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CAN WE REPLACE TRANSIENT ELASTOGRAPHY I.E. FIBRO SCAN WITH CHEAP BIOMARKERS? A CROSS SECTIONAL STUDY AT LAHORE GENERAL HOSPITAL, LAHORE

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Abstract:

Objective: *To assess the validity ofSerum Aminotransferase Levels i.e. ALT & AST,Albumin and Bilirubinas fibrosis biomarker, we compared their serum levels with fibro scan for the fibrosis staging and predicting its progression in Pakistani population.*

Methods: *The prospective cross sectional study was conducted in medicine unit 1 and hepatitis clinic of Lahore General Hospital, Lahore starting from11Feb 2017–29Dec2018. We studied 1376 HCV infected patients which were got CBC, LFTs, ELISA, PCR and fibro scan done to perfectly diagnose ongoing hepatitis C infection. In order to differentiate HCV fibrosis progression, we compared the effectiveness of readily available serum aminotransferase Levels i.e. ALT & AST, Albumin and Bilirubin with fibro scan.*

Results: *Area Under the Curve for F0-F3 was less than 0.6 except ALT indicating thatneither of Albumin, Bilirbin, ALT or ASTcan be used as diagnostic biomarker for predicting F0-F3.But,An ALT for F4 stage, the sensitivity was 57.3, specificity 50.0 with AUC=0.698.AST>40 had sensitivity of 82.6 and specificity of 79.0 for F4 stage with AUC=0.707. Bilirubin >0.95 had sensitivity of 45% and specificity of 79.0 for F4 stage with AUC=0.651. Albumin can neither be used as diagnostic biomarker for all the stages of fibrosis (F0-F4).*

Conclusion: *An AST, ALT and Bilirubin can predict cirrhosis in patients with chronic hepatitis C infection. In these patients, a liver biopsy and fibro scan may not be necessary in these patients.*

Keywords: *Hepatitis C, Blood Platelets, Fibro scan score.*

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INTRODUCTION:

Hepatitis C is the leading cause of cirrhosis and cirrhosis associated complications and affects more than 170 million population worldwide. Pakistan has the second highest prevalence of hepatitis C after Egypt [1]. Due to widespread prevalence of disease and treatment is very expensive it has a huge cost burden. Medicines for hepatitis are quite expensive. in USA burden of HEP C exceeds $10 annually. according to an estimate cost of only liver transplant is $200000 in USA and subsequent yearly care is quite expensive [2]. HCV is RNA virus belongs to FLAVIVIRIDAE [3]. It is enveloped RNA virus whose genome encodes a polyprotein of more than 3000 amino acids that is cleaved co and post translationally at the endoplasmic reticulum by host and viral proteases which yields 3 structural and 7 nonstructural proteins.HCV nonstructural proteins forms membrane associated replication complex [4]. HEPATITIS C virus infection is cause of chronic progressive liver disease. HCV infection is leading cause of cirrhosis and hepatocellular carcinoma [5,6,29]. HCV is self-limiting disease in 15 to 20 % of cases, 75% to 85% develop chronic disease, rate of chronic disease is influenced by many factors including age at the time of infection, gender, ethnicity. Cirrhosis develop in 10 to 15 % of individuals with HCV infections. [7-10]. There are multiple external and host factor which affect the progression of disease for example chronic alcohol use is a major external risk factor for progression of chronic hepatitis c to fibrosis to hepatocellular carcinoma. Host factor also effect the progression of disease for example infection in older age, coinfection with HIV, poor compliance and hepatitis B. [11,12,30]. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Albumin, bilirubin and platelets are produced mainly from liver cells. AST is mainly found in liver and heart cells. In normal individuals AST levels in the blood are low but after liver damage AST levels in the blood rise. AST is found mainly in the liver and kidneys. It is an enzyme that catalyzes the transfer the amino group to form the hepatic metabolite oxaloacetate [13]. ALT is found abundantly in cytosol of hepatocytes. ALT activity in the liver is 3000 times higher than in the serum. So, in the case of hepatocellular damage ALT is released from liver cells and its measurement in the blood can predict hepatocellular. ALT is specific to liver though it is released in minute quantities from other cells [15,16]. In acute hepatic injury AST levels are usually higher than ALT because of higher activity of AST in hepatocytes and its release from live. In chronic hepatocellular injury ALT levels are usually higher than AST until fibrosis starts. As fibrosis progresses ALT levels decline and ratio of AST to ALT increases.so by the time cirrhosis is present AST levels are usually higher than ALT [17,18]. In hepatocellular damage the synthetic function is also affected which is reflected by deceasing levels of albumin and platelet count and rise in bilirubin [19,20].

