

# STATUS OF INFLAMMATORY AND ANTI-INFLAMMATORY MARKERS IN MIGRAINE

**Sumitra Bhoi <sup>a</sup>, Rajiv Kumar Nanda <sup>b</sup>, Satarupa Dash <sup>b</sup>, Sujata Panda <sup>1,c</sup>, Chandrakant Poddar <sup>d</sup> and Bimal Krushna Panda <sup>e</sup>**

<sup>a</sup> Dept of Biochemistry, VIMSAR, <sup>b</sup> Dept of Physiology, VIMSAR, Burla, <sup>c</sup> Dept of ENT, VIMSAR, Burla, <sup>d</sup> Dept Of Pediatrics, VIMSAR, Burla, <sup>e</sup> Dept Of Anaesthesiology, VIMSAR, Burla

**ABSTRACT Background:** Migraine is a Primary Headache disorder, the exact aetiology of which to date is not established. Various neuroinflammatory conditions, cytokines, oxidative stress, neuropeptides and vasomotor changes have been hypothesized for the pathogenesis of migraine headaches. **Aim and Objective:** This study aimed to evaluate inflammatory and non-inflammatory marker levels in patients with migraine and compare them with levels measured in controls. **Materials and Methods:** This is a case-control study of 45 cases of migraine between the age group 15-50 years of either sex attending the outpatient department (OPD) of E.N.T, VIMSAR Burla between July 2019 to December 2019. After proper history and clinical evaluations, we analysed the serum level of inflammatory markers like Neutrophil-to-lymphocyte ratio (NLR), Malondialdehyde (MDA), C-Reactive Protein (CRP) and anti-inflammatory marker Adiponectin in migraine patients. We compared blood levels of these parameters of migraine patients with 45 controls attending the same OPD for ENT diseases other than migraine during the same period. **Results:** The Unpaired t-test between case and control groups showed a significant increase in serum levels of NLR, MDA and CRP in migraine patients compared to controls( $P \leq 0.05$ ). The case and control groups did not show a significant difference in the value of adiponectin. **Conclusion:** This study indicates that oxidative stress markers (MDA) and inflammatory markers like NLR and CRP may potentially be risk factors for migraine. So, early prediction of inflammatory markers might be beneficial in managing migraine patients.

**KEYWORDS** Migraine, Inflammatory markers, MDA, CRP, NLR

## Introduction

Migraine is a primary headache disorder characterised by recurrent headaches that are moderate to severe. Typically, the headaches affect one half of the head, pulsating in nature, last for 2 to 72 hours and may be associated with nausea, vomiting, and sensitivity to light, sound or smell. Several large epidemiological studies showed that the prevalence of migraine in the general population ranges from 6-13%.<sup>[1]</sup>

In one-fifth of migraineurs, a neurological disturbance (visual, sensory or motor) appears during or before the migraine aura's

development.<sup>[2]</sup> The accepted duration of most aura symptoms is 1hr, but motor symptoms are often longer lasting. Visual aura is the most common form of migraine aura<sup>[3]</sup>, which consists of increased photophobia, dry eye, negative transient visual symptom changes(scintillating scotoma, obscuration of vision, tunnel vision, hemianopia) and positive, persistent visual phenomenon(visual snow, floaters or metamorphopsia with distorted contours and lightening symptoms).<sup>[4]</sup>Usually, the visual symptoms come first during a migraine attack, followed by a pain attack.<sup>[5]</sup>

The exact aetiology of migraine to date is not established. It has an environmental and genetic predisposition. It is associated with obesity, stress and depression.<sup>[6,7]</sup> For most of the twentieth century, the prevailing theory of migraine held that pain results from abnormal dilatation of the intracranial blood vessels leading to mechanical excitation of the innervating sensory fibres.

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<sup>1</sup>Corresponding author: Sujata Panda (drbksujata@yahoo.co.in)

This vascular theory has never been validated as there is no difference in blood flow velocity exists in vertebral and middle cerebral arteries during migraine attacks.[8] Again consistent relationship between vessel calibre, cerebral blood flow and headache has not been established.

There has recently been mounting evidence of endothelial activation and dysfunction associated with migraine.[9] Stress is the most common trigger factor of migraine. Oxidative stress is an imbalance between Reactive Oxygen Species (ROS) production and their elimination by antioxidants.[10] The decreasing antioxidant level increases oxidative stress, thus leading to inflammation and changes in the pathophysiology of vessels.[11] In response to stress, the number of neutrophils increases in circulation and the number of lymphocytes decreases. The proportion (NLR) is a useful marker of the pathophysiology of migraine.[12] Thus stress leads to sterile inflammation of cerebral neurovasculature.

In this study, we aimed to evaluate the levels of inflammatory parameters like Neutrophil to lymphocyte Ratio (NLR), oxidative stress marker Malondialdehyde (MDA), C-Reactive Protein (CRP) and anti-inflammatory parameters like Adiponectin levels in patients with migraine and compare with levels measured in controls.

