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Multi-Element Salivary Biomarker Profiling for Non-Invasive Stratification of Periodontal Disease

Electrolyte-Trace Element Interactions and Diagnostic Performance

Elaf Salam Sabeth, Abbas Fadhil Al-Hashimi

College of Medicine, Al-Nahrin University, Baghdad, Iraq

Abstract

This study aimed to evaluate salivary sodium, potassium, cobalt, and iron as a multi-element biomarker profile for non-invasive stratification of periodontal disease and to investigate electrolyte–trace element interactions in relation to periodontal inflammatory and structural parameters. This comparative cross-sectional study included 90 adults equally classified into periodontally healthy, gingivitis, and periodontitis groups. Unstimulated whole saliva samples were collected using the passive drool method, and salivary sodium, potassium, cobalt, and iron concentrations were measured by atomic absorption spectrophotometry. Clinical periodontal parameters included bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL). Group comparisons, post-hoc testing, correlation analysis, inter-element correlation analysis, and receiver operating characteristic (ROC) curve analysis were performed. Salivary sodium increased progressively from periodontal health to gingivitis and periodontitis (16.64 ± 2.33, 20.50 ± 2.55, and 22.39 ± 3.23 mmol/L, respectively; $p < 0.001$), whereas potassium decreased across the corresponding groups (12.36 ± 1.32, 10.65 ± 1.77, and 10.38 ± 2.30 mmol/L, respectively; $p < 0.001$). Cobalt and iron were also reduced in disease groups compared with healthy controls, decreasing from 0.28 ± 0.09 to 0.21 ± 0.09 and 0.18 ± 0.09 µg/dL for cobalt, and from 47.63 ± 11.03 to 34.40 ± 8.89 and 30.77 ± 11.35 µg/dL for iron, respectively (both $p < 0.001$). Sodium showed the clearest progressive discriminatory pattern across the three periodontal groups, while potassium, cobalt, and iron mainly distinguished periodontal health from disease. Sodium was positively correlated with BOP ($r = 0.565$, $p < 0.001$), whereas potassium ($r = -0.316$, $p = 0.002$), cobalt ($r = -0.314$, $p = 0.003$), and iron ($r = -0.468$, $p < 0.001$) were negatively correlated with BOP. None of the salivary elements showed significant correlations with PPD or CAL within the periodontitis group. Inter-element analysis showed that sodium and potassium, cobalt, and iron were all negatively correlated whereas potassium, cobalt, and iron were positively correlated with each other. ROC analysis showed the best diagnostic performance for sodium, with the most significant difference between healthy controls and periodontitis noted (AUC = 0.932, sensitivity = 83.3%, specificity = 86.7%). Periodontal disease was correlated with a coordinated multi-element salivary profile characterized by elevated sodium levels and concurrent reductions in potassium, cobalt, and iron. This elemental remodeling pattern was more related to periodontal inflammatory burden rather than structural periodontal destruction. Multi-element salivary biomarker profiling may serve as a promising non-invasive adjunct for periodontal disease stratification, although further validation in larger and demographically balanced cohorts is required.

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Email: elaf.s.sabih.med24@ced.nahrainuviv.edu.iq

Introduction

Periodontal diseases are chronic inflammatory disorders characterized by dysbiotic dental biofilm and maintained by a complex host immune–inflammatory response [1,2]. Gingivitis is a reversible inflammatory process that is limited to gingival tissues, and periodontitis is characterized by irreversible destruction of the tooth-supporting structures, such as periodontal ligament breakdown, alveolar bone loss, periodontal pocket formation, and clinical attachment loss [3-5]. However, conventional clinical periodontal examination is currently considered the diagnostic foundation, yet probing pocket depth (PPD), clinical attachment level (CAL), bleeding on probing (BOP), and radiographic bone loss do not fully capture the biological activity of periodontal disease. PPD and CAL represent mainly the burden of accumulated structural damage, while inflammatory parameters and molecular biomarkers inform us about the current activity of disease and the host response to it [6,7].

Saliva has become a powerful non-invasive diagnostic biofluid for periodontal assessment because it is readily available, safe to obtain, acceptable to patients, and suitable for continuous sampling [8]. These features render saliva a potential candidate tool for screening, monitoring, and risk stratification of oral inflammatory diseases [6,9]. In the context of periodontal disease, saliva reflects local biological changes in the oral environment via such inflammatory mediators, microbial products, enzymes, oxidative stress markers, metabolites, electrolytes, and trace elements [7,10,11]. Thus, salivary diagnostics may provide complementary biochemical information in harmony with, not in direct replacement to, conventional clinical periodontal assessment.

With this potential, however, translation into clinical practice of salivary biomarkers remains limited by biological and methodological variability. Salivary composition can be affected by various pre-analytical and analytical factors including method of saliva collection, stimulation status, flow rate, circadian rhythm, hydration, diet, smoking, systemic health conditions, sample processing, and laboratory technique [6,12-14]. These limitations have fostered movement from single-biomarker methods to multi-marker and omics-based approaches that may be more appropriate to effectively represent the biological complexity of periodontal disease. Multi-biomarker panels, microbiome signatures, metabolomic strategies, and ionic profiling are increasingly being investigated as tools to improve periodontal diagnostic accuracy and biological interpretation [6,7,12,15].

Electrolytes and trace elements are of interest to salivary biomarker groups, due primarily to their relevance to ionic homeostasis, epithelial transport, enzyme activity, oxidative balance, immune control, and microbially mediated interactions [16,17]. The ionic profiling provides insights into the evolution of disease-related modifications in the elemental compositions of biological fluid and may identify linked biochemical alterations that are not discernible with separate readings for many individual markers [12]. In periodontal disease, salivary elemental content may be affected by gingival inflammation, increased vascular permeability, disruption of epithelial barrier, changes in salivary gland transport, oxidative stress, tissue degradation, and microbial metabolic activity [12,18,19]. Nonetheless, available evidence is heterogeneous and the diagnostic relevance of combined electrolyte–trace element profiles has to be further elaborated.

Sodium and potassium are significant salivary electrolytes associated with the maintenance of oral ionic homeostasis and salivary gland regulation. Sodium concentration is partly regulated through ductal reabsorption, whereas potassium is actively secreted into saliva through glandular epithelial mechanisms [19,20] . Inflammatory exudation, epithelial permeability and host–microbial interactions may lead to disruption of local ionic equilibria in the face of periodontal inflammation. Salivary sodium and potassium in periodontal disease have shown variable findings, with some work indicating increased sodium in disease conditions but potassium findings being less consistent across populations and methodologies [12,14,18]. These discrepancies indicate that sodium and potassium might be informative, not as separate biomarkers to be used, but rather through consideration of a full salivary elemental profile.

Other trace elements like cobalt and iron might also add to the biochemical landscape of periodontal disease. Cobalt is biologically significant as part of vitamin B12 and indirectly participates in redox-related and inflammatory pathways, but its salivary involvement in periodontal disease has not been adequately explored [12,21]. Iron has received greater attention because of its involvement in oxidative stress, microbial growth, and inflammatory tissue injury. Iron homeostasis may be dysregulated and, via oxidative damage and host–microbial interactions, result in periodontal inflammation, while salivary iron concentrations can differ according to periodontal status, systemic metabolic conditions, and analytical methodology [22-25].Thus, quantifying trace elements along with major electrolytes could give a more holistic picture of the salivary biochemical remodeling in periodontal disease.

A major gap in existing studies is that many studies of salivary elements have only examined it as independent entities, in contrast to this holistic profile at some biochemical level. A solitary salivary change in periodontal disease is unlikely to mirror a solitary isolated salivary alteration; it is expected to be associated instead with coordinated alterations of a variety of inflammatory, oxidative stress, epithelial transport and tissue response elements. Evaluating electrolyte–trace element interactions may therefore offer a more informative model than single-marker analysis. In this context, the relationships among sodium, potassium, cobalt, and iron may help determine whether periodontal disease is associated with a coordinated salivary elemental remodeling pattern and whether this pattern is more closely related to inflammatory activity, represented by BOP, or to structural periodontal destruction, represented by PPD and CAL.

Accordingly, the present study aimed to evaluate salivary sodium, potassium, cobalt, and iron as a multi-element biomarker profile for non-invasive stratification of periodontal disease. Specifically, it compared salivary elemental levels among periodontally healthy individuals, gingivitis patients, and periodontitis patients; assessed their associations with clinical periodontal parameters; investigated electrolyte–trace element interactions; and evaluated the diagnostic performance of individual salivary elements using receiver operating characteristic curve analysis. By focusing on multi-element salivary profiling rather than a single-biomarker approach, this study seeks to provide a broader understanding of salivary ionic remodeling in periodontal disease and its potential value as a non-invasive adjunct to conventional periodontal assessment.