MATERIALS AND METHODOLOGY:

This study was conducted at medicine wards and Hepatitis Clinic, Lahore General Hospital, Lahore, Pakistan. HCV RNA positive patients were identified among hepatitis patients having anti-HCV antibodies and viral genotype was noted. It was prospective cross-sectional study aimed at effective diagnosis of liver fibrosis without reliance on expensive transient elastography, the availability of which is also problematic in developing countries where hepatitis is relatively more prevalent.

This study was carried out from 11 Feb 2017–29 Dec 2018. HBV patients and those patients on which any clinical findings of liver cancer were present, were excluded from study. Total 1376 patients were engaged over this period and informed consent was obtained. The study was approved by Institutional Ethical Review Board (IERB), LGH. Quantitative determination of the Fibro scan score, biomarkers (liver function tests (LFTs), albumin and bilirubin, was done and fibrosis stages were determined from fibro scan score.

Statistical analysis:

The data was analyzed using statistical package SPSS windows version 22. A p value of less than 0.05 was considered statistically significant. To determine the significant association between continuous variables and liver fibrosis stages, Spearman's rank correlation was used. The student t-test was used to compare arithmetic means and parameters while Chi-square(X2) test was used to compare categorical data. The univariate regression analysis were done for different biomarkers. Receiver Operating Curves (ROC) were performed and area under the receiver operating characteristic curves (AUROCs) was used to compare and deduce the diagnostic accuracies of the selected biomarkers along with their cutoff points, sensitivities and specificities.

RESULTS:

Patients:

A total of 1376 patients, comprising of 516(37.5%) men and 860(62.5%) women, with mean age of 42±13, were enrolled. Of these 1280 were married and 91 were unmarried. Of these 738(53%) have fibrosis stage F0-F1, 55(4%) F2, 177(12.9%) F3 and 406(29.5%) F4. Other characteristics of patients are

given in table 1.
Table # 1

Correlation of fibrosis stage with serum ALT
The linear Curve Estimation Analysis and Pearson correlation showed a linear relationship between stages of fibrosis by Fibro scan and serum ALT levels. Table 2.

By applying independent sample T test, the relationship between stage of fibrosis predicted by fibro scan and rise in ALT levels was found to be significant($p<0.05$). table 3 and 4.

