ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
Index Copernicus Value: 68 . 16
PubMed - National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 4, Issue – 4, July - 2019, Page No. : 71 - 75

Bone-specific Alkaline Phosphatase (BAP) in Primary Hypertension-a pilot study

Dhivya Selvaraj[1], Sukanya Gangopadhyay[2], Pradeep Kumar S[3], BC Kabi[4]

[1]Senior Resident, Department of Biochemistry, VMMC and Safdarjung Hospital, New Delhi-110029

[2]Assistant Professor, Department of Biochemistry, VMMC and Safdarjung Hospital, New Delhi-110029

[3]Senior Resident, Department of GI Surgery, AIIMS, New Delhi-110023

[4]Director-Professor, Department of Biochemistry, VMMC and Safdarjung Hospital, New Delhi-110029

Corresponding Author: Sukanya Gangopadhyay, Assistant Professor, Department of Biochemistry, VMMC and Safdarjung Hospital, New Delhi-110029

Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

Background Primary Hypertension is a very common, chronic health problem. It is a significant risk factor for atherosclerosis, cardiovascular diseases, etc.BAPis up-regulated in vascular calcification, CAD, CKD associated cardiovascular events but its association with Primary Hypertention has not been established yet.

Objectives To estimate serum BAP level to evaluate cardiovascular risk in patients with Primary Hypertension.

Materials and methods: We conducted a pilot studyin 75 patients with Primary Hypertension, in the Department of Biochemistry, VMMC &Safdarjung Hospital, New Delhi, focusing on BAP marker in Primary Hypertension. The study group was divided into cases of 75 patients with Primary Hypertension and controls of 30 healthy individuals without Primary Hypertension. Serum BAP was measured using Sandwich - ELISA method. The mean of serum BAP in cases and controls were 656.36pg/ml (range 63.99-3966.97pg/ml) and 120.32pg/ml(range 20.15-563.97pg/ml) respectively. The cases and controls were compared by using Welch's t-test.

Results: Serum BAP was very significantly raised in cases as compared to controls (p value < 0.0001).

Conclusion Our study concludes high serum BAP levels in Primary Hypertension may be related to its pathology. So, these patients should be followed up regularly to assess the risk for cardiovascular events and BAP levels. But, further prospective studies are required to establish this fact.

Introduction Primary Hypertension is a very common, chronic health problem, being the most important etiology of preventable death and disability in the world (1). Its prevalence is estimated as 30–45% of the world population(1). It is a significant risk factor for atherosclerosis, cardiovascular diseases, nephropathy, stroke, etc. It accounts for 45%, 51% of deaths due to heart diseases, stroke respectively and also accounts for 16.5% (9.4 million) of all deaths annually (2).

The new classification of HTN is defined as having consistent Systolic Blood Pressure (SBP) greater than or equal to 130 mmHg and Diastolic Blood Pressure (DBP) greater than or equal to 80 mmHg (3). Primary hypertension constitutes 90% of HTN and has no known etiology (4).There are many defined biomarkers of

hypertension which denotes its pathogenesis and etiology, progression and control, complications.

The different measures of blood pressure areassociated with significant calcification in multiple vascular beds, and that these associationsvary with age.Pulse pressure and isolated systolic hypertension are robust and important correlates for calcified atherosclerosis in different vascularbeds. Isolated systolic hypertension may be clinically relevant in diagnosing or preventing calcified atherosclerosis.(18)

Since vascular calcification contributes to cardiovascular risk in various population subsets (10), makers of vascular calcification canbe an attractive option. Recently, it was suggested thatalkaline phosphatase (ALP) plays a pivotal role in mineral metabolism (11) and might be a molecular marker of vascular calcification.(12) Accordingly, the role of ALP has been highlightedin terms of its effects on vascular disease. The level of ALP was associated with the severity of coronarycalcification; higher ALP level was correlated with obvious and heavy calcification. The levels of tissue-non-specific ALP were also correlated with that of bone-specific ALP, which has been reported to be a useful marker of vascular calcification (17). Recently, several studies reported a significant link between ALP and adverseoutcome in patients with chronic kidney disease or those under haemodialysis(13) Cardiovascular complications are the leading cause of death in patients with chronic kidney disease (CKD). Vascular calcification is a common complication inCKD, and investigators have demonstrated that the extent and histoanatomic type of vascular calcification are predictors of subsequent vascular mortality.(19) Furthermore, two recent papers showed thathigher ALP levels are associated with an excess risk of deathamong survivors of stroke(14) or MI.(15)

