International Journal of Medical and Health Research
Online ISSN: 2349-4182 Print ISSN: 2349-5979
www.medicalsjournals.com
Volume 2; Issue 2; February 2016; Page No. 39-43

Serum Alkaline Phosphatase Level in the Liver of Heroin Addicts

[1, 2] Sahrish Farooqi, [2] Tooba Altaf, [1] Hira Mubeen, [1] Shahid Raza

[1] Department of Biotechnology, Faculty of Biological Sciences, University of South Asia, Lahore, Pakistan
[2] Department of Biochemistry, Kinnaird College for Women, Lahore, Pakistan

Abstract
Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. This study aimed to evaluate whether serum alkaline phosphatase level is disturbed in the liver of heroin addicts or not. Data was collected from the patients who were admitted in the addiction center of Punjab institute of Mental Health. Alkaline phosphatase level, HCV and HBV were checked in heroin addicts and control subjects. Study was conducted on total 25 heroin addicts. Nearly 36% heroin addicts showed elevated level of alkaline phosphatase as compared to normal level of this enzyme. Only 4% addicts showed value below normal.

Keywords: Serum Alkaline Phosphatase, Heroin Addicts

Introduction

Alkaline phosphatase (ALP) is a ubiquitous membrane-bound glycoprotein that catalyzes the hydrolysis of phosphate monoesters. ALP is classified into four isozymes in humans. This classification is based according to the specificity of the tissue to be expressed (Sligbrand, 1984) [42]. It is expressed in many tissues throughout the body and is especially abundant in hepatic, skeletal, and renal tissue (McComb *et al,* 1979; Tsai *et al.,* 2000) [28, 47]. This enzyme is product of at least three ALP gene loci (placental, intestinal and L/B/K) (Henthorn *et al.,* 1982; Millan *et al.,* 1995; Muller et al., 2000) [30, 32] which are distinguishable in man by a variety of structural, biochemical and immunologic methods (Mulivor *et al.,* 1978; McKenna *et al.,* 1978; Harris, 1986) [31, 12]. Liver ALP is located near the end of the short arm of chromosome 1 (Smith *et al.,* 1988; Orimo, 2010) [43, 36]. Slight differences in electrophoretic mobility and thermo stability between the Liver/bone/kidney ALPs from various tissues are attributed to differences in post-translational modification. More than 95% of serum ALP activity is derived from hepatocytes and osteoblasts (Shimazak *et al.,* 2005) [41]. Anchored liver ALP is used as a canalicular membrane marker in hepatocytes and is localized to relatively rigid sphingomyelin-rich patches on that surface (Imjeti *et al.,* 2011; Bertone *et al.,* 2011; Ismair *et al.,* 2009; Inoue *et al.,* 1983; Nichols *et al.,* 2003) [16, 3, 18, 17, 34]. ALP plays an active role in down regulating the secretory activities of the intrahepatic biliary epithelium, including decreasing bile flow and biliary bicarbonate excretion (Alvaro *et al.,* 2000) [2]. It also carries out endotoxin detoxification and can reduce organ injuries (i.e., in the lungs, liver, and kidneys) in conditions such as ischemic-reperfusion damage or septic shock (Poelstra *et al.,* 1997; van Veen *et al.,* 2006; Heemskerk *et al.,* 2009) [37, 48, 13]. Growing children, pregnant women, people consuming a high-fat diet and healthy individuals who have the blood group O or B and secrete the H-blood group substance in the postprandial period have been found to have abnormally high ALP levels (Cho *et al.,* 1995).
The activity of liver and bone ALP in serum has been applied extensively in routine diagnosis. Estimation of ALP is significant to score reflecting liver function reserve (the Child-Pugh class and the Model for End-stage Liver Disease (MELD)) (European Association for the Study of the Liver and European Organisation for Research and Treatment of Cancer, 2012; The Cancer of the Liver Italian Program (CLIP) Investigators, 1998; [45] Kudo *et al.,* 2004; [24] Kamath *et al.,* 2001; [22] chan *et al.,* 2001). The discriminatory power of ALP in predicting overall and disease-free survival by showing highest c-indices has been reported (Chan *et al.,* 2015) [5].
The goal of this study is to identify the proportion of serum ALP levels in heroin addicts. Secondary goals are to identify the proportion of HCV in those heroin addicts.