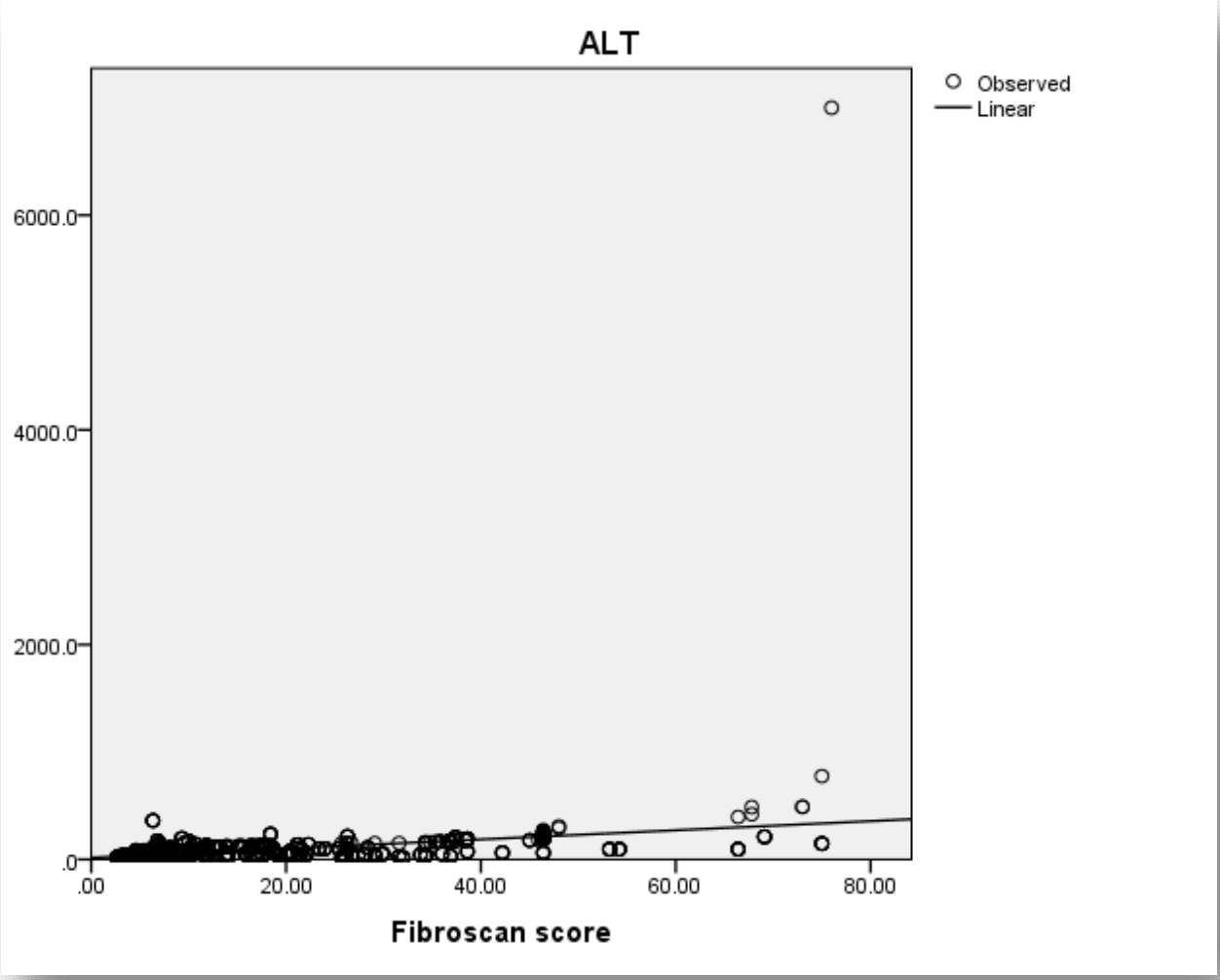
Table # 2. Pearson correlation

	Frequency	Percent	Valid Percent	Cumulative Percent
Gender				
Female	860	62.5	62.5	62.5
Male	516	37.5	37.5	100.0
Total	1376	100.0	100.0	
Occupation				
Housewife	471	34.2	34.2	34.2
Laborer	826	60.0	60.0	94.3
Working Lady	79	5.7	5.7	100.0
Total	1376	100.0	100.0	
Marital Status				
Married	1280	93.0	93.0	93.0
Unmarried	91	6.6	6.6	99.4
3.0	5	.4	.4	100.0
Total	1376	100.0	100.0	
Fibrosis stage				
F0-F1	738	53.6	53.6	53.6
F2	55	4.0	4.0	57.6
F3	177	12.9	12.9	70.5
F4	406	29.5	29.5	100.0
Total	1376	100.0	100.0	

Dependent Variable: ALT

Equation	Model Summary					Parameter Estimates	
	R Square	F	df1	df2	Sig.	Constant	b1
Linear	.088	132.363	1	1374	.000	16.226	4.253

The independent variable is Fibro scan score.



