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ORIGINAL ARTICLE

Evaluation of Diagnostic Role of Alkaline Phosphatase in the Patients of Cerebral Stroke

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ABSTRACT

Background: The cerebral stroke is one of the most common disorders in South Asia. The Pakistani adult population suffered from cerebral strokes in more than 10% of cases. The TNAP isoform of Alkaline Phosphatase is also found in the brain. It is possible to diagnose cerebral stroke patients based on their serum ALP level. In this study, the objective is to evaluate the level of ALP in patients suffering from cerebral strokes.

Methodology: This cross-sectional study was conducted at the Department of Medicine at the LUMHS Jamshoro. A total of 55 patients (26 males & 29 females) with cerebral stroke between the ages of 40-70 years were selected according to the inclusion & exclusion criteria between the ages of 40-70 years. By using the AMP (2-amino, 2-methyl, 1 propanol) method, we were able to estimate the serum level of ALP.

Results: In 29.1% of the patients, the mean serum ALP level was 85.13 ± 10.93 U/L, while in 70.9% of the patients, the mean serum ALP level was 231 .42 ± 5.76 U/L. As a result of this study, it can be concluded that approximately 70% of cerebral stroke patients had elevated levels of serum ALP levels, which can be used as a diagnostic tool for cerebral stroke patients.

Conclusion: Cerebral stroke patients had elevated serum ALP levels, which are a cheap and easily quantifiable biomarker.

Keywords: Cerebral Stroke, Alkaline Phosphatase, TNAP, Isoenzyme

INTRODUCTION

An event in which the brain lacks oxygen and glucose, resulting in a loss of memory and motor function.[1] Among the types of cerebral stroke that most commonly occur in our region, ischemic stroke is by far the most common.[2] In addition to diabetes mellitus, hypertension, hypercholesterolemia, and stress, diabetes mellitus is the leading predisposing factor for strokes.[3]

Globally, cerebral stroke is the second leading cause of death after myocardial infarction.[4] Globally, deaths in 2016 accounted for 10.2% of all deaths.[2] In the world there are 5.2 % of people who suffer from disabilities caused by strokes, which is a staggering number.[4,5] Stroke prevalence has increased in underdeveloped countries over the past thirty years, reaching 14.3%. [5, 6]

South Asian countries have a significantly higher prevalence of stroke than any other region of the world because of a combination of factors such as high mortality rate, young age incidence, stroke provoking factors, and a high involvement of factors causing the morbidity of stroke patients. [7,8]

The alkaline phosphatase enzyme (ALP) belongs to the ectophosphatase family of enzymes. [9] It is mainly responsible for bone mineralization as well as a number of other functions in bones.[10] There are four isoenzymes of human ALP on electrophoresis; intestinal (IAP), placental (PLAP), germinal (GCAP), and tissue non-specific (TNAP). [11] TNAP is found in brain tissues and the nervous system. [12] In the brain, TNAP is responsible for the development of various tissues, such as the myelin sheath. Under the influence of TNAP, different neurotransmitters are synthesized and regulated. [13,14]

In different regions of the world, researchers are investigating the levels of ALP and specific TNAP in brain tissues, cerebrospinal fluid samples, and blood samples to determine different variations in TNAP levels in animal and human models. In the brain, ALP is also involved in phosphorylation.[15] The ALP levels in patients with epilepsy and neurological disorders have been studied in past decades, and it has been hypothesized that those patients have elevated levels of ALP.[16]

It is the purpose of this study to study the level of ALP that may be present in patients suffering from cerebral strokes.

METHODOLOGY

From November 2020 to April 2021, a descriptive cross-sectional study was conducted at the Department of Medicine Liaquat University of Medical & Health Sciences Jamshoro, Sindh. A total of 55 patients with cerebral stroke were enrolled in this study with their own consent and consent of their attendants from different medical wards of LUMHS Hospital Jamshoro and Hyderabad. A total of 40 patients between the ages of 40 and 70, males and females, with hemiplegia, paraplegia, bilateral paralysis, lower extremity paralysis, upper extremity paralysis, diabetic or non-diabetic, and hypertensive or non-hypertensive were included in this study. All patients aged less than 40 years or more than 70 years, with any history or evidence of bony disorder, liver dysfunction, hepatitis B & C, cirrhosis of liver, pregnancy, any history of carcinoma of bones, prostate, breast, liver, or lung, as well as any history of medication that altered the level of ALP, were excluded. Non-probability sampling was used for the sampling.

