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A study of serum arginase activity in diagnosis of liver diseases

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Abstract
Background: *Liver disease presents as a spectrum of clinically asymptomatic liver disease to end stage liver disease. Various serum markers used in liver diseases but are not specific or sensitive and influenced by other factors. We do a battery of investigations - liver function tests. Liver biopsy is the gold standard investigation, but is invasive. There is a still a search for a non invasive and a better marker for diagnosis of liver diseases. Serum arginase an enzyme of urea cycle gets elevated in liver damage and can be used in diagnosing liver diseases.*
Objective: *To assess the serum arginase activity and state that it can be a marker of diagnosis of liver disease. To assess the severity of liver disease using serum arginase*
Materials and Methods: *This was a rural based teaching hospital based cross sectional study of 80 patients with liver diseases. The serum arginase level (by elisa method) in the patients were compared with the novel liver function tests. Also we analysed whether serum arginase can be used as a indicator of disease severity by comparing it with the Child-Pugh score and MELD score.*
Results: *The mean serum arginase value of the study subjects by ELISA method was 92.38 ng/ml with a p value of 0.01 and was found to be statistically significant. When compared with AST & ALT using Child Pugh and Meld score, serum arginase was found to have a poor correlation with severity with a p value of 0.976 with CP score and 0.83 with MELD score which was statistically insignificant. Serum AST values were better predictor of severity with a p value of 0.432 with CP score and 0.018 with MELD score which was statistically significant.*
Conclusion: *Serum arginase can be used in diagnosis of liver diseases but a poor indicator of the severity when compared with scoring systems. Serum AST correlated better with severity in this study.*

Introduction
Liver plays the central role in metabolism and liver diseases are in rising trend all over the world responsible for around 700 thousand deaths per year, the 14th commonest cause of death worldwide. The diagnosis of liver diseases is made by a battery of serological investigations and imaging techniques. The novel liver function

tests include measurement of serum bilirubin, liver enzymes – Alaline transaminase (ALT) and Aspartate transaminase (AST), Alkaline phosphatise (ALP). The synthetic function is measured by serum protein and albumin, prothrombin time (PT) and internationalised ratio (INR). Most of the above investigations though reflect the liver functions are influenced by various factors. Witnessing normal patients with elevated liver enzymes is common in clinical practice and it leads to unnecessary investigations and treatment.

The values of the liver function tests also do not correlate with the disease severity. The best investigation for diagnosing the liver disease, its stage and aetiology is liver biopsy. But liver biopsy is a invasive procedure and requires expertise to perform it and has lot of complications. There is always a search for a non invasive and a better marker for the diagnosis of liver diseases and various parameters are under trial.

Arginase an enzyme of urea cycle is involved in the final step and converts arginine to ornithine and urea. It exerts in two isoforms and arginase I is found in the liver. Some studies have shown that serum arginase can be a better marker for diagnosing liver diseases and gets elevated early in the disease from the damaged hepatocytes. The normal serum arginase value is 1-30 ng / ml and can increase several folds in patients with liver diseases. But still the studies on serum arginase are less and there are very few Indian studies.

Aim of the study
1. To assess the serum arginase activity in patients with liver diseases.
2. To state that serum arginase can be used in diagnosis of liver diseases.
3. To assess the severity of patient with liver diseases using serum arginase levels.

Methodology
It was a rural based teaching hospital study of 80 patients with liver disease. After getting informed consent from patient and attenders, history taking, examination and serum sample collected for complete hemogram, RFT & LFT and ultrasonogram. The concentration of serum arginase level was determined by an Enzyme Linked Immunosorbent Assay (ELISA) method by using commercially available enzyme-linked immunosorbent assay kits. The normal significant level of detection in serum is set by the manufacturer at 1-30 ng/ml of arginase. Values greater than mean of 15 ng/ml were taken as significantly elevated. Determination of biochemical parameters (LFTs and creatinine) was performed using micro 600 semi auto analyzer from MERC.

Statistical analysis
All results are expressed as mean ± standard deviation (SD) for continuous variables and as frequencies for categorical variables. The difference in the age and gender between groups is disproved using independent student t-test and chi-square test, respectively. The relationship between the variables of liver function tests serum levels (liver enzymes and arginase) was estimated using the correlation graphs. All analysis was done using Statistical Package for the Social Sciences (SPSS) version 16 for windows.

Results
During the study period of 15 months in this cross sectional study of 80 patients with liver diseases due to various causes, the basic characteristics of the study patients are given in the table 1. In this study 95% (76 numbers) of them had elevated serum arginase value while only 5% of the study subjects had normal value. The mean arginase value of the study patients was 92.38ng/dl and when compared with the normal mean (15 ng/ml) was statistically significant with a p value of < 0.01(shown in table 2, figure 1).