Group Statistics (stage F0-F2)

Fibrosis Stage		N	Mean	Std. Deviation	Std. Error Mean
ALT	F0-F1	738	50.306	35.2310	1.2969
	F2	55	67.673	42.3832	5.7149

Table # 3. Independent sample t test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
ALT F0-F2	Equal variances assumed	1.298	.255	-3.474	791	.001
	Equal variances not assumed			-2.964	59.693	.004

Group statistics (stage F3-F4)

Fibrosis Stage		N	Mean	Std. Deviation	Std. Error Mean
ALT	F3	177	76.497	40.0657	3.0115
	F4	406	126.874	352.4840	17.4935

Table # 4. Independent sample t test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
ALT (F3-F4)	Equal variances assumed	4.132	.043	-1.895	581	.059
	Equal variances not assumed			-2.838	428.495	.005

Correlation of stage of fibrosis ad serum AST level:

The linear curve Estimation Analysis and Pearson Correlation Coefficient showed a linear relationship between stage of fibrosis and increase in serum AST level (R value is 0.307) given in table 5

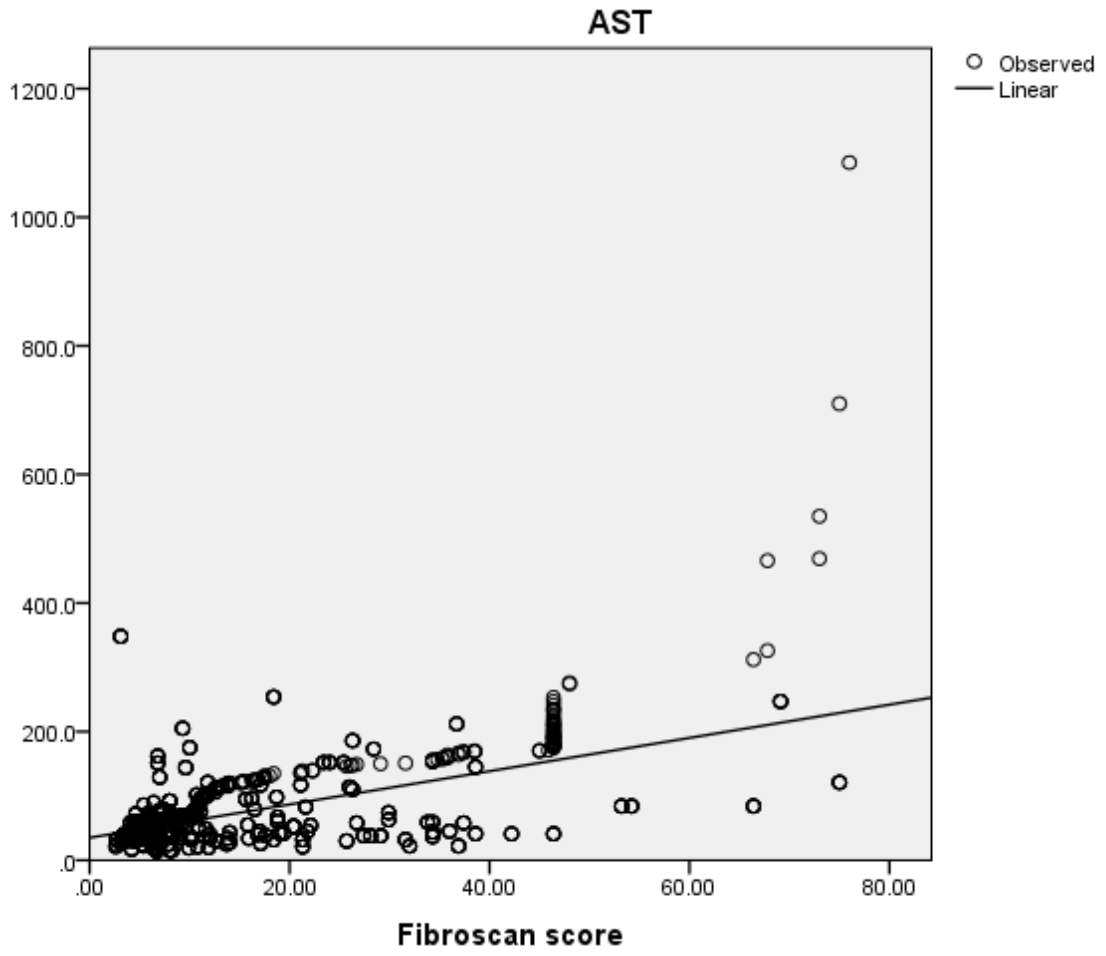
By applying independent sample T test, the relationship between stage of fibrosis predicted by Fibro scan and rise in serum AST level was found to be significant ($p < 0.05$) table 6,7.

