EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 2
36-39
Year: 2016

ESTIMATION OF SERUM ASPARATATE AMINOTRANSFERASE IN HEROIN ADDICTS

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

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

***Author for Correspondence: Sahrish Farooqi**
Department of Biotechnology, Faculty of Biological Sciences, University of South Asia, Lahore, Pakistan

Article Received on 01/12/2015 **Article Revised on 22/12/2015** **Article Accepted on 13/01/2016**

ABSTRACT
This study sought to investigate the effect of heroin drug on the serum level of Aspartate transaminase in heroin addicts. The liver function tests were conducted on 25 serum samples of heroin addicts and 25 control serum samples. Marked elevation in serum AST were observed in heroin addicts. Nearly 68% heroin dependents showed elevated level of Aspartate aminotransferase. The results from this study suggest that heroin use elevates the level of serum Asparatate aminotransferase.

KEYWORDS: Heroin, serum, Aspartate transaminase, liver function tests.

INTRODUCTION

Aspartate transaminase (AST) is also known as aspartate aminotransferase or serum glutamic oxaloacetic transaminase. It was the first purified transaminase family member (Gelfand & Steinberg, 1977). AST is generally localized within the liver and heart (Jones & Blackmore, 1982). The mammalian aspartate aminotransferase enzyme exists in two isoenzymic forms: mitochondrial and cytosolic aspartate aminotransferase. These two isoenzymes are produced from an identical ancestral AST gene and share approximately 45% amino acid sequence homology (Rej, 1978). AST belongs to the class I of pyridoxal-phosphate dependent aminotransferase. This class comprises 11 proteins in the human proteome. AST is a dimer consisting of two identical subunits. It has about 400 amino acid residues with a molecular mass of about 45 kDa (Kirsch *et al.*, 1984). AST carries out transformation of the amino group from aspartate or glutamate to the corresponding ketoacid. Although the chemical reaction mediated by a transaminase was initially described in 1950 (Meistera & Tice, 1950).

AST enzymatic activity measured in circulation actually reflects relevant aspects of the physiology and pathophysiology of the liver function beyond hepatocyte membrane disruption (Sookoian & Pirola, 2012). Changes in the levels of serum AST are useful serological markers for the diagnosis of hepatic diseases and for the evaluation of therapeutic efficacy and adverse hepatic effects of drug treatment (Pratt & Kaplan, 2000). The liver plays a central role in the pharmacokinetics of most drugs. Liver damage results in reduction in drug-metabolizing activities. (Williams & Mamelok, 1980; Westphal & Brogard, 1997; Verbeeck & Horsmans, 1998; Delcò *et al.*, 2005; Verbeeck, 2008; Periáñez-Párraga *et al.*, 2012; Ali *et al.*, 2013). Intravenous heroin intake leads to significant morphological changes in the liver tissue (vesicular changes, fat changes, chronic hepatitis, and cirrhosis). The intensity of these changes increases with duration of heroin usage.

The goal of this study is to identify the proportion of serum AST 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 AST using preprepared buffer available in kit. The 2 ml reagent provided in kit was taken and mixed with 8 ml buffer to make 10 ml substrate. Then after keeping this substrate test tube in water bath, nearly 50µl of serum (AST) was mixed with the enzyme substrate and

then subjected to humalyzer. 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 AST 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 Aspartate aminotransferase (AST), after preparing substrate from buffer and reagent, 50µl serum was added into the test tube. Its absorbance was measured in the humalyzer. The principle used was
2-oxoglutarate + L-alanine →L-Glutamate + private
Pruvate + NADH + H^+ → L-lactate + NAD^+

Procedure

Pipette into cuvettes	25˚C, 30˚C	37˚C
Sample	200µl	100µl
Buffer	1000µl	1000µl

It was mixed, incubated for 5 min at 37˚C

Substrate	250µl	250µl

It was mixed and its absorbance was read 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.

RESULTS
Aspartate Aminotransferase
Maximum number of heroin addicts had elevated level of aspartate aminotransferase. Nearly 68% heroin dependants showed elevated level of aspartate aminotransferase, 4% showed below normal values and 28% showed normal level of enzyme. The mean serum aspartate aminotransferase in heroin dependants was 75.00U/L. The control subjects had the mean value of 39.40U/L. Statistically significant difference was observed in both the values.

Table1: Levels of AST in heroin addicts and control group.

Group	Mean (U/I)	N	Std. Deviation
Heroin	75.0000	25	48.96257
Control	39.4000	25	4.42531

Level of SGOT (AST) in HCV positive patients
Addicts who were positive for HCV tests had elevated AST level. The values of AST are taken on the x-axis and count or number of addicts is taken on the y-axis.

