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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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Содержание:

PEPTIDYLARGININE DEIMINASE 4 AND FUSOBACTERIUM NUCLEATUM: A HIDDEN ALLIANCE IN PERIODONTAL DISEASE PROGRESSION

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Abstract.

Background: *Fusobacterium nucleatum (F. nuclatum)* has been recognized as a bridging pathogen within subgingival biofilms, in the same arrow, Peptidyl Arginine Deiminase 4 (PAD4), a host protein responsible for histone citrullination, confers to the neutrophil extracellular traps formation and subsequent tissue damage. Therefore, the current study aimed to explore the association of PAD4 activity and *F. nucleatum* prevalence in patients; with periodontal disease and evaluate their diagnostic potential role in disease progression.

Methods: Ninety participants aged from (25–55) years were categorized equally into 3 groups: (Group I) healthy controls, (Group II) patients with gingivitis, and (Group III) patients with severe periodontitis /stage III. Clinical periodontal parameters plaque index (PLI); gingival index (GI), bleeding on probing (BOP); probing pocket depth (PPD); clinical attachment loss (CAL) was recorded. Unstimulated saliva analyzed for PAD4 level using ELISA, and subgingival plaque was examined for *F. nucleatum* prevalence using qPCR. Data were analyzed using Kruskal–Wallis, Spearman's correlation, and ROC curves.

Results: Patients with severe periodontitis demonstrated markedly elevated salivary PAD4 levels compared with patients with gingivitis and controls ($P < 0.001$). There was no interconnection between PAD4 and clinical parameters. On another hand, *F. nucleatum* counts were significantly higher in diseased groups than controls ($P < 0.001$), demonstrating highly significant correlations with clinical periodontal parameters especially in severe periodontitis patients. ROC curve analysis revealed excellent diagnostic accuracy for PAD4 (AUC=0.98) and *F. nucleatum* (AUC=1.00) in discriminating between health and disease, confirming their value as reliable biomarkers for periodontal disease.

Conclusion: PAD4 overexpression together with the increased prevalence of *F. nucleatum* possibly crucial in the development and progression of periodontal diseases. They can act as reliable biomarkers for periodontal inflammation. Screening salivary PAD4 levels and *F. nucleatum* counts may facilitate early recognization of patients susceptible for periodontal disease, providing a basis for timely intervention and more precise disease monitoring.

Key words. Periodontitis, gingivitis, salivary biomarkers, peptidyl arginine deiminase 4, *F. nucleatum*, periodontal disease.

Introduction.

Periodontal diseases regarded as the most widespread chronic condition in adults, contributing substantially to worldwide burden of oral problems. Nearly half of the adult people are affected, and periodontitis remains a leading cause of tooth loss [1,2]. At its core, periodontitis reflects a destructive inflammatory response against microbial biofilms that colonize tooth surfaces, ultimately causing breakdown of periodontal support tissues [3,4]. The development of periodontitis is not the consequence of bacteria alone but rather the outcome of a dynamic interaction between "dysbiotic" microbial communities and the host's immune defenses [5]. Within these communities, *F. nucleatum* has drawn particular attention. This gram-negative anaerobe functions as a "bridging" species, linking early colonizers to late colonizers in the biofilm [6]. By doing so, it not only promotes structural maturation of the plaque but also amplifies the inflammatory burden in surrounding periodontal tissues [7,8]. A growing body of research has connected *F. nucleatum* with both the severity of periodontal destruction and extra-oral complications [9]. Parallel to the microbial challenge, the host immune system—especially neutrophils—plays a decisive role in disease progression. Neutrophils release web-like cofiguration known as "neutrophil extracellular traps (NETs)" which can immobilize and kill microbes. However, massive or dysregulated NET formation has been involved in collateral tissue injury within the periodontium [10]. A central driver of this process is the enzyme Peptidyl Arginine Deiminase 4 (PAD4), which citrullinates histones and facilitates chromatin decondensation during NETosis [11]. Elevated PAD4 activity has been associated with chronic inflammatory conditions and is increasingly suspected to contribute to periodontal tissue damage [12]. Linking *F. nucleatum* with PAD4-driven immune responses offers a novel perspective on the pathogenesis of periodontitis. High PAD4 expression in the periodontal environment may signal an exaggerated neutrophil reaction triggered by microbial dysbiosis, positioning it as a potential biomarker of disease activity [13]. Despite this theoretical framework, there is still limited evidence exploring the combined assessment of salivary PAD4 and *F. nucleatum* in patients with periodontal diseases. Thus, the study aimed to inspect this relationship that may provide new diagnostic insights for monitoring periodontal health and disease.