Table # 5. Pearson correlation

Dependent Variable: AST

Equation	Model Summary					Parameter Estimates	
	R Square	F	df1	df2	Sig.	Constant	b1
Linear	.307	608.527	1	1374	.000	35.011	2.587

The independent variable is Fibro scan score.



Group statistics (stage F0-F2)

Fibrosis Stage		N	Mean	Std. Deviation	Std. Error Mean
AST	F0-F1	738	51.005	32.4268	1.1936
	F2	55	67.582	45.6483	6.1552

Table # 6. independent sample t test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
AST	Equal variances assumed	4.991	.026	-3.541	791	.000
	Equal variances not assumed			-2.644	58.132	.011

Group statistics (stage F3-F4)

Fibrosis Stage		N	Mean	Std. Deviation	Std. Error Mean
AST	F3	177	72.158	39.4682	2.9666
	F4	406	110.512	93.4620	4.6384

Table # 7.independent sample t test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
AST	Equal variances assumed	34.446	.000	-5.257	581	.000
	Equal variances not assumed			-6.966	580.562	.000

Correlation of stages of fibrosis with serum albumin There was linear relationship between stages of fibrosis by fibro scan and decrease in serum albumin level according to Linear Curve Estimation Analysis and Pearson Coefficient as shown in table 8.

By applying independent sample T test ,the relationship between stages of fibrosis and serum albumin level was found to be non-significant($p>0.05$) table 9,10

Table # 8.pearson correlation

	Model Summary					Parameter Estimates	
Equation	R Square	F	df1	df2	Sig.	Constant	b1
Linear	.172	285.895	1	1374	.000	4.353	-.042

Group statistics(F0-F2)

Fibrosis		N	MEAN	Std. deviation	Std error mean
Albumin	F0-F1	738	4.1020	.84328	.03104
	F2	55	4.6809	3.44039	.46390

Table # 9. independent sample T test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
Albumin	Equal variances assumed	115.172	.000	-3.416	791	.001
	Equal variances not assumed			-1.245	54.485	.218

Group statistics (F3-F4)

Fibrosis Stage		N	Mean	Std. Deviation	Std. Error Mean
Albumin	F3	177	3.4305	1.06145	.07978
	F4	406	3.1531	1.51788	.07533

Table # 10.independent sample T test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
Albumin	Equal variances assumed	36.433	.000	2.207	581	.028
	Equal variances not assumed			2.528	468.036	.012

