https://doi.org/10.48047/AFJBS.6.13.2024.5909-5925

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Comparative Cross-Sectional Study of Neopterin Levels in Patients with Chronic Periodontitis and Chronic Migraine

Dr. Vinu Ramasundaram S MDS[1],Dr. Deepa Ponnaiyan MDS[2*] , MFDS RCPSG(UK), Dr. C M Anitha MDS[3], Dr. Prakash P.S.G MDS[4], Dr. Akanksha Singh MDS[5]

1. Post graduate, SRM Dental college, Bharathi Salai, Ramapuram, Chennai
2. Professor, SRM Dental college, Bharathi Salai
3. Assistant professor, SRM Dental college, Bharathi Salai
4. Professor, SRM Dental college, Bharathi Salai
5. Post graduate, SRM Dental college, Bharathi Salai, Ramapuram, Chennai

Volume 6, Issue 13, Aug 2024

Received: 15 June 2024

Accepted: 25 July 2024

Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.13.2024.5909-5925

ABSTRACT:

Introduction Migraine, a chronic neurovascular condition influenced by chronic inflammation, shares cytokines and inflammatory mediators with periodontitis, which can exacerbate migraine intensity. Neopterin, a purine nucleotide influenced by inflammation and free radicals, can serve as a marker for both diseases. ***Aim:*** This study aimed to measure serum and salivary neopterin levels in chronic periodontitis and chronic migraine patients to establish an association. ***Materials And Methods:*** Sixty-eight subjects were randomly divided into two groups of 34 each: chronic periodontitis (CP) and chronic periodontitis with migraine (CP+CM). Clinical parameters and serum and salivary neopterin levels were measured and compared using an independent samples t-test and Mann-Whitney U test. Data analysis was done using SPSS, with a P-value <0.05 considered statistically significant. ***Results:*** The mean values of PPD, CAL, and PI were higher in group 2 (CP+CM) than in group 1 (CP), while BI was higher in group 1 (3.52) compared to group 2 (2.94) with all significant differences. Mean serum and salivary neopterin levels were significantly higher in group 2 (447.1 pg/ml; 445.4 pg/ml) than in group 1 (85.20 pg/ml; 94.5 pg/ml). Neopterin can be considered a biomarker for the interrelationship between chronic migraine and chronic periodontitis.

KEYWORDS: Biomarker, Headache, Inflammation, Migraine, MSQ, Neuroinflammation, Neurovascular, Saliva, Serum.

INTRODUCTION

Chronic periodontitis is an inflammatory response that is caused by periodontopathogenic microorganism primarily anaerobic gram negative bacteria that leads to dysregulation of adaptive and innate immune response, thus triggering release of inflammatory mediators that result in loss of supporting periodontal tissues in susceptible individuals. (Berezow AB, 2011) Although, periodontal disease is considered a localized disease the ensuing release of various inflammatory mediators, there is a microbial and inflammatory spill over into systemic circulation that results in conditions such as diabetes, cardiovascular disease, neuro degenerative diseases and neurological disorder such as migraine. (Fenol A, 2017)(Lavigne SE, 2020)

Migraine, a chronic neurovascular condition primarily associated with recurrent headaches that trigger autonomic symptoms and its thought to be genetically transmitted which affects women two to three times more frequently than males (Rejdak K, 2006). Research has shown prevalence of migraines ranges from 2.6% to 21.7% globally, with an average of about 12%.of people are afftected worldwide (Lipton RB, 2007)

Migraine is frequently seen with or without aura, with aura is characterized by the presence of visual, auditory, speech-related, or motor aura symptoms whilst, without aura (Motaghi M, 2013) is described by the International Headache Society as a unilateral, throbbing headache that lasts for four to seventy-two hours, that gets worse during normal physical activity, and can result in nausea, vomiting, photophobia. (Christiansen I, 1999)

The pathology of migraine entails multi-factorial theories, among these hypothesis include neurogenic inflammation, (Huang YK, 2021) cerebral blood vessel contractile dysfunctions, and neurovascular inflammatory events that are initiated in the cerebral cortex and disseminate systemically causing secretion of neuropeptides like leptin, adiponectin, resistin, pentraxin and neopterin (Abbasi M, 2019)

Neopterin, which is a byproduct of the catabolism of guanosine triphosphate (GTP), a purine nucleotide, a member of the pteridine family is known to be influenced by the presence of excessive inflammation or free radical formation being a nitric oxide (NO) derivative. (Scarel-Caminaga, 2017) Human macrophages and immune cells produce neopterin and it is a cellular immune system marker. (Ghisoni K, 2015)

