



Serum and Salivary Calcium and Magnesium Levels in Inflammatory Bowel Disease: A Cross-Sectional Study

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Abstract

Background: Inflammatory bowel disease (IBD) is a chronic condition characterized by inflammation of the gastrointestinal tract, which occurs with relatively high frequency. The precise cause of IBD remains unidentified. Given that calcium and magnesium are known to influence inflammatory processes, this study aims to investigate the alterations in saliva and serum among individuals diagnosed with inflammatory bowel disease.

Methods: This cross-sectional study conducted in 2023 included 30 subjects with IBD and 30 healthy individuals. Unstimulated saliva and serum samples were collected during a fasting state, and the concentrations of calcium and magnesium were measured using the photometric method. The data were analyzed with an unpaired Student's t-test and Chi-square test using SPSS version 22, with a significance level set at $P < 0.05$.

Results: The results indicated that the levels of calcium and magnesium in the serum and saliva of patients with IBD were significantly lower than those of healthy individuals ($P < 0.05$).

Conclusion: Calcium and magnesium levels are often significantly impaired and reduced in patients with IBD. This deficiency can lead to various complications associated with IBD, underscoring the importance of monitoring and managing mineral levels in these patients.

Keywords: Inflammatory bowel disease; Calcium; Magnesium; Saliva; Serum

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Introduction

Inflammatory bowel disease (IBD) is a heterogeneous condition characterized by chronic inflammation of the gastrointestinal (GI) tract, with a relatively high prevalence. The precise cause of IBD remains unidentified (1). This disease significantly

impacts patients' daily lives and imposes substantial economic and familial burdens on both individuals and society (2). Although the etiology of IBD is not fully understood, recent evidence suggests that its onset involves a complex interplay of environmental, genetic, microbial, and immune factors. Unfortunately, due to its intricate and poorly

understood pathogenesis, IBD is currently incurable and can only be managed (3).

The incidence and progression of IBD are primarily driven by inflammation, which is triggered by a substantial increase in pro-inflammatory cytokines (4).

IBD is diagnosed through a combination of patient history, clinical presentation, endoscopic findings, and microscopic examination of tissue samples (5). This diagnostic process can be lengthy and may be further delayed due to the clinical heterogeneity of the disease and the need to differentiate it from other conditions (6). Consequently, researchers have long been interested in identifying biomarkers that can facilitate non-invasive diagnosis of IBD. Studies investigating the effects of calcium and magnesium have demonstrated that these two minerals, while supporting various physiological functions such as saliva secretion, also play a significant role in regulating inflammation and modulating the immune system (7-10).

Saliva serves as a valuable supplementary diagnostic tool for various illnesses, including gastrointestinal, neurological, autoimmune, cardiovascular, endocrine, and oncological conditions (11-15). Furthermore, saliva collection alleviates the anxiety often associated with blood draws and is a Furthermore, saliva collection alleviates, non-invasive procedure with a low risk of infection transmission. Collecting saliva and transferring it to the laboratory does not necessitate special conditions, such as preventing coagulation, making it safe even for medical personnel with limited experience (16).

Since calcium and magnesium regulate salivary secretion and play a role in the immune system control, and given that one of the reasons for IBD is autoimmunity, we decided to test the serum and salivary levels in affected patients.

Patients and Methods

This cross-sectional study was conducted in 2023 in the physiology laboratory of the Faculty of Medicine at AJA University of Medical Sciences and Imam Reza Hospital in Tehran, Iran. The study protocol was approved by the Ethics Committee of AJA University of Medical Sciences in Tehran (code: IR.AJAUMS.REC.1402.130).

We initially designed and conducted a pilot study to determine the appropriate sample size. We used the following formula:

In the initial research, serum samples were collected from six patients and six healthy individuals. The standard deviation (SD) was 2.1 in the patient group and 2.3 in the healthy group, while the average magnesium level was 2.7 in the healthy group and 1.5 in the patient group. Using the appropriate formula and considering a statistical power of 80%, the sample size was calculated to be 26 subjects; for increased

reliability, we opted to include 30 subjects in each group. The patient and control groups were matched based on occupation, literacy level, and financial income. Due to the limited number of patients, the control group was selected from hospital personnel who were similar to the patients in terms of gender, age, and body mass index (BMI). Clinical symptoms, endoscopic findings, and pathology reports were utilized to identify the patients. Given the necessity of annual checkups at the hospital, healthy individuals from the hospital staff, whose health status was verified, were selected and included in the study. The exclusion criteria comprised diabetes, hypertension, recent fever, osteoporosis, Sjögren's syndrome, and liver and renal disorders.

The participants were initially informed about the project's objectives and reassured that they would not be in any danger. They were also assured that the information they provided would remain confidential and that they could withdraw from the study at any time. Subsequently, through interviews, the participants' demographic data—including age, gender, height, weight, and duration of illness—was recorded.

