Open Access

Original Article

Comparative Analysis of Human Cystatin C Levels in Migraine without Aura and Healthy Controls

Mariyam Khan, Maham Tahir*, Sunia Hameed, Muhammad Umar Ali Khan***, Mehwish Qamar*, Mamoona Shafiq***

Department of Physiology, Nishtar Medical University, Multan Pakistan, *Department of Physiology, Islam Medical and Dental College, Sialkot Pakistan, **Department of Radiology, Mukhtiar A Sheikh Hospital, Multan Pakistan, ***Department of Histopathology, Bakhtawar Amin Hospital, Multan Pakistan

ABSTRACT

Objective: To compare the levels of human cystatin c (novel biomarker) as well as the association of other parameters in migraine patients without aura and healthy controls.

Study Design: Cross-sectional, comparative study.

Place and Duration of Study: The research was conducted at IMBB Physiology Department, University of Lahore, Lahore Pakistan, from May 2021 to Jun 2022.

Methodology: After taking history, a detailed clinical examination was conducted of participants in both cases and controls of the study. A proforma was filled and a non-probability convenience sampling technique was used. The blood samples were drawn, centrifuged to isolate serum and analyzed concentration of serum cystatin C levels were measured by using enzyme linked immunosorbent assay (ELISA). SPSS version 22 was used for statistical analysis. Mann-Whitney U test was used to determine the median IQR values of both groups and *p*-value<0.05 was considered significant.

Results: Among cases 19(47.5%) were male and 21(52.5%) were female. Mean age of cases and controls was 25.57±5.93 and 26.15±7.19 years. The most frequent occupation among cases and controls was student, doctors, nurses and house wives. Among cases only 3(7.5%) patients were currently smokers while among controls 3(7.5%) were currently smokers. Among cases body mass index of 11(27.5%) patients were 30 and 4(10%) participants in controls had body mass index as 30. The most frequent symptom was photophobia followed by nausea, phonophobia, vomiting and atypical features. All these features were significantly higher. Mean cystatin C level was significantly higher among cases when compared with controls. i.e., Cases: 0.97 mg/l vs. Controls: 0.60 mg/l, *p*-value= 0.003.

Conclusion: Study concluded that patients with migraine without aura have elevated levels of cystatin C in their blood when compared with healthy controls.

Keywords: Cystatin C, Migraine, Protective role, Oxidative stress.

How to Cite This Article: *Khan M, Tahir M, Hameed S, Khan MUA, Qamar M, Shafiq M. Comparative Analysis of Human Cystatin C Levels in Migraine without Aura and Healthy Controls. Pak Armed Forces Med J 2023; 73(4): 1195-1199. DOI: https://doi.org/10.51253/pafmj.v73i4.10867*

INTRODUCTION

Migraine headache is a complex cerebrovascular disorder that impacts life significantly including reduced productivity, family life, mental health, leisure activities and the economy. The annual prevalence of migraine in the general population is close to 15%.[1] Migraine headache is the second most prevalent cause of global impairment in all age groups ,irrespective of gender and the first most common cause of global disability in women aged 15-49, according to the most recent GBD stud.[2] Symptoms of migraine mainly unilateral, throbbing headache that can last anywhere from four to seventy-two hours and associated with neurological, gastrointestinal, and autonomic problems.[3] Unbalanced and moderate to severe discomfort on one side of the body is typical, and it is made worse by specific triggers. Acute photophobia and phonophobia are also associated to it. One-third of migraine sufferers encounter an aura phase, which includes visual, sensory and cognitive difficulties as well as a change in their mood. According to a study, migraines without aura account for the vast majority of all cases.[4] The particular pathophysiological mechanism behind this debilitating disorder is yet unsolved, however, genetic, epigenetic, and environmental factors all contribute to migraine's multi-factorial nature.