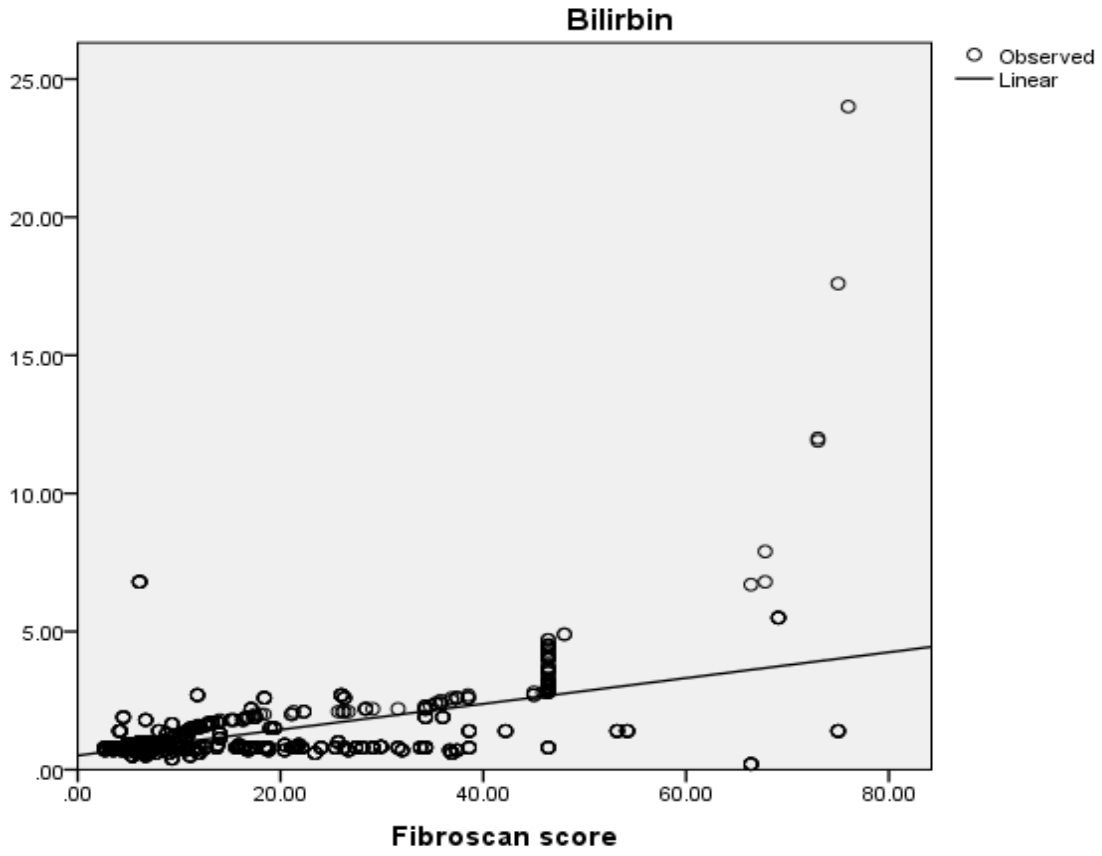
Correlation of fibrosis stages and serum bilirubin level.

According to our analysis inthe Linear Curve Estimation and Pearson Analysis, there was a linear correlation between stages of fibrosis and rise in serum bilirubin level table 11.

By applying independent sample T test, we found that serum bilirubin rise was not significantly correlated with FIBRO scan stage F1-F2, but correlation in stage F3-F4 was significant. Table 12,13.

Table # 11.pearson correlation

	Model Summary					Parameter Estimates	
Equation	R Square	F	df1	df2	Sig.	Constant	b1
Linear	.281	538.226	1	1374	.000	.513	.047



Group statistics(F0-F2)

Fibrosis Stage		N	Mean	Std. Deviation	Std. Error Mean
Bilirubin	F0-F1	738	.8839	.52249	.01923
	F2	55	.9245	.34101	.04598

Table # 12. independent sample T test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
Bilirubin	Equal variances assumed	2.416	.121	-.568	791	.570
	Equal variances not assumed			-.816	74.382	.417

Group statistics(F3-F4)

Fibrosis Stage		N	Mean	Std. Deviation	Std. Error Mean
Bilirubin	F3	177	1.1494	.45279	.03403
	F4	406	1.7673	1.95597	.09707

Table # 13. independent sample T test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	T	Df	Sig. (2-tailed)
Bilirubin	Equal variances assumed	38.091	.000	-4.152	581	.000
	Equal variances not assumed			-6.006	493.526	.000

