Evaluation of the Diagnostic Potential of Liver Aminotransferases and Alkaline Phosphatase in Patients with Cardiovascular Diseases

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ABSTRACT

Background

Cardiovascular diseases (CVDs) are the major cause of morbidity and mortality, particularly in developing countries. Early diagnosis with the best diagnostic marker is highly desired for the prevention and timely treatment of CVDs. However, there is still a dearth of an ideal marker for the detection of CVDs.

Objective

To explore the diagnostic potential of liver aminotransferases (AST and ALT), and alkaline phosphatase for the diagnosis of CVDs without liver involvement.

Method

This was a cross-sectional study conducted among 200 adult patients with CVDs, who visited the cardiology and emergency units of Manipal Teaching Hospital, Pokhara, Nepal. The study was conducted from January 2018 to December 2020. The baseline data on family history, anthropometry, baseline biochemical parameters, liver enzymes, and cardiac biomarkers were collected using standard and validated methods. The data were analyzed using SPSS version 21 and MedCalc software 2021.

Result

The diagnostic sensitivity of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were 53.7%, 52.6%, and 33.7% and specificity were 99%, 90%, and 90% respectively. The area under the curve (AUC) of AST, ALT, and ALP were 0.78, 0.73, and 0.52 respectively. ROC curve indicated that serum AST and ALT activity was a better reliable marker than the serum ALP activities.

Conclusion

Our study suggests that serum aspartate aminotransferase and alanine aminotransferase but not alkaline phosphatase could have some diagnostic potential to diagnose the risk of CVDs. However, they could not replace the currently adopted cardiac biomarkers such as cTnI and CK-MB.

KEY WORDS

Alkaline phosphatase, Aminotransferases, Cardiovascular diseases, Diagnostic potential

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of premature death worldwide, particularly with the increasing trend in low and middle-income countries.[1-3] In 2019, 17.9 million people were died from CVDs which indicate 32% of all global deaths. The South Asian region is considered to be the hotbed of CVDs.[4,5] The prevalent CVDs in Nepal include coronary heart disease, rheumatic heart disease, hypertensive heart disease, heart failure and infective endocarditis.[6] The novel biomarkers associated with CVDs risks, including B-type natriuretic peptide (BNP), N-terminal prohormone BNP (NT-proBNP), troponin I, creatine-kinase-MB, high sensitivity C-reative protein, myeloperoxidase (MPO), fibrinogen, cystatin C and homocysteine. BNP and NT-proBNP use in a diagnosis of heart failure and tropoini-I and CK-MB used as a cardiac biomarker for diagnosis of acute coronary syndrome. Circulating level of MPO used to predict risks of coronary heart disease.[7] The traditional biomarkers of CVDs including liver enzyme, lactate dehydrogenase and creatine kinase. The advancement in biomarker research and search of an ideal cardiac biomarker is thus still in the progress.[8,9]

Many studies have shown that the liver-derived serum aminotransferase and alkaline phosphatase are significantly associated with incident CVDs and thus could potentially be used as risk markers for CVDs.[10-12] Hence, the study of the diagnostic potential of the liver enzymes viz aminotransferase (AST and ALT) and alkaline phosphatase (ALP) could have a significant impact on the differential diagnosis of CVDs, especially in subjects with non-alcoholic fatty liver disease (NAFLD). However, there have been no such previous studies in the Kaski population with CVDs that have investigated the diagnostic efficiency of these enzymes. The main aim of this study was, to study the potential of three liver enzymes for the diagnosis of CVD risks among the adult population of Kaski district, Nepal.

METHODS

This was a descriptive cross-sectional hospital-based study conducted among 200 adult patients with CVDs, who visited the cardiology and emergency units of Manipal Teaching Hospital, Pokhara, Nepal. The CVDs included in this study were ST-elevation myocardial infarction (STEMI) (152), Non-ST-elevation myocardial infarction (Non-STEMI) (14), unstable angina (22), heart failure (11) and rheumatic heart disease (1). The study was conducted from January 2018 to December 2020. The presence of CVDs in admitted subjects was diagnosed by the treating physicians based on sign and symptoms, cardiac marker profile (serum troponin I and CK-MB) and an electrocardiogram finding. Patients who had CVDs together with acute or chronic liver (liver damage, jaundice, hepatitis, cirrhosis of liver), intestinal disease (inflammatory bowel disease), and musculoskeletal diseases (myopathies, muscular dystrophies, and celiac disease) were excluded from the study.

Data regarding age, gender, family history, dietary habits, physical activity, smoking habits and drinking habits were collected. Bodyweight, height, waist and hip circumferences, waist-hip ratio (WHR), body mass index (BMI), and blood pressure of all the patients were measured using the standard techniques and protocols.[13]

Blood collection, separation, storage and Biochemical Analysis

Five milliliters of venous blood samples were collected from the forearm of each study participant by venipuncture using a disposable syringe and kept in 12''x75'' plain gel tubes. The blood samples were then allowed to clot at room temperature, centrifuged at 4000 rpm for about 10 minutes and the supernatant serum samples were collected. The serum samples so obtained were analyzed immediately whenever possible or stored at -20°C until analyzed in case of delay.