Methodology

Total 25 patients of 25-45 years who were heroin addicts were included. Their behavioral parameters and clinical history were recorded. Nearly 21 heroin addicts were taken from the outdoor of Punjab Institute of Mental Hospital. The rest of the heroin addicts were taken from the psychiatric outdoor of Sir Ganga Ram Hospital. Control samples were taken from male individuals having healthy liver with no other significant disease. Then 5 ml of blood sample of each individual was taken from each participant. Then blood was centrifuged and serum was kept for storage in container at -20 °C. This serum is then utilized for performing anti HCV and HBsAg test screening. The rest of serum was then utilized for performing liver function tests using humalyzer 3000. The enzyme substrates were prepared for ALP using pre-prepared buffer available in kit. The 2ml reagent provided in kit was taken and mixed with 8ml buffer to make 10ml substrate. Then after keeping this substrate test tube in water bath, nearly 10 µl of serum was mixed with the enzyme substrate and then subjected to humalyzer. The results were recorded. This equipment is provided with standard values for enzyme levels and thus provide direct clue about the range of normal and elevated levels in the form of graph and numeric value. After checking the level of ALP and testing for HBV and HCV in heroin addicts, blood samples of controls were taken and same tests were performed. Then the results came were compared and analyzed between heroin takers and controls.

For estimation of alkaline phosphatase (ALP), after preparing substrate from buffer and reagent, 10µl serum was added into the test tube. Its absorbance was measured in the humalyzer. The principle used for ALP:

P-Nitrophenylphosphate+H_2O → Phosphate + p-nitrophenol

Procedure

Pipette into cuvettes	25 ˚C, 30 ˚C, 37 ˚C
Sample	20 µl
Buffer	1000 µl

It was mixed and incubated for 1 min at 37 ˚C.

Substrate	250 µl

It was mixed and absorbance was read exactly after 1 minute.

Anti HCV and HBsAg test screening

This screening was carried out with the help of screening strips. A small drop of serum was poured into the well of strip. The serum moved on the strip. It left mark of positive or negative result.

Result

Many heroin addicts had elevated serum alkaline phosphatase. Nearly 36% heroin addicts showed elevated level of alkaline phosphatase as compared to normal level of this enzyme. Only 4% addicts showed value below normal. The maximum number of heroin dependents had elevated serum alkaline phosphatase 255.32 U/l which was significantly higher than the mean values 224.2 U/l in control subjects.

Table1: Levels of ALP in heroin addict and control group.

Group	Mean(U/l)	N	Std. Deviation
Heroin	255.3200	25	65.31763
Control	224.2000	25	43.66921

Level of ALP in HCV positive patients

Addicts with positive HCV tests showed significantly high alkaline phosphatase level. The values of ALP are taken on the x-axis and count or number of addicts is taken on the y-axis.