Migraine attacks can also be triggered by a variety of external events. A number of risk factors, such as fasting or skipping meals, dehydration, hypoxia, inadequate sleep, and intense activity, have been shown to be linked with increase in oxidative stress levels in the body.[5] Chronic migraines are thought to be caused by cytokines and neuropeptides that are released in response to a local inflammatory reaction known as neurogenic inflammation.[6] Signs of inflammation in this illness include arterial vasodilation, increased vascular permeability, and mast cell degranulation. A rise

Correspondence: Dr Mamoona Shafiq, Physiology Department, Islam Medical College, Sialkot Pakistan

in arachidonic acid levels, caused by inflammatory mediators and pro-inflammatory cytokines produced by mast cells near primary nociceptive neurons is the proposed etiology of migraine and its neurological symptoms. IHS criteria have been used to diagnose migraine, but this disorder remains a complex one that is both under and over-recognized because of its clinical heterogeneity. This evident clinical variance has impeded the finding of effective treatments.[7] That is why biomarkers have recently been the focus of several research endeavors.[8]

Human cystatin C is a peptide that belongs to the cystatin superfamily of proteinase inhibitors. It is secreted into the bloodstream by practically all human cells.[9] To reduce inflammatory response, extracellular matrix disintegration, as well as phagocytic activities, cysteine C inhibits lysosomal proteinase activity and cysteine protease. Chronic inflammation and cell death are linked to oxidative stress, and Cystatin C is a good indicator of this. Inflammation and oxidative stress have been implicated in migraine causation in many studies.[10]

A 122 amino acid, single polypeptide chain, non-glycosylated cysteine proteinase inhibitor known as human Cystatin C. The "housekeeping type" Cystatin 3 (CST3) gene expresses it, and all nucleated cells reliably produce it at a steady rate. This protein is freely filtered in the renal glomeruli, where it is then reabsorbed and catabolized. All parts of the human body, including the adenohypophysis, thyroid, pancreas, adrenal medulla, and cortical neurons of the brain, express Cystatin C.[11]

Human cystatin C modulates and regulates inflammatory responses, growth, neurodegeneration, and repair.[12] Numerous studies have revealed that it has neuroprotective properties in the face of damage and stress. The rationale of the study is to investigate the link between migraine without aura and human cystatin C (a novel biomarker).

METHODOLOGY

The study was conducted with the aim to compare the levels of human cystatin c (novel biomarker) as well as the association of other parameters in migraine patients without aura and healthy controls to help understand its pathophysiology.

This study was designed as a cross sectional compa-rative study. The research was conducted at IMBB Physiology Department, University of Lahore, Lahore Pakistan, from 21st May 2022 till 30th June 2022. Sample size was calculated using the study of T. AkdaĞ and A.Uca with the help of following formula:

$$n = \frac{\sigma(z1-\alpha + z1-\beta)}{(\mu o - \mu\beta)2}$$

The estimated sample size was 9 per group but for the better power of study we took 40 samples per group.[13]

Inclusion Criteria: For subjects (n=40) are migraine patients without aura, all ages and for controls (n=40) are healthy controls without migraine and all ages were included in the study.

Exclusion Criteria: Absence of all other acute and chronic illness were excueded in the study.

Two groups were formed with a sum of 80 patients in this study with half of the patients having migraine without aura and the other 40 with healthy controls. According to International Headache criteria, Migraine patients without aura were diagnosed on the basis of history and clinical examination. Informed consent was taken and performa was filled documenting detailed demographic data, associated symptoms, triggering factors and treatment. World health organization reported Body mass (BMI) were calculated and considered as weight (Kg) divided by the square of height. blood samples (10mL) were taken from each patient to be transferred to biochemistry tubes. After coagulation, samples was separated, centrifuged at 3000rpm for approximately 10 whole minutes and then stored in deep freeze at -80C until assay. After the initial procedure, serum levels of Cys C were measured by enzyme linked immunosorbent assay (ELISA). Concentration of serum Cys C levels were measured by using a commercially available ELISA kit (Elabscience -E1104HU) for human cystatin as per manufacturer s instructions.

The statistical analysis software utilized was SPSS version 22. Data normality was checked using the Shapiro-Wilk test. Means and percentages were used to express continuous variables and numbers, respectively. To compare the levels of serum cystatin C in patients and controls, the Man Whitney U test had been used. *p*-value greater than 0.05 was regarded as significant.