Materials and Methodos

Study Design and Reporting

The present cross-sectional study compared salivary sodium, potassium, cobalt, and iron

as a multi-element biomarker profile for the non-invasive stratification of periodontal disease. Subjects were initially divided into three parallel groups based on periodontal status which were periodontally healthy controls, patients with gingivitis, and patients with periodontitis. Salivary concentrations of sodium, potassium, cobalt, and iron were the main outcomes. Secondary outcomes included the associations between salivary elements and clinical periodontal parameters, electrolyte–trace element interactions, and the diagnostic performance of individual salivary elements in differentiating periodontal status groups. The manuscript was prepared in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations.

Study Setting and Ethical Considerations

Participants were enrolled from the patients who presented to the Dental Teaching Clinics, College of Dentistry, University of Baghdad, Baghdad, Iraq, for periodontal examination and assessment. Saliva samples were analyzed in laboratories at the Poisoning Consultation Center, Surgical Specialty Hospital, Baghdad, Iraq. The study was conducted from October 2025 to February 2026 under standardized clinical and laboratory conditions. Ethical approval was obtained from the Institutional Review Board of the College of Medicine, Al-Nahrain University (Approval No. 20251098; 28 October 2025). The study was carried out in accordance with the ethical principles of the Declaration of Helsinki [26]. All participants were notified about the study objectives and procedures. To this end, written informed consent was obtained before their enrollment.

Study Population

A total of 90 adult participants were included and allocated equally into three groups: periodontally healthy controls (n = 30), gingivitis patients (n = 30), and periodontitis patients (n = 30). The sample size was guided by the expected ability to detect differences in salivary electrolyte and trace-element levels among periodontal status groups, with 80% statistical power and a significance level of 0.05, based on previous related studies [18,27].

Inclusion criteria included adulthood age (18–55 years), more than 20 natural teeth, and willingness to provide written informed consent. Exclusion criteria were systemic diseases or conditions with known effects on mineral or electrolyte metabolism (e.g. uncontrolled diabetes mellitus, renal disease, or endocrine disorders); use of antibiotics or anti-inflammatory medications within the previous three months; pregnancy or lactation; periodontal therapy within the previous six months; heavy metal occupational exposure; and the presence of metallic dental prostheses, especially cobalt–chromium restorations, due to their anticipated impact on salivary cobalt concentrations.

Periodontal Examination and Group Classification

All patients received a full-mouth periodontal examination from one calibrated examiner. Periodontal measurements were recorded at six sites per tooth, excluding third molars. Clinical periodontal assessments included bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL). Measurements were performed using a standardized UNC-15 periodontal probe. PPD was defined as the distance from the gingival margin to the base of the periodontal pocket, whereas CAL was defined as the distance from the cement-enamel junction to the base of the pocket or sulcus. BOP was assessed approximately 15 seconds after gentle probing.

Participants were categorized according to standardized periodontal diagnostic criteria. Periodontally healthy participants were defined as individuals with BOP <10%, PPD ≤3 mm, and no clinical attachment loss. Gingivitis was defined as the presence of gingival inflammation and BOP >10% without clinical attachment loss or radiographic evidence of alveolar bone loss. Periodontitis was diagnosed according to the 2017 World Workshop classification criteria, based on the presence of interdental clinical attachment loss at two or more non-adjacent teeth, or buccal/oral CAL ≥3 mm with pocketing >3 mm at two or more teeth [5,28]. Cases of periodontitis were categorized according to stage, grade, and extent for descriptive purposes.

Before the main work, examiner calibration was carried out. A preliminary examination was conducted on about ten individuals, not in the final analysis. PPD, CAL, and BOP were measured twice with a one-hour interval between measurements to assess intra-examiner consistency.

Saliva Collection and Processing

Unstimulated whole saliva specimens were taken from all participants applying the passive drool method. Participants were then instructed to stop eating, drinking, smoking, chewing gum, toothbrushing, or using mouthwash for at least 60 min prior to saliva collection. These samples were preferably collected in the morning to minimize the effect of diurnal variation and were obtained before clinical periodontal examination [14,29]. In collection, participants were sitting comfortably, with their heads tilted slightly forwards, and were instructed to let saliva accumulate in the floor of their mouth and drool gently into sterilized trace-element-free polypropylene tubes. Saliva was collected for about five minutes, or until a volume of 2–5 mL was withdrawn. Samples were placed on ice and transported to the laboratory within one hour of collection. The samples were centrifuged (3000 × g for 10 min at 4 °C) to remove cellular debris and particulate matter. Supernatant was then carefully aliquoted into trace-element-free tubes and stored at −80°C until the biochemical analysis [30].

Laboratory Analysis of Salivary Elements

The concentrations of sodium, potassium, cobalt, and iron in saliva were determined using atomic absorption spectrophotometry. The values of sodium, potassium, and iron were determined by flame atomic absorption spectrophotometry, and cobalt by flameless atomic absorption spectrophotometry, due to its trace quantity contained in saliva. Salivary frozen samples were thawed at room temperature and vortexed lightly prior to analysis. One appropriate volume of saliva was added to acid-cleaned digestion vessels; concentrated ultrapure nitric acid, with or without hydrogen peroxide, was added for organic component digestion. After digestion, the samples were cooled and diluted to a constant final volume with ultrapure deionized water. The prepared samples were kept temporarily in acid-washed, trace-element-free polypropylene vials until instrumental measurement.

Based on known concentrations of sodium, potassium, cobalt, and iron, calibration curves were prepared for each element. Elemental concentrations of salivary extract absorbance values were compared with corresponding calibration curve(s). All measurements were conducted in triplicate to enhance data quality and reproducibility.

Quality Control and Quality Assurance

At all stages of lab work, trace-metal analytical grade reagents and ultrapure deionized water were used. To keep contamination to a minimum, acid-washed, trace-element-free containers were placed at this stage. Procedural blanks are included to observe for possible contamination during sample preparation. Quality control samples and spiked samples were analyzed for method accuracy and analytical recovery. Standard laboratory quality assurance practices were referenced when calibrating and validating the tests [31].

Statistical Analysis

The data were entered, coded, checked, and statistically analyzed via SPSS version 26.

The normality of continuous variables was checked through the Shapiro–Wilk test, supported by visual inspection of histograms and Q–Q plots. Normally distributed variables were reported as mean ± standard deviation (SD) while non-normally distributed variables were described using median and interquartile range (IQR). Categorical variables were presented as frequencies and percentages.

Between-group comparisons were performed according to data distribution. One-way analysis of variance (ANOVA) was used to compare normally distributed salivary element levels among the three periodontal groups. When significant group differences were detected, Games–Howell post-hoc testing was used for pairwise comparisons. Non-normally distributed variables were compared using the Kruskal–Wallis test, followed by pairwise comparisons when appropriate. The magnitude of group differences was estimated using eta squared (η^2) as a measure of effect size.

Because demographic differences were observed among the study groups, particularly in age and sex distribution, these variables were considered during interpretation of salivary biomarker findings. Where applicable, sensitivity to potential demographic confounding was assessed descriptively by examining whether the direction and magnitude of salivary elemental differences were biologically consistent with periodontal status.

Correlation analyses were performed to assess associations between salivary sodium, potassium, cobalt, and iron and clinical periodontal parameters. Spearman correlation analysis was used for associations involving BOP, PPD, and CAL when non-normal distribution was present or when non-parametric confirmation was required. Correlations between salivary elements and BOP were assessed across the total study sample, whereas correlations with PPD and CAL were restricted to the periodontitis group because these parameters were clinically relevant in that group. Inter-element correlations among sodium, potassium, cobalt, and iron were also evaluated to explore electrolyte–trace element interaction patterns.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of salivary sodium, potassium, cobalt, and iron in differentiating periodontally healthy controls from gingivitis patients, healthy controls from periodontitis patients, and gingivitis patients from periodontitis patients. The area under the curve (AUC), sensitivity, specificity, and optimal cutoff values were calculated for each salivary element. For sodium, higher values were considered disease-positive, whereas for potassium, cobalt, and iron, lower values were considered disease-positive according to the observed direction of group differences. A p-value <0.05 was considered statistically significant.

Results

Study Population and Clinical Periodontal Profile

A total of 90 participants were included in the final analysis and were equally distributed into three periodontal status groups: periodontally healthy controls (n = 30), gingivitis patients (n = 30), and periodontitis patients (n = 30). All included participants had complete demographic, clinical periodontal, and salivary elemental measurements. Participant allocation is summarized in Figure 1.

