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Research Article

Effects of pyridoxal phosphate in analysis of aminotransferase activity in patients undergoing hemodialysis

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Abstract

Background and Aims: Factors like hemodilution, pyridoxine deficiency, hepatocyte growth factor and uremic toxins have been proposed for low aminotransferase activity in chronic kidney disease (CKD) patients undergoing haemodialysis. This may be a concerning factor in making diagnosis of liver disorders like hepatitis in patients undergoing haemodialysis. In present study we attempted to find out the cause of hypoamino transferasemia in chronic kidney diseases and biochemical principle of analysis.

Materials and Methods: Serum levels of various biochemical parameters are measured in CKD patients undergoing haemodialysis. The serum activities of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) are determined (with and without the addition of PLP) in a group of 176 patients undergoing hemodialysis

Results: Serum AST and ALT activities are significantly lower in the CKD patients compared to the control group (P value 0.0003 and 0.001 respectively). Measurement of activity with pyridoxal phosphate containing reagent resulted into a significant increase in values comparison to without pyridoxal phosphate. The percentage activation is higher in patients as compared to controls.

Conclusion: The upper limit of reference range for aminotransferases in CKD patients undergoing hemodialysis should be considered lower as compared to healthy subjects. Reagent containing pyridoxal phosphate is considered appropriate for aminotransferase activity measurement.

Keywords: chronic kidney disease (CKD), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), (HGF)- Hepatocyte growth factor, PLP-Pyridoxal phosphate.

1. Introduction

Serum activities of Alanine aminotransferase (ALT, EC 2.6.1.2) and Aspartate aminotransferase (AST, EC 2.6.1.1) are commonly used in diagnosis and monitoring of hepatocellular damage[1]. Activity of these aminotransferases is dependent upon the active form of vitamin B6 i.e. pyridoxal phosphate[2]. Low aminotransferase activity is a common finding among chronic kidney disease patients undergoing hemodialysis[3,4]. So there is confusion on use of aminotransferases as reliable indicators for hepatocellular damage associated with CKD[5].

In developing countries like India, reagent kits used for routine investigations like AST and ALT do not contain pyridoxal phosphate and the measurement of activity is done without added pyridoxal phosphate which is against the guidelines issued by International Federation of Clinical Chemistry (IFCC) for the measurements of the aminotransferases[6].

In CKD there is retention of metabolic end products and their intermediary products such as urea, creatinine, uric acid, creatine, polyamines, cyanates, hippurate, carnitine, sulphates and phosphates[10]. These toxins may directly hamper the activity of various enzymes or may affect synthetic functions of liver. Hemodialysis is also associated with increased production of growth factors like hepatocyte growth factor (HGF) which causes protection against apoptosis and inhibiting release of enzymes in serum[7,8]. In a study Edmando PL reported that ALT levels increase by 25% in post dialysis samples after ultra filtration in comparison to predialysis ALT levels. The authors attributed this increase to correction of hemodilution with dialysis.[9]

In view of the conflicting reports we tried to measure serum aminotransferase levels *in-vitro* in patients with chronic kidney disease undergoing hemodialysis and to analyse if addition of pyridoxal phosphate has got any impact on the results.

2. Materials and Methods

104 CKD patients undergoing hemodialysis and 69 age and sex matched healthy control subjects were included for the study. Informed consent is taken from all the subjects. Ethical clearance has also been obtained from the ethical committee of the hospital. Exclusion criteria include history of jaundice, alcohol intake over the past 6 months, pregnant women and women taking oral contraceptive pills. The control group included hospital staff and healthy relatives of patients. All patients and controls were negative for viral markers like hepatitis B, C and HIV. All the patients and controls are evaluated and compared for different biochemical parameters like kidney function tests and the liver function tests.

Serum of CKD patients and healthy controls is collected and processed in presence and absence of pyridoxal phosphate. The reagent without pyridoxal phosphate is procured from Roche diagnostics (AST cat no-10851124-216 and ALT cat no 10851132-216). The reagent with pyridoxal phosphate (AST ref no 8433815 and ALT ref no-1655281) is supplied by orthoclinical diagnostics and is analysed on Vitros 350 automated analyser (ortho clinical diagnostics, Inc., Rochester, NY, USA). 11 µg of pyridoxal phosphate is used per slide which is enough to

saturate all apoaminotransferase molecules in sample dispensed upon slide. Other biochemical parameters like serum urea, creatinine, uric acid, gamma glutamyl transferase, alkaline phosphatise and lactate dehydrogenase are also measured in fully automated analyzer (Olympus AU400) with IFCC approved methods.