Analysis of variance (ANOVA) was done to find out whether the serum arginase value can be used to assess the severity of the disease by comparing with CP score and MELD score. In this study, the liver enzymes AST, ALT and Arginase was not

able to assess the severity of liver disease statistically when compared with CP score and Arginase had the week correlation with severity of the disease as shown in table 3. The liver enzymes AST, ALT and Arginase was assessed with the MELD score, AST was found to be statistically significant in assessing severity with a P value of 0.018 whereas ALT and arginase had poor correlation with the severity of the disease shown in table 4.

Assessing the role of MCV as a predictor of chronic alcohol intake we - 39.4% of the alcoholics had a MCV >100 and only 7.14% of the non alcoholics had a MCV >100. The p value is < 0.01 and the difference was found to be statistically significant (shown in table 4).

Table 1: Baseline characteristics of the study patients

Patient characteristics		
Sex wise distribution n=80	Males – n= 74 (92.5%) Females – n= 6 (7.5%)	
Age wise distribution n=80	20-35 yrs – n= 9 (11.25%) 35-50 yrs – n =38 (47.5%) 50 – 70 yrs – n= 33 (41.25%)	
Etiology of liver disease in study subjects n=80	Alcoholics – n= 66 (82.5%) Viral hepatitis B- n= 11 (13.75%) Viral hepatitis C –n= 1 (1.5%) NASH – n= 1 (1.5%) Wilson's disease – n= 1(1.5%) Cardiac cirrhosis – n= 1(1.5%)	
Complications n=80	Ascites – n= 54 (67.5%) Hepatic – n= 7 (8.75%) encephalopathy	
Thrombocytopenia n=80	Yes – n= 45 (57.5%) No – n= 35 (42.5%)	
Baseline LFT of the patients n=80	**Serum bilirubin** –	< 1.3 mg/dl – n= 20 (25%) 1.3 – 2.0 – n= 11 (13.75%) > 2 – n= 49 (61.25%)
	AST – normal Elevated	5- 37 IU/L – n= 5 (6.25%) > 37 IU/L – n= 75 (93.75%)
	ALT - normal Elevated	5- 50 IU/L – n= 34(42.5%) > 50 IU/L – n= 46 (57.5%)
	Sr.albumin normal decreased	n= 29 (36.25%) n= 51 (63.75%)

Table 2: Serum arginase level in the study patients

Patient characteristics	Serum Arginase level		
Study subjects n=80	Normal value (1-30 ng/dl) Elevated (> 30 ng/dl)	n=4 (5%) n= 76 (95%)	
Sex wise distribution of mean serum arginase level (ng/dl)	Males Females **Total**	n= 74 n = 6 **n=80**	**92.54 ± 33.36** **90.33 ± 16.80** **92.38 ± 32.35**
Age wise distribution of mean serum arginase level (ng/dl)	20-35 yrs 35-50 yrs 50 – 70 yrs **Total**	n= 9 n=38 n= 33 **n=80**	**101.11 ± 29.89** **87.68 ± 35.13** **96.81 ± 30.67** **92.38 ± 32.35**
Etiology wise distribution of mean serum arginase level (ng/dl)	Alcoholics Viralhepatitis B Viralhepatitis C NASH Wilson'sdisease Cardiac cirrhosis **Total**	n= 66 n= 11 n= 1 n= 1 n= 1 n= 1 **n=80**	**93.60 ± 34.34** **93.27 ± 24.90** **80** **118** **80** **72** **92.38 ± 32.35**
Mean serum arginase level (ng/dl) in complication of liver disease	Ascites present Asites absent Hepatic encephalopathy present Hepatic encephalopathy absent	n= 54 n= 26 n= 7 n= 73	**93.19± 34.19** **90.69 ±28.71** **86.86 ± 40.57** **92.90 ±31.74**

Table 3: Comparison of AST, ALT & Arginase with the Child Pugh score

Serum enzymes	Child Pugh score severity class			Test of Significance ANOVA (p value)*
	Class A	Class B	Class C	
Serum AST (IU/L) mean± SD	125.57 ± 82.6	196.7 ± 241.85	146.5 ± 94.37	0.432
Serum ALT (IU/L) mean± SD	98.43 ± 60.63	133 ± 173.12	109.93 ± 102.44	0.432
Serum Arginase (IU/L) mean± SD	91.43 ± 30.24	91.81 ± 33.45	93.4 ± 32.23	0.976