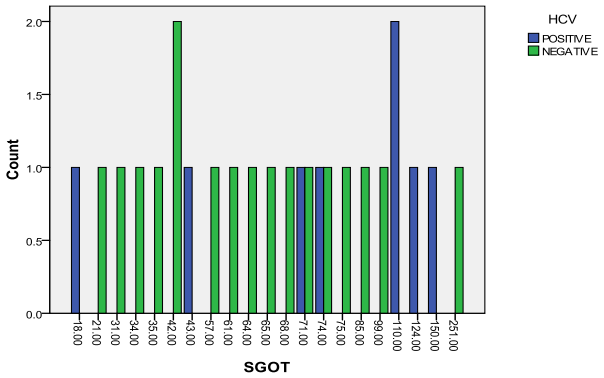

Figure1: Comparison of AST in HCV positive and HCV negative heroin addicts.

Level of SGOT (AST) in positive HBsAg addicts
Addicts carrying positive HBsAg tests had high AST level than normal AST level. The values of AST are taken on the x-axis and number of addicts is taken on the y-axis.

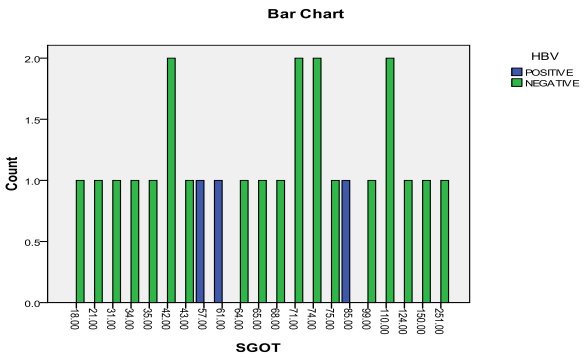

Figure 2: Comparison of SGOT in HBsAg positive and HBsAg negative heroin addicts.

DISCUSSION
Serum level of AST is regarded as markers of liver injury, including a wide range of etiologies from viral hepatitis to fatty liver (Pratt & Kaplan, 2000). The first report of the role of liver transaminases in the prediction of liver cellular damage was published in 1955 by Molander and colleagues, after noticing that the levels of AST were elevated after acute myocardial infarction (Molander *et al.* 1995). This high serum AST activity is mostly attributed to the accompanying acute central necrosis of the liver associated with circulatory changes (Killip, 1960). Notably, while the correlation between the degree of hepatocyte injury and amininotransferase levels is poor (Pratt & Kaplan, 2000). It is accepted that blood level of AST is a consequence of the liver cell membrane damage, with the subsequent leakage of intracellular enzymes into the circulation, especially the cytosolic ones (Kamiike *et al.*, 1989; Reichling & Kaplan, 1988). The serum level of transaminases is affected by demographic ones, such as sex, age and ethnicity; anthropometric features, such as waist circumference and body mass index; and drug abuse

(Ioannou *et al.*, 2006). AST levels are highly heritable, with additive genetic effects accounting for 48% and 32% of the variation, respectively (Whitfield *et al.*, 2002). Serum AST was shown to be the factor most strongly associated with significant HCV recurrence within one year. Serum AST had the significant impact on progression of fibrosis as compared to ALT (Tanaka *et al.*, 2013). Association between AST and graft fibrosis in post-liver transplant settings has also been suggested. It may be because AST is present in both mitochondria and cytoplasm while ALT is located only in cytoplasm, and thus the recurrent hepatitis C which is usually more aggressive than in non-liver transplant settings may lead to more AST release with mitochondrial damage (Benlloch *et al.*, 2005; Cross *et al.*, 2010). Abnormal levels of liver enzymes reflect high levels of hepatic transamination of amino acids in the organ (Sookoian & Pirola, 2012). AST elevation may result from a myriad of causes, ranging from muscle injury to hemolysis (Giannini *et al.*, 2005). Spurious elevation of AST occurs during treatment for acne (Duffy *et al.*, 2014). 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). Abusers of both alcohol and parenteral drugs have an increased risk of developing cirrhosis (Novick *et al.*, 1986).