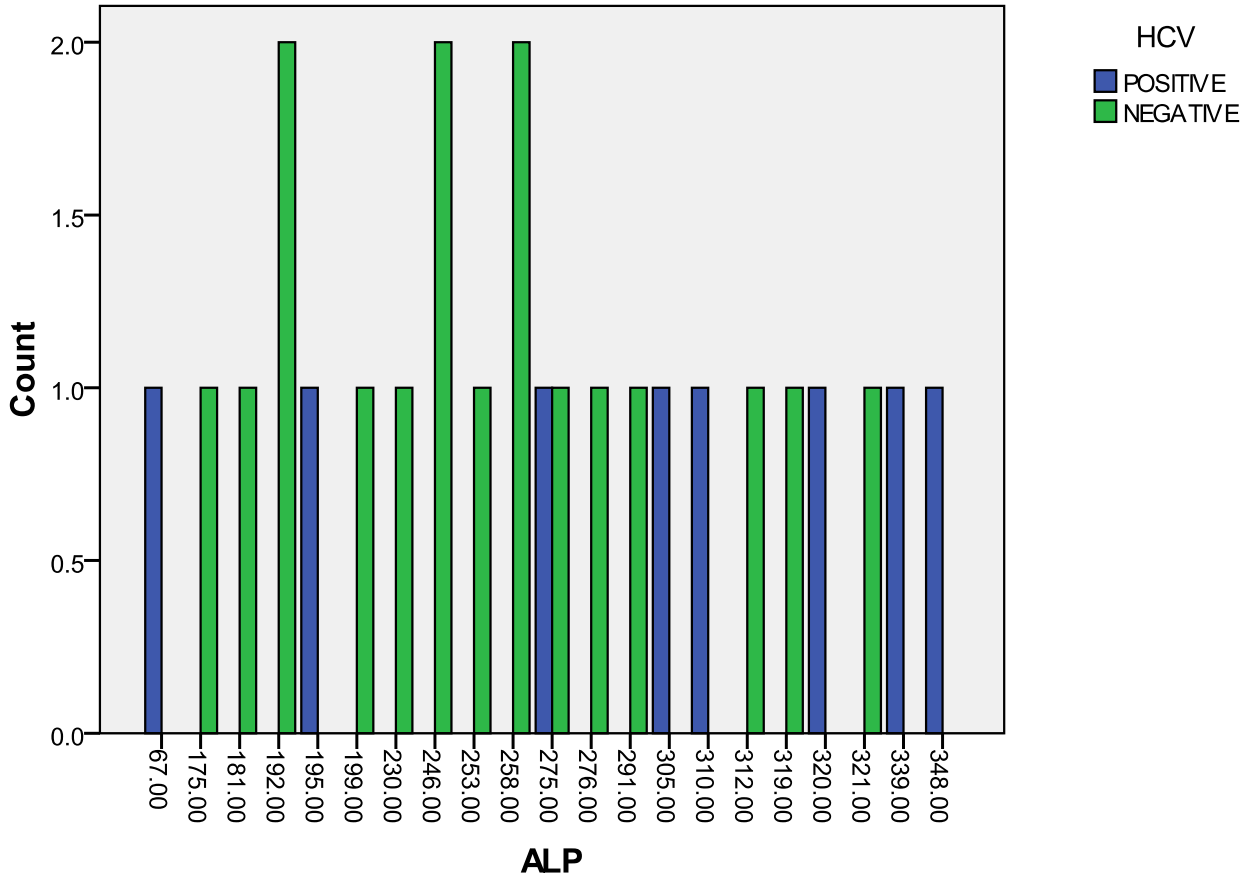

Fig 1: Comparison of ALP levels of HCV positive and HCV negative heroin addicts.

Level of ALP in HBsAg positive patients: Addicts with positive HBsAg test showed high ALP level. The values of ALP are taken on the x-axis and number of addicts is taken on the y-axis.