Table 4: Comparison of AST, ALT & Arginase with the MELD score

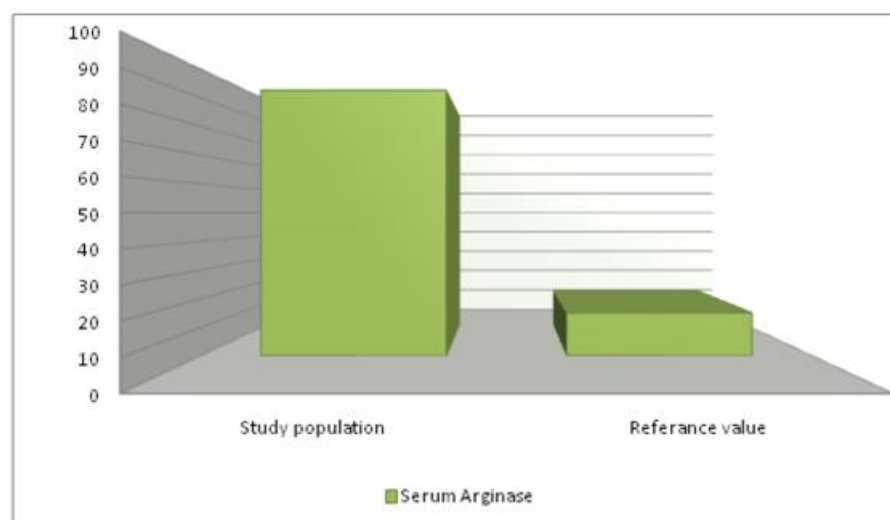| Serum enzymes	MELD score severity class				Test of Significance ANOVA (p value)*
	Class A	Class B	Class C	Class D	
Serum AST (IU/L) mean± SD	122.18 ± 124.92	165.83 ± 154.96	172.23 ± 102.13	447 ± 581.56	**0.018**
Serum ALT (IU/L) mean± SD	68.76 ± 76.48	132. 04 ± 163.65	145.15 ± 122.38	145.25 ± 139.54	0.39
Serum Arginase (IU/L) mean± SD	95.06 ±23.97	93.57 ±38.02	84.77 ±24.04	92 ± 13.36	0.83

Figure 1: Serum arginase value in liver diseases compared with reference mean value

Discussion

There are studies implicating the use of serum arginase for diagnosing liver diseases. Previous studies have shown that serum arginase is more specific and sensitive marker for diagnosing liver damage. However there are only one to two studies of serum arginase in our country.

In this study, we evaluated in 80 patients with liver diseases the serum arginase activity by ELISA method. Also we evaluated whether serum arginase can indicate the severity of the liver disease by comparing it with the Child Pugh score and MELD score. Most of the study subjects were present in the age groups of 36-50 years with 38 cases (47.5%) with predominant male subjects as in most of the studies on liver diseases. In this study consumption of alcoholic beverages was the most common cause forming the main bulk of study population. 66 numbers (82.5%), and belonged to male gender. There were no studies of serum arginase activity in ALD and in our study it was the major population of study subjects. It also

shows the pattern of liver diseases in this region needing health education. The demographic variables of the study population is listed in table 1.

MCV as a marker of chronic alcoholism: We compared the mean corpuscular volume (MCV) of RBCs of ALD subjects with other patients. 26 ALD subjects (39.4%) of them had macrocytosis (MCV >100 fl) and only 1 study subjects in the non alcoholic group had macrocytosis. Based on the Chi square test the p value was < 0.01 and was found to be statistically significant.

Serum arginase and liver diseases

Serum arginase activity was elevated in most of them suffering from liver diseases. The minimum and maximum level of serum arginase in the study was 26 ng/ml and 200 ng/ml. Serum arginase was able to diagnose liver diseases in 76 subjects (95% of study population) and only 4 subjects had normal serum levels shown in figure 1. . In this study mean serum arginase value of the study population was 92.38 ng/dl. Comparing it with the reference mean value the p value was <0.01 and it was found to be statistically significant Kimura et al in patients with autoimmune hepatitis concluded the superiority of arginase to liver specific autoantibodies. In another study in HCC patients by Chrzanowska et al serum arginase was elevated significantly and post resection came to normal levels. There was no significant difference in among the alcoholic group and viral hepatits B group, could not differentiate among various etiologies

Assessment of severity of liver diseases: When compared with MELD score, serum arginase was a poor indicator of severity with a p value of 0.83. Serum AST was a better indicator with a p value of 0.018 statistically significant. Similar findings were observed in a recent study by Chhabra et al found serum arginase was significantly elevated in patients with liver diseases. On follow up of these patients serum arginase was poorly correlating with severity of liver disease whereas AST and ornithine carbomyl transferase (OCT) were better

indicators of severity. To conclude it is clear from the study that serum arginase can be used as a marker for the diagnosis of liver diseases but was a poor indicator of the severity of liver diseases compared with scoring systems. Serum AST levels was better marker of severity of liver disease. Limitations of the study: A cross sectional study, causal relationships cannot be established. A larger sample size might have explained the outcome in a better manner. Also a follow up could better assess the prognostic value of serum arginase.

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