Neopterin concentrations are found to be high in body fluids like serum, saliva and urine and other neurological disorders when the cellular immune system is active such as Alzheimer's, depression , cerebral vasculopathy and migraine. (Dantzer R, 2008) (Klaus F, 2021) It's also

been observed neopterin concentrations were higher in inflammatory conditions such as periodontitis (Vrecko K, 1997)

Neopterin concentrations can therefore be measured to gauge the level of immune system activation within the periodontium and neurologic inflammatory changes.(Altamura C, 2021)(Davis BE, 2017) The presence of neopterin has been remarkably elicited and correlated with the progression of periodontal diseases as well as its role in migraine.(Burstein R 2015) Hence, it can be hypothesized that neopterin maybe closely associated with the progression of both periodontitis and migraine. In the light of these facts, it needs to be determined whether there exists a correlation between migraine and periodontitis (Ameijeira P, 2019)(Leira Y, 2019) by assessing the biomarker neopterin levels and to investigate if this biomarker might reflect the extent of periodontal destruction.

*MATERIALS AND METHODS:*In this cross-sectional comparative study sixty eight subjects (35 males and 33 females) aged 30 to 70 years were selected randomly for the study from the outpatient department of SRM General Hospital, Ramapuram, divided equally into 34 per group . Group 1 chronic periodontitis (CP) and Group 2 chronic periodontitis with migraine (CP+CM). All procedures followed were in accordance with the ethical standards of the Institutional Ethical Review board, SRM Dental College and Hospital and all procedures were done in accordance with the Helsinki Declaration of 1975 that was revised in 2013. A written informed consent was obtained from those who agreed to participate, and ethical clearance was obtained (SRMU/M&HS/SRMDC/2022/PG/016). Patients were recruited for the study that was conducted in September 2022 to October 2023. Based on GPower version 3.1.9.2. sample size was set at sixty eight with α err prob 0.05 and power 0.90. Subjects with minimum 15 teeth or more exhibiting generalized chronic periodontitis patients (pocket depth $\geq$6mm and CAL of $\geq$ 5mm in more than 30% diseased sites,(According to the AAP classification 2017 stage III grade B in group I and II)(Caton J, 2018) and the selection of migraineurs are based on previously documented patients to be able to predict migraines from pre-monitory symptoms as defined by IHS (International Headache Society) (Arnold M, 2018) considered to have CM if they presented headache occurring on 15 or more days per month for more than 3 months were included in group II. Furthermore, we documented the kind of migraine (aura-or without aura), the duration of the migraine(in months), the intensity of pain using the visual analogue scale (VAS), and the total number of headache days each month were included in the study. Exclusion criteria were diabetes, blood dyscrasias, gross oral pathology,

smoking, alcoholism, anomalies of the immune system, medications that would affect periodontal status, and previous or current periodontal therapy.

Periodontal assessment:

All subjects were periodontally assed using a (UNC-15, Hu friedy, Germany) at six sites per tooth. Pocket depth >6mm and CAL of >5mm were defined as periodontitis. Bleeding on probing (BOP) was calculated as the percentage of positive sites per subject. Each subject underwent a full-mouth periodontal probing and charting, along with orthopantomagram (OPG) to assess periodontal bone loss. The subjects were categorized into two groups of 34 each based on clinical examination, Bleeding index (BI) (Ainamo and bay, 1975), plaque index (PI) (Sillness and Loe, 1964), probing pocket depth (PPD) (Greenstein G, 1997), clinical attachment loss (CAL)(Philstrom BL, 1992) and radiographic evidence of bone loss.

Assessing migraine specific quality of life (MS-QoL)

Migraine Specific Quality questionnaire version 2.1(MSQ) was recorded in the CM patients. The MSQ is a 14-item PRO instrument that measures the impact of migraine across three essential aspects of a patient's health related quality of life (HRQOL) over the past 4 weeks in standardize response categories to a six-point Likert-type scale with seven items in role function-restrictive (RR), four items in role function-preventive (RP), and three items in emotional function (EF) which was developed by (Martin BC, 2000) The raw score for each domain is the sum of the responses for the items in that domain. These raw scores are then transformed into a 0–100 scale, where a higher score indicates better quality of life.

Clinical samples:

Blood collection:

The venipuncture technique was used to withdraw 5ml of venous blood which was then transferred to a non EDTA tube and was inclined for 45 minutes till the blood coagulated naturally.(World Health Organization, 2010) The blood was centrifuged at 3000rpm for 10 minutes and micropipette was used to separate the serum which was then stored in 0.5ml Eppendorf tube at -80°C in a deep freezer until ELISA is performed.

Saliva collection:

To prevent any interference with the results, the patients were instructed to sit up straight and advised to refrain from eating and drinking at least three hours before the procedure. Unstimulated whole saliva in a quantity of 5 ml was collected using the passive drool method in a sterile uricup and transferred to a Tarsons conical end centrifuge tube, and centrifuged for 10 minutes at 2700 rpm.(Kaufman E, 2014) The supernatant was separated by centrifugation, subsequently separated into three 0.5 ml eppendorf tubes and stored at -80°C until the ELISA process was carried out.