Subjects, Materials and Methods.

Study design: This an "observational case-control study" conducted in Baghdad between December 2024 and May 2025. Participants included in this study were individuals seeking care from the periodontal department. This study conforms with the Helsinki`s Declaration. Participants were given a detailed information regarding the study's goals; gave their informed consent by filling out a consent form approved by the dental college ethic`s committee under reference number of REC186 and study number MUOSU-202126.

To find out the appropriate number of participants for the study, salivary PAD4 was employed as a primary outcome measure. By Using G power (authored by Franz-Faull, University of Kiel,

Germany) with a power of 0.90 for the research and (a two-sided alpha error of probability of 0.05), an effect size of F is 0.4 "large effect size"]14,15[, under these condition the required sample size is 84 subjects; we expand the sample size to 90 subjects.

Therefore, the total sample size in this study will be 90 subjects their age range from 25–55 years divided into three distinct groups as follows: group I, the control group, comprising 30 volunteers with clinically healthy periodontium: had no (CAL), and (PPD) ≤3 mm, with bleeding on probing (BOP) less than 10%. Group II, consisting of 30 patients with gingivitis have an intact periodontium (no CAL), PPD ≤3 mm, BOP >10% [16[and group III severe periodontitis/stage III had detectable interdental (CAL) at ≥ 2 non-adjacent teeth, of ≥ 5mm on the buccal (facial) or lingual/palatal surfaces associated with pocketing ≥ 6 mm at ≥ two teeth]17[. In addition, all periodontitis cases were generalized and unstable, with no risk factors, e.g., diabetes mellitus (DM) and/or smoking]18,19[**.** All participants should demonstrate excellent general health, lack of any past systemic conditions, and have 20 teeth present.

Contrarily, the exclusion criteria: were the existence of any inflammatory oral illness (other than periodontitis) that may affect the levels of biomarker being studied, individuals suffering from systemic illnesses, a history of alcohol use or smoking, pregnant and nursing women, and who had administered any medications within the previous three months, or have an extensive periodontal therapy and using vitamins, antioxidants, or contraceptives were also excluded. The clinical parameters used to assess periodontal health of participants were: PLI, GI, BOP%, PPD, and CAL, measurements were documented using William periodontal probe.

Four surfaces for PLI, GI and BOP were examined]20,21 [and six sites for PPD and CAL values (mesio-lingual, mid-lingual, disto-lingual, disto-buccal, mid-buccal and mesio-buccal) were examined for each tooth, the third molars were excluded.

Saliva Sampling: Prior to saliva collection, participants were directed to abstain from consuming anything except the water, for minimum one hour. To avoid contamination, samples were obtained before any oral clinical procedures. A volume of 5 ml of, whole saliva (unstimulated) was gathered between (9:00 a.m. to 12:00 p.m.) using the passive drooling technique. During the procedure, subjects were advised not to swallow and to allow saliva to accumulate and drip over the lower lip into a sterile plastic container. The collected samples were then centrifuged for 10 minutes at 3000 rpm to separate the clear supernatant. To inhibit bacterial growth and reduce biomarker degradation caused by protein denaturation, the specimens were stored at –20 °C until ELISA Testing was conducted. Before analysis, all samples were thawed to room temperature]22,23[.

Dental Plaque Sampling: Subgingival plaque specimens were acquired from the "deepest" periodontal pockets within the patient groups, while in the control group, samples were collected from the gingival sulcus. To minimize contamination from saliva or supragingival plaque, each sampling site was scaled first supragingivally with a sterile curette, isolated with cotton rolls, and cleaned gently using sterile cotton pellets]24,25[. A fine, sterile Gracey curette was then placed into the pocket along the tooth surface without applying pressure. Upon encountering apical tissue resistance, a single vertical stroke was executed to collect the plaque sample. The material was immediately placed into an eppendorf tube containing 0.5 ml of TE buffer (10 mM Tris-HCl and 1 mM EDTA with pH ~7.6), and the instrument tip was vigorously agitated within the solution. Then, plaque samples were stored at –40°C till DNA extraction]24,26[.

