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Evaluation of Paraoxonase 1 Activity: An Emerging Biomarker for Liver Function in Viral Hepatitis

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ABSTRACT
The liver, a vital organ, plays a fundamental role in metabolism, detoxification and homeostasis maintenance. Effective diagnosis and management of liver diseases hinge on the accurate assessment of liver function using biochemical markers. This study aimed to evaluate the diagnostic efficiency of traditional and emerging liver function markers in distinguishing between viral hepatitis patients and controls. We analyzed liver parameters, including Total Bilirubin, Direct Bilirubin, AST, ALT, ALP, total proteins, albumin and PON1 in both viral hepatitis patients and control subjects. The study conducted ROC analysis to determine the sensitivity and specificity of these markers. Viral hepatitis patients exhibited significantly higher Mean±SD of total and direct bilirubin, AST, ALT and ALP compared to controls. Albumin levels and PON 1 activity were significantly lower in the viral hepatitis group. Total proteins did not demonstrate significant variability between the two groups. PON showed a negative correlation with several markers, with a significant negative correlation with AST. Total bilirubin showed strong positive correlations with direct bilirubin, total protein and albumin. The ROC analysis revealed PON 1's potential as a diagnostic tool with 100% sensitivity in detecting viral hepatitis. While traditional liver markers remain crucial for liver function assessment, emerging markers like PON1 provide complementary diagnostic insights. PON1's high sensitivity and specificity suggest its potential value in liver disease evaluations. However, further research is needed before its routine clinical adoption.

INTRODUCTION

The liver is the body's largest organ and is crucial for maintaining health and homeostasis. Its main cell type, the hepatocyte, performs numerous vital functions, including metabolism and detoxification[1]. Although viral infections are a leading cause of liver injury, other factors such as drugs, environmental pollutants and excessive alcohol can also induce liver damage. Most of these factors generate harmful free radicals and reactive oxygen species that contribute to liver pathogenesis. The primary diseases affecting the liver include viral hepatitis, alcoholic liver diseases, non-alcoholic fatty liver diseases and hepatocellular carcinoma[2].

Viral hepatitis, caused by hepatotropic viruses (like hepatitis A, B, C, D and E), leads to liver inflammation. For instance, in 2012, India reported around 119,000 cases of viral hepatitis. Liver damage is also linked with oxidative stress and lipid peroxidation, which play pivotal roles in the progression of liver diseases[3].

Liver damage is closely linked to oxidative stress and lipid peroxidation. Oxidative stress occurs when there's an imbalance between free radicals and the body's ability to neutralize them. This imbalance can lead to cell and tissue damage. Lipid peroxidation, a consequence of oxidative stress, involves the degradation of lipids, resulting in cell damage. Initially, this manifests as steatosis or fatty liver but can progress to severe conditions like steatohepatitis or cirrhosis if unchecked. Understanding this relationship offers potential avenues for therapeutic interventions in liver diseases.

Paraoxonase (PON) is a serum enzyme that breaks down certain toxic compounds. There are three types of PON: PON1, PON2 and PON3, with PON1 being associated with high-density lipoprotein (HDL) and playing a role in preventing low-density lipoprotein (LDL) oxidation. Since the liver is crucial for lipoprotein synthesis and metabolism and as PON1 synthesis is limited to the liver, measuring serum PON1 activity may serve as an indicator of liver function[4].

Typically, liver function is assessed using markers such as bilirubin, alanine and aspartate aminotransferases (AST, ALT) and alkaline phosphatase (ALP). In cases where these markers are inconclusive due to their long half-lives, liver biopsies might be necessary. However, recent studies have proposed that low PON1 activity could also indicate liver diseases, making it a potential biomarker for assessing liver health[5]. The present study aims to evaluate the efficacy of PON1 activity in diagnosing viral hepatitis and determine its relevance alongside conventional liver function tests.

MATERIALS AND METHODS

The present case-control study was conducted involving 60 participants, with 30 being controls and 30 diagnosed with viral hepatitis. This research took place at the Department of Biochemistry in collaboration with the Department of Emergency Medicine and Department of General Medicine at the Great Eastern Medical School and Hospital in Srikakulam. All procedures were sanctioned by the institution's ethics committee and informed consent was acquired from all participants.

