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Evaluating the Interplay between Pentraxin-3 and Cystatin C in Migraine Patients

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ABSTRACT

Objective: Evaluate the levels of serum pentraxin-3 and human cystatin C in migraine patients and the relationship between various parameters in migraine patients to healthy controls.

Study Design: Cross-sectional comparative study.

Place and Duration of Study: Department of Physiology, University of Lahore, Lahore Pakistan, from Apr 2021 to Apr 2022.

Methodology: Following the collecting of histories, participants in the study - both cases and controls - were given a thorough clinical examination. A proforma was filled out by each candidate and a non-probability convenience sampling approach was chosen. After drawing blood samples, the serum was separated by centrifugation. The enzyme-linked immunosorbent assay (ELISA) was used to determine the concentrations of serum pentraxin-3 and cystatin C. Utilizing SPSS version 22, statistical analysis was conducted. The median IQR values of both groups were ascertained using the Mann-Whitney U test, with a *p*-value of less than 0.05 being deemed significant.

Results: Cases include 52.5% female patients and 47.5% male patients. Cases and controls had average ages of 25.57 5.93 and 26.15 7.19 respectively. BMI falls in the category 18–25 in most cases. Photophobia was the most common symptom, which was followed by phonophobia, nausea and vomiting. When comparing cases to controls, the mean cystatin C and pentraxin-3 levels were much higher in patients. Mean value of PTX-3 was 145.57±157.7 pg/l in cases and 43.65±33.01 pg/l in controls. Mean value of cystatin C was 0.97±0.67 mg/l in cases and 0.60±0.44 mg/l in controls.

Conclusion: Levels of serum pentraxin-3 and human cystatin C were raised in migraine cases as compare to control group.

Keywords: Pentraxin-3, Cystatin C, Migraine, Neuroinflammation, Neuroprotection, Antioxidant.

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INTRODUCTION

Recurrent, severe headaches that can cause severe pain and incapacity are the hallmark of migraines, a complicated neurological condition. It can be unilateral, temporal, throbbing or pulsating, cyclical, and sometimes lasts for four to seventy-two hours.[1] It can also cause disruptions to everyday activities. Although the precise aetiology of migraines is unknown, a mix of neurological, environmental, and hereditary factors are thought to be involved. Early symptoms, or prodromal phase, are common in migraines, then occur headache phase and lastly postdromal phase.[2] According to the most current GBD survey, migraine headaches are the second most common cause of global impairment across all age categories, regardless of gender. It is also known as the most common cause of worldwide cognitive impairment among women aged 15-49.[3]

The central nervous system is exposed to inflammatory chemicals during migraine episodes, which is linked to neuroinflammation. This involves the activation of glial cells, including microglia and astrocytes. When a migraine occurs, the trigeminal nerve pathways become more sensitive due to this neuroinflammation, which can result in the experience of pain.[4] In migraine research, biomarkers are essential for comprehending the fundamental causes of the condition, enhancing diagnostic, forecasting therapeutic outcomes, and creating focused treatments.[5]

A class of proteins implicated in inflammation and the immune response that have been conserved throughout evolution are called pentraxins. Given its longer amino acid sequence than that of small pentraxins like CRP, PTX-3 in particular is regarded as a long pentraxins. C-reactive protein (CRP) and serum amyloid P component (SAP) are two other members of the pentraxin family of proteins, which also contains pentraxin 3 (PTX3). The vascular endothelial cells secrete Pentraxin-3 in response to inflammatory events.[6] Numerous nociceptors are triggered by the inflammatory mediators generated by damaged endothelium, which in turn activates the trigeminovascular pathway and sensitizes different parts of the brain. This information is relevant to the research of the inflammatory element of migraine.[7,8]

Small proteins called cystatin C are generated by all of the body's cells, including the kidney's cells. It is

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an inhibitor of proteases that belongs to the cystatin superfamily and is involved in controlling the activity of enzymes known as cysteine proteases. Although the processes are complicated, cystatin C has in fact shown possible neuroprotective implications.[9] Cystatin C possesses antioxidant qualities, which aid in scaven-ging detrimental reactive oxygen species (ROS) inside the brain. Overabundance of ROS can result in oxidative stress, which damages neurons. Cystatin C may help shield neurons from oxidative injury by lowering oxidative stress.[10]

The balance between neuroinflammation and neuroprotection is crucial for maintaining brain health. As biomarker pentraxin-3 has neuroinflammatory roles and Cystatin C has neuroprotective roles, so we to evaluate that either both of the biomarkers have any influence in context of migraine.