As shown in Table 1, age differed significantly among the three groups (Kruskal–Wallis H = 37.962, p < 0.001). The periodontitis group was older than both the healthy and gingivitis groups, with a median age of 53.50 years and a mean age of 46.90 ± 9.95 years. Sex distribution also differed significantly among groups (χ^2 = 15.512, p < 0.001), with males representing 70.0% of the periodontitis group compared with 26.7% in both the healthy and gingivitis groups. BOP differed significantly among groups (Kruskal–Wallis H = 60.128, p < 0.001). Mean BOP was 4.50 ± 1.94% in healthy controls, 53.71 ± 25.57% in gingivitis patients, and 63.73 ± 14.83% in periodontitis patients. PPD and CAL were evaluated only in the periodontitis group, with mean values of 5.43 ± 1.32 mm and 4.69 ± 1.63 mm, respectively.

Among the periodontitis group, Stage III was the disease stage most reported, presenting in 14 patients (46.7%), followed by Stage II in 12 patients (40.0%), Stage IV in 3 patients (10.0%), and Stage I in 1 patient (3.3%). Grade B was the most common grade occurring in 20 patients (66.7%), followed by Grade C in 9 patients (30.0%) and Grade A in 1 patient (3.3%). Generalized periodontitis was observed in 17 patients (56.7%), while localized disease was observed in 13 patients (43.3%).

Multi-Element Salivary Profile Across Periodontal Groups

The salivary multi-element profile differed significantly across periodontal status groups. As shown in Table 2 and Figure 2, sodium demonstrated a progressive increase from healthy controls to gingivitis and periodontitis, whereas potassium, cobalt, and iron were lower in disease groups compared with healthy controls.

Mean salivary sodium concentration increased from 16.64 ± 2.33 mmol/L in healthy controls to 20.50 ± 2.55 mmol/L in gingivitis patients and 22.39 ± 3.23 mmol/L in periodontitis patients. In contrast, potassium decreased from 12.36 ± 1.32 mmol/L in healthy controls to 10.65 ± 1.77 mmol/L in gingivitis and 10.38 ± 2.30 mmol/L in periodontitis. Cobalt decreased from 0.28 ± 0.09 µg/dL to 0.21 ± 0.09 µg/dL and 0.18 ± 0.09 µg/dL across the same groups, while iron decreased from 47.63 ± 11.03 µg/dL to 34.40 ± 8.89 µg/dL and 30.77 ± 11.35 µg/dL, respectively. One-way ANOVA showed statistically significant differences among the three groups for sodium (F = 34.500, p < 0.001), potassium (F = 10.191, p < 0.001), cobalt (F = 10.747, p < 0.001), and iron (F = 21.515, p < 0.001). Effect-size analysis demonstrated large group effects for sodium (η^2 = 0.442), potassium (η^2 = 0.190), cobalt (η^2 = 0.198), and iron (η^2 = 0.331).

Games–Howell post-hoc testing showed that sodium differed significantly across all pairwise comparisons, including healthy controls versus gingivitis (p < 0.001), healthy controls versus periodontitis (p < 0.001), and gingivitis versus periodontitis (p = 0.038). Potassium was significantly lower in both gingivitis and periodontitis compared with healthy controls (p < 0.001 for both comparisons) but did not differ significantly between gingivitis and periodontitis (p = 0.861). Similarly, cobalt and iron significantly differentiated healthy controls from disease groups, while no significant differences were observed between gingivitis and periodontitis (Table 3).

Associations Between Salivary Elements and Periodontal Parameters

Correlation analysis was performed to assess the relationship between salivary elements and clinical periodontal parameters. As shown in Table 4, BOP correlations were evaluated across all participants, whereas PPD and CAL correlations were restricted to the periodontitis group. Salivary sodium showed a significant positive correlation with BOP (r = 0.565, p < 0.001), as illustrated in Figure 3. In contrast, potassium (r = −0.316, p = 0.002), cobalt (r = −0.314, p = 0.003), and iron (r = −0.468, p < 0.001) showed significant negative correlations with BOP. The negative correlation between salivary iron and BOP is illustrated in Figure 4.

Within the periodontitis group, none of the salivary elements showed statistically significant correlations with PPD or CAL. Sodium showed weak non-significant positive correlations with PPD (r = 0.161, p = 0.395) and CAL (r = 0.181, p = 0.338), while potassium, cobalt, and iron showed weak non-significant negative correlations with both parameters.

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Electrolyte–Trace Element Interaction Pattern

Inter-element correlation analysis demonstrated a coordinated salivary elemental interaction pattern. As shown in Table 5 and Figure 5, sodium was inversely correlated with potassium ($r = -0.697$, $p < 0.001$), cobalt ($r = -0.633$, $p < 0.001$), and iron ($r = -0.687$, $p < 0.001$). In contrast, potassium, cobalt, and iron were positively correlated with each other. Potassium was positively correlated with cobalt ($r = 0.508$, $p < 0.001$) and iron ($r = 0.468$, $p < 0.001$), while cobalt was positively correlated with iron ($r = 0.545$, $p < 0.001$).

Diagnostic Performance of Salivary Elements

ROC curve analysis was performed to evaluate the diagnostic performance of salivary sodium, potassium, cobalt, and iron in differentiating periodontal status groups. Higher sodium values were considered disease-positive, whereas lower potassium, cobalt, and iron values were considered disease-positive according to the observed direction of group differences. ROC results are summarized in Table 6 and illustrated in Figure 6.

For differentiating healthy controls from gingivitis, sodium demonstrated the highest discriminatory performance (AUC = 0.870). At an optimal cutoff value of ≥18.7 mmol/L, sodium showed 76.7% sensitivity and 83.3% specificity. Iron also showed good diagnostic performance (AUC = 0.827), followed by potassium (AUC = 0.786) and cobalt (AUC = 0.700).

For differentiating healthy controls from periodontitis, sodium showed the strongest diagnostic performance, with an excellent AUC of 0.932. At a cutoff value of ≥19.6 mmol/L, sodium showed 83.3% sensitivity and 86.7% specificity. Iron showed good diagnostic accuracy (AUC = 0.860), while cobalt and potassium showed acceptable discriminatory performance, with AUC values of 0.796 and 0.760, respectively.

For differentiating gingivitis from periodontitis, sodium showed modest but statistically significant performance (AUC = 0.672, $p = 0.023$), with 43.3% sensitivity and 90.0% specificity at a cutoff value of ≥22.8 mmol/L. Potassium, cobalt, and iron showed weak-to-modest non-significant performance in this comparison.

Discussion

The present study evaluated salivary sodium, potassium, cobalt, and iron as a multi-element biomarker profile for non-invasive stratification of periodontal disease. The principal finding was that periodontal disease was associated with a coordinated salivary elemental remodeling pattern characterized by progressive sodium elevation and concurrent reductions in potassium, cobalt, and iron. Instead, this trend represented a concerted electrolyte–trace element transition across periodontal health, gingivitis, and periodontitis, and did not occur in isolated differences in individual elements. Sodium exhibited the most pronounced progressive change between the three periodontal groups and showed the best diagnostic performance, especially for periodontal health vs periodontitis. In contrast, potassium, cobalt, and iron predominantly separated periodontal health from disease, though they exhibited limited ability to separate gingivitis from periodontitis. More significantly, salivary elemental changes were more strongly related to bleeding on probing (BOP) than to probing pocket depth (PPD) or clinical attachment level (CAL), indicating that the observed multi-element profile could represent periodontal inflammatory burden more closely than accumulated structural destruction.

One of the key findings of this study is the progressive increase of salivary sodium in periodontal health, gingivitis, and periodontitis. Sodium is a major salivary electrolyte which is regulated partly by ductal transport and epithelial reabsorption mechanisms, and its concentration could be affected by salivary gland function, epithelial permeability, and local inflammatory fluid shifts (19, 20). Periodontal inflammation is defined by vascular dilation, increased gingival crevicular fluid flow, disruption of epithelial barrier, and infiltration of inflammatory cells, which can impact the ionic composition of whole saliva [12,18]. The present result of increasing levels of sodium from health to gingivitis and periodontitis further suggests that salivary sodium may be sensitive to the rising periodontal inflammatory activity.