ELISA Detection of (PAD4): The ELISA technique for estimation of PAD4 was a sandwich enzyme immunoassay; the kit applicable for in vitro quantitative measurement of PAD4 (My BioSource/USA; Catalog No. (MBS3802).

DNA Extraction: The DNA extracted from samples of dental plaque according to the manufacturer protocol of ABIO pure Extraction. A spectrophotometer used to check the DNA quantity in each sample and afterwards stored at -20°C until qPCR.

Quantitative PCR (qPCR) Screening: To detect and quantify *F. nucleatum* bacteria with high accuracy, species-specific primers directed against the 16S rRNA gene were selected according to recent studies [26,27]. The primer sequences were then evaluated using the NCBI Primer-BLAST tool to confirm their accuracy and to ensure they did not amplify non-target oral microorganisms (Table 1).

The standard curve method uses a series of dilution of known "template copy number" in the qPCR assay. Linear regression of log concentration (copy μl^{-1}) versus C^T gives the standard curve, and this is then used to calculate copy number (copy μl^{-1}) in the sample (Figures 1).

Statistical Analysis: Study data assessed using the computerized analysis (SPSS) software program (version 28, IBM, USA). Descriptive data were expressed as mean, standard deviation (SD), minimum, maximum, and standard error. Data normality tested by Shapiro-Wilk test which indicated a non-parametric distribution; thus, multi-group comparisons were conducted using the **Kruskal–Wallis test** and in case of significance, additional intra-group comparisons were performed using Dunn's test. ROC (receiver operating characteristic) and AUC (area under curve) evaluate the diagnostic accuracy of the studied markers. P-value of ≤ 0.05 was significant.

Results.

Demographic and clinical data of participants:

The differences in age range and gender among the study groups were statistically non-significant (P=0.3). Statistically, significantly higher differences were observed in the percentage of lost teeth, PLI, GI and BOP%, among the study groups (P <0.001). PPD and CAL were assessed only in Group III, as this group exclusively included patients with severe periodontitis (Table 2).

Salivary Level of PAD4 and Quantification of *F. nucleatum* Bacteria

The current results showed a highly significant difference (P <0.001) in the mean of PAD4 level among study groups, patients with severe periodontitis recorded the highest mean of the salivary PAD4 (46.2 ±7.6 µg/ml) followed patients with gingivitis and controls. The highest (P < 0.001) *F. nucleatum*

Table 1. *Primers Used for F. nucleatum Detection.*

Primers	**Sequence `5-3`**	**Annealing Temp. (°C)**
F. nucleatum -F	GGATTTATTGGGCGTAAAGC	50
F.nucleatum -R	GGCATTCCTACAAATATCTACGAA	
F.nucleatum -P	FAM-CTCTACACTTGTAGTTCCG	

Table 2. *The demographic data of the study population.*

Parameters	All groups	G I	G II	G III	p value
		N=30	**N= 30**	**N= 30**	
Age, years	46.1± 3.5	45.2±2.3	46.2±2.4	47.1±4.9	0.3 ^{NS}
Gender, Male: Female	54 (60%):36 (40%)	16 (53.3%):14 (46.7%)	17 (56.7%):13(43.3%)	21 (70%):9 (30%)	0.3 ^{NS}
PLI	-	0.2 ±0.09	1.5 0.7	2.5 ±0.2	<0.001^{HS}
GI	-	0.2 ±0.06	2.3 0.4	2.6 ±0.1	<0.001^{HS}
BOP, % of score 1	-	4.3%	51.4%	69.0%	<0.001^{HS}
PPD	-	NA	NA	9.0 ±0.7)	-
CAL	-	NA	NA	6.0 ±0.7)	-
Teeth loss, n(%)		12 (1.5%)	76 (9.1%)	143 (17.1%)	<0.001^{HS}
PLI: Plaque Index, GI: Gingival Index, BOP: Bleeding on Probing, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss, NA: Not Applicable, G: Group; N: Number; SD: Standard Deviation, %: Percentage, HS: Highly Significant, *Kruskal-Wallis Test,* Chi Square Test					

Table 3. *Levels of Salivary PAD4 and Subgingival F. nucleatum.*

Parameters		PAD4, µg/ml	*F. nucleatum*
Groups, mean±SD	I (n=30)	11.2 ±8.0a	460.8±292.7a
	II(n=30)	29.0 ±7.0b	1473.0±699.5b
	III(n=30)	46.2 ±7.6c	4364000 ±1438.4c
P value		<0.001	<0.001
PAD4: Peptidyl Arginine Deiminase4, N: Number, SD: Standard Deviation, *Kruskal-Wallis Test, different letters indicate highly significant differences using Dunn's test			