The concentrations of serum AST, ALT, ALP, and CK-MB were measured using a fully automated dry chemistry-based analyzer (VITROS® 350 chemistry system, Ortho clinical diagnostics, UK) while troponin-I was measured using VITROS ECiQ Immunodiagnostic according to the protocols provided by the manufacturer. The values of these parameters were interpreted using the normal ranges provided by the manufacturer in the reagent kit insert. The processed sera were assayed in a single batch after proper standardization and quality control of the analyzers and using the same lots of reagents. The within run CVs% for AST, ALT, ALP, CK-MB, and troponin-I were 3.05, 3.74, 9.95, 4.51, and 8.91% respectively, and were within the acceptable ranges. Internal quality control was run every day and observed values were within ±2SD. Moreover, our laboratory also takes part in the monthly External Quality Assessments Scheme (EQAS) conducted by the Christian Medical College (CMC) Vellore, India which further guaranteed the accuracy and reliability of our laboratory results.

The ethical clearance was obtained from the Institutional Review Committee (IRC) of the Manipal College of Medical Sciences before the enrollment of the study subjects. The written informed consent was obtained from each study subject for interviews, questionnaires, and sample collection.

All the statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 21 (SPSS, IBM, Chicago, IL) except for the determination of diagnostic efficiency. The later analysis was performed using MedCalc software 2021. Continuous data were expressed as mean ± SD whereas categorical data were presented as numbers and percentages. The difference in the means of categorical variables was compared by the independent student's t-test while those of categorical variables were compared with the Chi-square tests. ROC curve were used to study

the diagnostic potential of liver enzymes. The diagnostic efficiency of these liver enzymes was expressed in the form of sensitivity, specificity, diagnostic cut-off values, and area under the curve (AUC). P-value (two-tailed) < 0.05 was considered statistically significant.

RESULTS

A total number of 200 study subjects were enrolled in the present study, out of which 130 (65%) were male and 70 (35%) were female. The age of patients with CVDs ranged between 32-86 years, with the mean value of 61.29±12.67 years. Among patients with CVDs, 49% were smokers, and 52.5% were drinkers. The gender-based frequency of socio-demographic variables such as smoking habits, and drinking habits was significantly higher ($p < 0.05$) in patients with CVDs (table 1).

Table 1. Baseline socio-demographic and biochemical characteristics of the study subjects (N=200)

Variables	Male (n=130)	Female (n=70)	Total	p-value
Age (years)	60.4±12.3	63.0±13.3	61.3±12.7	0.161
Blood pressure, mmHg				
Systolic	121.1±16.0	124.2±13.5	122.2±15.2	0.167
Diastolic	77.4±10.0	79.5±10.5	78.2±10.2	0.171
Physical data				
BMI	25.5±2.9	25.9±3.3	25.6±3.1	0.361
Waist to Hip ratio	1.0±0.1	1.0±0.4	1.0±0.4	0.451
Smoking habits				
Smoker	86 (43)	12 (6)	98 (49)	0.000
Non-smoker	44 (22)	58 (29)	102 (51)	
Alcohol consumption				
Drinker	82 (41)	23 (11.5)	105 (52.5)	0.000
Non-drinker	48 (24)	47 (23.5)	95 (47.5)	
AST, IU/L	124.6±160.1	124.6±184.8	124.6±168.7	0.999
ALT, IU/L	56.9±73.2	52.8±69.1	55.5±71.6	0.699
ALP, IU/L	94.7±49.6	110.7±114.2	100.3±78.6	0.171
CK-MB, IU/L	86.8±96.2	89.4±94.3	87.7±95.3	0.852
Troponin I, µg/L	11.7±23.3	10.8±22.8	11.4±23.1	0.789

The continuous variables were expressed as mean ± SD while categorical variables were expressed as n(%). CVDs (cardiovascular diseases), AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), ALP (Alkaline phosphatase), CK-MB (Creatine kinase-MB)

The mean serum activity of AST was significantly higher in 107 (53.5%) patients with CVDs. However, the levels of ALT were significantly higher only in 64 (32%) patients with CVDs. The serum levels of all biochemical variables such as AST (p= 0.999), ALT (p= 0.699), ALP (p=0.171), CK-MB (p=0.852) and troponin-I (p=0.789) were insignificant among male and female CVD patients, it indicates no difference among gender. However, the serum level of AST, ALT, CK-MB, and troponin-I was significantly higher ($p < 0.05$) among patients with CVDs (table 1).