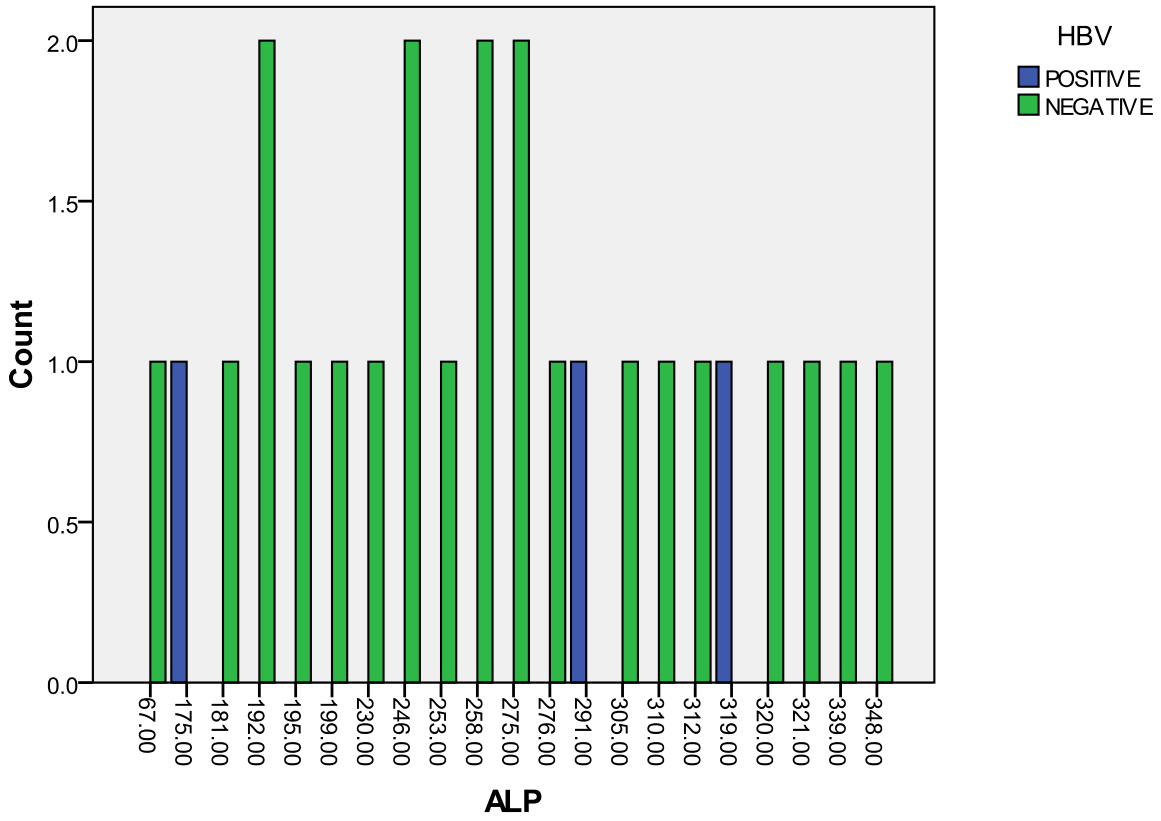

Fig 2: Comparison of ALP in HBsAg positive and HBsAg negative heroin addicts.

Discussion

Elevation of ALP level may be due to toxic and allergic manifestations of heroin on liver (Louria *et al.,* 1967) [26]. Elevated ALP indicates damaged liver cells (Rodan & Rodan, 1984) [39]. High ALP levels can show that the bile ducts are obstructed. Patients with leukemia have increased Alp activity (Massey et al., 1996) [27]. The serum ALP level is a marker of activated osteoblast activity (Ho *et al.,* 2013) [15]. Alkaline phosphatases are elevated in obese persons and strong predictors of poor health outcomes, including greater risk for mortality and cardiovascular events in older adult (Wannamethee *et al.,* 2013; Golik *et al.,* 1991) [49, 11]. Enhanced production and excretion of ALP into the bile has been observed after auxiliary liver transplantation. Chronic exposure of ALP cause further increase in ALP and gamma-glutamyl transpeptidase activities in the intrahepatic bile ducts and hepatocyte canalicular pole. This promotes enlargement of bile canaliculi, and decreases bile flow and biliary bicarbonate excretion (Jersky et al., 1974) [19]. Some drugs like cyclosporine use is associated with increased serum ALP levels after renal transplantation (Briner *et al.,* 1993; Loertscher *et al.,* 1983) [4, 25]. Lowered levels of ALP are less common than elevated levels. Serum alkaline phosphatase level fell with antibiotic treatment (Natsag *et al.,* 2012) [33]. Drugs like oral contraceptives have been demonstrated to reduce alkaline phosphatase (*Schiele et al., 1998*) [40]. Abnormal liver enzymes were observed in serum in intravenous heroin addicts. Liver function abnormalities found in intravenous heroin addicts (Tennant & Moll, 1995; Cooper, *et al.,* 1975; Pollock *et al.,* 1978) [44, 8, 38]. Abusers of both alcohol and parenteral drugs have an increased risk of developing cirrhosis (Novick *et al.,* 1986) [35].

Acknowledgements

The authors would like to thank the administration of Sir Ganga Ram Hospital for allowing us to perform the laboratory work at their place. We are also thankful to Dr. Muhammad Waseem of Punjab Institute of Mental Hospital for granting us the permission to get blood samples of heroin addicts from the psychiatric outdoor of Punjab Institute of Mental Hospital. We would also appreciate the support given by head of the Department and teachers of Biochemistry department of Kinnaird College.