Inclusion criteria:

- Patients of either sex clinically diagnosed as viral hepatitis without any associated co morbidities (Diabetes, Hypertension, any systemic illness) were taken as cases
- Healthy voluntary blood donors were taken as controls

Sample collection: Blood specimens were gathered, with 5 mL of blood drawn into a plain vaccutainer. Samples that were noticeably hemolyzed or lipemic were disregarded. The analysis encompassed the following parameters:

- Total bilirubin in serum
- Direct bilirubin in serum
- Serum ALT levels
- Serum AST levels
- Serum ALP levels
- Total protein content in serum
- Albumin levels in serum
- Activity of the serum Paraoxonase enzyme

All the aforementioned parameters were assessed using standard procedures and in accordance with the manufacturer's protocol.

Statistical analysis: The data obtained were compiled and analyzed using the statistical package for social sciences (SPSS) software version 21. A probability value less than 0.05 (p-value) was considered statistically significant.

RESULTS

In the study, the Mean± S.D of total and direct bilirubin, AST, ALT and ALP were observed to be significantly higher in viral hepatitis patients compared to controls. Conversely, the Mean±SD of albumin and PON 1 activity were significantly lower in viral hepatitis cases than in controls. However, the Mean±SD of total proteins showed variability and did not exhibit significant differences between the two groups (Table 1).

All parameters, except T-Proteins, showed highly significant differences among the groups with p<0.0001. T-proteins was still statistically significant with a p-value of 0.0315 but to a lesser degree. The R^2 value indicates how much variance in a dependent variable is explained by independent variables. Notably, 41.43% of the variability in PON activity is attributed to group differences. In essence, there are marked differences in the parameter values across the groups, with PON activity showing the highest variance explained by these differences (Table 2).

Table 3 presents Pearson's correlation coefficients for liver parameters in controls, including Total Bilirubin, Direct Bilirubin, AST, ALT, ALP, Total Proteins, Albumin and PON.

PON negatively correlated with total bilirubin, direct bilirubin, ALT, total protein and albumin, though these were not statistically significant. However, it had a significant negative correlation with AST (p = 0.047) and a non-significant positive correlation with ALP. Total Bilirubin had significant positive correlations with direct bilirubin, total protein (p = 0.020) and albumin (p = 0.001). AST showed a significant positive correlation with ALT (p = 0.001).

Table 4 presents Pearson's correlation coefficients and associated p-values for various liver parameters in individuals with viral hepatitis. The parameters being compared include Total Bilirubin (T. BIL), Direct Bilirubin (D.BIL), AST, ALT, ALP, Total Proteins (T.PRO), Albumin (ALB) and PON.

PON's correlation

Negative correlations (not statistically significant): PON showed negative correlations with total bilirubin, direct bilirubin, AST, ALT, ALP and total protein. These correlations weren't statistically significant, as their p-values were greater than 0.05.

Positive correlation (not statistically significant): PON was positively correlated with albumin but this wasn't statistically significant.

Total bilirubin's correlation: Positive Correlations (statistically significant): Total bilirubin had significant positive correlations with direct bilirubin, ALT and total protein, as indicated by their p-values less than 0.05.

Table 1: Mean±SD of various parameters in controls and in cases

LFT parameters	Controls	Ciral hepatitis
	Mean±SD	
T. Bilirubin	0.82±0.51	9.05±10.7
D. Bilirubin	0.36±0.31	3.27±4.52
ALT	24.31±14.36	376.80±311.9
AST	27.71±13.44	403.50±292.4
ALP	70.63±15.01	93.03±44.36
Total proteins	7.21±0.96	6.66±2.47
Albumin	4.30±0.72	3.06±1.17
PON activity	988.00±238	213.30±186.5

Table 2: Anova, p-value, f-value and R^2 in controls and cases

Parameters	F-value	p-value	R^2
T.Bilirubin	6.369	<0.0001	0.1486
D.Bilirubin	7.754	<0.0001	0.1752
ALT	10.620	<0.0001	0.2254
AST	21.630	<0.0001	0.3721
ALP	12.990	<0.0001	0.2624
T.Proteins	2.728	0.0315	0.0695
Albumin	21.680	<0.0001	0.3726
PON activity	25.640	<0.0001	0.4143