Analysis:

The samples were assayed for neopterin levels by enzyme immunoassay (ELISA) kit as instructed by the manufacturer Abbkine Scientific Co., Ltd., (Wuhan, China). The detection range was 45 pg/mL-720 pg/mL. The minimum detectable dose (MDD) of Human Neopterin (NEOP) was typically less than 1.0 pg/mL. Preparation of the enzyme conjugate, neopterin antiserum, and washing buffer were carried out prior to the assay. After adding 40 μL sample diluent, 10 μL sample was added to appropriate wells and one hundred microliters of the enzyme conjugate and, subsequently, 50 μL neopterin antiserum (using a multi-pipette plus a pipette tip) were placed in each well. The plate was covered and incubated for 90 minutes at room temperature. After rinsing wash buffer the plate was inverted to remove remaining liquid. The substrate reaction was stopped by adding 100 μL TMB (tetramethylbenzidine) stop solution to each well and the colour changed was observed from blue to yellow. The levels of neopterin in the tested samples were estimated using the standard enzyme-linked immunosorbent assay reader at an optical density of 450 nm and standard curve were plotted

Statistical analysis :

The data was analysed using parametric and non-parametric test as the normality tests Kolmogorov-Smirnov and Shapiro-Wilks tests results revealed that clinical parameters followed Normal distribution and Neopterin values did not follow Normal distribution. To compare the mean clinical parameter and proportions between groups independent samples t-test was applied. Neopterin values between groups was compared using independent samples Mann Whitney U test. Data was analysed using SPSS (IBM SPSS Statistics for Windows,

Version 26.0, Armonk, NY: IBM Corp. Released 2019) and P-value <0.05 was considered to be statistically significant.

RESULTS

The gender distribution in study subjects showed that, 20(58.8%) were males and 14(41.2%) were females in group 1 whereas in group 2, 15(44.1%) were males and 19(55.9%) were females with mean age of (43.4 ± 11.20) in group 1 and (32.7±10.05) in group 2 respectively. The comparison of mean of PPD, CAL, PI and BI between group 1 and group 2 (p-value <0.05 statistically significant) is shown in Table/Fig 1.

Table 1: Comparison of clinical parameters between the Groups

Clinical Parameters (Sample t-test)	**Mean ± SD**		**Confidence interval (95%) t test (UL-LL)**		**P value**
	Group 1(CP)	**Group 2 (CP+CM)**	**Group 1**	**Group 2**	
PPD	6.11 ± 0.66	7.14 ± 0.66	6.64 (0.71-1.34)		<0.001**
CAL	4.43 ± 0.49	5.51 ± 071	6.48 (0.74 -1.41)		<0.001**
PI	2.47 ± 0.26	2.67 ± 0.46	2.13 (0.00 - 0.38)		0.035*
BI	3.52 ± 0.35	2.94 ± 0.31	8.05 (0.72 - 0.433)		<0.001**

§ Independent sample t test

PPD - Periodontal Probing Depth , CAL - Clinical Attachment Level , PI - Plaque Index BI- Bleeding Index , SD – Standard Deviation , LL- Lower Limit , UL- Upper Limit CP – Chronic Periodontitis , CM – Chronic Migraine. * Significant ** Highly Significant

FIG 1*:* Representation of correlation serum and salivary neopterin with periodontal pocket depth and clinical attachment level in group 1 and group 2