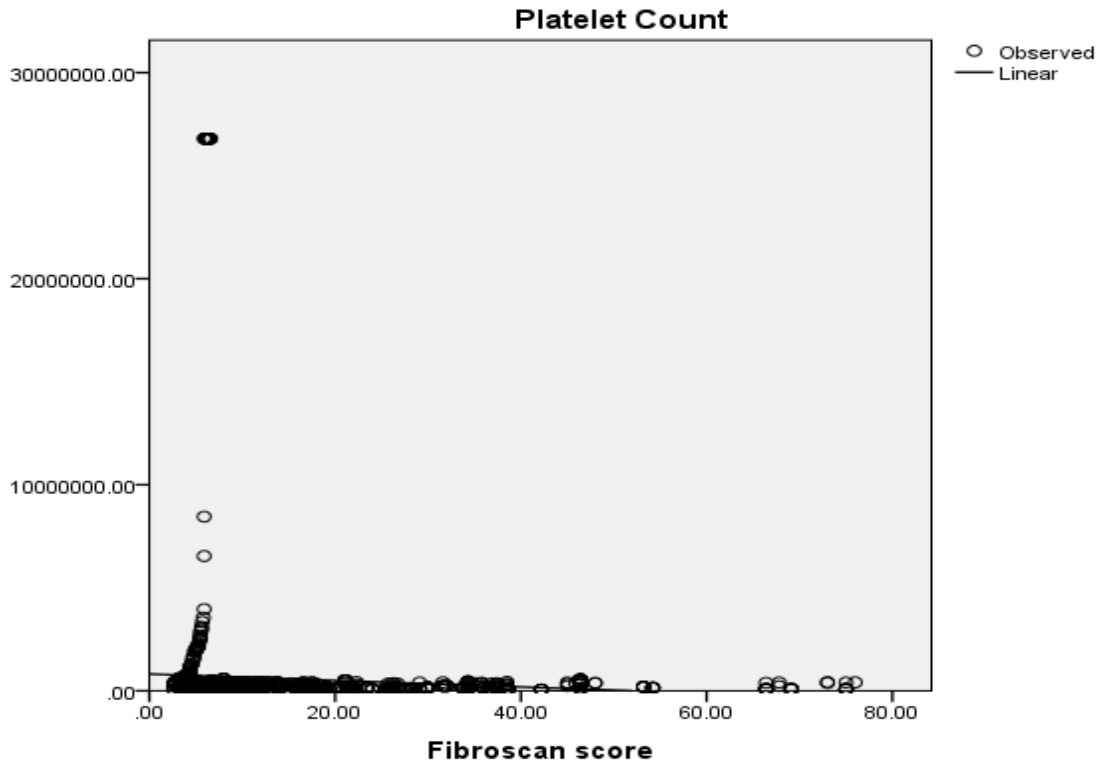
Correlation of fibrosis stages with platelet count:

the linear curve estimation analysis and Pearson Correlation Coefficient showed a linear correlation between stages of fibrosis and decrease in platelet count. Table 13

by applying independent sample T test relationship between stages of fibrosis and decrease in platelet count was found to be significant ($p < 0.05$) table 14 ,15

table # 14. Pearson correlation

	Model Summary					Parameter Estimates	
Equation	R Square	F	df1	df2	Sig.	Constant	b1
Linear	.006	8.789	1	1374	.003	814449.604	-15680.904



Group statistics (F0-F2)

Fibrosis Stage		N	Mean	Std. Deviation	Std. Error Mean
Platelet Count	F0-F1	738	902486.0786	3650689.45321	134383.73585
	F2	55	248509.0909	106855.71316	14408.42142

Table # 15. independent sample T test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	T	Df	Sig. (2-tailed)
Platelet Count	Equal variances assumed	4.982	.026	1.328	791	.185
	Equal variances not assumed			4.839	752.685	.000

Group statistics (F3-F4)

Fibrosis Stage		N	Mean	Std. Deviation	Std. Error Mean
Platelet Count	F3	177	260547.0339	100764.30262	7573.90878
	F4	406	213694.5813	142127.17421	7053.65322

Table # 16. independent sample T test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	T	Df	Sig. (2-tailed)
Platelet Count	Equal variances assumed	19.621	.000	3.971	581	.000
	Equal variances not assumed			4.527	462.504	.000

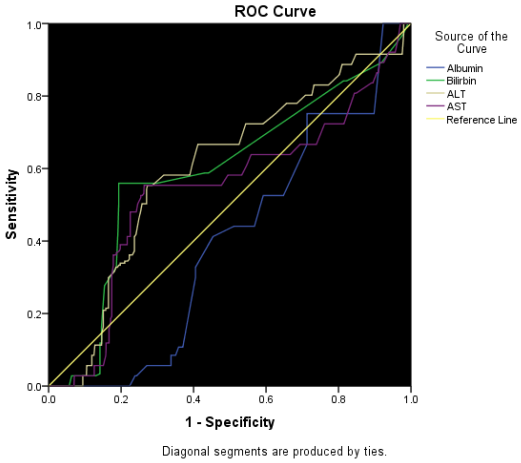
ROC curves Interpretation:

Area Under the Curve for F0-F3 was less than 0.6 except ALT indicating thatneither of Albumin, Bilirbin, ALT or ASTcan be used as diagnostic biomarker for predicting F0-F3.