This finding is in line with previous salivary ionic investigations of periodontal disease showing that changes to macro elements can coexist with periodontal disease, but published studies presenting findings were not uniform [12]. Pampani et al. also reported changes in salivary sodium in periodontal disease, suggesting sodium may be considered a relevant biochemical contributor to the assessment of periodontal condition [18]. Still, the current study extends previous work by assessing sodium within a broader multi-element framework rather than as just an isolated electrolyte. This matters because periodontal disease is a biologically complex disease and more than likely won't be represented by a single salivary marker. The strong performance of sodium in this study, especially its excellent diagnostic accuracy for distinguishing healthy controls from periodontitis, implies that sodium may represent a dominant component of the salivary elemental response to periodontal inflammation.

Compared to healthy controls, salivary potassium levels decreased in the gingivitis and periodontitis groups, as opposed to sodium. Potassium is actively secreted into saliva and depends on the transport modes of glandular epithelium [19,20]. Potassium findings by previous studies in periodontal disease were inconsistent with some suggesting increased levels and others showing no stable pattern across disease states [12,14,18,32,33]. The reduction observed in the present study may reflect population-specific characteristics, unstimulated saliva collection, analytical methods, dietary or hydration-related factors, or changes in salivary gland electrolyte handling during inflammatory states. These inconsistencies highlight why potassium may be less reliable as an isolated biomarker. Nevertheless, when interpreted as part of a multi-element profile, the reduction in potassium contributed to a broader electrolyte imbalance associated with periodontal disease.

The decrease in cobalt and iron levels in disease groups further supports the presence of a coordinated salivary trace-element shift. Cobalt has been rarely investigated as an independent salivary periodontal biomarker, and most available evidence comes from broader ionic or trace-element studies [12]. Its biological importance is mainly linked to vitamin B12-dependent pathways, metal homeostasis, and potential redox-related mechanisms [21]. Therefore, the lower salivary cobalt levels observed in gingivitis and periodontitis should be interpreted cautiously. At present, cobalt may be more appropriately viewed as one component of a broader salivary elemental profile rather than as an independent diagnostic marker.

Iron is more biologically relevant due to its role in oxidative stress, microbial growth, immune regulation, and inflammatory tissue injury. Iron homeostasis is highly interlinked with inflammatory pathways and oxidative stress-response pathways, and altered iron metabolism may modify periodontal tissue damage through reactive oxygen species generation, affecting host immune responses, and influencing microbial virulence mechanisms [22-25,34,35]. Nevertheless, the results of salivary iron in periodontal disease have been conflicting as salivary iron may depend on gingival bleeding, systemic iron status, microbial utilization, analytical techniques, and population factors [12,14,25]. In this study, salivary iron was lower in gingivitis and periodontitis relative to healthy controls with good diagnostic performance to differentiate healthy controls versus disease groups. This observation

indicates that in periodontal inflammation, iron depletion, redistribution, or changed availability can take place in saliva, although further studies with systemic iron markers are also needed to determine whether this is in fact the result of local oral changes, systemic iron regulation, or methodological effects.

The assessment of electrolyte–trace element interactions is one of the essential strengths of this study. Sodium was inversely correlated with potassium, cobalt, and iron, whereas potassium, cobalt, and iron were positively correlated with each other. Based on this interaction pattern, periodontal disease might reflect a coordinated elemental remodeling process, rather than random individual analyte changes. The inverse relationship of sodium to the other elements may be indicative of changes in salivary ionic and trace-element dynamics during inflammation. Possible mechanisms could be epithelial transport modulation, inflammatory exudation, oxidative stress, salivary gland response, microbial metabolism, and alterations in oral fluid composition. Such interpretation is also consistent with disease states that might appear via multi-element signatures instead of biomarker changes alone [6,7,12,36].

These correlation findings give a further perspective on the biological significance of the salivary elemental profile observed. BOP was found to be positively correlated with sodium and negatively correlated with potassium, cobalt, and iron. On the other hand, none of the measured elements showed significant correlations with PPD or CAL within the periodontitis group. This distinction is clinically significant because BOP captures ongoing gingival inflammatory activities, whereas PPD and CAL primarily reflect periodontal pocketing and accumulated attachment loss [4,5,7]. The greater association with BOP is indicative that the salivary elemental profile identified in this study probably has a more direct relation with active inflammatory burden than with irreversible tissue destruction. This suggests that salivary elemental biomarkers should be considered as auxiliary markers for inflammatory status as opposed to clinical markers for periodontal destruction.

In the context of biomarker biology, the lack of significant correlations with PPD and CAL should be interpreted. PPD and CAL serve as critical clinical parameters for diagnosing and staging periodontitis, but these clinical parameters themselves do not necessarily reflect real-time inflammatory activity. Salivary biomarkers, on the other hand, may capture dynamic changes in the oral inflammatory environment [6,7,36,37]. The same concern has also been reported in recent salivary diagnostic literature, where clinical periodontal readings remain indispensable but may be biologically incomplete for assessing current disease activity [7,11,15]. Accordingly, the present results favor an adjunctive protocol in which salivary elemental profiling is supplementary to clinical periodontal examination and adds biochemical information regarding inflammation.

The potential diagnosing significance of salivary elemental analysis was also upheld by the ROC analysis. Sodium demonstrated the best diagnostic performance, as it displayed good accuracy distinguishing healthy controls from gingivitis and excellent accuracy distinguishing healthy controls from periodontitis. Iron indicated similar diagnostic performance, as it distinguished healthy controls from disease groups, while potassium and cobalt had acceptable but weak performance. These results indicate that sodium may rank as the most informative feature in the studied profile, while iron may have additional diagnostic value. Nonetheless the poor classification of all components for distinguishing gingivitis from periodontitis suggests that the multi-element salivary profile is more suitable to differentiate periodontal health from disease rather than separating inflammatory disease states. This distinction is important to prevent overinterpretation of diagnostic utility.

The modest diagnostic success for gingivitis versus periodontitis is likely due to a few hypotheses. Gingivitis and periodontitis are both characterized by inflammatory components, mainly gingival bleeding, and both involve host inflammatory responses induced by biofilm [4,5,38]. While periodontitis is characterized by irreversible attachment loss and pocketing, the salivary elemental alterations are likely to represent a feature of the inflammatory disorder seen in both gingivitis and periodontitis, rather than structural destruction exclusive of periodontitis. This interpretation is corroborated by the robust correlations with BOP and the lack of significant correlations with PPD and CAL. Hence, salivary sodium, potassium, cobalt, and iron may be more accurately conceptualized as markers of periodontal inflammatory status rather than as meaningful markers of periodontitis severity.

Such findings are in line with the general direction of current salivary diagnostic research. Recent reviews highlight that saliva continues to hold the promise of non-invasive periodontal screening, monitoring, and precision-based care. However, no single salivary biomarker is expected to replace conventional periodontal examination [6,7,10,37]. Multi-marker panels and omics-based approaches are increasingly recommended because they may better capture the multifactorial nature of periodontal disease [12,15]. The present study contributes to this direction by showing that a four-element salivary profile can reveal a biologically coherent pattern involving electrolytes and trace elements. This may be particularly relevant in settings where non-invasive, low-cost, and repeatable screening tools are needed.

This study has numerous clinical and research implications. First, sodium may represent a strong signal of periodontal inflammatory burden in saliva, particularly in association with other elemental changes. Second, decreases in potassium, cobalt, and iron should not be excluded because their combined pattern may enhance the biological picture of salivary elemental remodeling. Third, inter-element relationships may represent more informative relations than concentration changes per component. Salivary elemental profiling, it should be concluded, rather than a standalone diagnostic substitute for periodontal probing, radiographic evaluation, and clinical classification, should become an adjunctive approach for inflammatory assessment and periodontal risk stratification.

There are several limitations of the present study. First, the cross-sectional design precludes causal inference, and it is not possible to establish whether salivary elemental changes precede, accompany, or are a direct result of periodontal inflammation. Longitudinal investigation is necessary to determine whether these markers change with the advance of disease or with periodontal treatment. Second, the sample size was of moderate value (30 participants per group), and large cohorts are needed to confirm cut-off values and enhance generalizability. Thirdly, ages and sex varied widely among study groups, and periodontitis was the most marked group, which might influence salivary composition. While these demographic differences were considered during the interpretation, further research should design study populations into demographically matched groups or modified multivariable models. Fourth, salivary analytes may be affected by hydration, diet, circadian variability, smoking, flow rate, oral hygiene behavior, systemic factors, and laboratory procedures [6,13,14]. While standardized saliva sampling and processing processes were employed, residual pre-analytic variability cannot be ruled out. Fifth, systemic serum concentrations of sodium, potassium, cobalt, and iron were not assessed, limiting the ability to differentiate between regional oral changes and systemic elemental status. Lastly, the single-center nature of recruitment can limit generalizability to various populations.

