inflammatory mediator that cause oxidative stress and cause cellular damage and tissue destruction and at last loss of toot. The pathological cascade mechanism of Periodontal disease (PD) triggered by bacterial toxins. Frist step is activation of major pro-inflammatory mediator inducible nitric oxide synthase enzyme (iNOS) which introns increase production of nitric oxide gas. Elevated nitric oxide concentration cause vasodilation of local blood vessels, leading to increased vascular permeability and leakage of fluid into the surrounding gingival tissues. Consequently, resulting redness and swollen of gums. Because NO contains an unpaired electron, excessive production contributes to oxidative and nitro-oxidative stress. (Morou etal., 2022).The massive increase in of NO gas rapidly combines with local superoxide radicals (O_2^-) to create incredibly nitrogen species Peroxynitrite ($ONOO^-$) and initiates widespread lipid peroxidation and directly ruptures host cell membranes. Higher level of NO also activate Matrix Metalloproteinases that aggressively degrade collagen matrix of periodontal ligaments and result is structure collapse and formed periodontal pocket and due to dissolve of periodontal ligament teeth lose stability. (Asad etal.,2024; Mendoza etal., 2025). Higher serum nitric oxide levels in periodontitis patients indicate increased inflammatory and oxidative activity, suggesting that NO is positively associated with periodontal disease progression and tissue destruction.

A major finding of the present investigation (Figure 2) demonstrates significantly lower level of serum magnesium in patients with periodontitis compared with healthy controls. The approximately 29% reduction in serum magnesium levels among periodontitis patients suggests a potential association between magnesium deficiency and periodontal disease.

A major finding of the present investigation was the significant reduction in serum

magnesium levels among periodontitis patients. This observation agrees with the findings of Meisel et al. (2005), who reported that higher serum magnesium concentrations were associated with reduced probing depth, less attachment loss, and a greater number of remaining teeth. Similarly, Khan et al. (2017) demonstrated significantly lower serum magnesium levels in chronic periodontitis patients.

The relationship between magnesium deficiency and periodontal disease may be explained by the anti-inflammatory properties of magnesium. Meisel et al. (2005) proposed that magnesium acts as a natural calcium-channel blocker and suppresses activation of nuclear factor-kappa B (NF-κB), a key regulator of inflammatory cytokine production. Furthermore, Nielsen (2018) reported that magnesium deficiency promotes the release of inflammatory mediators such as TNF-α, IL-1β, and IL-6 concluding that an inadequate magnesium status is directly associated with chronic low-grade inflammation and increased oxidative stress. The inverse relationship between magnesium and nitric oxide observed in the present study is supported by mechanistic evidence. Normal magnesium levels may help control calcium entry, reduce blood vessel stress, and maintain nitric oxide balance**.** (Nielsen, 2018; Houston, 2011)

Almousa et al. (2018) suggested that magnesium-deficient environments facilitate bacterial lipopolysaccharide-induced activation of inducible nitric oxide synthase, leading to excessive nitric oxide production. Consequently, reduced magnesium levels may remove an important regulatory mechanism controlling inflammatory responses, thereby amplifying nitric oxide-mediated tissue injury. Recent epidemiological evidence further supports the importance of magnesium in periodontal health**.** (Wu et al., 2024)**.**

Shang et al. (2023) reported that individuals with high magnesium depletion scores were approximately twice as likely to develop moderate-to-severe periodontitis, suggesting that magnesium depletion may represent an independent risk factor for periodontal disease progression. Oxidative stress is increasingly recognized as a central mechanism in periodontal tissue destruction. Wang et al. (2017) demonstrated that persistent oxidative stress contributes to periodontal ligament degradation and alveolar bone resorption. Similarly, Cepeda et al. (2025) demonstrated that magnesium supplementation improved antioxidant enzyme activity, including Superoxide dismutase and Catalase.

According to recent advances in periodontal immunology have highlighted the role of inflammation resolution pathways. Van Dyke and Sima (2020) proposed that failure of inflammation treatment contributes significantly to persistent periodontal tissue damage and loos of tooth. Inflammation can control by using enzymatic and nonenzymatic antioxidants.