References

1. Abramowitz M. Serum alkaline phosphatase and phosphate and risk of mortality and hospitalization. Clin J Am Soc Nephrol. 2010; 5(6):064-1071.
2. Alvaro D. The function of alkaline phosphatase in the liver: regulation of intrahepatic biliary epithelium secretory activities in the rat. Hepatology. 2000; 32:174e84.
3. Bertone V. Altered alkaline phosphatase ctivity in obese zucker rats liver respect to lean zucker and wistar rats discussed in terms of all putative roles ascribed to the enzyme Eur. J Histochem. 2011; 55:24-32.
4. Briner VA. Cyclosporin A-induced transient rise in plasma alkaline phosphatase in kidney transplant patients. Transpl Int. 1993; 6:99e107.
5. Chan. Albumin-to-Alkaline Phosphatase Ratio: A Novel Prognostic Index for Hepatocellular Carcinoma Dis Markers. 2015: 564057.
6. Chan SL. Prospective validation of the Chinese University Prognostic Index and comparison with other staging systems for hepatocellular carcinoma in an Asian

population. J of Gastroenterology and Hepatology. 2011; 26(2):340-347.
7. Cho SR. Unusually high alkaline phosphatase due to intestinal isoenzyme in a healthy adult. Clin Chem Lab Med. 2005; 43:1274e5.
8. Cooper AD. Liver disease in nonparenteral drug abusers. JAMA. 1975; 233(9):964-6.
9. European Association for the Study of the Liver and European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J of Hepatology. 2012; 56(4):908-943.
10. Fishman WH. Alkaline phosphatase isozymes: recent progress. Clin Biochem. 1990; 23:99-104.
11. Golik A. Elevated serum liver enzymes in obesity: a dilemma during clinical trials. Int J Obes. 1991; 15(12):797-801.
12. Harris H. The harvey lectures: series 76. New York: Academic. 1986, 95-123.
13. Heemskerk S. Alkaline phosphatase treatment improves renal function in severe sepsis or septic shock patients. Crit Care Med. 2009; 37:417e23. e1.
14. Henthorn PS. Different missense mutations at the tissue nonspecific alkaline phosphatase genes locus in autosomal recessively inherited forms of mild and severe hypophosphatasia. Proc Natl Acad Sci. 1992; 89:9924-9928.
15. Ho. Isolated increase in serum alkaline phosphatase after liver transplantation: Risk factors and outcomes analysis International Journal of Surgery. 2013; 11:92e95.
16. Imjeti NS. N-glycosylation instead of cholesterol mediates oligomerization and apical sorting of GPI-APs in FRT cells. Mol. Biol. Cell, 2011; 22:4621-4634.
17. Inoue M. Rat liver canalicular membrane vesicles. Isolation and topological characterization J Biol Chem. 1983; 258:5183-5188.
18. Ismair MG. Stieger ABC-transporters are localized in caveolin-1-positive and reggie-1-negative and reggie-2-negative microdomains of the canalicular membrane in rat hepatocytes. Hepatology. 2009; 49:1673-1682.
19. Jersky J. Alkaline phosphatase in auxiliary liver transplantation. Gut. 1974; 15:636e43.
20. Jono S. Vascular calcification in chronic kidney disease. J Bone Miner Metab. 2006; 24(2):176-181.
21. Kalantar-Zadeh K. Kidney bone disease and mortality in CKD: Revisiting the role of vitamin D, calcimimetics, alkaline phosphatase, and minerals.Kidney Int Suppl. 2010; (117):S10-21.
22. Kamath PS. A model to predict survival in patients with end-stage liver disease. Hepatology. 2001; 33(2):464-470.
23. Kim JM. The effect of alkaline phosphatase and intrahepatic metastases in large hepatocellular carcinoma. World J Surg Oncol. 2013; 21(11):40.
24. Kudo M. Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with the CLIP score. Hepatology. 2004; 40(6):1396-1405.
25. Loertscher R. Persistent elevation of alkaline phosphatase in cyclosporine-treated renal transplant recipients. Transplantation. 1983; 36:115e6.