These findings should be validated in larger, multicenter, and demographically balanced cohorts in the future. Longitudinal studies are needed to determine whether salivary elemental profiles change after periodontal therapy and whether they can predict disease progression or treatment response. Future investigations should also consider the application of combinational multi-marker models including electrolytes, trace elements, inflammatory cytokines, oxidative stress markers, microbiome profiles, and clinical parameters. Integration of such models might enhance diagnostic accuracy and take salivary diagnostics further to clinical practice for the use in precision periodontology. Further, the salivary flow rate and serum elemental measurements and standardized pre-analytical protocols should also be implemented in future studies, which would lead to increases in reproducibility and clarify the biological source of salivary elemental changes.

Collectively, the current study shows that periodontal disease is accompanied by an intricate multi-element saliva profile with a higher sodium concentration and lower concentrations of potassium, cobalt, and iron. The pattern was significantly more associated with BOP compared to PPD or CAL, which supports the assumption that salivary elemental remodeling represents the periodontal inflammatory burden more than structural periodontal destruction. Sodium showed the strongest diagnostic performance, particularly for distinguishing periodontal health from periodontitis, while iron provided additional diagnostic value. These results support the feasibility of multi-element salivary biomarker profiling in the context of periodontal disease stratification as a non-invasive approach and represent a basis for confirming electrolyte–trace element interactions in the field of salivary periodontal diagnostics.

Conclusion

This study demonstrated that periodontal disease is associated with a coordinated multi-element salivary profile characterized by increased sodium and concurrent reductions in potassium, cobalt, and iron. Sodium showed the clearest progressive pattern across periodontal health, gingivitis, and periodontitis, and had the strongest diagnostic performance, particularly for distinguishing periodontal health from periodontitis. In contrast, potassium, cobalt, and iron mainly differentiated periodontal health from disease rather than clearly separating gingivitis from periodontitis.

The stronger association of salivary elements with bleeding on probing than with probing pocket depth or clinical attachment level suggests that this elemental profile may reflect periodontal inflammatory burden more closely than accumulated structural destruction. Inter-element correlation analysis further supports the presence of coordinated electrolyte–trace element remodeling in periodontal disease. Overall, multi-element salivary biomarker profiling may serve as a promising non-invasive adjunct to conventional periodontal assessment, although larger longitudinal and demographically balanced studies are required before clinical implementation.

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Author Contributions

Elaf Salam Sabeh contributed to participant recruitment, clinical periodontal examination, saliva sample collection, data collection, and preparation of the initial manuscript draft. Dr. Abbas Fadhil Al-Hashimi contributed to study conception and design, supervision of clinical and laboratory procedures, statistical interpretation, critical revision of the manuscript, and approval of the final manuscript.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

Ethical approval was obtained from the Institutional Review Board of the College of Medicine, Al-Nahrain University, Iraq (Approval No. 20251098; 28 October 2025).

Informed Consent

Written informed consent was obtained from all participants before enrollment in the study.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Table 1. Demographic and clinical periodontal characteristics of the study groups.

Variable	Healthy controls n = 30	Gingivitis n = 30	Periodontitis n = 30	Statistical test	p-value
Age, years, median (IQR)	26.00 (23.25–29.50)	30.00 (25.00–35.00)	53.50 (38.00–55.00)	Kruskal–Wallis H = 37.962	<0.001
Age, years, mean ± SD	28.20 ± 8.06	31.97 ± 9.55	46.90 ± 9.95	—	—
Male, n (%)	8 (26.7%)	8 (26.7%)	21 (70.0%)	χ^2 = 15.512	<0.001
Female, n (%)	22 (73.3%)	22 (73.3%)	9 (30.0%)	—	—
BOP, %, mean ± SD	4.50 ± 1.94	53.71 ± 25.57	63.73 ± 14.83	Kruskal–Wallis H = 60.128	<0.001
PPD, mm, mean ± SD	—	—	5.43 ± 1.32	Descriptive only	—
CAL, mm, mean ± SD	—	—	4.69 ± 1.63	Descriptive only	—

Note: Data are presented as mean ± SD, median (IQR), or n (%). BOP = bleeding on probing; PPD = probing pocket depth; CAL = clinical attachment level. PPD and CAL were assessed only in the periodontitis group.

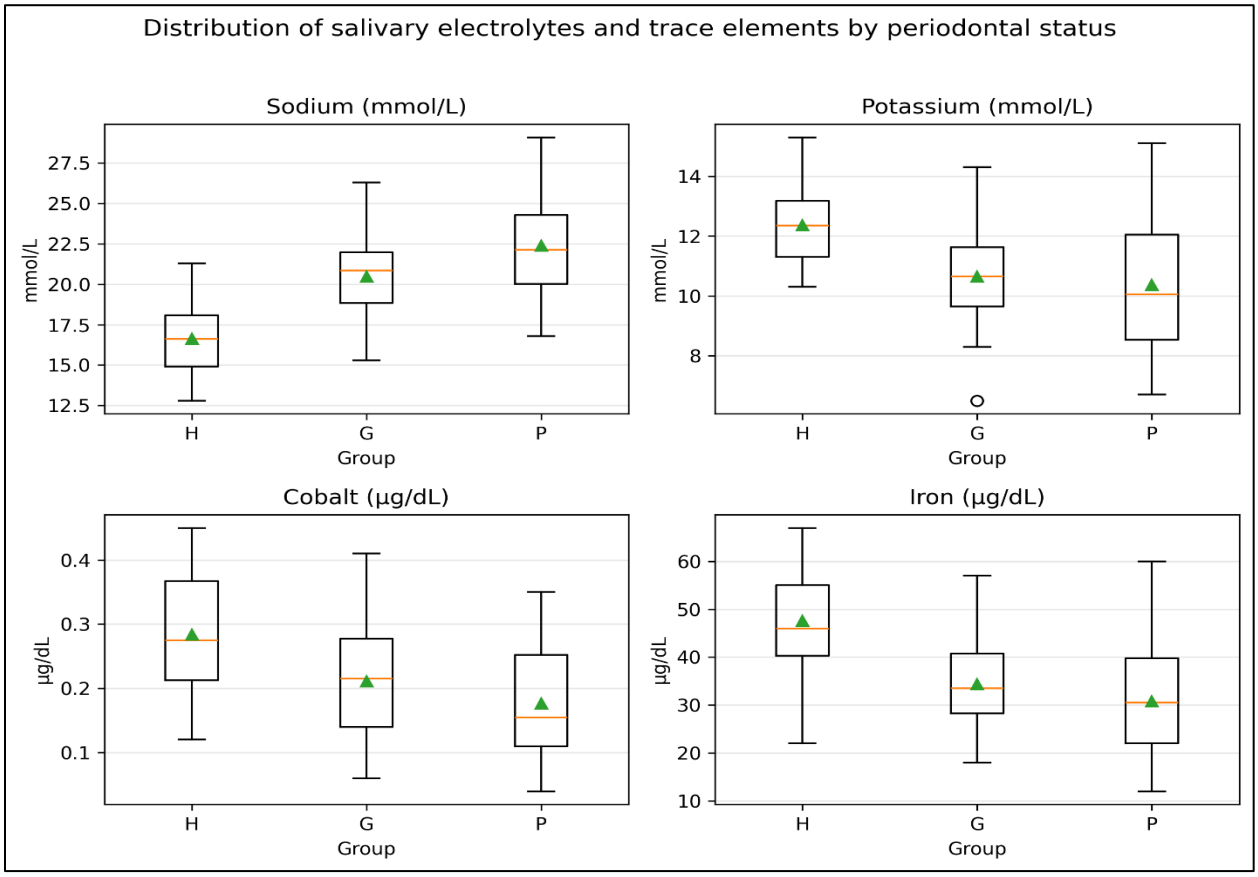

Figure 2. Distribution of salivary sodium, potassium, cobalt, and iron levels across periodontal status groups. Sodium showed a progressive increase from periodontally healthy controls to gingivitis and periodontitis, whereas potassium, cobalt, and iron were reduced in disease groups compared with healthy controls.

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Table 2. Salivary sodium, potassium, cobalt, and iron levels among the study groups.