Table 4. *Correlation between PAD4 levels and clinical periodontal parameters.*

Parameters	**PAD4**					
	G I		**G II**		**G III**	
	r	**P value**	**r**	**P value**	**r**	**P value**
N. of lost teeth	-0.3	0.08	0.02	0.8	-0.2	0.2
PLI	0.06	0.7	-0.01	0.9	-0.09	0.6
GI	-0.07	0.6	0.1	0.3	0.1	0.5
BOP 1 %	0.08	0.6	-0.1	0.5	0.01	0.9
PPD	-	-	-	-	-0.05	0.7
CAL	-	-	-	-	-0.05	0.7
G: Group, PLI: plaque Index, GI: Gingival Index, BOP: Bleeding on Probing, %: Percentage, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss, NS: Non-Significant, *r: Sperman rank correlation.						

Table 5. *The Correlations of F. nucleatum Counts with Salivary and Clinical Parameters.*

Parameters	***F. nucleatum***					
	G I		**G II**		**G III**	
	r	P -value	r	P- value	r	P -value
N. of lost teeth	-0.4	0.01^{S}	0.3	0.06	0.07	0.7
PLI	0.8	<0.001^{HS}	0.7	<0.001 ^{HS}	0.6	<0.001 ^{HS}
GI	0.7	<0.001^{HS}	0.3	0.02 ^{S}	0.3	0.55
BOP 1 %	0.7	<0.001^{HS}	0.4	0.01 ^{S}	0.9	<0.001 ^{HS}
PPD	-	-	-	-	0.7	<0.001 ^{HS}
CAL	-	-	-	-	0.6	<0.001 ^{HS}
PAD4	-0.003	0.9	0.08	0.6	0.07	0.6
G: Group, N: Number, PLI: Plaque Index, GI: Gingival Index, BOP: Bleeding on Probing, %: Percentage, PPD: Probing pocket depth, CAL: Clinical attachment loss, S: Significant, HS: Highly Significant, *r: Spearman rank correlation						

Table 6. *Diagnostic performance of F. nucleatum and PAD4 in differentiating oral health, gingivitis, and severe periodontitis based on ROC curve analysis.*

Biomarkers	G	AUC	P-value	Optimal cutoff	Sensitivity	Specificity
F. nucleatum	G I – G II	0.9	<0.001 HS	755.500	0.80	0.80
PAD4		0.9	<0.001 HS	18.1	0.96	0.90
F. nucleatum	G I – G III	1.0	<0.001HS	1558000	1.0	1.0
PAD4		0.98	<0.001HS	30.2	0.96	0.97
G: group, AUC: area under the curve, HS: highly significant						