RESULTS

Mean age of cases and controls was 25.57±5.93 and 26.15±7.19 years. Minimum and maximum age among cases was 18 and 38 years while among controls minimum and maximum age was 18 and 43 years. Female patients were more prevalent, and the

typical BMI ranged from 18-25. Marriage status and smoking history had no effect on migraine frequency. The detailed data is shown in Table-I.

Table-I: Frequency distribution of demographic parameters

Demographic Categories	Cases n(%)	Controls n(%)
Gender		
Male	19(47.5%)	19(47.5%)
Female	21(52.5%)	18(52.5%)
Marital Status		
Yes	16(40%)	14(35%)
No	24(60%)	26(65%)
Bmi		
<18	10(25%)	10(25%)
18-25	24(60%)	20(50%)
>25	6(15%)	10(25%)
Smoking Status		
Yes	3(7.5%)	8(20%)
No	37(92%)	32(80%)
Use of Prophylactic Drugs		
Yes	5(12.5%)	0(0%)
No	35(87.5%)	40(100%)

Each patient exhibited a different set of signs and symptoms; 95% of the individuals we recruited, reported having photophobia, 75% had phonophobia, 72% had nausea, 42.5 reported with vomiting and 25% complained of unusual abdominal pain. As described in Figure-1.

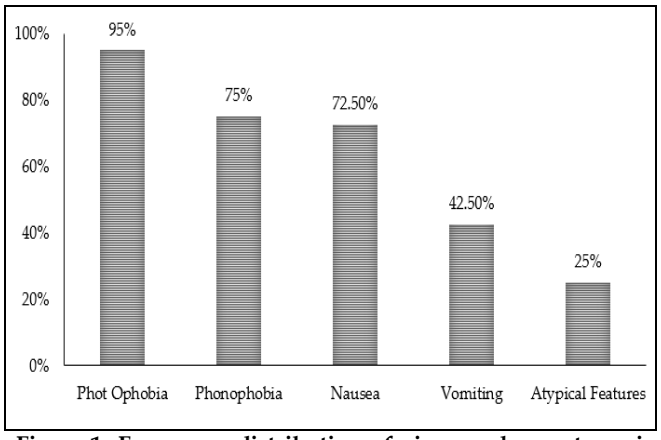

Figure-1: Frequency distribution of signs and symptoms in migraine patients

Mean cystatin C level was significantly higher among cases when compared with controls. i.e., Cases: 0.97mg/l and Controls: 0.60 mg/l, *p*-value=0.003 Minimum and maximum value of cystatin C level among cases was 0.06-2.99mg/l and among control it was 0.16-2.83mg/l respectively. *p*-value is 0.003 Table-II.

Table-II: Comparison of Cystatin C levels between cases and controls

Results	Cases	Controls
Total participants	40	40
Mean±SD	0.97±0.67	0.60±0.44
Median (IQR)	0.79 (0.95)	0.43(0.38)
Minimum value of cystatin C	0.06	0.16
Maximum value of Cystatin C	2.99	2.83
p-value*	0.003**	

**using Mann Whitney U test,*
*** p-value less than 0.05 considered statistically significant*

DISCUSSION

A common neurological condition known as migraine affects 10-20% of people worldwide. A person's quality of life is negatively impacted by migraines. Although the precise etiology of migraines is still unknown, it is thought that multiple factors contribute to their pathophysiology. Thus, migraines may develop because of vascular, neurogenic, or metabolic processes.[14]

According to Xiao *et al.* common migraine symptoms without aura include the following: Pain on one side of the head, throbbing in nature, photophobia, sensitivity to sound, phonophobia and pain or discomfort exacerbated by physical activity.[15] In our study the most frequent symptom was photophobia followed by nausea, Phonophobia, vomiting and atypical features. All these features were significantly higher.