The purpose of study was to investigate the levels of serum pentraxin-3 and human cystatin C and the relationship between various parameters in migraine patients to healthy controls in order to better understand the pathophysiology and diagnostic approach.

METHODOLOGY

This cross sectional comparative study conducted at the department of Physiology, University of Lahore, Pakistan, granted the study's ethical permission. Using the formula of means $n=\frac{\sigma 2(Z1-\alpha+Z1-\beta)}{(\mu o-\mu a)2}$ and the research of Dominguez *at el*, the sample size was determined to be one per group. For improved study power and utilization of purchased kits, we collected 80 samples.

Inclusion Criteria: For cases (n=40), individuals with diagnosed migraine but no aura and all age groups. For Controls (n=40), well-maintained healthy controls, free of migraine and all age groups.

Exclusion Criteria: The patients with following disorders were not included in the list of diagnosed cases; hypertension, diabetes, hypertrophic cardiomyopathy, cardiac illness, additional neurologic conditions, a mental health issue, diabetes mellitus, git disorders or liver illnesses, chronic illnesses or any other metabolic disease.

Forty migraine diagnosed cases and forty healthy controls were included in this study. The International Headache Disorders Beta Version criteria were used to diagnose migraine. Every one of them took over a written informed consent. Both the controls and the patients answered a pro-forma. Measurements of blood pressure and blood glucose were made in order to rule out hypertension and diabetes. A 5 millilitre blood sample was drawn via the antecubital vein for every individual. The serum was obtained by centrifuging the sample for 15 minutes, and it was then kept in Eppendorf vials at -200 C for use in ELISA.

We used ELISA kits i.e. (Elabscience -E1104HU) and (Elabscience Human PTX3/TSG-14 (pentraxin 3) ELISA kit) for the measurement of serum cyststin C and serum pentraxin-3 respectively, by following the directions provided by the manufacturers.

For statistical analysis, SPSS version 22 is utilized. For data normality of data, the Shapiro-Wilk test is applied. Numbers (%) are used to indicate categorical variables while means are used to express continuous variables. The Mann–Whitney U test is used to compare the serum levels of PTX-3 and cystatin C in patients and controls. The *p*-value of >0.05 is taken as significant.

RESULTS

The mean age of the patients was 25.57±5.93, whereas the controls were 26.15±7.19 years. For cases, the minimum and maximum ages were 18 and 38 years, whereas for controls, they were 18-43 years. The majority of patients were female, and their average BMI fell between the category 18-25. Individual patients present with different signs and symptoms, i.e,. 95% of our patients report photophobia, 75% phonophobia, 72.5% nausea, 42.5% vomiting, and 25% unusual gastrointestinal discomfort. Frequency distribution of different demographic parameters are illustrated in Table-I.

Table-I: Demographic parameters in cases and controls

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%)

There are several elements that contribute to the onset of a migraine headache. Stress was confirmed as a triggering factor by most migraine sufferers in our study. Also the patients had migraine headache as a

result of skipping meals and being exposed to bright lighting. In addition to this, migraine also occurred as a result of sleep disruption, travel, loud noises, strong odours, hormonal disturbances, physical exercise, and change in weather. The frequency of patients that were triggered by various factors are described in the Table-II.

Table-II: The Frequency Distribution of Migraine Triggering Variables

Triggering Variables	n (%), (n=40)	
	Yes	No
Stress	36 (90%)	4 (10%)
Skipping meals	31 (77.5%)	9 (22.5%)
Sleep disturbances	27 (67.5%)	13 (32.5%)
Hormones	8 (20%)	32 (80%)
Traveling	25 (62.5%)	15 (37.5%)
Loud noise	25 (62.5%)	55 (37.5%)
Intense lights	31 (77.5%)	9 (22.5%)
Strong odours	17 (42.5%)	23 (57.5%)
Physical exercise	10 (25%)	30 (75%)
Weather changes	14 (35%)	26 (65%)
Specific food	13 (32.5)	27 (67.5)

Serum pentraxin-3 and serum cyststin C levels were compared in patients and controls using the Mann-Whitney U test. Levels of serum PTX-3 and cyststin C were higher in cases as compare to controls. For pentraxin-3 the median IQR was calculated to be 100.55 (84) in cases while in the control group, it was 31.15 (38.8). Mean PTX-3 was 145.57 ±157.7 pg/l in cases and 43.65 ±33.01pg/l in controls. The *p*-value of 0.0001 suggests it is significant. The mean cystatin C level in Cases: 0.97 mg/l and Controls: 0.60 mg/l. The *p*-value was 0.003 and it was considered significant.