2.1 Statistical Analysis

Analysis of data is done using statistical software Stata/IC 12.1 (Stata Corp LP, Texas 77845 USA). Normality test is used to determine whether a dataset is well modelled by a normal distribution. Log transformation is applied so that it appears more closely to the assumption of normality. The student's t-test is used to compare the means between the patient and the control groups. A p value of less than 0.05 is considered as a statistically significant. The quantification of linear relationship among different parameters is explored by using Pearson's correlation coefficient. By using aminotransferase activities with and without pyridoxal phosphate the percentage activations are calculated by using the formula: Activation (%)= [(B-A) / A] x 100, in which B is the enzyme activity with PLP, and A without it. Rank sum test is applied to compare the average level of percentage activation.

3. Observation and Results

A total 104 CKD stage-V patients, (60 males and 46 females) of more than 18 years of age and 69 age and sex matched healthy controls (45 males and 24 females) who fulfilled the criteria are recruited for this study. The mean age of the patients is 41.01 ± 9.26 years and the controls 39.9 ± 11.5 years.

Table-1:- Biochemical analysis of case and control values.

Parameters	Patients(N=104)	Controls(N= 69)	p-value
Urea	134.2±66.2(mg/dl)	25.6±8.0(mg/dl)	0.001
Creatinine	6.1±3.6(mg/dl)	0.7±0.1(mg/dl)	0.001
Uric Acid	6.5±2.2(mg/dl)	4.4±1.2(mg/dl)	0.001
AST	21.1±9.5(IU/L)	25.8±5.8(IU/L)	0.001
ALT	15±8.7(IU/L)	28.4±9.9(IU/L)	0.001
ALP	279.8±188.1(IU/L)	218.7±69.0(IU/L)	0.021
GGT	31.5±43.3(IU/L)	27.9±16.4(IU/L)	0.391
LDH	541.7±232.9(IU/L)	370.8±100.2(IU/L)	0.001

Table 1 shows levels of AST and ALT to be significantly lower among the CKD patients compared to controls (p value <0.0003 and <0.001 respectively.) The alkaline phosphatase (ALP) and LDH levels in serum of CKD patients are significantly higher compared to controls. However, no significant difference is found in Gamma Glutamyl transferase (GGT) activity in the patient group compared to the control group.

Table- 2: Correlation between different biochemical parameters in CKD patients

Parameter (p value)	AST	ALT	ALP	GGT	LDH
Urea	-0.30* (0.001)	-0.36* (0.001)	0.24* (0.002)	0.14 (0.060)	0.40* (0.001)
Creatinine	-0.25* (0.001)	-0.40* (0.001)	0.29* (0.001)	0.13 (0.080)	0.36* (0.001)
Uric Acid	-0.12(0.110)	-0.20* (0.008)	0.09 (0.222)	0.07 (0.382)	0.30* (0.001)

*Significant correlation

Table-2 depicts AST levels in CKD patients are lower compared to healthy controls and shows negative correlation with urea and creatinine levels, though the decrease is not significant. The levels of ALT, ALP, LDH and GGT also doesn't show any significant correlations with increase in none of the uremic parameters like urea, creatinine and uric acid.