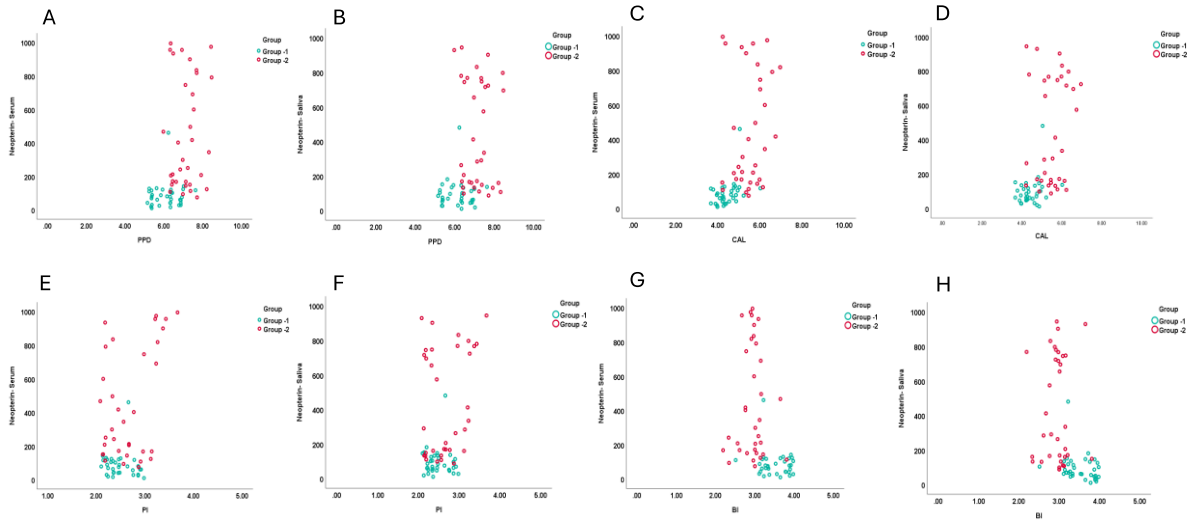

Fig A and B shows correlation of serum neopterin range with PPD and CAL in group 1 and group 2, Fig C and D shows correlation of salivary neopterin level with PPD and CAL in group 1 and group 2

Table 2 and Fig 1, shows neopterin values of serum and saliva between the groups. Comparing the mean levels, both serum and salivary neopterin was found to be higher in group 2 (447.1 pg/ml ; 445.4 pg/ml) than group 1 (85.20 pg/ml ; 94.5 pg/ml) which was highly significant.

Table 2: Comparison of serum and salivary Neopterin levels between periodontitis group and migraineurs group

Neopterin (pg/ml)	**Median**	**Mean± Std.Dev (pl)**		**P Value**
		GROUP 1	GROUP 2	
SERUM	76.8	85.2 ± 76.60	447.1 ± 325.70	<0.001*
SALIVA	72.2	94.5 ± 80.96	445.4 ± 308.39	<0.001*

§ *Mann Whitney U test*

While assessing the periodontal pocket depth (PPD), clinical attachment level (CAL), and bleeding index (BI), the mean values were found to be highly significant in group 2 than group 1 respectively(<0.001) While assessing the plaque index (PI), the mean was found to be

higher in group 1 (3.52) than group 2 (2.94) with the p-value of (<0.035) was found to be significant between group 1 and group 2. Table 3 signifies the correlation which indicates among neopterin serum and saliva group, CAL and BI shows strong correlation whereas PPD shows moderate correlation and PI shows weak correlation.

Table 3: Correlations between Clinical parameters and Neopterin values amoung group1 and group 2

		Neopterin-Serum	Neopterin-Saliva
PPD	Correlation	0.494	0.393
	P-value	<0.001*	<0.001*
	N	68	68
	R	0.963	0.960
CAL	Correlation	0.642	0.564
	P-value	<0.001*	<0.001*
	N	68	68
	R	0.987	0.981
PI	Correlation	0.104	0.170
	P-value	0.397	0.165
	N	68	68
	R	0.958	0.956
BI	Correlation	-0.595	-0.615
	P-value	<0.001*	<0.001*
	N	68	68
	R	0.944	0.950

§ Spearman's Nonparametric

PPD - Periodontal Probing Depth , CAL - Clinical Attachment Level , PI - Plaque Index BI-Bleeding Index , . * Significant ** Highly Significant.

Table 4: Descriptive MSQ values, domain scores and overall scores in migraineurs with periodontitis (Group II)

Table 4 shows descriptive statistics of migraine specific quality of life questionnaire (MSQ), domain 1,2,3 and the overall score in the migraineurs with periodontitis. The overall MSQ was (55.17) among the study participants and it was observed that the domain mean scores were higher for domain 1 role function-restrictive (2.08) followed by domain 2 role function-preventive (RP) (0.66) and least in domain 3 emotional function (EF) (0.42). The assessment of overall score was found to have a mean value of 7.52.

Variables	**Domain 1**	**Domain 2**	**Domain 3**	**Overall Score**
Mean	2.0841	0.6691	0.4265	3.17
Std. Deviation	.57724	.16118	.14853	0.72
Range	1.84	.58	.55	2.50