Serum alkaline phosphatase (ALP) is a member of a family of zinc metalloprotein enzymes that function to split off a terminal phosphate group from an organic phosphate ester.Active center of ALP enzymes includes a serine residue. Mg and Zn ions are required for minimal activity. Many things may cause increases of ALP activity in serum, the most common being obstructive liver disease and metabolic bone disease. There are four different genes coding ALP isozymes in humans. Of these, three are tissue specific (TSALP), the ALPI, coding the intestinal ALP, ALPP, coding the placental ALP, andALPPL, coding the germ-cell ALP. The fourth gene locus, ALPL, is expressed in differenttissues and therefore termed tissue-nonspecific ALP (TNALP) (8).An increase of the liver or particularly the boneisoform (bone specific ALP) in serum can provide valuable diagnostic information. BAP is mainly secreted by osteoblast cells and has an important role in bone formation and skeletal mineralization. Bone isoenzyme is elevated as a result of increased osteoblastic activity (6-9).

BAP is up-regulated in vascular calcification, CAD, CKD associated cardiovascular events but its association with Primary Hypertention has not been established yet.

Considering that vascular calcification contributes to atherosclerosis, vascular hardening, and ageing (16) serum BAP levels mayalso be linked with the risk of cardiovascular events in the population of Primary Hypertension. Moreover, no previous study has investigated the relationbetween BAP andPrimary Hypertension.To evaluate the potential role of BAP as a marker in Primary Hypertension, we assessed the hypothesis thathigher BAP levels are related with poor clinical outcomes interms of all-cause mortality.

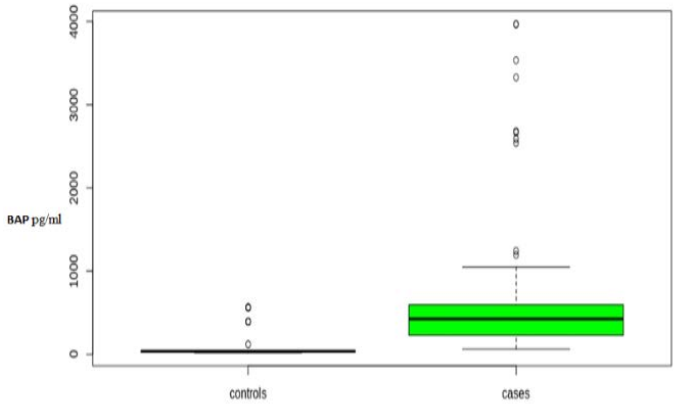
Materials and Methods

The present study was carried out in the Department of Biochemistry in association with the Department of Medicine, Vardhman Mahavir Medical College and Safdarjung Hospital,75 hypertensive patients and 30 healthy volunteers were recruited into the study. The study was a clinical based case control study and was carried out after obtaining a clearance from Institute human ethical committee and informed consent from each study subjects in their local language. Inclusion criteria: Patients's age more than 30 years. The age of the study groups was between 30and 60 years. Exclusion criteria:Pregnant and lactating women, patients with Diabetes mellitus, chronic alcohol abuse, secondary hypertension causes like chronic kidney disease, primary aldosteronism, renovascular disease, chronic steroid therapy, etc were excluded.

Brief history was taken from all the subjects and clinical examination including BP measurement was done. Blood pressure was measured by auscultatory method using an aneroid sphygmomanometer. After BP measurement, subjects were divided into hypertensives if SBP> 140 and DBP>90 and normotensives if SBP< 140 and DBP< 90(according to JNC 8 guidelines).