Table- 3: Activities of AST and ALT with and without PLP cases and controls

Liver Function	Without PLP(IU/L)	With PLP(IU/L)	p value
AST (Control)	28.5 ± 11.74 24 (21 – 32)	31.6 ± 11.33 27 (24 – 35)	0.001
ALT (Control)	28.4 ± 14. 95 24 (17 – 35)	34.6 ± 16.5 30.5 (23 – 40)	0.001
AST (Case)	18.6 ± 8.2 17 (14 – 22)	28.0 ± 12.5 26 (20 – 32)	0.001
ALT (Case)	11.8 ± 5.2 24 (17 – 35)	28.0 ± 12.5 26 (20 – 32)	0.001

Table 3 shows activities of aminotransferase with and without addition of PLP. The median and their range for AST is 17 (14 – 22) IU/L without PLP and 26 (20 – 30) IU/L with PLP and for ALT 24 (17 – 35)IU/L without PLP and 27 (24 – 35)IU/L with PLP in patients undergoing dialysis. In control patients, the median activity of AST is 24 (21 – 32)IU/L and 27 (24 – 35)IU/L and for ALT 24 (17 - 35)IU/L and 30.5 (23 – 40)IU/L respectively in without PLP and with PLP. The differences are highly significant ($p < 0.001$).

Table- 4: Percentage activation of case and control

Liver Function	Control(IU/L)	Case(IU/L)	p value
SGOT	13.03 ± 9.1 10.4 (6.2 – 18.2)	53.7 ± 37.2 45.4 (34.9 – 88.8)	0.001
SGPT	25.2 ± 16.9 24.7 (12.5 – 35)	119.3 ± 67.2 111.1 (76.4 – 145)	0.001

Table 4 shows that the percentage activation of AST and ALT are found to be significant ($p < 0.001$) while comparing between normal adults and haemodialysis patients. The median % activation of AST and their range is 10.4IU/L (6.2 – 18.2) % and 45.4IU/L (34.9 – 88.8) and for ALT it is 24.7IU/L (12.5 – 35) and 111.1IU/L (76.4 – 145) % respectively in healthy controls and haemodialysis patients.

4. Discussion

CKD patients on chronic hemodialysis with HCV infection have raised AST and ALT levels with a frequency of 4-67%[11-13]. On the other hand, the frequency of raised AST and ALT levels in patients positive for HCV antibodies without CKD is much higher around 54-75%[14]. So the ALT levels are assumed to be poor predictors of hepatocellular damage in the chronic haemodialysis population. Possible causes of low

aminotransferase activity may be pyridoxine deficiency, increased levels of hepatocyte growth factor, uremic toxins, and hemodilution. The level of transaminases in patients with advanced azotemia should be taken in lower range[15].

Figure 1: Effect of PLP addition to the reagent (BMY) on AST values in patients undergoing haemodialysis

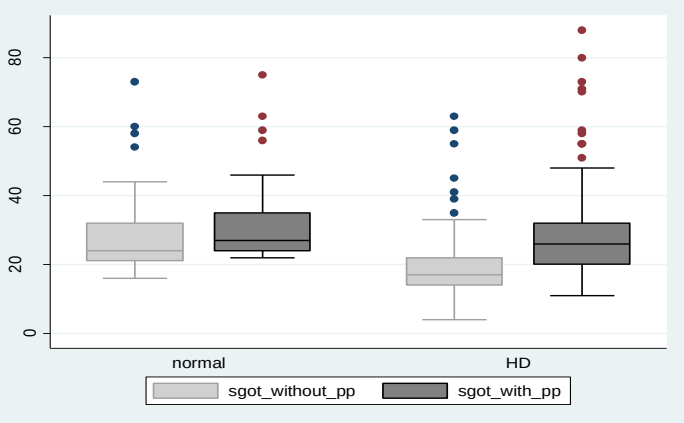
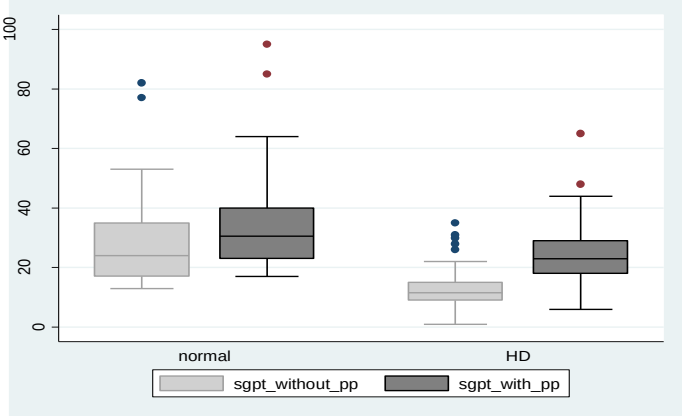