DISCUSSION

In the present study, neopterin production by activated macrophages has been demonstrated with clinical significance. (Rubino E, 2017) There is limited literature (Ozmeric N, 2002) about neopterin activity in saliva and serum. However, there are certain biomarkers as leptin, adiponectin, resistin, galactin-3, pentraxin-3, TNF- α and interleukin-1 which have been found in migraine in few studies (Ameijira P, 2019) (Leira Y, 2020) and therefore there could be a possible association which needs further research.(Leira Y, 2017) In the present study, serum and salivary neopterin in a group of chronic periodontitis patients with and without migraine was correlated with corresponding clinical parameters. Serum and salivary neopterin levels were elevated in chronic periodontitis patients with migraine and it was comparatively lesser in chronic periodontitis patient without migraine. Similarly, (Zhao ZN, 2002) determined that the mean total amount of neopterin in salivary concentration was higher in periodontitis patients compared to health. The reason for elevated neopterin levels in periodontitis could be higher cell mediated macrophage infiltration and activation which release neopterin and are known to be characteristic of chronic inflammation such as periodontitis, provoked by pathogenic bacteria. (Yan J, 2023)(Heneberk O, 2023) This study is the first to compare serum and salivary levels to establish a correlation between a systemic neurological condition migraine and periodontitis. It was observed that there were equally high levels of neopterin in both serum and saliva suggesting that periodontitis could have an impact on chronification and worsening of migraine by inflammatory changes. A study conducted by (Heneberk O, 2022)

observed that the saliva and serum levels of neopterin was much higher in periodontally compromised patients and periodontal therapy which was associated with decrease in neopterin levels in serum and saliva. It was established that the increase in the Neopterin concentrations before treatment as compared to after periodontal therapy supports the role of macrophages in the resolution of periodontal inflammation and the maintenance of periodontal health. (Rahimi M, 2018)(Varma SV, 2022)

According to the potential etiological origins of periodontal disease, this immune-inflammatory disease can coexist or be predisposed by an infection with one or more virulent bacteria, an impairment in the host immune system, or a combination of both. (Mahendra L, 2014)(Bisetti L, 2022)(Ponnaiyan D, 2024) Since, macrophage infiltration and activation are recognised indicators of chronic inflammatory processes caused by pathogenic bacteria, such as periodontitis.(Pradeep AR, 2007) Human macrophages produce neopterin, a hallmark of cellular immunological activation, after being stimulated by IFN-y released by T cells, which are also crucial in periodontal disorders and hence neopterin is regarded as a reliable marker of cell-mediated immune status.(Leira Y, 2019) The synthesis of nitric oxide (NO) by neopterin is known to include nitric oxide synthase (NOS)(Ramroodi, 2017)

Saliva naturally has antibacterial abilities because of the presence of NO, which is mostly released by salivary glands and is a potent antimicrobial agent produced in response to periodontopathogens and local inflammatory changes (Prasanna JS, 2017)

Furthermore, in a study conducted by (Hedef A, 2023) it was shown that the that serum neopterin levels were found to be higher in the attack period compared with the interictal period. The high neopterin levels detected during the attack period, especially when migraine headaches are present, suggest that neopterin and other possible intermediates in the BH4 pathway may play a role in the formation of headaches by stimulating neuropathic and inflammatory pain, thereby inhibiting this pathway reduces migraine pain as reported by (Latremoliere A, 2015). The fact that saliva and serum neopterin were found to be higher in our study with chronic periodontitis and migraineurs as compared to non-migraineurs is thus consistent with the current literature.

It was indicated that chronic periodontitis (CP) in patients with CM was associated with significantly higher serum leptin concentrations. In view of the above literature evidence, it can be suggested that elevated levels of neopterin in chronic periodontitis and migraine as

compared to without migraine might help understand the relationship between the two conditions. (Yago Leira, 2019) studies the relationship between chronic periodontitis and migraine using a serum biomarkers (CGRP, IL-6,IL-10) and observed elevated calcitonin gene related peptide (CGRP, IL-6) levels in periodontitis patients with migraine suggesting periodontitis is involved in the pathophysiology of migraine. Periodontal inflammation that occurs within the gingiva releases a great number of pro - inflammatory mediators that could play a pivotal role in the development of migraine attacks, which may result in chronic migraine (CM)(Jassar H, 2019)(Peskersoy C, 2016) showed that mean serum leptin levels were significantly and positively associated with greater mean PPD and mean CAL. Therefore, it was suggested that CP via the biomarker may be involved in the process of migraine chronification. The present study has established the co-relation of chronic periodontitis and migraineurs and their consequent neopterin values which were believed to be higher in patients with both this condition than the ones without it.

With respect to our study, a Migraine Specific Quality questionnaire (MSQ) was recorded in the CM patients.(Asawavichienjinda T, 2017)(Tassorelli C, 2018) The MSQ values for the first domain that is role function-restrictive was the highest as compared to the other domains in the survey conducted suggesting that migraine significantly affects their ability to perform daily activities

population-based studies are needed to further investigate neopterin involvement in migraine with a cross sectional study design. A comparison between neurogenic markers could have shown a comparable data for a better understanding of the presence of this biomarkers in such conditions.