Biomarker	Healthy controls n = 30 Mean ± SD	Gingivitis n = 30 Mean ± SD	Periodontitis n = 30 Mean ± SD	ANOVA F	p-value	η^2	Effect size
Sodium (mmol/L)	16.64 ± 2.33	20.5 ± 2.55	22.39 ± 3.23	34.5	<0.001	0.442	Large
Potassium (mmol/L)	12.36 ± 1.32	10.65 ± 1.77	10.38 ± 2.3	10.191	<0.001	0.19	Large
Cobalt (µg/dL)	0.28 ± 0.09	0.21 ± 0.09	0.18 ± 0.09	10.747	<0.001	0.198	Large
Iron (µg/dL)	47.63 ± 11.03	34.4 ± 8.89	30.77 ± 11.35	21.515	<0.001	0.331	Large

Note: Data are presented as mean ± SD. Comparisons were performed using one-way ANOVA. η^2 = eta squared.

Table 3. Pairwise post-hoc comparisons of salivary elements among study groups.

Biomarker	Healthy vs Gingivitis	Healthy vs Periodontitis	Gingivitis vs Periodontitis
Sodium (mmol/L)	<0.001	<0.001	0.038
Potassium (mmol/L)	<0.001	<0.001	0.861
Cobalt (µg/dL)	0.009	<0.001	0.311
Iron (µg/dL)	<0.001	<0.001	0.358

Note: Pairwise comparisons were performed using Games–Howell post-hoc testing. A p-value <0.05 was considered statistically significant.

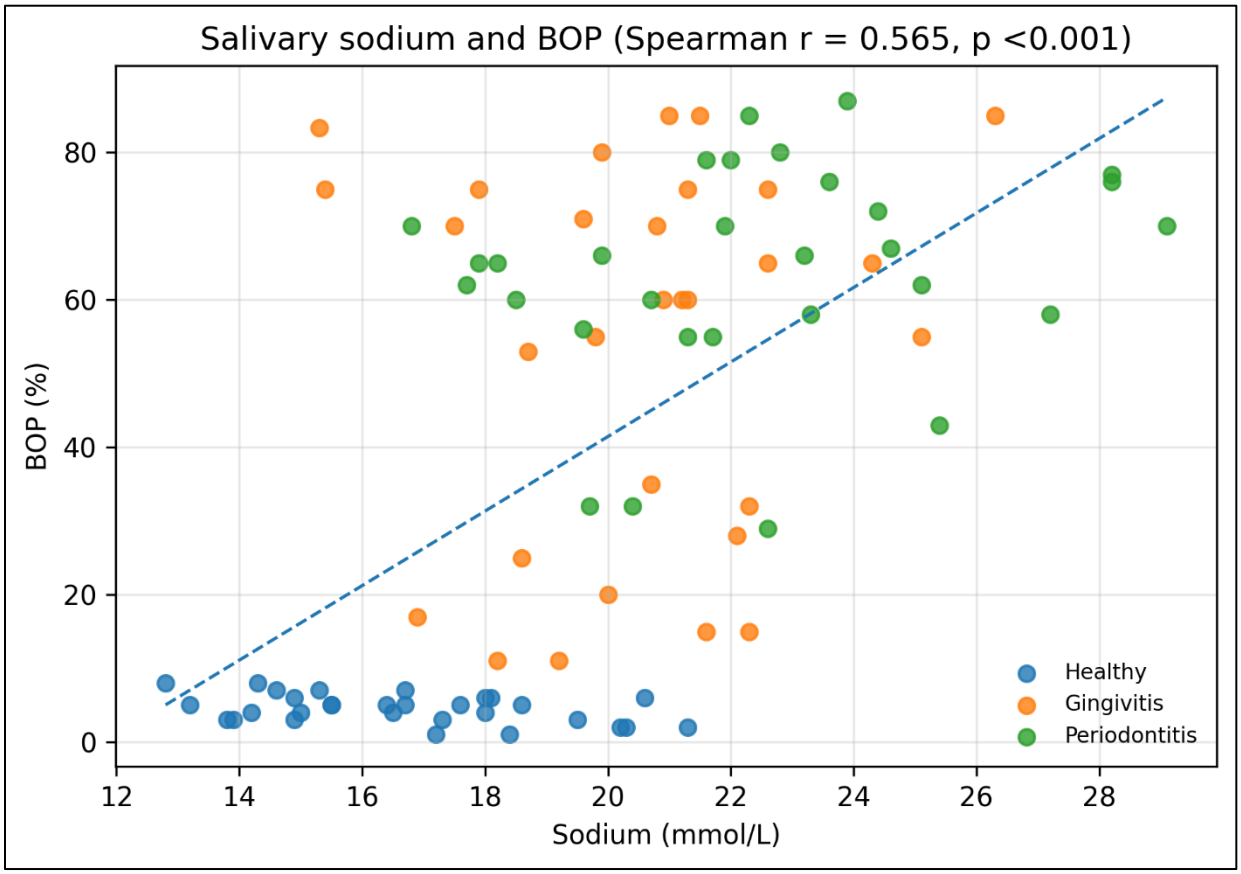

Figure 3. Correlation between salivary sodium level and bleeding on probing across the total study sample. Salivary sodium showed a significant positive correlation with bleeding on probing, indicating that higher sodium levels were associated with greater periodontal inflammatory burden.

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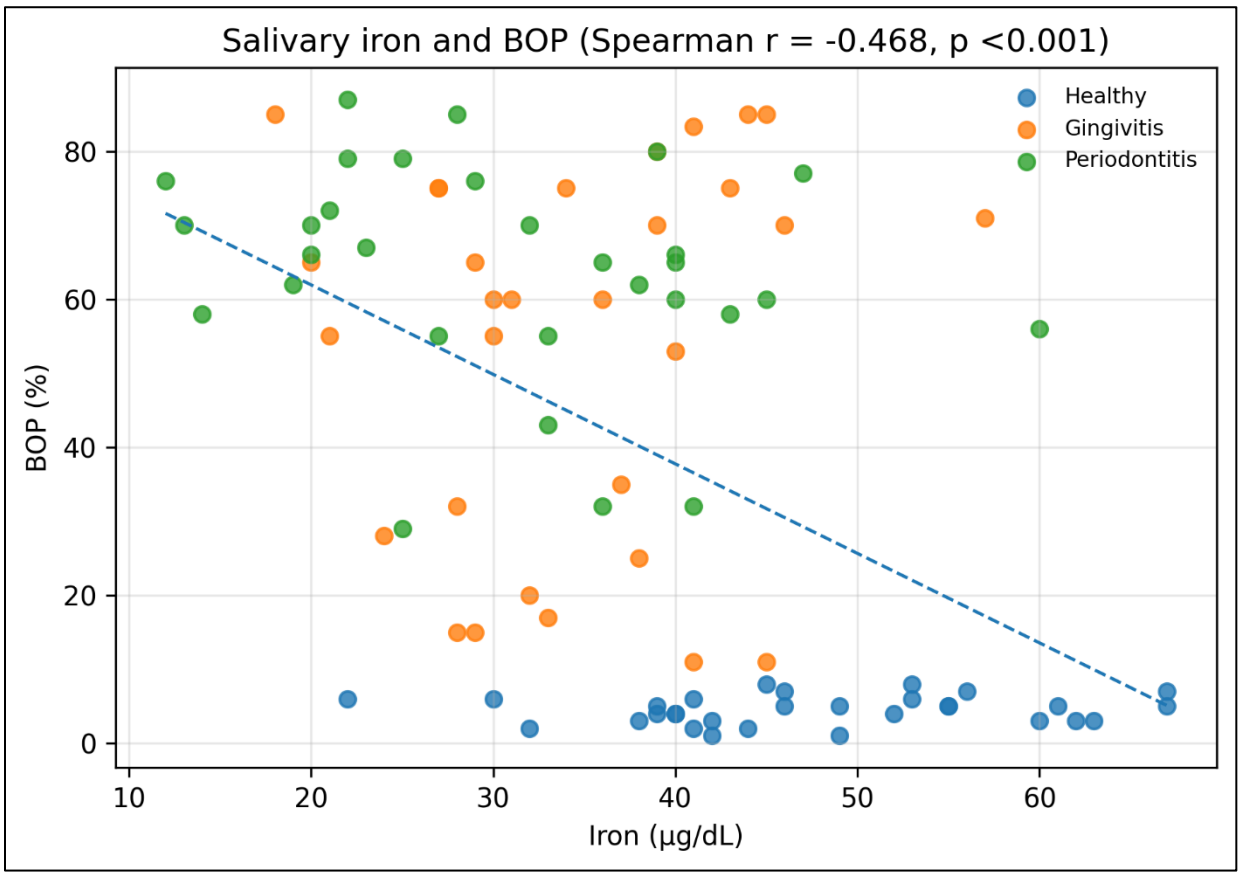

Figure 4. Correlation between salivary iron level and bleeding on probing across the total study sample. Salivary iron showed a significant negative correlation with bleeding on probing, indicating that lower iron levels were associated with greater periodontal inflammatory burden.