By taking 3 cc of venous blood under aseptic conditions, the serum ALP level was measured using the AMP (2-amino, 2-methyl, 1 propanol) method.[17] An ALP level of 40 to 120 units per liter is considered normal. Other investigated records were obtained from the patient's admission hospital file record, like L.F., T, RBS, HbA1c%, HBsAg, and anti-HCV levels.

RESULTS

During the study, 55 patients who had suffered a cerebral stroke were selected from the medical wards of the LUMHS hospital Jamshoro and Hyderabad. Using the age as a criterion, the patients were divided into three groups according to their ages. Group A enrolled 13 patients whose ages ranged from 41 to 50 years of age, Group B enrolled 18 patients whose ages ranged from 51 to 60 years, and Group C enrolled 24 patients whose ages ranged from 61 to 70 years old. According to gender demography, 26 males and 29 females were enrolled in group-I and group-II respectively. On the day of the research visit, the serum ALP level was measured at the patient's bedside. In 16 out of 55 patients, the serum ALP level was normal, while in 39 patients, the level was elevated. The normal level of ALP was observed in 29.1% of our research population, while the elevated level of ALP was observed in 70.9%. The mean serum ALP level in 29.1% of the patients was 85.13 ± 10.93 U/L, while the mean serum ALP level in

70.9% of the patients was 231 .42 ± 5.76 U/L. More than 70% of our research population with cerebral stroke has elevated levels of ALP, showing a strong descriptive relationship between ALP and cerebral stroke patients.

Table 1: Gender Distribution

Gender Distribution	
Male (Group-I)	Female (Group-II)
26 (47.27%)	29 (52.72%)

Table 2: Distribution of patients according age groups

Distribution of Groups according to Age		
Group –A (41-50years)	Group-B (51-60 years)	Group-C (61-70 years)
13 (23.63%)	18 (32.72%)	24 (43.63%)

Table 3: Serum ALP levels mean & % with Normal & Elevated Levels

Serum ALP (U/L) Levels	
Group - A (with Normal Levels)	Group –B (with Elevated Levels)
No: of patients 16 (29.1%)	No: of patients 39 (70.9%)
Mean ALP levels = 85.13 ± 10.93	Mean ALP levels = 231 .42 ± 5.76

DISCUSSION

An ischemic stroke is basically an infarction or ischemia of a selected area of the brain caused by inadequate oxygen and blood supply.[18,19] There is a higher incidence of cerebral stroke among older people and more cases are reported among females.[20] In the development of cerebral stroke, hypertension, diabetes mellitus, alcoholism, elder age, smoking, use of narcotic drugs, and dyslipidemia are the risk factors.[3] In clinical practice, multiple blood tools are used for the assessment of cerebral stroke, but none is proven as an authentic biomarker for its diagnosis. For diagnosing cerebral stroke, it is necessary to evaluate some blood markers that can be easily accessed and cheap. Animal studies have shown that Alkaline Phosphatase plays a diagnostic and prognostic role in cerebral stroke.

ALP was first discovered in 1923 by Robert Robison as a highly abundant enzyme found in the bones of animals. [21,22] Mineralization of bones is a basic function of ALP in humans.[22] Electrophoresis shows that ALP contains different isoenzymes. An enzyme called Tissue Non Alkaline Phosphatase (TNAP) is considered to be an isoenzyme of ALP.[11] The TNAP protein is commonly found in bones, intestines, livers and brains.[23] As well as playing a role in inflammatory processes, ALP causes cerebral stroke, which is also an inflammatory injury to brain cells.[24] ALP deactivates ATP production, which is a dangerous signal for injury of cells.[25] TNAP also regulates gene expression in brain and spinal cord cells.[23,26] Furthermore, TNAP plays a crucial role in neurotransmitter synthesis and spinal cord myelination.[13,14] Thus, ALP plays an important role in the development of different brain cells directly or indirectly. The level of ALP varies with age, gender, and pathological conditions. ALP disrupts the hemostasis of brain cells at abnormal levels. Studies on animals and humans have shown that ALP levels vary depending on the type of brain injury.