This study concluded that neopterin can be considered as a biomarker to assess the interrelationship between chronic migraine and chronic periodontitis stating that there is a positive correlation while measuring the serum and salivary levels of the patients. In the light of these facts, further long term studies might be required to study the relationship in detail

DECLARATION:

1. CONFLICT OF INTEREST : All authors do not have any conflicts of interest to declare
2. FUNDING : This study was self-funded

3. ETHICAL APPROVAL : This study has been approved by the Institutional Review Board and Institutional Ethical Committee. **IRB APPROVAL NUMBER:** SRMU/M&HS/SRMDC/2022/PG/016
4. INFORMED CONSENT : Written informed consent was taken from all patients before the commencement of the study

AUTHORS CONTRIBUTION :

VR - conducted the entire study from Sample collection till processing. Manuscript writing and statistical analysis and interpretation.

DP- The conceptualisation and visualisation of the study as well as the Data curation along with supervision and guidance for the study.

CMA- Supervision and guidance along with curation of various data.

PSG- Supervision and guidance along with validation and approval.

AS- Proof Reading and assistance in manuscript writing and recruiting of patients for the study.

REFERENCES:

1. Abbasi M, Noori-Zadeh A, Seidkhani-Nahal A, Kaffashian M, Bakhtiyari S, Panahi S. Leptin, adiponectin, and resistin blood adipokine levels in migraineurs: Systematic reviews and meta-analyses. Cephalalgia. 2019 Jul;39(8):1010-21.
2. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J. 1975;25(4):229–35.
3. Altamura C, Corbelli I, De Tommaso M, Di Lorenzo C, Di Lorenzo G, Di Renzo A, Filippi M, Jannini TB, Messina R, Parisi P, Parisi V. Pathophysiological bases of comorbidity in migraine. Front Hum Neurosci. 2021 Apr 20;15:640574.
4. Ameijeira P, Leira Y, Dominguez C, Leira R, Blanco J. Association between periodontitis and chronic migraine: a case–control study. Odontology. 2019 Jan 25;107:90-5.

5. Arnold M. Headache classification committee of the international headache society (IHS) the international classification of headache disorders. Cephalalgia. 2018;38(1):1-211.
6. Asawavichienjinda T, Vorasayan P, Noiwattanakul J, Phanthumchinda K. Validity, reliability, and sensitivity to change of the Thai version of the Migraine-Specific Quality of Life Questionnaire version 2.1. Asian Biomedicine. 2017 Aug 1;11(4):331-42.
7. Berezow AB, Darveau RP. Microbial shift and periodontitis. Periodontology 2000. 2011 Feb;55(1):36.
8. Biscetti L, De Vanna G, Cresta E, Bellotti A, Corbelli I, Letizia Cupini M, Calabresi P, Sarchielli P. Immunological findings in patients with migraine and other primary headaches: a narrative review. Clinical and Experimental Immunology. 2022 Jan 1;207(1):11-26.
9. Burstein R, Noseda R, Borsook D. Migraine: multiple processes, complex pathophysiology. Journal of Neuroscience. 2015 Apr 29;35(17):6619-29.
10. Caton J, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. *J Periodontol*. 2018; 89(Suppl 1): S1–S8.
11. Christiansen I, Thomsen LL, Daugaard D, Ulrich V, Olesen J. Glyceryl trinitrate induces attacks of migraine without aura in sufferers of migraine with aura. Cephalalgia. 1999 Sep;19(7):660-7.
12. Dantzer R, O'connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nature reviews neuroscience. 2008 Jan;9(1):46-56.
13. Davis BE. Assessing the relationship between periodontal disease and subsequent inflammatory responses and headache disorders. 2017. (Master's thesis). Available from: https://scholarcommons.sc.edu/etd/4382.
14. Fenol A, Swetha VR, Krishnan S, Perayil J, Vyloppillil R, Bhaskar A, Shereef M, Balakrishnan B, Puzhankara L. Correlation of salivary neopterin and plasma fibrinogen levels in patients with chronic periodontitis and/or type 2 diabetes mellitus. Pteridines. 2017 Dec 20;28(3-4):177-83.
15. Ghisoni K, de Paula Martins R, Barbeito L, Latini A. Neopterin as a potential cytoprotective brain molecule. Journal of psychiatric research. 2015 Dec 1;71:134-9.