Table 3: Pearson's correlation between different parameters in controls

		T. BIL	D.BIL	AST	ALT	ALP	T.PRO	ALB	PON
T. BIL	Pearson r		0.888	0.209	0.079	-0.070	0.422	0.558	-0.226
	p-value		5.87	0.268	0.677	0.712	0.020	0.001	0.155
D.BIL	Pearson r	0.888		0.249	0.156	-0.030	0.448	0.579	-0.295
	p-value	5.876536e011		0.184	0.410	0.876	0.013	0.001	0.114
AST	Pearson r	0.209	0.249		0.562	0.165	0.250	0.261	-0.365
	p-value	0.267745	0.183		0.001	0.384	0.183	0.163	0.047
ALT	Pearson r	0.079	0.156	0.562		0.270	0.001	0.062	-0.221
	p-value	0.676635	0.40	0.001		0.149	0.994	0.744	0.241
ALP	Pearson r	-0.070	-0.030	0.165			-0.333	-0.071	0.201
	p-value	0.712129	0.875	0.384	0.149		0.072	0.708	0.287
T. PRO	Pearson r	0.422	0.448	0.250	0.001	-0.333		0.629	-0.179
	p-value	0.020305	0.013	0.183	0.944	0.072		1.976	0.345
ALB	Pearson r	0.558	0.579	0.261	0.062	-0.071	-0.629		-0.138
	p-value	0.001341	0.000803	0.163	0.744	0.708	1.976		0.467
PON	Pearson r	-0.266	-0.295	-0.365	-0.221	0.201	-0.179	-0.138	
	p-value	0.1549648	0.113824	0.047	0.241	0.287	0.345	0.467	

Table 4: Pearson's correlation between different parameters in viral hepatitis

		T. BIL	D.BIL	AST	ALT	ALP	T.PRO	ALB	PON
T. BIL	Pearson r		0.888	0.307	0.402	0.182	0.687	0.291	-0.170
	p-value		0.874521	0.099	0.028	0.336	2.760	0.119	0.368
D.BIL	Pearson r	0.888		0.167	0.279	0.120	0.694	0.253	-0.073
	p-value	0.874521		0.377	0.136	0.529	2.123	0.178	0.701
AST	Pearson r	0.307	0.167		0.626	0.133	0.161	0.065	-0.197
	p-value	0.098717	0.376637		2.131	0.484	0.394	0.734	0.296
ALT	Pearson r	0.402	0.279	0.626		0.049	0.0329	0.212	-0.231
	p-value	0.027798	0.135609	2.131		0.798	0.076	0.261	0.219
ALP	Pearson r	0.182	0.120	0.133	0.049		0.105	0.079	0.077
	p-value	0.336	0.528	0.484	0.0798		0.582	0.680	0.685
T. PRO	Pearson r	0.687	0.694	0.161	0.329	0.105		0.679	-0.055
	p-value	0.000028	0.000021	0.394	0.076	0.582		3.751	0.772
ALB	Pearson r	0.291	0.253	0.065	0.212	0.079	0.679		0.030
	p-value	0.118769	0.178200	0.734	0.261	0.680	3.751		0.875
PON	Pearson r	-0.170	-0.073	-0.0197	-0.231	-0.077	-0.055	0.030	
	p-value	0.368340	0.701361	0.296	0.219	0.685	0.772	0.085	

Table 5: Sensitivity (Sens), specificity (Spf) and best cut off value (bcv) in discriminating cases and controls

	Total bilirubin			Direct bilirubin			PON		
	Sens	Spf	Bcv	Sens	Spf	Bcv	Sens	Spf	Bcv
VH	100	83.33	>1.13	73.33	93.33	>0.9	96.67	100	<575.1
	AST			ALT			PON		
	Sens	Spf	Bcv	Sens	Spf	Bcv	Sens	Spf	Bcv
VH	90.00	100	>76.90	100	96.55	>48.5	96.67	100	<575.1
	ALP			PON					
	Sens	Spf	Bcv	Sens	Spf	Bcv			
VH	40.00	93.33	>93.00	96.67	100	<575.1			
	Total protein			Albumin			PON		
	Sens	Spf	Bcv	Sens	Spf	Bcv	Sens	Spf	Bcv
VH	60	93.33	<6.16	56.67	100	<3.10	100	96.67	<575.1

Table 6: Area under curve (AUC), diagnostic efficiency (DE) and Significance in discriminating analyzed parameters in controls and cases

	Total bilirubin			Direct bilirubin			PON		
	AUC	DE	p-value	AUC	DE	p-value	AUC	DE	p-value
VH	0.9661	91.6	<0.0001	0.9056	83.3	<0.0001	0.9856	98.3	<0.0001
	AST			ALT			PON		
	AUC	DE	p-value	AUC	DE	p-value	AUC	DE	p-value
VH	0.9822	94.9	<0.0001	0.9966	98.3	<0.0001	0.9856	98.3	<0.0001
	ALP			PON					
	AUC	DE	p-value	AUC	DE	p-value			
VH	0.6456	66.64	0.0528	0.9856	98.3	<0.0001			
	Total protein			Albumin			PON		
	AUC	DE	SIG	AUC	DE	SIG	AUC	DE	SIG
VH	0.7222	76.6	0.0031	0.8417	69.9	<0.0001	0.9856	98.3	<0.0001

Direct bilirubin's correlation:

Positive correlation (statistically significant): Direct bilirubin was positively correlated with total protein, which was statistically significant.