Table 4. Correlations between salivary elements and clinical periodontal parameters.

Biomarker	BOP r (p-value), all participants	PPD r (p-value), periodontitis group	CAL r (p-value), periodontitis group
Sodium (mmol/L)	0.565 (<0.001)	0.161 (0.395)	0.181 (0.338)
Potassium (mmol/L)	−0.316 (0.002)	−0.277 (0.138)	−0.153 (0.42)
Cobalt (µg/dL)	−0.314 (0.003)	−0.205 (0.277)	−0.249 (0.185)
Iron (µg/dL)	−0.468 (<0.001)	−0.190 (0.315)	−0.243 (0.196)

Note: Correlations were assessed using Spearman correlation analysis. BOP = bleeding on probing; PPD = probing pocket depth; CAL = clinical attachment level.

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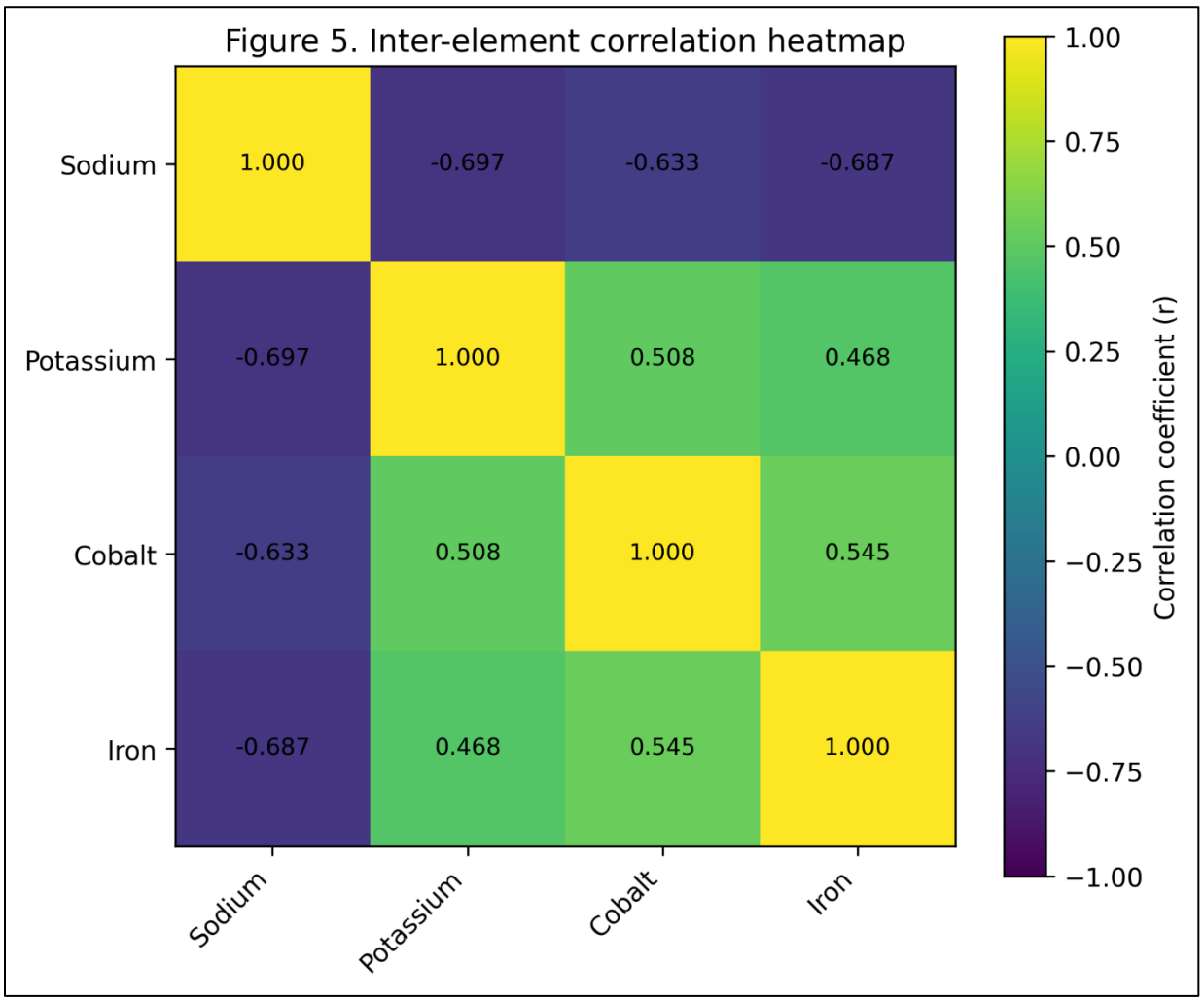

Figure 5. Inter-element correlation heatmap showing electrolyte–trace element interaction patterns among salivary sodium, potassium, cobalt, and iron. Sodium showed inverse correlations with potassium, cobalt, and iron, whereas potassium, cobalt, and iron showed positive correlations with each other.

Table 5. Inter-element correlations among salivary sodium, potassium, cobalt, and iron.

Biomarker pair	r	p-value
Sodium vs Potassium	−0.697	<0.001
Sodium vs Cobalt	−0.633	<0.001
Sodium vs Iron	−0.687	<0.001
Potassium vs Cobalt	0.508	<0.001
Potassium vs Iron	0.468	<0.001
Cobalt vs Iron	0.545	<0.001

Note: Inter-element correlations were assessed using Pearson correlation analysis.

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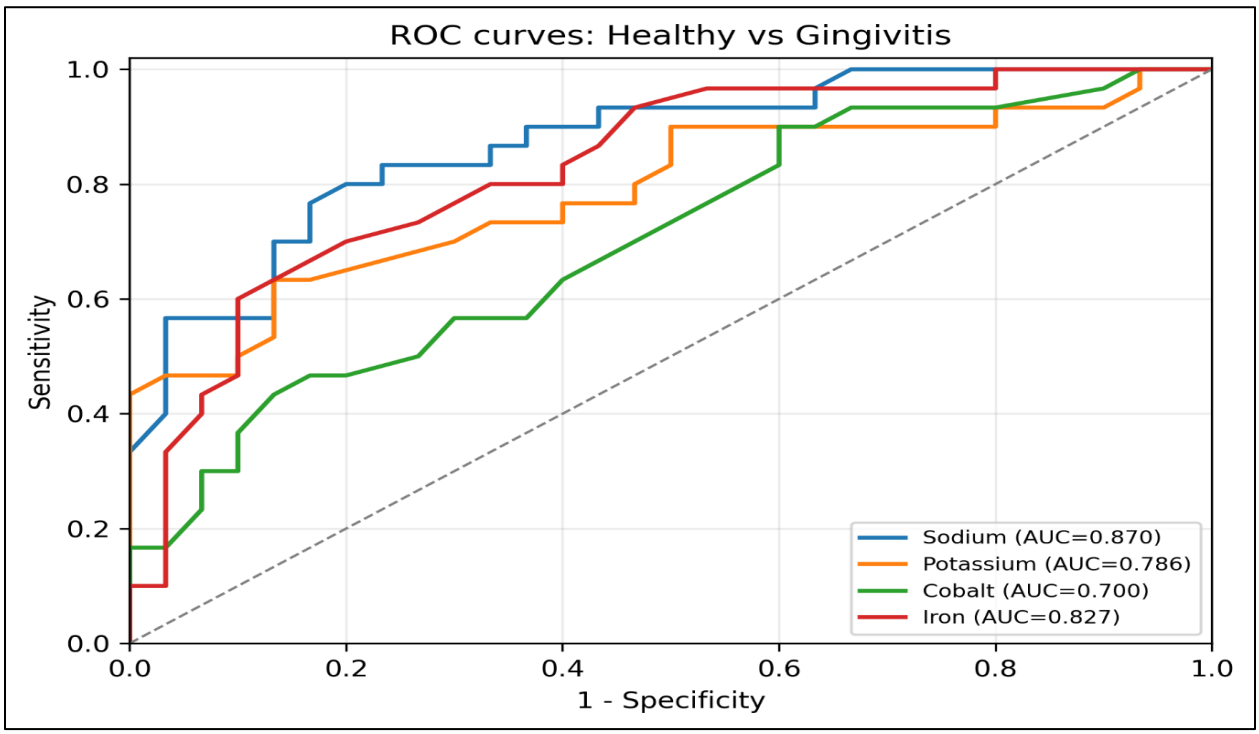

Figure 6A. Healthy controls vs Gingivitis.