Table 2. The AUCs, cut off values, Youden Indices, sensitivity and specificity of AST, ALT, and ALP for the diagnosis of CVDs

	AUC (95% CI)	Cut off	Youden Index	Sensitivity (%)	Specificity (%)	LR+	LR-	p-value
AST	0.78 (0.71-0.83)	52	0.5	53.7	99	5.4	0.5	<0.001
ALT	0.73 (0.66-0.79)	36	0.4	52.6	90	5.3	0.5	0.001
ALP	0.52 (0.45-0.59)	96	0.2	33.7	90	3.37	0.7	0.804

Results are represented as %, AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), ALP (Alkaline Phosphatase), AUC (Area under the curve), LR+ (Positive likelihood ratio), LR- (Negative likelihood ratio), CVDs (Cardiovascular diseases)

The cut-off values of AST, ALT, and ALP were 56 U/L, 36 U/L, and 96 U/L respectively. The diagnostic sensitivities of AST, ALT, and ALP were 53.7%, 52.6%, and 33.7% and specificity were 99%, 90%, and 90%, respectively for CVDs. The AUCs of AST, ALT, and ALP were 0.78, 0.73, and 0.52, respectively which suggest them as the fair diagnostic biomarkers for cardiovascular diseases (table 2). ROC curves indicate that serum AST and ALT activity were better markers than the serum ALP activity for the diagnosis of CVDs (fig. 1).

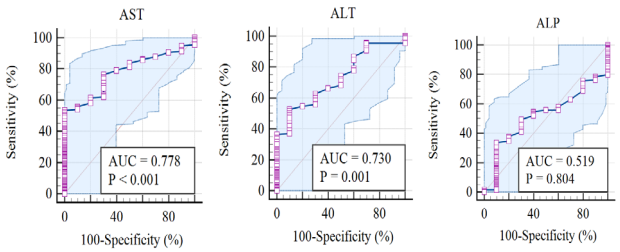

Figure 1. Receiver operating characteristics (ROC) curve of liver enzyme activity for a diagnostic biomarker of CVDs. AUC (Area under curve) AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase)

DISCUSSION

In our study, the majority of patients were from the elderly age group and males. The serum level of AST and ALT were higher than the reference range in half to one-third of patients. The study conducted by Loftus et al and Moon et al also showed an increase in liver enzymes in ST elevated myocardial infarction.[14,15] The study conducted by Lee et al showed that increased ALT and AST are associated with CVD mortality.[11] Similarly, the study from Korea also reported that elevated ALT levels were associated with increased CVD mortality.[16,17] The reason for elevated aminotransferase in CVDs may be due to hypoxic hepatitis caused by cardiac diseases or due to associated risk factors like obesity, insulin resistance, and metabolic syndrome.[18,19]

Increased aminotransferase levels have also been indicated with increased risk of CVD through underlying endothelial dysfunction, inflammation, and impaired hemostasis.[20]

In some of the patients, the level of serum ALP activity was elevated in our study subjects. Some studies have reported that elevated ALP is related to CVDs mortality and hospitalization.[21,22] An elevated serum ALP level may promote vascular calcification which may damage vascular integrity and promote atherosclerosis. The main link between elevated ALP level and CVDs through inflammation.[23,24] In our study, the serum activity of these enzymes showed a weak to moderate association with serum levels of cardiac biomarkers (cTnI and CK-MB). The diagnostic sensitivity of AST, ALT, and ALP were 53.7%, 52.6%, and 33.7% respectively for the diagnosis of CVDs.

This is the first study from Nepal that investigates the diagnostic efficiency of three liver enzymes for CVDs by analyzing the ROC curves. The AUC value 0.9-1.0 indicates excellent, 0.8-0.9 indicates good, 0.7-0.8 indicates fair, 0.6-7.0 indicates poor and 0.50-0.60 indicates fail discriminatory ability of the diagnostic test.[25] Our study revealed that AST and ALT had some level of diagnostic potential for the diagnosis of CVDs. The cut-off value of AST was 56 U/L with 53.7% sensitivity and 99% specificity while the cut-off value of ALT was 36 U/L with 52.6% sensitivity and 90% specificity for CVDs. In the same way, ALP cut-off value was 96 U/L with 33.7% sensitivity and 90% specificity. Youden index (J) indicates the maximum potential effectiveness of diagnostic biomarkers. It combines sensitivity and specificity into a single measure and has a value between 0 and 1. Youden's index equals 1 in perfect diagnostic tests.[26] In our study, Youden index for AST, ALT, and ALP were 0.5, 0.4, and 0.2 respectively. These findings suggested that liver enzyme activity had not more than 50% perfect diagnostic potential for CVDs. Therefore, it can be concluded that the serum values of liver enzymes should not be used alone as the biomarkers for the diagnosis of CVDs. However, they may add up in better differential diagnosis of CVDs when assayed in a panel together with established cardiac biomarkers. Though our study provides the preliminary data on the diagnostic potential, a multi-centre study with larger and well-characterized patients with CVDs should be conducted in order to obtain more robust and reliable data on the diagnostic potential of these three liver enzymes.

The limitation of this study is a small sample size, aged population and single center study.

CONCLUSION

Our study suggests that only serum aminotransferases have some moderate diagnostic potential for the CVDs and thus could not replace the currently adopted cardiac biomarkers such as cTnI and CK-MB. However, they could be included in the existing panel of cardiac biomarkers, particularly when an inflammation of the liver is also suspected in a patient with CVD.

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