According to Kim et al. (2017)[27], Makil et al. (2017)[28], stroke patients with cardiovascular problems have disturbed ALP levels. According to Tan et al. (2017)[29], serum ALP levels increased in stroke patients with hypertension. However, another study, published by Ndrepepa et al. (2017)[30], showed that ALP levels were significantly altered in stroke patients who underwent cardiac bypass surgery as a result of stroke.

The results of our study are in agreement with that of Zhong et al. (2018)[31], who reported that serum ALP levels were highly raised in patients with acute ischemic attacks of cerebral stroke as well.

There are some limitations in our study, including our selection of subjects from one hospital, so a larger sample size study is required in the future, along with analysis of serum ALP in patients with cerebral stroke who have underlying different associated risk factors. It is also necessary to compare serum ALP levels between age groups and evaluate the variation of serum ALP levels between genders.

CONCLUSION

As a result, the serum ALP levels increased in patients who suffered a cerebral stroke, which can be a cheap and easily assessable biomarker that can provide insight into the diagnosis of the disease.

REFERENCES

1. Shukla V, Shakya AK, Perez-Pinzon MA, Dave KR. Cerebral ischemic damage in diabetes: an inflammatory perspective. Journal of neuro- inflammation. 2017 Dec;14(1):1-22.
2. Fekadu G, Chelkeba L, Kebede A. Risk factors, clinical presentations and predictors of stroke among adult patients admitted to stroke unit of Jimma university medical center, south west Ethiopia: prospective observational study. BMC neurology. 2019 Dec;19(1):1-1.
3. Jehan S, Farag M, Zizi F, Pandi-Perumal SR, Chung A, Truong A, Tello D, McFarlane SI. Obstructive sleep apnea and stroke. Sleep medicine and disorders: international journal. 2018;2(5):120.
4. Global Health Estimates 2016:Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016. Geneva, World Health Organization. 2018. cited on April 20 2020.
5. Sherin A, Ul-Haq Z, Fazid S, Shah BH, Khattak MI, Nabi F. Prevalence of stroke in Pakistan: Findings from Khyber Pakhtunkhwa integrated population health survey (KP-IPHS) 2016-17. Pak J Med Sci. 2020 Nov-Dec;36(7):1435-1440.
6. Ezejimofor MC, Chen Y-F, Kandala N-B, Ezejimofor BC, Ezeabasili AC, Stranges S, et al. Stroke survivors in low-and middle-income countries:a meta-analysis of prevalence and secular trends. J Neurol Sci. 2016;364:68–76.
7. Martinez-Amezcua P, Haque W, Khera R, Kanaya AM, Sattar N, Lam CS, Harikrishnan S, Shah SJ, Kandula NR, Jose PO, Narayan KV. The upcoming epidemic of heart failure in South Asia. Circulation: Heart Failure. 2020 Oct;13(10):e007218..
8. Ravindranath V, Menesgere AL, Wallin MT. Burden of neurological disease. InNeurobiology of Brain Disorders 2023 Jan 1 (pp. 1047-1064). Academic Press.
9. Zhou X, Khusbu FY, Chen H, Ma C. A turn-on fluorescence assay of alkaline phosphatase activity based on an enzyme-triggered conformational switch of G-quadruplex. Talanta. 2020 Feb 1;208:120453.
10. Wu GJ, Chen KY, Yang JD, Liu SH, Chen RM. Naringin Improves Osteoblast Mineralization and Bone Healing and Strength through Regulating Estrogen Receptor Alpha-Dependent Alkaline Phosphatase Gene Expression. Journal of Agricultural and Food Chemistry. 2021 Nov 1;69(44):13020-33.
11. Komaru K, Ishida-Okumura Y, Numa-Kinjoh N, Hasegawa T, Oda K. Molecular and cellular basis of hypophosphatasia. Journal of oral biosciences. 2019 Sep 1;61(3):141-8.
12. Nwafor DC, Brichacek AL, Ali A, Brown CM. Tissue-Nonspecific Alkaline Phosphatase in Central Nervous System Health and Disease: A Focus on Brain Microvascular Endothelial Cells. International Journal of Molecular Sciences. 2021 May 17;22(10):5257.
13. Sekaran S, Vimalraj S, Thangavelu L. The Physiological and Pathological Role of Tissue Nonspecific Alkaline Phosphatase beyond Mineralization. Biomolecules. 2021 Oct 21;11(11):1564.
14. Bartlett CL, Cave EM, Crowther NJ, Ferris WF. A new perspective on the function of Tissue Non-Specific Alkaline Phosphatase: from bone mineralization to intra-cellular lipid accumulation. Molecular and Cellular Biochemistry. 2022 Apr 26:1-4.
15. Wang H, Chen S, Zhang Y, Xu H, Sun H. Electroacupuncture ameliorates neuronal injury by Pink1/Parkin-mediated mitophagy clearance in cerebral ischemia-reperfusion. Nitric Oxide. 2019 Oct 1;91:23-34.
16. Rawat C, Kukal S, Kushwaha S, Agarwal R, Sharma S, Srivastava AK, Kukreti R. Elevated serum alkaline phosphatase in epilepsy: effect of age and treatment. British Journal of Biomedical Science. 2020 Jan 2;77(1):44-7.
17. Kanta P, Ghosh T, Kaur A, Muthukumarappa T. An innovative and cost-effective way to estimate alkaline phosphatase activity in in vitro cellular model systems. International journal of biochemistry and molecular biology. 2021;12(1):1.
18. Puig J, Shankar J, Liebeskind D, Terceño M, Nael K, Demchuk AM, Menon B, Dowlatshahi D, Leiva-Salinas C, Wintermark M, Thomalla G. From “time is brain” to “imaging is brain”: a paradigm shift in the