The International Headache Society's (IHS) diagnostic guidelines were used to identify migraine as a clinical illness. According to one study, migraine without aura accounts for about 64% of headaches.[16] There are vascular, neurogenic, and biochemical theories for the pathophysiology of migraine attacks. The fundamental causes of migraine pathophysiology are yet unknown, though. For the early diagnosis, prognosis, and treatment of migraine, specific and favorable biomarkers are crucial.[17]

Numerous inflammatory features of migraine have been studied, and relationships between different inflammatory biomarkers and migraine have been found. C-reactive protein (CRP), an inflammatory biomarker, has previously been suspected of contributing to coronary heart disease and stroke and has also been discovered to be higher in migraine sufferers.[18]

Multiple research came to the conclusion that Cystatin C plays a strong regulator function in the inflammatory process and in the body's defense against bacterial and viral infections. Cystatin C has been proposed to serve a regulatory role in the inflam-

matory process by modulating leukocyte chemotaxis and phagocytosis in a prior study.[19] Thus increased cystatin C can have a protective role in migraine patients, and our study also support this. Another study also concluded that Cystatin C is a significant factor in endogenous neuroprotection. It is also a cutting-edge option for the treatment of stroke by preserving the integrity of the lysosomal membrane.[20]

In our study mean cystatin C level was significantly higher among cases when compared with controls. Value of cyststin C was as, in Cases: 0.97 mg/l and in Controls: 0.60 mg/l, *p*-value was found significant, *p*-value=0.003. Minimum and maximum value of cystatin C level among cases was 0.06-2.99 mg/l and among control it was 0.16-2.83 mg/l respectively. In a recent study, Akda *et al*. also found that the patients with migraine showed greater serum levels of cystatin C than healthy controls.[13]

Contrary to our study, a previous study concluded that inflammation and endothelial dysfunction are linked to elevated cystatin C concentrations, which play a role in the etiology of atherosclerosis. These elements could be the cause of the connection between poor vasodilation and microcirculation issues. Additionally, a high concentration of cystatin C has been linked to an increased metabolic rate and may indicate the persistence and severity of other risk factors such hypertension, other neurological conditions and preclinical renal failures.[21]

Cystatin C must also be able to distinguish between amyotrophic lateral sclerosis (ALS) patients and people with neurologic conditions that closely resemble ALS, or people with ALS "mimic diseases," in order to be therapeutically effective as a diagnostic biomarker. In the study of Nakane *et al.* cyststin C levels were decreased and this was also contrary to our study.[10]

Given the diversity of migraine patients, it is likely that a panel of many biomarkers rather than a single protein biomarker will be necessary to distinguish migraine pathophysiology from other neurological conditions with sufficient diagnostic certainty.

The diagnosis of migraine and the assessment of prognosis may be aided by the prediction of circulating biomarkers. As far as we are aware, the study we describe is the first to be conducted in Pakistan on patients with migraine without aura to ascertain the serum levels of Cystatin C. We do, however, accept that our study has several drawbacks, with the small sample size being the key one.

RECOMMENDATION

Without the support of Prof. Dr. Tania Shakoori this research would have never been possible. We all thank her for being our North Star.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

MK & MT: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

SH &, MUAK: Data acquisition, data analysis, approval of the final version to be published.