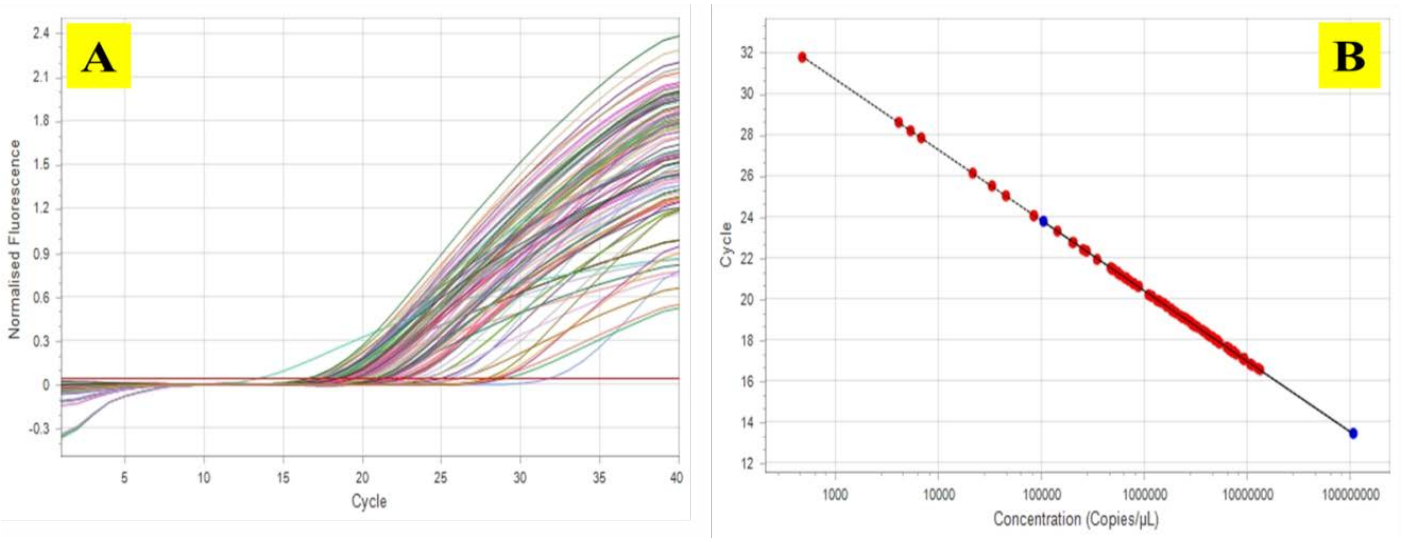

Figure 1. *PCR curves (A) Amplification plot of F. nucleatum genomic DNA serial dilutions (B) F. nucleatum standard curve.*

counts were observed in patients with severe periodontitis (Group III) than that of gingivitis (Group II) and controls (Group I) (Table 3).

Correlation between Salivary PAD4 Levels and Clinical Parameters

The findings of this study demonstrated a non-significant correlations regarding salivary PAD4 levels with periodontal parameters among the study groups (P>0.05) (Table 4).

Correlation of *F. nucleatum* Counts with Measured Parameters & Salivary PAD4

The present study used spearman's coefficient correlation to find the possible relationships among the studied parameters. In control group, *F. nucleatum* bacterial count revealed a negative significant correlation with the number of lost teeth (r=-0.4, P=0.01), and positive highly significant correlation with PLI (r=0.8, P<0.001), GI (r=0.7, P<0.001), and bleeding percentage (BOP 1%) (r=0.7, P<0.001). Furthermore, in patients with gingivitis, a positive highly significant associations were also found between bacteria and PLI (r = 0.7, P< 0.001), and another positive significant correlation regarding GI (r=0.3, P=0.02), and BOP 1% (r=0.4, P=0.01), while the correlation with the number of lost teeth was marginally non-significant (r=0.3, P=0.06). Finally, in patients with severe periodontitis, *F. nucleatum* count was strongly associated with PLI (r= 0.6, P<0.001), BOP 1% (r=0.9, P<0.001), PPD (r=0.7, P<0.001), and CAL (r=0.6, P<0.001), respectively (Table 5).

Table (6) provides crucial data on the diagnostic accuracy of *F. nucleatum* bacteria and salivary PAD4 in discriminating between healthy controls (Group I) and gingivitis group (Group II), the biomarkers demonstrated excellent diagnostic performance. *F. nucleatum* shown (AUC) of 0.90 (P< 0.001), with an optimal cutoff value of 755.5 yielding 80% sensitivity and 80% specificity. PAD4 achieved the highest accuracy in this comparison, with an AUC of 0.90 (P< 0.001), an optimal cutoff of 18.1, and a sensitivity of 96% with 90% specificity. Interestingly, in comparison of healthy controls (Group I) with severe periodontitis (Group III); again, *F. nucleatum* achieved perfect discrimination with an AUC of 1.00 (P < 0.001) at a cutoff of 1,558,000, resulting in 100% sensitivity and specificity. While PAD4 showed an excellent diagnostic behavior with an AUC of 0.98 (P < 0.001), and at a cutoff value of 30.2, sensitivity and specificity were 96% and 97%, respectively.

Discussion.

Periodontists should consider PAD4 as a critical nuclear enzyme involved in the host--microbe interaction that drives periodontal tissue breakdown. PAD4 activity is essential for the process of histone citrullination, which facilitates chromatin uncoiling and the release of extracellular nets (NETs) by neutrophil. Although NETs be a constituent of the innate immune defense system, excessive PAD4 activation has been linked to collateral tissue injury and amplification of the local inflammatory response within the periodontium [11,28].

At the same time, F. nucleatum continues to be among the most consistent bacterial species associated with periodontal disease progression. This microorganism acts as a bridge in subgingival biofilms and possesses virulence factors capable of disrupting epithelial barriers and stimulating pro-inflammatory cytokine release [7,8]. More recently, experimental studies have indicated

that F. nucleatum may directly promote PAD4-mediated NET formation, thereby strengthening the link between microbial challenge and host-derived destructive pathways [29,30].