16. Greenstein G. Contemporary interpretation of probing depth assessments: diagnostic and therapeutic implications. A literature review. Journal of periodontology. 1997 Dec;68(12):1194-205.
17. Hedef A, Baylan FA, Dülgeroğlu Y, Berktaş DT, Tolun Fİ. Serum Levels of Neopterin, Galectin-3, Tissue Necrosis Factor Alpha, and Interleukin-10 During the Interictal and Attack Periods of Patients with Migraine. Turkish Journal of Neurology/Turk Noroloji Dergisi. 2023 Mar 1;29(1):24-29.
18. Heneberk O, Vernerova A, Kujovska Krcmova L, Wurfelova E, Radochova V. Neopterin Levels in Periodontitis and after Nonsurgical Periodontal Therapy: Evaluation of Gingival Crevicular Fluid, Oral Fluid, Serum and Urinary Samples—A Case-Control Study. Biomedicines. 2022 Dec 9;10(12):3200.
19. Heneberk O, Wurfelova E, Radochova V. Neopterin, the Cell-Mediated Immune Response Biomarker, in Inflammatory Periodontal Diseases: A Narrative Review of a More than Fifty Years Old Biomarker. Biomedicines. 2023 Apr 27;11(5):1294.
20. Huang YK, Yang LC, Wang YH, Chang YC. Increased risk of migraine in patients with chronic periodontitis: a population-based cohort study. International Journal of Environmental Research and Public Health. 2021 Feb;18(4):1921.
21. Jassar H, Nascimento TD, Kaciroti N, DosSantos MF, Danciu T, Koeppe RA, Smith YR, Bigal ME, Porreca F, Casey KL, Zubieta JK. Impact of chronic migraine attacks and their severity on the endogenous μ-opioid neurotransmission in the limbic system. NeuroImage: Clinical. 2019 Jan 1;23:101905.
22. Kaufman E, Lamster IB. Analysis of saliva for periodontal diagnosis: a review. Journal of clinical periodontology. 2000 Jul;27(7):453-65.
23. Klaus F, Guetter K, Schlegel R, Seifritz E, Rassi A, Thöny B, Cathomas F, Kaiser S. Peripheral biopterin and neopterin in schizophrenia and depression. Psychiatry research. 2021 Mar 1;297:113745.
24. Latremoliere A, Latini A, Andrews N, Cronin SJ, Fujita M, Gorska K, Hovius R, Romero C, Chuaiphichai S, Painter M, Miracca G. Reduction of neuropathic and inflammatory pain through inhibition of the tetrahydrobiopterin pathway. Neuron. 2015 Jun 17;86(6):1393-406.
25. Lavigne SE, Forrest JL. An umbrella review of systematic reviews of the evidence of a causal relationship between periodontal disease and cardiovascular diseases: Position paper from the Canadian Dental Hygienists Association. Canadian Journal of Dental Hygiene. 2020 Feb;54(1):32.

26. Leira Y, Ameijeira P, Domínguez C, Leira R, Blanco J. The role of leptin as a biomarker in the relationship between periodontitis and chronic migraine. Journal of Clinical Periodontology. 2017 Dec;44(12):1208-14.
27. Leira Y, Ameijeira P, Domínguez C, López-Arias E, Ávila-Gómez P, Pérez-Mato M, Sobrino T, Campos F, D'Aiuto F, Leira R, Blanco J. Severe periodontitis is linked with increased peripheral levels of sTWEAK and PTX3 in chronic migraineurs. Clinical oral investigations. 2020 Feb;24:597-606.
28. Leira Y, Ameijeira P, Dominguez C, López-Arias E, Ávila-Gómez P, Pérez-Mato M, Sobrino T, Campos F, D'Aiuto F, Leira R, Blanco J. Periodontal inflammation is related to increased serum calcitonin gene-related peptide levels in patients with chronic migraine. Journal of periodontology. 2019 Oct;90(10):1088-95.
29. Leira Y, Pozo-Rosich P, Torres-Ferrús M, Orlandi M, Láinez JM, Álvaro LC, Monzón MJ, Guerrero Á, García-Azorín D, Belvís R, González-Oria C. Self-reported periodontitis and migraine: results from a multicenter, cross-sectional survey in Spain. Odontology. 2019 Oct;107:530-5.
30. Lipton RB, Bigal ME, Diamond M, Freitag F, Reed ML, Stewart WF. Migraine prevalence, disease burden, and the need for preventive therapy. Neurology. 2007 Jan 30;68(5):343-9.
31. Mahendra L, Mahendra J, Borra SK, Nagarajan A. Estimation of salivary neopterin in chronic periodontitis. Indian Journal of Dental Research. 2014 Nov 1;25(6):794.
32. Martin BC, Pathak DS, Sharfman MI, Adelman JU, Taylor F, Kwong WJ, Jhingran P. Validity and reliability of the migraine-specific quality of life questionnaire (MSQ Version 2.1). Headache: The Journal of Head and Face Pain. 2000 Mar 27;40(3):204-16.
33. Motaghi M, Haghjooy Javanmard S, Haghdoost F, Tajadini M, Saadatnia M, Rafiee L, Zandifar A. Relationship between vitamin D receptor gene polymorphisms and migraine without aura in an Iranian population. Biomed Res Int. 2013;2013:351942.
34. Ozmeriç N, Baydar T, Bodur A, Engin AB, Uraz A, Eren K, Sahin G. Level of neopterin, a marker of immune cell activation in gingival crevicular fluid, saliva, and urine in patients with aggressive periodontitis. Journal of periodontology. 2002 Jul;73(7):720-5.
35. Peşkersoy C, Peker Ş, Kaya A, Ünalp A, Gökay N. Evaluation of the relationship between migraine disorder andoral comorbidities: multicenter randomized clinical trial. Turkish journal of medical sciences. 2016;46(3):712-8.