AST's correlation

Positive correlation (statistically significant): AST exhibited a significant positive correlation with ALT.

Total proteins' correlation

Positive correlation (statistically significant): Total protein had a significant positive correlation with albumin.

ROC curve analysis: In order to assess the maximum sensitivity, specificity and diagnostic efficiency of various parameters in identifying abnormality, the best cut off values are calculated using ROC analysis. Best cut off values are established by selecting a point closer to the left hand curve that provides greatest sum of sensitivity and specificity.

Diagnostic efficiency is defined as the portion of all currently classified as having or not having disease.

$$\text{Diagnostic efficiency} = \frac{\text{True positive+true negatives}}{\text{Total no of patients evaluated}} \times 100$$

Area under curve provides unbiased estimates of sensitivity and specificity. It is a comprehensive representation of pure accuracy discriminating ability over the entire range of the test.

The ROC analysis revealed that PON 1 had 100% sensitivity in identifying viral hepatitis. In viral hepatitis and COL, its specificity was comparable to bilirubin, AST and ALT and better than other parameters. PON 1 demonstrated higher diagnostic accuracy over other markers such as bilirubin, AST and ALT. In HCC, while PON 1's sensitivity surpassed several parameters, its specificity was less compared to bilirubin and AST but remained equivalent to ALP and superior to ALT (Table 5).

Table 6 shows, PON 1 outperform other parameters in differentiating between cases and controls, showcasing superior diagnostic efficiency. In the context of viral hepatitis, PON 1's diagnostic capability is on par with ALT.

DISCUSSIONS

The liver plays a crucial role in metabolism, detoxification and maintenance of homeostasis. Evaluating liver function using biochemical markers is imperative to diagnose and manage liver diseases effectively. Our findings reiterate the significance of some well-established liver markers while also emphasizing the potential utility of others in distinguishing between diseased states and controls.

From our observations, there's an evident increase in the Mean±S.D of total and direct bilirubin, AST, ALT and ALP in viral hepatitis patients relative to controls. This is consistent with previous studies, emphasizing the role of these markers in liver injury. Elevations in these markers typically indicate hepatocellular

damage, cholestasis, or a combination of both[6]. Conversely, albumin levels were significantly lower in the viral hepatitis group, which aligns with its known decline in chronic liver disease due to reduced synthetic function of the liver[7]. Interestingly, PON 1 activity was also significantly diminished in the viral hepatitis cohort. This is noteworthy as recent literature has shed light on PON 1's potential role in oxidative stress and liver pathogenesis[8].

Total proteins, though, didn't demonstrate significant variability between the two groups, which can be attributed to the balance between its individual components, albumin and globulins[9]. Our correlation analysis further deepens the understanding of the interplay between various markers. The negative correlation of PON with several parameters, albeit not always significant, indicates potential underlying mechanisms that warrant further exploration. Its significant negative correlation with AST, an enzyme indicative of hepatocellular damage, might suggest a possible protective or compensatory role of PON in liver injury. This is a novel observation aligning with some emerging studies on the antioxidant role of PON[10].

Total bilirubin's strong positive correlations with direct bilirubin, total protein and albumin reinforce the interconnected nature of these markers in liver metabolism and function[11]. AST's positive correlation with ALT, a well-documented observation, re-emphasizes their joint elevation in hepatocellular injury[12].

The ROC analysis findings underscore PON 1's potential diagnostic prowess, with it showing a 100% sensitivity in detecting viral hepatitis. This high sensitivity, combined with specificity comparable to established markers like bilirubin, AST and ALT, suggests that PON 1 could be a valuable addition to the current liver function test panel. This is further supported by PON 1's diagnostic efficiency being on par with ALT, as observed in our study.

In conclusion, while traditional markers remain indispensable in liver function assessment, emerging markers like PON 1 offer complementary insights. Incorporating them into diagnostic workflows could enhance the precision and comprehensiveness of liver disease evaluations. However, further studies and validation are essential before their routine clinical adoption.

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