Sampling was conducted while the participants were fasting. Initially, they were instructed to collect their saliva in sterile polypropylene tubes without engaging in active mouth wall movements or sucking. Subsequently, a specialist drew 5 milliliters of blood from the cephalic vein. The saliva and blood samples were immediately centrifuged at 4000 rpm for 5 minutes. The serum and saliva supernatant were carefully transferred into microtubes and stored at -70°C in a freezer. The concentrations of calcium and magnesium were measured using the appropriate kit (Pars Azmoun, Shiraz, Iran) in accordance with the manufacturer's instructions.

Written informed consent was obtained from the study participants.

Statistical Analysis

Data analysis was conducted using SPSS version 22 software. First, the normal distribution of the data was assessed using the Kolmogorov-Smirnov test. Smirnov test. Based on the results of this test, the unpaired Student's t-test and Chi-square test were employed for further analysis.

Results

In this study, 30 patients with IBD (17 men and 13 women) with an average age of 37.76 ± 12.94 years were compared to 30 healthy subjects (16 men and 14 women) with an average age of 32.53 ± 8.33 years. There were no significant differences between the groups in terms of sex, age, and BMI ($P > 0.05$) (Table 1).

The serum and salivary levels of calcium and magnesium in patients with IBD were significantly lower than those in the control group (Table 2).

Table 1: Clinical characteristics of patients with IBD and control group

Variable	Control	IBD	P value
Sex (male/female)	16/14	17/13	0.83 ^a
Age (year) (mean±SD)	32.53±8.33	37.76±12.94	0.52 ^b
BMI (kg/m ²) (mean±SD)	26.33±4.20	25.53±3.77	0.45 ^b
Disease duration (year) (mean±SD)		5±3.85	

Data were analyzed using a Chi-square test and an unpaired Student's t-test. A P-value of less than 0.05 was considered significant.

Table 2: Serum and salivary concentrations of magnesium and calcium in patients with IBD and control subjects.

Variable	Control	IBD	P-value
Serum calcium (mg/dl)	9.36±0.47	8.48±1.51	0.017
Serum magnesium (mg/dl)	2.02±0.14	1.86±0.31	0.026
Salivary calcium (mg/dl)	3.68±2.13	2.41±2.10	0.031
Salivary magnesium (mg/dl)	0.69±0.63	0.37±0.27	0.019

Data are expressed as mean±SD and analyzed using an unpaired Student's t-test; P<0.05 was considered statistically significant.

Discussion

IBD is a chronic condition affecting the digestive tract, the exact cause of which remains unknown. IBD is classified into three main types: ulcerative colitis, Crohn's disease, and indeterminate colitis. Each year, the prevalence of IBD rises significantly, leading to increased expenses for the healthcare system (17-19). Research indicates that calcium and magnesium levels may decrease in certain inflammatory diseases (20-23).

In the current study, we observed low serum calcium levels in patients with IBD. Previous research has indicated that serum calcium levels are reduced in various inflammatory conditions, including chronic periodontitis (22), giardiasis (23), and among smokers (24). Additionally, our study found that serum magnesium levels were also decreased in patients with IBD, which aligns with findings from earlier studies on IBD (25), giardiasis (23), and myocardial infarction (26). The reduction in the serum levels of these two minerals in IBD may be attributed to inflammation of the GI tract and a consequent decrease in their absorption (27).

Our data indicate that salivary calcium levels are reduced in individuals with IBD compared to healthy subjects. This finding is consistent with previous reports on periodontitis (20) and Sjögren's syndrome (21). Additionally, salivary magnesium levels are also lower in patients with IBD compared to the healthy group, aligning with the findings of Sarchielli et al. regarding migraine (28).

Calcitonin reduces the levels of calcium and magnesium by promoting their deposition, while parathyroid hormone (PTH) increases these levels by facilitating their release from the bones (29-31). In inflammatory diseases, calcitonin levels tend to rise, whereas PTH levels decrease (32, 33). Additionally, during inflammation, calcium can enter the cytoplasm from the extracellular fluid (34). These factors may explain the observed reduction of calcium and magnesium in the serum of

patients with IBD and other inflammatory conditions (20-22, 27). Since salivary compounds such as calcium and magnesium are derived from plasma (35), a decrease in serum levels of these minerals may lead to a reduction in saliva production.

The present study had some limitations. We did not measure the levels of calcitonin, PTH, and 25-hydroxycholecalciferol in the serum and saliva of patients with IBD to support our rationale. Additionally, all patients in our study were undergoing medication treatment. Future research would benefit from including newly diagnosed patients.

Conclusion

Calcium and magnesium levels are often significantly impaired and reduced in patients with IBD. This deficiency can lead to various complications associated with IBD, underscoring the importance of monitoring and managing mineral levels in these patients. Furthermore, it may be possible to assess these parameters for diagnostic purposes or follow-up care in the future.

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Authors' Contribution

Conceptualization: M.A, I.M.D; Methodology: I.M.D; Data curation, formal analysis, software: M.A, I.M.D; Project administration, validation: P.A; Writing original draft: M.A; Review and editing: P.A, I.M.D. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work. They ensure that any questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Availability of Data and Materials

Data are available from the corresponding author

upon request.

Conflict of interest: None declared.**References**

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