management of acute ischemic stroke. Journal of Neuroimaging. 2020 Sep;30(5):562-71.
19. Demeestere J, Wouters A, Christensen S, Lemmens R, Lansberg MG. Review of perfusion imaging in acute ischemic stroke: from time to tissue. Stroke. 2020 Mar;51(3):1017-24.
20. Freisinger E, Sehner S, Malyar NM, Suling A, Reinecke H, Wegscheider K. Nationwide routine-data analysis of sex differences in outcome of acute myocardial infarction. Clinical cardiology. 2018 Aug;41(8):1013-21.
21. Brichacek AL, Brown CM. Alkaline phosphatase: a potential biomarker for stroke and implications for treatment. Metabolic brain disease. 2019 Feb;34(1):3-19.
22. Whyte MP. Hypophosphatasia and how alkaline phosphatase promotes mineralization. InGenetics of bone biology and skeletal disease 2018 Jan; 01; 481-505.
23. Nwafor DC, Brichacek AL, Ali A, Brown CM. Tissue-Nonspecific Alkaline Phosphatase in Central Nervous System Health and Disease: A Focus on Brain Microvascular Endothelial Cells. International Journal of Molecular Sciences. 2021 May 17;22(10):5257.
24. Cui C, Shen D, Zuo D, Ye X. Neuroprotective effects of sonochemical-synthesized SiO2 nanoparticles in vivo models of ischemic/reperfusion injury in stroke. Arabian Journal of Chemistry. 2021 Nov 1;14(11):103416.
25. Villanueva-Paz M, Morán L, López-Alcántara N, Freixo C, Andrade RJ, Lucena MI, Cubero FJ. Oxidative stress in drug-induced liver injury (DILI): from mechanisms to biomarkers for use in clinical practice. Antioxidants. 2021 Mar 5;10(3):390.
26. Lawlor L, Yang XB. Harnessing the HDAC–histone deacetylase enzymes, inhibitors and how these can be utilised in tissue engineering. International journal of oral science. 2019 Jun 10;11(2):1-1.
27. Kim J, Song T-J, Song D, et al. Serum Alkaline Phosphatase and Phosphate in Cerebral Atherosclerosis and Functional Outcomes after Cerebral Infarction. Stroke; 2013; 44:3547–9.
28. Makil ES, Tang X, Frazier EA, Collins RT Alkaline Phosphatase: A Biomarker of Cardiac Function in Pediatric Patients. Pediatric Cardiology 2017; 38:762–9.
29. Tan L-M, Wang L, Chen J-J. Diagnostic performance of bone metabolic indexes for the detection of stroke. Saudi Medical Journal 2017; 37:30–35
30. Ndrepepa G,Xhepa E, Braun S, et al. Alkaline phosphatase and prognosis in patients with coronary artery disease. European Journal of Clinical Investigation; 2017; 47:378–387.
31. Zhong C, You S, Chen J, et al. Serum Alkaline Phosphatase, Phosphate, and In-Hospital Mortality in Acute Ischemic Stroke Patients. Journal of Stroke and Cerebrovascular Diseases; 2018; 27:257–266.