## Materials and Methods

It was a hospital-based case-control study conducted after getting institutional ethical clearance. Patients attending the outpatient department of ENT, Veer Surendra Sai Institute of Medical Sciences and Research, Burla, Odisha, India, were taken for study during the period from July 2019 to December 2019. Subjects were described in the study, and written consent was taken. Clinically diagnosed migraine patients were selected as cases, and subjects attending the same OPD for ENT diseases other than migraine were selected as control.

Patients with complaints of intermittent headaches with or without aura associated with nausea or vomiting or visual disturbances triggered by stress were included in the study.

Patients with headaches diagnosed with fever, sinusitis, refractive error, intracranial complications, diabetes, hypertension, malignant disease, pregnant women, children below 15 years and elderly patients more than 50 years were excluded from the study.

NLR, MDA, CRP and Adiponectin were studied in the Biochemistry department and compared in study groups. 8ml of venous blood was drawn from all participants after taking aseptic precautions and 12 hours of fasting.

A complete blood count (CBC) was done using an automated haematology analyser(ACCULAB CBC-360). NLR was calculated by dividing the absolute neutrophil count(ANC) value by absolute lymphocyte count(ALC). Malondialdehyde (MDA) was evaluated by the Satoh et al. method.[13] Estimating C-Reactive Protein (CRP) was done using the standard nephelometry procedure. The assay of Adiponectin was performed by ELISA Method (Erba) as per the manufacturer's instructions.

The data were analyzed using statistical software SPSS (Statistical Package for the Social Sciences, IBM Corporation, Armonk, New York) Version 20. Data were presented as mean  $\pm$  standard deviation. Statistical analysis was done by unpaired t-test. The corresponding values in different groups were compared statistically by determining the "p" value.  $p < 0.05$  was considered to be significant statistically.

## Results

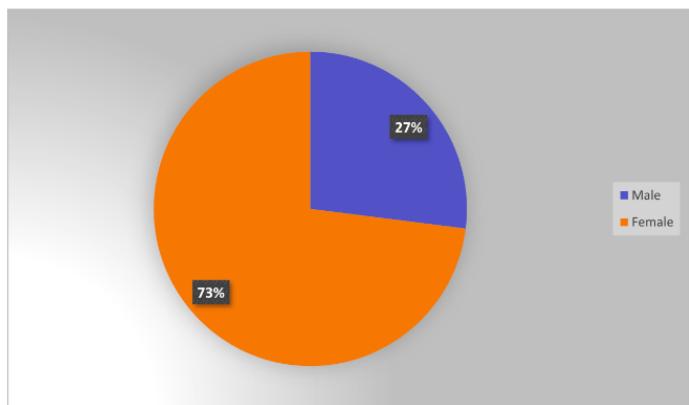
We recruited 90 subjects for the study. Forty-five subjects clinically diagnosed with migraine constituted the case group, and 45 subjects attending the ENT outpatient department for diseases other than migraine of similar age and sex constituted the control group.

The study included 45 migraine patients with a mean age of 32.9 years ranging from 15-50 years. There were 12 males and 33 females. Out of 33 females, 5 had had a history of aura i.e., visual disturbances before a migraine attack. The mean age of the control group was 32.6 years, with 18 males and 27 females. The mean value of BMI was  $24.3 \pm 2.4$  and  $24.62 \pm 2.5$  in cases and controls, respectively, which was statistically insignificant. (Table 1).

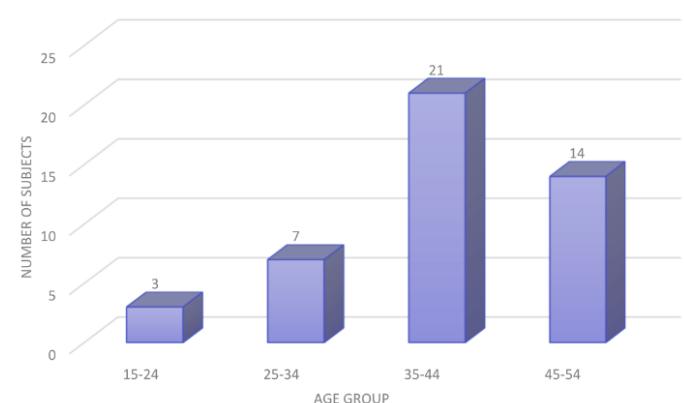
The mean Haemoglobin level in cases and control groups were  $13.7 \pm 2.2$  and  $13.8 \pm 2.3$ , respectively, which is not significant statistically. (Table 2)

The mean level of NLR in cases was  $2.16 \pm 0.35$ , higher than the control group with a mean value of  $1.59 \pm 0.42$ . The level of MDA was higher in migraine patients with a mean value of  $3.0 \pm 0.58$ . Similarly, the mean value of CRP was  $6.08 \pm 1.04$ , which was also higher compared to the control group. (Table 2)

But the mean value of adiponectin was statistically insignificant between study groups, with mean values of  $7.06 \pm 1.02$  and  $7.15 \pm 1.07$ , respectively. (Table 2)



**Figure 1** Sex distribution in the cases.



**Figure 2** Distribution of migraine in different decades of the study group.

**Table 1** Demographic characteristics of migraine cases and controls.

Variable	Case (n=45)	Control (n=45)	P value
	(mean ± SD)	(mean ± SD)	
Age	32.9±6.7	32.6±6.8	0.834
BMI(kg/m <sup>2</sup> )	24.3±2.4	24.6±2.5	0.561

n: Number of subjects. P value < 0.05 was considered to be significant. SD: Standard deviation and BMI: Body mass index.