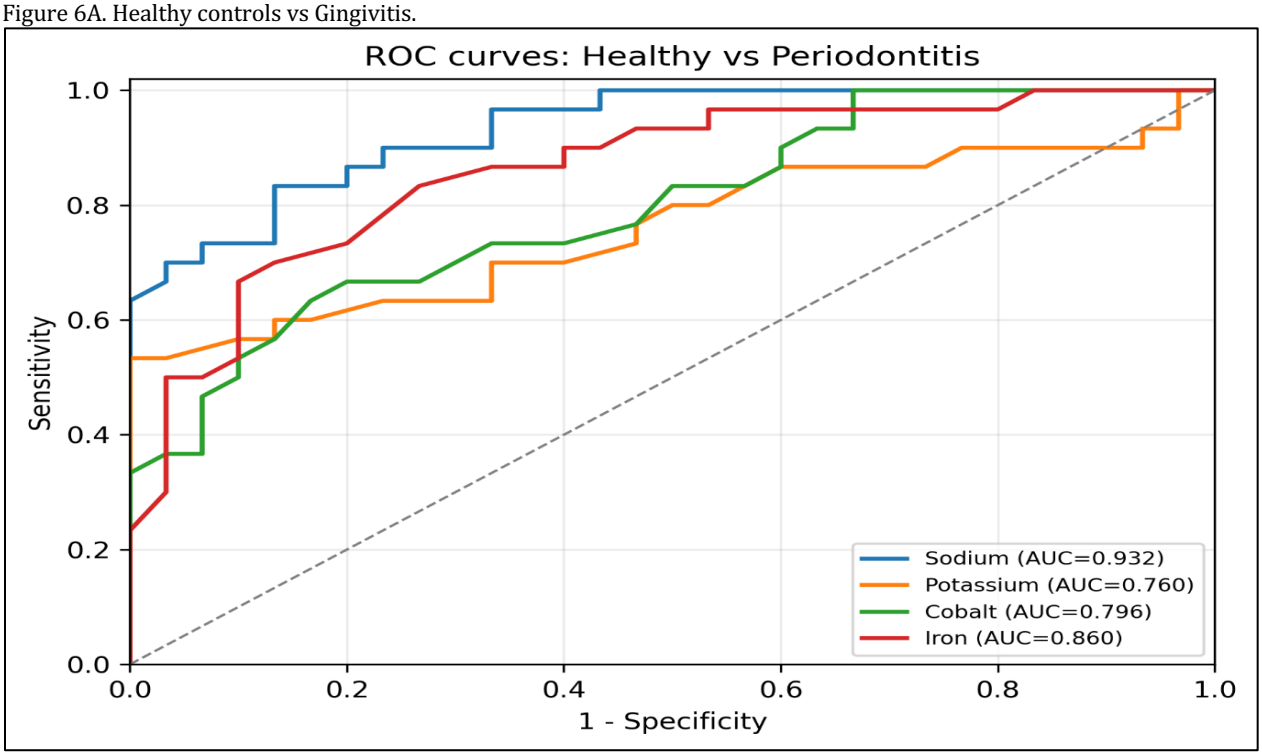

Figure 6B. Healthy controls vs Periodontitis.

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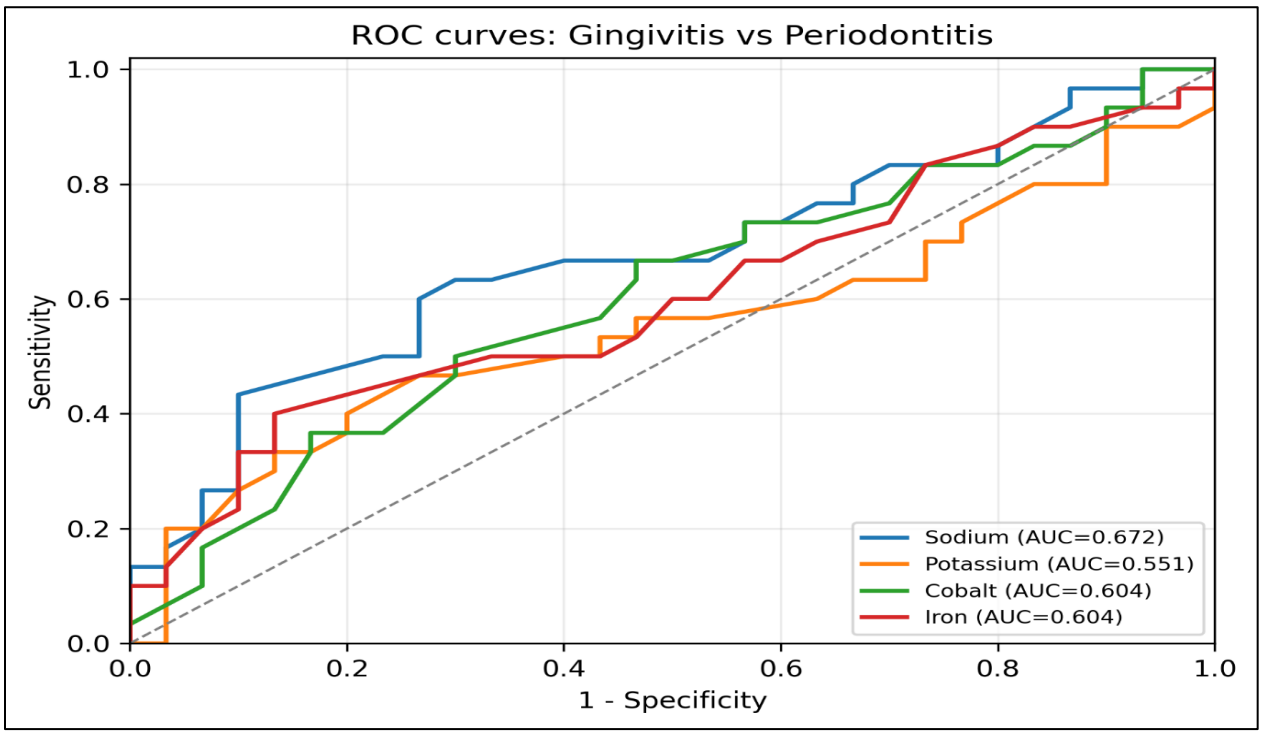

Figure 6C. Gingivitis vs Periodontitis.

Figure 6. Receiver operating characteristic (ROC) curves showing the diagnostic performance of salivary sodium, potassium, cobalt, and iron for differentiating periodontal status groups. (A) Healthy controls versus gingivitis. (B) Healthy controls versus periodontitis. (C) Gingivitis versus periodontitis.

Table 6. ROC diagnostic performance of salivary elements for differentiating periodontal status groups.

Comparison	Marker	AUC	Diagnostic level	p-value	Sensitivity (%)	Specificity (%)	Optimal cutoff rule
Healthy vs Gingivitis	Sodium (mmol/L)	0.87	Good	<0.001	76.7	83.3	≥18.7
Healthy vs Gingivitis	Potassium (mmol/L)	0.786	Acceptable	<0.001	63.3	86.7	≤10.8
Healthy vs Gingivitis	Cobalt (µg/dL)	0.7	Acceptable	0.008	43.3	86.7	≤0.17
Healthy vs Gingivitis	Iron (µg/dL)	0.827	Good	<0.001	60	90	≤37
Healthy vs Periodontitis	Sodium (mmol/L)	0.932	Excellent	<0.001	83.3	86.7	≥19.6
Healthy vs Periodontitis	Potassium (mmol/L)	0.76	Acceptable	<0.001	53.3	100	≤10.2
Healthy vs Periodontitis	Cobalt (µg/dL)	0.796	Acceptable	<0.001	63.3	83.3	≤0.18
Healthy vs Periodontitis	Iron (µg/dL)	0.86	Good	<0.001	66.7	90	≤36
Gingivitis vs Periodontitis	Sodium (mmol/L)	0.672	Modest	0.023	43.3	90	≥22.8
Gingivitis vs Periodontitis	Potassium (mmol/L)	0.551	Weak	0.501	40	80	≤9.3
Gingivitis vs Periodontitis	Cobalt (µg/dL)	0.604	Modest	0.169	50	70	≤0.15
Gingivitis vs Periodontitis	Iron (µg/dL)	0.604	Modest	0.169	40	86.7	≤25

Note: ROC = receiver operating characteristic; AUC = area under the curve. Higher sodium values and lower potassium, cobalt, and iron values were considered disease-positive directions according to observed group differences.