MQ & MS: Critical review, concept, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Steiner TJ, Stovner LJ. Global epidemiology of migraine and its implications for public health and health policy. Nat Rev Neurol 2023; 19(2): 109-117.
2. Steiner T, Stovner L, Jensen R, Uluduz D, Katsarava Z. Migraine remains second among the world's causes of disability, and first among young women: findings from GBD2019. BioMed Central 2020; 21(1): 137. https:// doi: 10.1186/s10194-020-01208-0
3. Wang X, Yin Z, Lian Y. Premonitory symptoms in migraine from China: a multi-clinic study of 4821 patients. Cephalalgia 2021; 41(9): 991-1003. https://doi: 10.1177 /0333102421997850.
4. Verhagen IE, Arend BW, Casteren DS, Thiermann NJ, Tange E, Brink A, et al. Migraine with and without aura in relation to the menstrual cycle and other hormonal milestones: A prospective cohort study. Cephalalgia. 2023; 43(6): 03331024231164322. https:// DOI: 10.1177/03331024231164322
5. Gross EC, Lisicki M, Fischer D. The metabolic face of migraine—from pathophysiology to treatment. Nat Rev Neurol 2019; 15(11): 627-643. https://doi: 10.1038/s41 582-019-0255-4
6. Safiai NI, Mohamad NA, Basri H, Mat LN, Hoo FK, Rashid AM, et al. High-frequency repetitive transcranial magnetic stimulation at dorsolateral prefrontal cortex for migraine prevention: A protocol for a systematic review of controlled trials. Plos one. 2021; 16(6): e0251528. https://doi: 10.1371/journal.pone.0251528
7. Ashina M, Terwindt GM, Al-Karagholi MA-M, De Boer I, Lee MJ, Hay DL, et al. Migraine: disease characterisation, biomarkers, and precision medicine. The Lancet 2021; 397(10283): 1496-1504. https://doi: 10.1016/S0140-6736(20)32162-0.
8. Pozo-Rosich P, Coppola G, Pascual J, Schwedt TJ. How does the brain change in chronic migraine? Developing disease biomarkers. Cephalalgia. 2021; 41(5): 613-630. https://doi: 10.1177/0333 102420974359.
9. Fernando S, Polkinghorne KR. Cystatin C: not just a marker of kidney function. Braz J Nephrol 2020; 42(1): 6-7. https://doi:10. 1590/2175-8239-JBN-2019-0240
10. Nakane S, Fujita K, Azuma S, Urushihara R, Kamada M, Harada M, et al. CSF cystatin C and diffusion tensor imaging parameters as biomarkers of upper motor neuron degeneration in amyotrophic lateral sclerosis. Clini Neurol Neurosurg 2018; 172(1): 162-168. https://doi: 10.1016/j.clineuro.2018.07.008

11. Zi M, Xu Y. Involvement of cystatin C in immunity and apoptosis. Immunol lett 2018; 196: 80-90. https://doi: 10.1016/j.imlet.2018.01.006.
12. Huang S, Zhang S, Wang J, Hou G, Xu S. Correlation between serum cystatin C level and retinal blood flow in patients with essential hypertension. Ophthalmic Res 2022; 65(3): 335-341. https://doi.org/10.1159/000522219
13. AkdaĞ T, Uca AU. Cystatin C as a potential biomarker to evaluate migraine. Arq Neuro-Psiquiatr 2020; 78: 337-341. https://doi: 10.1590/0004-282X20200005.
14. Frederiksen SD, Bekker-Nielsen Dunbar M, Snoer AH, Deen M, Edvinsson L. Serotonin and neuropeptides in blood from episodic and chronic migraine and cluster headache patients in case-control and case-crossover settings: A systematic review and meta-analysis. headache: J Head Face Pain 2020; 60(6): 1132-1164. https:// doi: 10.1111/head.13802.
15. Youn D-h. Trigeminal long-term potentiation as a cellular substrate for migraine. Medical Hypotheses 2018; 110: 27-30. https://doi: 10.1016/j.mehy.2017.10.026
16. Rammohan K, Shaji CV. Migraine and mood disorders: prevalence, clinical correlations and disability. J Neurosci in rural pract 2019; 10(01): 28-33. https://doi: 10.4103/jnrp.jnrp_146_18.
17. Yurekli BS, Kutbay NO. Low levels of cystatin C in patients with acromegaly. Endocrinol Metabolic Syndrome 2018; 7(5): 1-6. https://doi: 10.4172/2161-1017.1000290
18. Özdemir HH, Dönder A. Evaluation of neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and C-reactive protein in tension-type headache patients. J Neurosci Rural Pract 2021; 12(03): 566-570.
19. Leto G, Crescimanno M, Flandina C. On the role of cystatin C in cancer progression. Life Sci 2018; 202: 152-160. https:// doi: 10.1016/j.lfs.2018.04.013
20. Fang Z, Deng J, Wu Z, Dong B, Wang S, Chen X, et al. Cystatin C is a crucial endogenous protective determinant against stroke. Stroke 2017; 48(2): 436-444.
21. Kim TJ, Kang MK, Jeong H-G. Cystatin C is a useful predictor of early neurological deterioration following ischaemic stroke in elderly patients with normal renal function. Eur Stroke J 2017; 2(1): 23-30. https://doi: 10.1177/2396987316677197