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. Ali S & Fonseca V. Saxagliptin overview: special focus on safety and adverse effects. Expert Opin Drug Saf. 2013; 12: 103–9.
2. Benlloch S, et al. 2005. Prediction of fibrosis in HCV-infected liver transplant recipients with a simple noninvasive index. Liver Transpl. 2005 Apr; 11(4): 456-62.
3. Cooper AD. Liver disease in nonparenteral drug abusers. JAMA. 1975; 233(9): 964-6.
4. Cross TJ, et al. A simple, noninvasive test for the diagnosis of liver fibrosis in patients with hepatitis C recurrence after liver transplantation. J Viral Hepat. 2010; 17(9): 640-9.
5. Delcò F, et al. Dose adjustment in patients with liver disease. Drug Saf. 2005; 28: 529–45.
6. Duffy EK et al. Spurious elevation of aspartate aminotransferase in a patient on isotretinoin. Children's Hospital of Philadelphia, Pennsylvania. 2014; 71(4): e132-e133.
7. Gelfand DH, Steinberg RA. Escherichia coli mutants deficient in the aspartate and aromatic amino acid aminotransferases. J. Bacteriol. 1977; 130: 429-440.
8. Giannini, E.G., Testa, R., and Savarino, V. Liver enzyme alteration: a guide for clinicians. CMAJ. 2005; 172: 367–378
9. Ioannou GN, et al. The prevalence and predictors of elevated serum aminotransferase activity in the United States in 1999-2002. Am J Gastroenterol. 2006; 101(1): 76-82.
10. Jones S, Blackmore DJ. Observations on the isoenzymes of aspartate aminotransferase in equine tissues and serum. Equine Vet. J. 1982; 14: 311-316
11. Kamiike W, et al. 1989. Different patterns of leakage of cytosolic and mitochondrial enzymes. Clin Chim Acta. 1989 Dec 15; 185(3):265-70.
12. Killip T. High serum transaminase activity in heart disease. Circulatory failure and hepatic necrosis. PAYNE MA Circulation. 1960; 21: 646-60.
13. Kirsch JF, Eichele G, Ford GC, Vincent MG, Jansonius JN, Gehring H, Christen P. Mechanism of action of aspartate aminotransferase proposed on the basis of its spatial structure. J. Mol. Biol., 1984 174: 497-525.
14. Meistera A & Tice SV. Transamination from glutamine to alpha-keto acids. J Biol Chem., 1950; 187(1): 173-87.
15. Molander DW, et al. Serum glutamic oxalacetic transaminase as an index of hepatocellular integrity. J Lab Clin Med. 1955 Dec; 46(6):831-9.
16. Novick DM, et al. 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.
17. Periáñez-Párraga L, et al. Drug dosage recommendations in patients with chronic liver disease. Rev Esp Enferm Dig. 2012; 104: 165–84.
18. Pollock AA, et al. Hepatitis associated with high-dose oxacillin therapy. Arch Intern Med. 1978; 138(6): 915-7.
19. Pratt DS & Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med., 2000; 342(17): 1266-71.
20. Pratt DS & Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med. 2000; 342(17): 1266-71.
21. Reichling JJ & Kaplan MM. Clinical use of serum enzymes in liver disease. Dig Dis Sci. 1988; 33(12): 1601-14.
22. Rej R. Aspartate aminotransferase activity and isoenzyme proportions in human liver tissues. Clin. Chem. 1978; 24: 1971- 1979.
23. Sookoian S & Pirola CJ. Alanine and aspartate aminotransferase and glutamine-cycling pathway: their roles in pathogenesis of metabolic syndrome. World J Gastroenterol. 2012; 18(29): 3775-81.
24. Sookoian S & Pirola CJ. Alanine and aspartate aminotransferase and glutamine-cycling pathway: Their roles in pathogenesis of metabolic syndrome. World J Gastroenterol. 2012; 18(29): 3775–3781.

25. Tanaka T, et al. 2013. Serum aspartate aminotransferase level and previous histopathological findings enable reduction of protocol liver biopsies after liver transplantation for hepatitis C. Can J Gastroenterol. 2013 Mar; 27(3): 131–136.
26. 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.
27. Verbeeck RK & Horsmans Y. Effect of hepatic insufficiency on pharmacokinetics and drug dosing. Pharm World Sci., 1998; 20: 183–92.
28. Westphal JF & Brogard JM. Drug administration in chronic liver disease. Drug Saf. 1997; 17: 47–73.
29. Whitfield JB, et al. 2002. Genetic covariation between serum gamma-glutamyltransferase activity and cardiovascular risk factors.Clin Chem. 2002 Sep; 48(9): 1426-31.
30. Williams RL & Mamelok RD. Hepatic disease and drug pharmacokinetics. Clin Pharmacokinet. 1980; 5: 528–47.