The results of this study demonstrated no-meaningful differences among studied groups regarding to age and gender, suggesting well-matched characteristics among the study groups. The present study revealed that teeth loss was minimal among healthy controls (1.5%), whereas patients with periodontitis exhibited a higher proportion (9.1%), with the greatest percentage observed in the severe periodontitis group (17.1%). The intergroup differences were highly significant ($P<0.001$). These outcomes highlight that advancing periodontal disease is closely linked to an increased number of missing teeth, underscoring the destructive effect of chronic periodontal inflammation on the dentition and its supporting structures. Comparable evidence from recent investigations has confirmed that the degree of periodontal breakdown is positively associated with teeth loss burden [31-34].

Regarding the clinical periodontal parameters, PLI, GI, and BOP% showed statistically highly significant variations among the examined groups. This outcome is consistent with the well-established concept that dental plaque and gingival inflammation represent the primary etiological factors in the initiation and advancement of periodontal disease [35,36]. The stepwise elevation in plaque scores was paralleled by increased GI values, further supporting the close association between biofilm accumulation and gingival tissue inflammation. Similarly, BOP levels were markedly higher in diseased groups, confirming its role as a dependable indicator of current inflammatory activity and its predictive potential for future periodontal breakdown [37,38].

Chronic inflammation in periodontal tissues stimulates a cascade of host mediators, in which F. nucleatum has been recognized as a central microbial player. This organism possesses the ability to adhere, invade, and modulate immune responses, thereby intensifying periodontal tissue destruction. At the same time, PAD4 serves a crucial function in neutrophil extracellular trap (NET) formation through histone citrullination, a process that, while bactericidal, also causes collateral tissue injury. The present study demonstrated that patients with periodontitis exhibited significantly higher PAD4 expression and F. nucleatum counts compared with controls, suggesting that their interaction may accelerate periodontal breakdown. These results consistent with earlier studies highlighting the contribution of PAD4-mediated NETosis in inflammatory tissue damage and the F. nucleatum role in enhancing neutrophil activation and cytokine release [11,30]. Similar conclusions have been reached by studies that linked PAD4 activity with IL-6 and TNF-α upregulation during chronic inflammation [28].

In accordance with our study findings; Akkaya et al confirmed that PAD4 play an essential role in periodontitis pathogenesis, as the found in their study higher GCF PAD4 was found in severe periodontitis/ stage III patients in contrast to healthy controls [39], these results also agreed with another previous clinical studies who reported that PAD4 levels were elevated in gingival tissue samples, serum, gingival fluid, in periodontitis patients compared to healthy controls [40-42]. Furthermore; Laugisch et al. proposed that citrullination and PAD activity are responsible primarily for periodontal inflammation and bone destruction [40].

Citrullination is involved in physiological processes, including skin keratinization and physiological activity in the periodontal epithelium [43]. These findings plus subclinical gingivitis condition may describe PAD4 detection in the healthy controls [39].

In the current investigation, F. nucleatum was detected at a much higher frequency and load in patients with periodontitis compared with healthy individuals ($P<0.001$). Several molecular studies have confirmed the usage of quantitative detection methods, instead of simple presence/absence, increases the accuracy of identifying the microbial contribution to disease severity [29,8]. Our findings agree with recent evidence showing that F. nucleatum colonization is significantly elevated in severe periodontitis lesions compared to controls, supporting its role as a keystone pathogen in periodontal pathogenesis [7,30]. In terms of correlation, PAD4 didn't show any significant association with measured clinical parameters, these were in accordance with findings of previous study [39], the lack of significant correlation may be explained by that PAD4 have a threshold dependent effect on NETs production; once a critical enzyme level reached, additional PAD4 may not proportionally intensify tissue damage. Alternatively, it may serve dual roles participating in both immuno-physiological and pathological inflammation which could clarify its elevation regardless evident association with clinical periodontal parameters.

The present study demonstrated that F. nucleatum counts exhibited consistent and significant associations with key clinical periodontal parameters among healthy, gingivitis, and severe periodontitis study groups. In the control group, bacterial counts correlated positively with PLI, GI, and BOP%, indicating that even in clinically healthy individuals, the presence of F. nucleatum is linked to early signs of plaque accumulation and gingival inflammation. Interestingly, a negative correlation with tooth loss was observed, likely reflecting the preserved dentition in healthy subjects.