Five ml of blood sample (from both normal and hypertensive individuals) was collected in labelled clot activating tubes. The individual samples were centrifuged at 1200 rpm for 10 min and the serum was stored at 4°C for 24 hours or at 10°C or lower if the analysis were to be done at a later date. The separated serum was used for the analysis of Alkaline Phosphatase - ALP (Total), BAP. ALP assay was carried out using procedure approved by the IFCC. It was estimated based on a spectrophotometric method by a fully automated random access chemistry analyzer. The level of BAP was determined by ELISA. We used the kit of E lab science and it was based on the principle of Sandwich-ELISA method. The ELISA plate wells were pre-coated with the antibody specific BAP. Standards of samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for BAP and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain BAP, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour turns yellow. The OD is measured spectrophotometrically at a wavelength of 450nm. The OD value is proportional to the concentration of BAP. We calculate the concentration of BAP in the samples by comparing the OD of the samples to the standard curve.

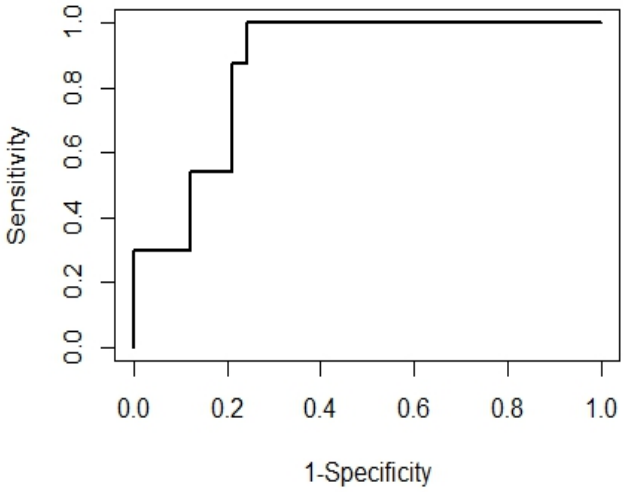
Results



The mean serum BAP in cases and controls were 656.36pg/ml (range 63.99-3966.97pg/ml) and 120.32pg/ml (range 20.15-563.97pg/ml) respectively.The cases and controls were compared by using Welch's t-test (R statistical software version 3.5.1).Serum BAP was

found to be significantly different in cases as compared to controls (p value < 0.0001).

ROC curve for BAP for the prediction of Primary Hypertension



Receiver Operator Characteristics analysis showed BAP have good predicting power, with the AUC of 0.87. The cutoff value was52.9 pg/mL with the sensitivity of 100% and specificity of specificity of 76%.

Discussion

This is first study to check BAP levels in Primary Hypertension, although ALP levels have been studied in Hypertensive disorders of Pregnancy, cerebral small vessel disease, CAD. Calcium, Albumin, ALP levels were normal in the study group. Bone disorders were ruled out as BAP levels were in normal range, and also with the absence of signs and symptoms of any bone disorder. Calcification of atherosclerotic plaques, media of the vessel wall causes the stiffness of the vessel wall in Primary Hypertension. Though BAP levels are in normal range, there is significant difference in the levels between the two groups, which is the important information that has to be explored in future. Enzymatic differences of the BAP isoforms with respect to ppi, rare reports concerning TNALP gene polymorphisms also have to be considered in this regard. Moreover, in ROC curve analysis, BAP showed increased sensitivity, better specificity and AUC. This suggests, the use of BAP (cutoff value > 52.9 pg/mL) as better predictor of vascular calcification in Primary Hypertension.

Biomarkers may or may not necessarily correlate with patient's clinical symptoms. As the diagnosis and treatment of HTN are mainly guided by conventional BP readings, BAP may prove to be useful in understanding the pathogenesis, progression, and therapeutic efficacy.

Limitations: The sample size of the study has to be increased. Vascular calcification has to be confirmed by investigations.BMD, calcium regulating hormones have to be checked in the study groups to rule out bone disorders.

Future perspectives: Further studies are needed to evaluate the possibility of a causal link of BAP to vascular calcification. To consider an BAP targeting strategy, in the treatment of Primary Hypertension could be the next step.