Figure 2: Effect of PLP addition to the reagent (BMY) on ALT values in patients undergoing haemodialysis



The present study shows low levels of serum transaminases among CKD patients. While levels of AST and ALT are lower, ALP levels are much higher among CKD patients in comparison to the controls. It is possible that higher ALP levels in the CKD patients in the present study reflects metabolic bone disease due to vitamin D deficiency. CKD patients also have higher LDH levels in comparison to the controls. Higher LDH levels in these patients may reflect hemoconcentration effect due to ultrafiltration and release from the formed elements within the extracorporeal circuit and complement-mediated leukocyte activation and pulmonary leukostasis. So it is advisable to ask for a pre dialysis sample for LDH estimation[16-17].

Vit-B6 deficiency has been a subject of concern in patients with CKD as symptoms of Vit-B6 deficiency like immune dysfunction, dermatologic disorders and peripheral neuropathies are similar hypothetically to the features of uremia [18-19]. The factors include inadequate intake or absorption, loss by dialysate, inhibition of the action or metabolism of pyridoxal phosphate by uremic toxins, impaired pyridoxal kinase mediated phosphorylation or increased activity of pyridoxal phosphatase[9]. Reportedly, pyridoxal-5'-phosphate may be hydrolyzed by alkaline phosphatase[20] and other enzymes of liposomal origin. This finding may be supported by the higher levels of alkaline phosphatase which may inactivate the pyridoxal-5'-phosphate and thus decrease the aminotransferase activity.

The fall of aminotransferases in hemodialysed patients could be supported by the pyridoxal phosphate deficiency which is lost during hemodialysis through dialysing membrane[9-10]. Aminotransferase activity is found to be increased when the patients on hemodialysis are supplemented with vit-B6 [18-19].

The aminotransferases are released normally in serum from liver by process of apoptosis. Hepatocyte Growth Factor (HGF) protects hepatocytes from apoptosis. It is known that, HGF levels are more in patients on regular haemodialysis[8]. Hemodialysis increases serum levels of HGF due to increased production from peripheral blood mononuclear cells. Reduced levels of apoptosis are observed in the liver of patients undergoing hemodialysis as this condition acts as a potent stimulus for (HGF) release, and thus protecting the hepatocyte from apoptosis[8,21].

Various studies have shown that urea and creatinine have cumulative enzyme inhibitory effect *in vivo*, better than in vitro. Retained urea in CKD patients are rapidly transformed into uremic toxins like cyanates and isocyanic acid which play a role in genesis of uremic syndrome. Uremic toxins lead to carbamoylation of various physiologically active proteins like enzymes. Carbamoylation results from the constant exposure and reaction with cyanates and isocyanic acid. Carbamoylation occurs *in vivo* and is associated with change in their molecular structure, charge and function[22-23]. Many hypotheses have been given for the inhibition of transaminase activity in serum by uremic toxins. When AST and ALT levels are measured by UV-light method, there may be some degree of underestimation due to absorption of UV light by uremic toxins.

The carbamoylation of protein is a slow process and it leads to alteration of charge, molecular structure and function *in vivo*. Uremic toxins may lead to carbamoylation of pyridoxal phosphate dependent enzymes AST, ALT and threonine dehydratase. Carbamoylation of the ε-amino group of AST and ALT leads to failure to react with pyridoxal phosphate responsible for incomplete catabolism and low activity of AST and ALT[24].

Serum AST have been shown to increase after dialysis; the rise is presumably due to removal of dialyzable inhibitor, increase release of the enzyme from erythrocytes in extracorporeal circuit or due to ultra filtration induced hemoconcentration.

5. Conclusion

In conclusion, the present study shows that renal dysfunction is strongly associated with significantly lower levels of the aminotransferases compared to healthy controls. The level of transaminases in patients with advanced azotemia should be taken in lower range. As serum aminotransferase levels are commonly used to screen for liver disease in the dialysis and predialysis CKD population, recognition of liver damage may be hampered by the reduction in aminotransferase values in these patients. While multiple mechanisms have been proposed earlier, the aetiology for such an effect remains unclear. A comprehensive study is required taking the different proposed factors altogether into consideration.

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