In the gingivitis group, strong positive correlations persisted between F. nucleatum and PLI, alongside weaker but significant associations with GI and BOP%. These findings supported the concept that F. nucleatum acts as a bridging organism within biofilms, enhancing microbial co-aggregation and promoting gingival inflammation. This is consistent with reports by Dorison et al. (2024), who have emphasized the central role of F. nucleatum in interspecies biofilm maturation and inflammatory priming [44].

In severe periodontitis, F. nucleatum correlated not only with plaque-related indices (PLI and BOP%) but also with measures of tissue destruction, (PPD) and (CAL). The strong correlation with BOP% ($r\approx0.9$) underscores the bacterium's association with active inflammatory sites, while significant correlations with PPD and CAL highlight its contribution to periodontal breakdown. These observations align with recent evidence from [45], who reported that elevated F. nucleatum levels in subgingival plaque were strongly associated with deeper pockets and higher attachment loss in periodontitis patients. Similarly, a study by [46]. Dharmayanti et al (2025) linked F. nucleatum

burden with immune system dysregulation, reinforcing its pathogenic role in periodontal tissue destruction [46]. Overall, these findings suggested that the PAD4-- F. nucleatum axis represents an important pathogenic pathway in periodontal disease, where microbial challenge and host enzymatic activity work synergistically to intensify tissue destruction.

In terms of diagnostic accuracy between periodontitis patients and healthy controls, F. nucleatum demonstrated excellent discriminatory efficacy with an AUC of 1.00 ($P < 0.001$), achieving 100% sensitivity and 100% specificity. Similarly, PAD4 provide outstanding diagnostic ability, with an AUC of 0.98 ($P < 0.001$); at the optimal cutoff value (30.2), sensitivity reached 96% and specificity was 97%. These results highlight the strong potential of both F. nucleatum detection and PAD4 expression as biomarkers for identifying periodontal disease.

When comparing severe periodontitis patients with healthy individuals, the diagnostic strength remained highly significant. F. nucleatum continued to show perfect discrimination (AUC=1.00, $P<0.001$), while PAD4 maintained a robust predictive value (AUC=0.98, $P<0.001$), further confirming their role in distinguishing advanced periodontal destruction. These results agreed with earlier research that emphasized the pathogenic role of F. nucleatum in periodontal disease and the PAD4-mediated host responses in disease progression [7,28-30,39].

The findings of the present study supported this biological framework, where both PAD4 and F. nucleatum demonstrated strong diagnostic performance in distinguishing periodontitis cases from healthy individuals. These results emphasize that the interaction between bacterial colonization and PAD4 upregulation is not incidental but may represent a pathogenic axis central to periodontal disease. Further research should investigate whether monitoring PAD4 expression alongside F. nucleatum detection could function as a reliable biomarker panel for early diagnosis and risk stratification clinically.

Study limitation that needs to be acknowledged, as the saliva can be influenced by dilution, some enzyme degradation that may affect the local enzymatic activity within pockets, so the impact of PAD4 in periodontal inflammation would be more evident if this study used gingival biopsy samples (gingival connective tissue) rather than salivary samples, the discrimination among groups may be more apparent. In the present study, the case group consisted of patients with clinically confirmed severe periodontitis, while the control group included clinically healthy individuals without any signs of periodontal inflammation. This sharp clinical contrast may have contributed to the nearly perfect discrimination observed in the ROC analysis. We acknowledge that this design likely increased the apparent diagnostic performance and limited the generalizability of the findings to broader clinical settings that include mild or moderate cases. We also recognize that the cutoff points and diagnostic metrics were derived and tested using the same dataset, which may introduce overfitting. Unfortunately, due to sample size limitations, an independent validation cohort was not available at this stage.

Conclusion.

The interaction between *F. nucleatum* and PAD4 appears to have essential role in periodontal disease pathogenesis. A bacterium's ability to promote inflammatory cascades, together with PAD4-mediated NET formation, provides a biological explanation for the significant diagnostic accuracy observed in this study. These findings indicated that the combined assessment of microbial and host-derived markers may enhance early detection and risk stratification of periodontitis. Further investigations are warranted to clarify the mechanistic pathways linking PAD4 activity with *F. nucleatum* colonization and to evaluate their potential as integrated biomarkers in routine periodontal practice.

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