Conclusion

Our study concludes serum BAP levels in Primary Hypertensive individuals may be related to its pathology. So, these patients should be followed up regularly to assess the risk for cardiovascular events and BAP levels. But, further prospective studies are required to establish this fact.

References

1. Schmieder RE. End organ damage in hypertension. DeutschesArzteblatt international 2010;107:866-73.
2. WHO. A global brief on hypertension. World Health Organization, 2013.
3. Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/

AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/ NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/ American Heart Association Task Force on Clinical Practice Guidelines. J Am CollCardiol 2017. [Epub ahead of print].

4. Kossaify A, Garcia A, Succar S, et al. Perspectives on the value of biomarkers in acute cardiac care and implications for strategic management. Biomark Insights 2013;8:115-26.
5. Griffiths G & Black J. Separation and identification of alkaline phosphatase isoenzymes and isoforms in serum of healthy persons by isoelectric focusing. Clin chem. 1987;33(12):2171-77.
6. Guyer P.B., Chamberlain A.T. (1980) BMJ, 280, 985.
7. Hata K., Tokuhiro H., Nakatsuka K., Miki T., NishizawaY., Morii H., Miura M. (1996) Ann. Clin. Biochem., 33,127-131.
8. Irving K., Jawad A. (2005) Grand Rounds, 5, 23-25.
9. Seabrook R.N., Bailyes E.M., Price C.P., Siddle K.,Luzio J.P. (1988) Clin. Chim. Acta, 172, 261-266.
10. Detrano R, Guerci AD, Carr JJ, Bild DE, Burke G, Folsom AR, Liu K, Shea S,Szklo M, Bluemke DA, O'Leary DH, Tracy R, Watson K, Wong ND,Kronmal RA. Coronary calcium as a predictor of coronary events in four racialor ethnic groups. N Engl J Med 2008;358:1336–1345.
11. Weiss MJ, Cole DE, Ray K, Whyte MP, Lafferty MA, Mulivor RA, Harris H. A missensemutation in the human liver/bone/kidney alkaline phosphatase gene causinga lethal form of hypophosphatasia. ProcNatlAcadSci USA 1988;85:7666–7669.
12. Johnson RC, Leopold JA, Loscalzo J. Vascular calcification: pathobiologicalmechanisms and clinical implications. Circ Res 2006;99:1044–1059.
13. 7. Regidor DL, Kovesdy CP, Mehrotra R, Rambod M, Jing J, McAllister CJ, VanWyck D, Kopple JD, Kalantar-Zadeh K. Serum alkaline phosphatase predicts mortalityamong maintenance hemodialysis patients. J Am SocNephrol 2008;19:2193–2203.
14. RyuWS, Lee SH, Kim CK, Kim BJ, Yoon BW. Increased serum alkaline phosphataseas a predictor of long-term mortality after stroke. Neurology 2010;75:1995–2002.
15. Tonelli M, Curhan G, Pfeffer M, Sacks F, Thadhani R, Melamed ML, Wiebe N,Muntner P. Relation between alkaline phosphatase, serum phosphate, and allcauseor cardiovascular mortality. Circulation 2009;120:1784–1792.
16. O'Neill WC. Pyrophosphate, alkaline phosphatase, and vascular calcification. Circ Res 2006;99:e2.
17. Jun-Bean Park, Do-yoon Kang, Han-Mo Yang, et al. Serum alkaline phosphatase is a predictor of mortality, myocardial infarction, or stent thrombosis after implantation of coronary drug-eluting stent. European Heart Journal 2013;34:920–931
18. Nicole E. Jensky1, Michael H. Criqui1, Michael C. Wright1, Christina L. Wassel1, Steven A.Brody2, and Matthew A. Allison1. Blood pressure and vascular calcification.Hypertension2010 April ; 55(4): 990–997.
19. Masahide Mizobuchi, Dwight Towler, and Eduardo Slatopolsky. Vascular Calcification: The Killer of Patients with Chronic Kidney Disease. J Am SocNephrol2009; 20: 1453–1464.