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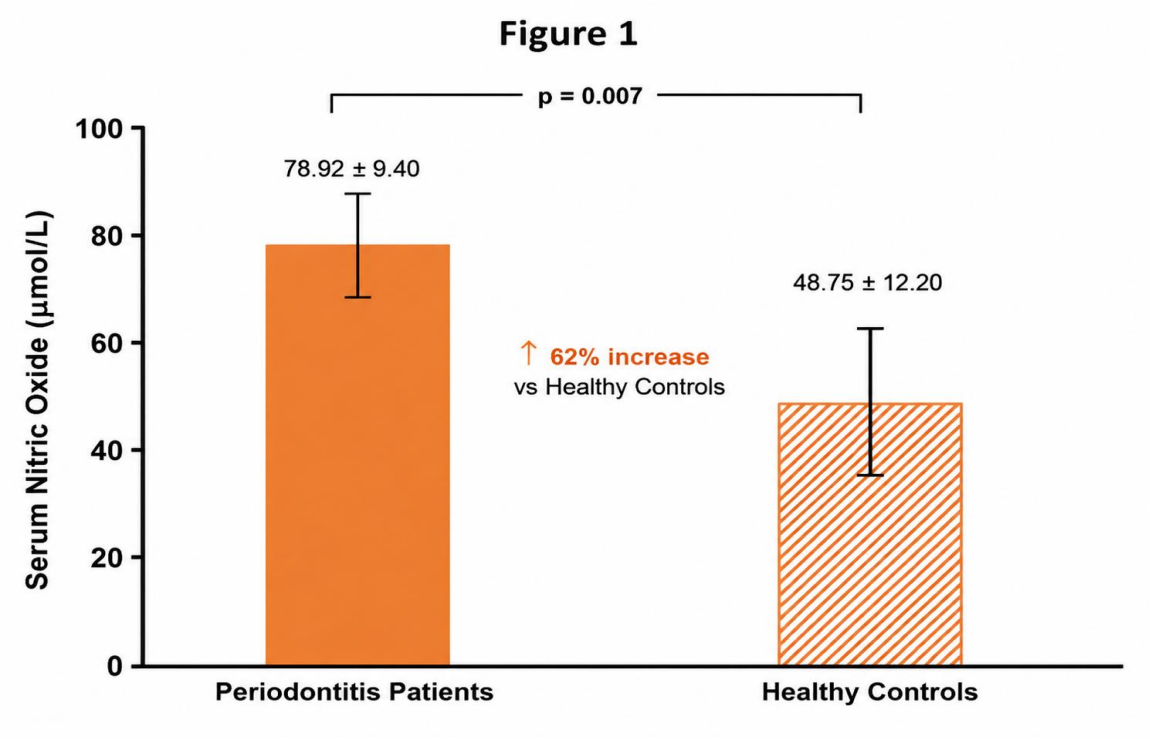

Figure 1: Serum levels of nitric oxide in Periodontitis patients and Controls. Values are shown in mean ± SD (n = 36). A significant difference was found between two groups by applying student "t" test with p-value = 0.007.

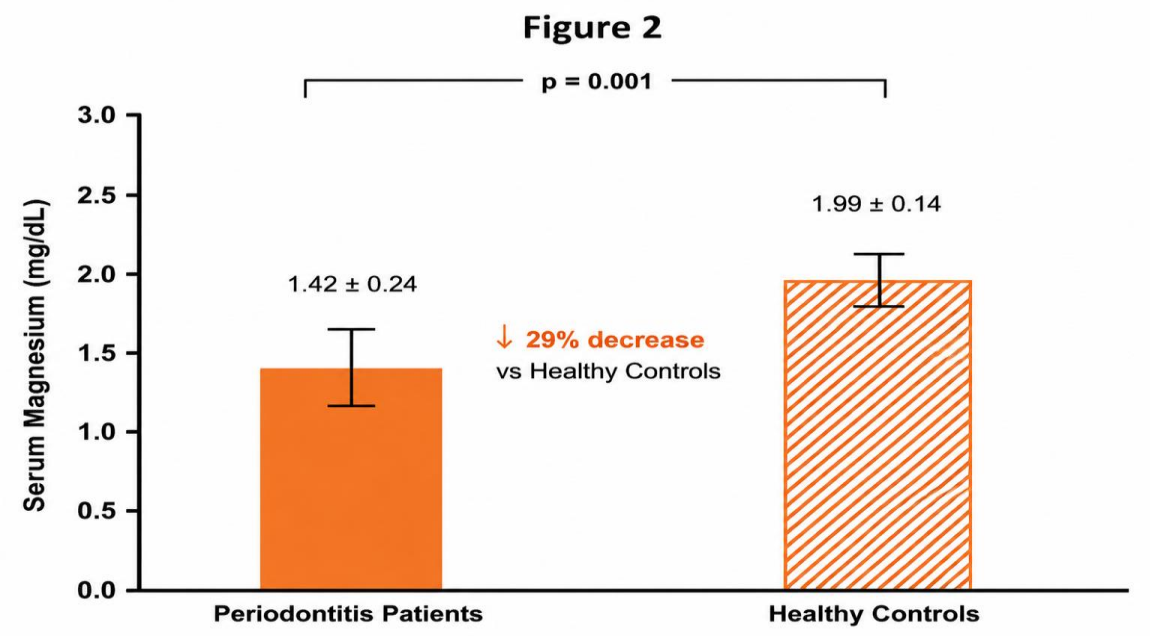

Figure 2: Serum levels of Magnesium in Periodontitis patients and Controls. Values are shown in mean ± SD (n=36). A significant difference was found between two groups by applying student 't', test with p-value=0.001.

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