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

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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 affected 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 ≥ 6 mm and CAL of ≥ 5 mm 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 assessed 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 orthopantomogram (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 standardized 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 $\pm$ 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 ± 0.71	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

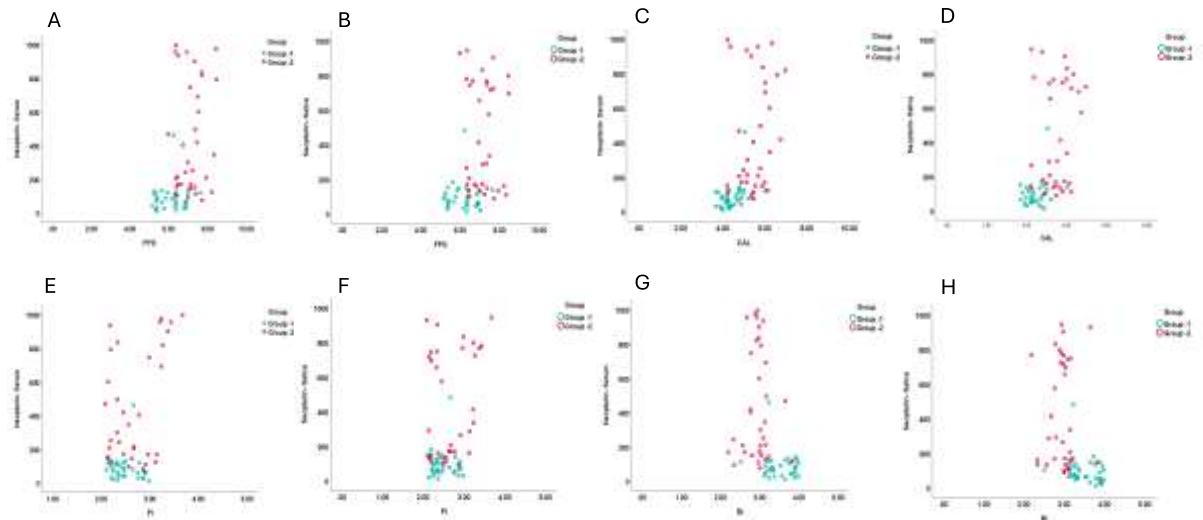


Fig A and B shows correlation of serum neopterin range with PPD and CAL in group 1 and group 2, Fig C and D shows correlation of salivary neopterin level with PPD and CAL in group 1 and group 2

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 among group 1 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- γ 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.

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