36. Pihlstrom BL. Measurement of attachment level in clinical trials: probing methods. Journal of periodontology. 1992 Dec;63:1072-7.
37. Ponnaiyan D, Rughwani RR, Victor DJ, Shetty G. Stem Cells in the Periodontium—Anatomically Related Yet Physiologically Diverse. Eur J Dent. 2024 Feb;18(1):1-13.
38. Pradeep AR, Kumar MS, Ramachandraprasad MV, Shikha C. Gingival crevicular fluid levels of neopterin in healthy subjects and in patients with different periodontal diseases. Journal of periodontology. 2007 Oct;78(10):1962-7.
39. Prasanna JS, Sumadhura C, Karunakar P. Neopterin as a diagnostic biomarker for diagnosis of inflammatory diseases like periodontitis. Journal of Oral Research and Review. 2017 Jan 1;9(1):45-9.
40. Rahimi M, Hosseini ZS. P150: The Effect of Periodontitis on Migraine Chronification. The Neuroscience Journal of Shefaye Khatam. 2018 Apr 10;6(2):181-.
41. Ramroodi N, Javan MR, Sanadgol N, Jahantigh M, Khodakheir TN, Ranjbar N. Association between interleukin-4 (IL-4), gene polymorphisms (C-589T, T+ 2979G, and C-33T) and migraine susceptibility in Iranian population: A case–control study. Egyptian Journal of Medical Human Genetics. 2017 Feb 27;18(1):29-34.
42. Rejdak K, Empl M, Giffin NJ, Afridi SK, Petzold A, Stelmasiak Z, Thompson EJ, Goadsby PJ, Kaube H, Giovannoni G. Increased urinary excretion of nitric oxide metabolites in longitudinally monitored migraine patients. European journal of neurology. 2006 Dec;13(12):1346-51.
43. Rubino E,Vacca A, Govone F, Gai A, Boschi S, Zucca M, De Martino P, Gentile S, Pinessi L, Rainero I. Investigating the role of adipokines in chronic migraine. Cephalalgia. 2017 Oct;37(11):1067-73.
44. Scarel-Caminaga RM, Cera FF, Pigossi SC, Finoti LS, Kim YJ, Viana AC, Secolin R, Montenegro MF, Tanus-Santos JE, Orrico SR, Cirelli JA. Inducible nitric oxide synthase polymorphisms and nitric oxide levels in individuals with chronic periodontitis. International journal of molecular sciences. 2017 Jun 15;18(6):1128.
45. Silness j, loe h. periodontal disease in pregnancy. ii. correlation between oral hygiene and periodontal condition. Acta Odontol Scand. 1964 Feb;22:121-35.
46. Tassorelli C, Diener HC, Dodick DW, Silberstein SD, Lipton RB, Ashina M, Becker WJ, Ferrari MD, Goadsby PJ, Pozo-Rosich P, Wang SJ. Guidelines of the International Headache Society for controlled trials of preventive treatment of chronic migraine in adults. Cephalalgia. 2018 Apr;38(5):815-32.

47. Varma SV, Varghese S, Priyadharsini VJ, Radhakrishnan J, Nair SV, Varma SV, Radhakrishnan J. Establishing the Role of Neurogenic Inflammation in the Pathogenesis of Periodontitis: A Systematic Review. Cureus. 2022 Jul 15;14(7):e26889.
48. Vrecko K, Staedtler P, Mischak I, Maresch L, Reibnegger G. Periodontitis and concentrations of the cellular immune activation marker neopterin in saliva and urine. Clinica chimica acta. 1997 Dec 10;268(1-2):31-40.
49. WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy. Geneva: World Health Organization; 2010. 2, Best practices in phlebotomy. Available from: https://www.ncbi.nlm.nih.gov/books/NBK138665/
50. Yan J, Kothur K, Mohammad S, Chung J, Patel S, Jones HF, Keating BA, Han VX, Webster R, Ardern-Holmes S, Antony J. CSF neopterin, quinolinic acid and kynurenine/tryptophan ratio are biomarkers of active neuroinflammation. EBioMedicine. 2023 May;91:104589.
51. Zhao ZN, Zhang ZQ, Wang QQ, Zhao BL, Wang H, Ge XJ, Yu FY. Genetic Predisposition to Periodontitis and Risk of Migraine: A Two-Sample Mendelian Randomization Study. Neurology and Therapy. 2023 Aug;12(4):1159-69.