Table-III: Levels of PTX-3 and Cystatin C in Cases and Controls

Participants	Mean±SD		Median IQR		*p*-value
	Cases	Controls	Cases	Controls	
PTX-3	145.57 ± 157.7 pg/l	43.65 ± 33.0 mg/l	100.55 (84)	31.15 (38.5)	<0.001
Cystatin C	0.97 ± 0.67 pg/l	0.60 ± 0.44 mg/l	0.79 (0.95)	0.43 (0.38)	0.003

DISCUSSION

Inflammatory processes in migraine headaches sensitize the cerebellum, meningeal arteries, & cortical material. They also produce different neurotransmitters that stimulate the trigeminovascular system. Typically, a patient's medical history, physical examination, subjective clinical criteria, and rule out other illnesses that might mimic a migraine are used to diagnose migraine.[11] Long-chain pentraxin, PTX-3 is released by vascular endothelial cells in response to inflammatory events. PTX-3, which plays a part in endothelial dysfunction and inflammatory disorders, is also considered as a unique biomarker in the pathogenesis of migraine headaches.[12]

Most of the patients in our study suffered with acute migraine. Acute migraine is diagnosed on the bases of frequency of migraine episodes per month i.e. the patients with <15 episodes per month for consecutive 3 months. Our study described that the level of PTX-3 were raised in the serum of migraine cases. According to a related research, individuals with acute migraines had higher levels of the biomarker PTX-3, but those with chronic migraines had lower levels. It was believed that it might be due to the early elevation of PTX-3 release and inflammatory reactions, which may result in migraine; however, as the condition becomes more chronic, PTX-3 release and inflammatory responses decrease.[13]

Migraine is a neuroinflammatory process and PTX-3 is an inflammatory biomarker. Neuroinflammation might be the cause of raised serum pentraxin-3 levels. Researches also indicate that PTX-3 is raised in inflammatory diseases. In the earlier research, individuals with multiple sclerosis, stroke, myocardial infarction, arthritis, and other inflammatory diseases also had elevated blood pentraxin-3 levels.[14,15]

We found that stress is the main cause for migraines, which primarily affect young women. Additional research has suggested that the female in reproductive age group might have more migraine headaches. This might be due to the hormonal fluctuations.[16] According to our research, there is no correlation between the onset of the disease and other factors such as smoking status, marital status, or BMI. On the other hand, Ornello *et al*. found that migraine attack onset is significantly correlated with elevated BMI.[17]

Cystatin C is a potent regulator of inflammation and an important component of the body's defensive mechanism against viral and bacterial infections. Our study's findings showed that serum cystatin C levels were elevated. This might be increased to promote neuroprotection and prevent further inflammation. In a previous study, it was suggested that Cystatin C regulates leukocyte chemotaxis and phagocytosis, which functions as a regulatory mechanism in the inflammatory process.[18]

Researchers have shown that cystatin C inhibits beta-amyloid peptides, which are believed to be involved in the development of Alzheimer's disease and do not aggregate or form fibrils. By preventing the

creation of dangerous aggregates, cystatin C has the ability to protect neurons from damage and delay the start of neurodegeneration.[19]

Cathepsins are among the cysteine proteases that are naturally inhibited by cystatin C. Cell damage and neuronal death may result from dysregulation of protease activity. Cystatin C may contribute to the preservation of cellular homeostasis and shield neurons from the damaging effects of proteases.[20]

In contrast to what we observed, another study found that increased cystatin C concentrations are associated with endothelial dysfunction and inflammation, both of which contribute to the pathogenesis of atherosclerosis. These factors may be the root of the relationship between microcirculation problems and insufficient vasodilation. A high concentration of cystatin C has also been associated with an elevated metabolic rate and may be a sign of the severity and persistence of additional risk factors, including preclinical renal failures, various neurological disorders, and hypertension.[21]

Predicting circulating biomarkers may help in migraine diagnosis and prognostic evaluation. To the best of our knowledge, the study we present is the first to measure serum levels of pentraxin-3 and cystatin C in patients with migraine without aura in Pakistan. However, we acknowledge that there are a number of limitations to our study, the main one being the small sample size.

CONCLUSION

Levels of serum pentraxin-3 and human cystatin C were raised in migraine cases as compare to control group.

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Conflict of Interest: None

Author's Contribution

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

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

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

NK & AF: 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.

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