26. Louria DB. The major medical complications of heroin addiction, Annuals Inernal Medicine. 1967; 67(1):1-21.
27. Massey GV. Benign transient hyperphosphatasemia in children with leukemia and lymphoma. Clin Pediatr. 1996; 35:501e4.
28. McComb RB. Alkaline phosphatase. New York: Plenum Publishing Corp, 1979.
29. McKenna MJ. Comparison of human alkaline phosphatase isoenzymes. structural evidence for three protein classes. Biochem J. 1979; 181:67-73.
30. Millan JL, Fishman WH. Biology of human alkaline phosphatase with special reference to cancer. Crit Rev Clin Lab Sci. 1995; 32:1-39.
31. Mulivor RA. Developmental change in human intestinal alkaline phosphatase. Ann Hum Genet. 1978; 42:1-13.
32. Muller H. Asp361Val mutant of alkaline phosphatase found in patients with dominantly inherited hypophosphatasia inhibits the activity of the wild-type enzyme. J Clinical Endocrinol Metabolism 2000; 85:743-747.
33. Natsag J. A mysterious gram-positive Case Rep Infect Dis. rods. 841834, 2012.
34. Nichols BJ. GM1-containing lipid rafts are depleted within clathrin-coated pits Curr. Biol. 2003; 13:686-690.
35. Novick DM. Chronic liver disease in abusers of alcohol and parenteral drugs: a report of 204 consecutive biopsy-proven cases. Alcohol Clin Exp Res. 1986; 10(5):500-5.
36. Orimo H. The mechanism of mineralization and the role of alkaline phosphatase in health and disease. J Nippon Med Sch. 2010; 77:4-12.
37. Poelstra K. A physiologic function for alkaline phosphatase: endotoxin detoxification. Lab Invest. 1997; 76:319e27.
38. Pollock AA. Hepatitis associated with high-dose oxacillin therapy. Arch Intern Med. 1978; 138(6):915-7.
39. Rodan GA, Rodan SB. In: Advances in bone and mineral research annual II. Peck WA, editor. Amsterdam: Excerpta Medica. 1984, 244-285.
40. Schiele F. Biological effects of eleven combined oral contraceptives on serum triglycerides, gamma-glutamyltransferase, alkaline phosphatase, bilirubin and other biochemical variables. Clin Chem Lab Med. 1998; 36(11):871-8.
41. Shimazaki C. High serum bone-specific alkaline phosphatase level after bortezomib-combined therapy in refractory multiple myeloma: possible role of bortezomib on osteoblast differentiation. Leukemia. 2005; 19:1102e3.
42. Sligbrand T. Present status and future trends of human alkaline phosphatases. Prog Clin Biol Res. 1984; 166:3-14.
43. Smith M. Regional assignment of the gene for human liver/bone/kidney alkaline phosphatase to chromosome 1 p36.1–p34. Genomics. 1988; 2:139-143.
44. Tennant F, Moll D. Seroprevalence of hepatitis A, B, C, and D markers and liver function abnormalities in intravenous heroinaddicts. J Addict Dis. 1995; 14(3):35-49.
45. The Cancer of the Liver Italian Program (CLIP) Investigators. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients. Hepatology. 1998; 28(3):751-755.

46. Tonelli M. Relation between alkaline phosphatase, serum phosphate, and all-cause or cardiovascular mortality. Circulation. 2009; 120(18):1784-1792.
47. Tsai LC. Expression and regulation of alkaline phosphatases in human breast cancer MCF-7 cells. Eur J Biochem. 2000; 267:1330-1339.
48. Van Veen SQ. Alkaline phosphatase reduces hepatic and pulmonary injury in liver ischaemiaereperfusion combined with partial resection. Br J Surg. 2006; 93:448e56.
49. Wannamethee SG. Alkaline pohsphatase, serum phosphate, and incident casriovascular disease and total mortality in older men. Areterioscler Thromb Vasc Biol. 2013; 33:1070-1076.
50. Wolf PL. The significance of transient hyperphosphatasemia of infancy and childhood to clinician and clinical pathologist. Arch Pathol Lab Med. 1995; 119:774e5.