**Table 2** Haematological and Biochemical findings in migraine cases and controls.

Variable	Case (n=45)	Control (n=45)	P value
	(mean ± SD)	(mean ± SD)	
Hb(gm/dl)	13.71±2.21	13.80±2.31	0.851
NLR	2.16±0.35	1.59±0.42	0.001
MDA (nmol/L)	3.01±0.35	1.93±0.63	0.001
CRP (mg/L)	6.08±1.04	3.65±1.06	0.001
Adiponectin(µg/ml)	7.06±1.02	7.15±1.07	0.684

n: Number of subjects. P value < 0.05 was considered significant, SD: Standard deviation, Hb: Haemoglobin, NLR: Neutrophil to lymphocyte ratio, MDA: Malondialdehyde and CRP: C-reactive protein.

## Discussion

The pathogenesis of migraine is very complex and not fully established. Various factors are responsible for the development of migraine. It is generally defined as a recurrent headache lasting 2-72 hours. Its worldwide prevalence is 13-15% affecting both genders.[14] Various mechanisms have been proposed for the pathogenesis of migraine, including vascular dysfunction, neurogenic, inflammatory and activation of the trigeminovascular pathway.[15]

The mean age of our patients was 32.9 years. Steiner TJ et al. and Stovener et al. concluded that migraine usually affects patients below 50 years.[16] In our study, females are more affected than males.

Previous studies showed that in the female sex, headaches are more common and affect approximately 20.3 million young and middle-aged women worldwide.[17]

The data presented in this study showed that NLR values in migraineurs were significantly higher compared with control subjects. Previous studies have also investigated the value of this marker in evaluating migraine patients.[18] A neutrophil is an active nonspecific inflammatory mediator of cellular immunity, while lymphocytes mediate inflammation's adaptive or protective aspect. So, an increased NLR value in migraine groups in our study suggests underlying inflammatory pathophysiology of migraine. However, NLR has been used in evaluating and prognosis of various disease processes, including coronary artery diseases, malignancies, and neurological and rheumatologic diseases.[19]

Our study showed a significant difference between the serum level of MDA in patients and the control group. MDA is a lipid peroxidation product, an acceptable biomarker of oxidative stress, and an indicator of increased production of reactive oxygen species(ROS). [20] Oxidative stress and neuroinflammation play an important role in migraine pathogenesis. Previous studies showed that migraine patients may have a higher level of NO- metabolites and Malondialdehyde(MDA) compared to healthy subjects, which are consistent with our study.[21]

Our study revealed that the value of CRP is higher in migraineurs than in control which is statistically significant and indicates inflammatory pathogenesis. Vanmolkot FH et al. concluded that increased CRP levels in young adult patients with migraines.[11] However, CRP is a marker of inflammation that may predict subclinical atherosclerosis. Its levels are increased in vascular diseases and healthy individuals without cardiovascular diseases.[22] High levels of this proinflammatory cytokine in our study may reflect the inflammatory status of migraine.

The present study found no significant association between migraine and serum adiponectin levels. No significant difference was detected in adiponectin levels between cases and control groups, consistent with Bernecker C et al. and Tietjen GE et al.[23,24] Adiponectin is a 244 amino acid protein discovered in 1995.[25] Studies show that adipokines like leptin and adiponectin play important roles in the pathophysiology of chronic headaches. Both have been associated with migraine episodes, particularly with aura patients with more prevalent inflammatory components. Again, previous studies showed that the anti-inflammatory and inflammatory role of adiponectin oligomer depends on its molecular weight. Low molecular weight(LMW) oligomer has been related to anti-inflammatory while high molecular weight(HMW) oligomer to pro-inflammatory effects and participates in migraine pathophysiology, particularly in chronic migraine.[26,27]

In our study, we measure the total adiponectin level without differentiating its isoforms, particularly low molecular weight(LMW) adipokine, which is a limitation in our study. Variation in serum adiponectin between individuals depends on various factors like BMI, age, physical activities, hormonal status etc. We have adjusted various parameters like weight, height, and age in our study except for the hormonal status of either sex. So further research is needed on migraine patients, particularly patients with aura, which is a limitation in our study due to the small number of (5) patients. Our study's lack of adiponectin variations might be related to the complex mechanism underlying migraine pathophysiology.

## Conclusion

Increased inflammatory biomarkers like NLR, MDA and CRP in our study suggest inflammatory pathophysiology of migraine. Early evaluation of these markers may help in early diagnosis and better treatment in migraine patients. A larger group with a longer period can be evaluated to get better results.

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## Conflict of Interest

There are no conflicts of interest to be declared by any of the authors of this study.

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