Area Under the Curve for F0-F3

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Albumin	.401	.019	.000	.363	.438
Bilirbin	.592	.024	.000	.544	.640
ALT	.602	.023	.000	.557	.647
AST	.544	.025	.061	.494	.593

The test result variable(s): Albumin, Bilirbin, ALT, AST has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.
a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5



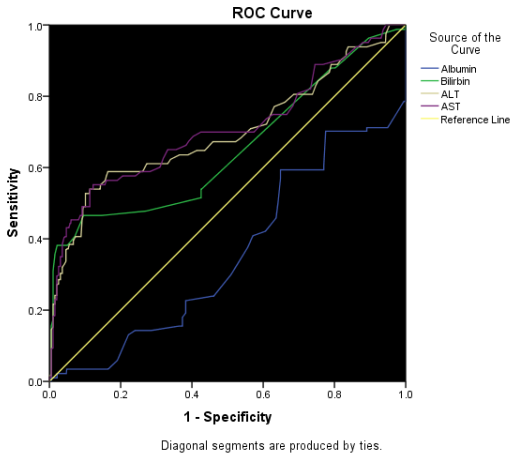
ROC Curve for F0-F3

But,An ALT for F4 stage, the sensitivity was 57.3, specificity 50.0 with AUC=0.698.
AST>40 had sensitivity of 82.6 and specificity of 79.0 for F4 stage with AUC=0.707.
Bilirubin >0.95 had sensitivity of 45% and specificity of 79.0 for F4 stage with AUC=0.651.
Albumin can neither be used as diagnostic biomarker for all the stages of fibrosis (F0-F4).

Area Under the Curve for F4

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Albumin	.356	.016	.000	.324	.389
Bilirbin	.651	.018	.000	.616	.686
ALT	.698	.017	.000	.663	.732
AST	.707	.017	.000	.673	.741

The test result variable(s): Albumin, Bilirbin, ALT, AST has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.
a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5



ROC Curve for F4

DISCUSSION:

Chronic infection with hepatitis c virus causes injury and inflammation of hepatocytes leading ultimately to fibrosis of liver [21]. Liver biopsy remains the gold standard for assessing the stages of fibrosis. Several systems for grading hepatic fibrosis have been proposed on the basis of seeing the collagen staining of liver biopsy. Most commonly or frequently used are Histology activity index (HAI or Knodell score [22], the Ishak modification [23] of HAI score and the Metavir score [24]. There are several limitations of taking liver biopsy like there should be an adequately sized biopsy as hepatic fibrosis may not be homogenous throughout the liver, a non-fragmented sample, correct histological method, proper use of staining [25]. These all limitation have led us to think that there should be other methods for scoring liver fibrosis like noninvasive markers. we used two serum markers (ALT and AST) in this study to estimate the stages of fibrosis. Specificity and sensitivity of ALT is good for prediction of hepatic injury though correlation between ALT levels fibrosis scoring is poor [26].

Our data concluded that 3a genotype is more common in Pakistan than other genotype and other studies augmented our results [27]. Our data showed that fibrosis due to hep C is more prevalent in male gender and augmented by other studies [28]. Our study concluded that there is positive relationship between hepatic fibrosis and serum ALT, AST and BILIRUBIN probably due to reason that in hepatic damage AST and ALT are released from hepatocytes and bilirubin is increased because of decreased conjugation of bilirubin. There is negative relationship between hepatic fibrosis and albumin and platelet count probably because of the reason that albumin is synthesized in liver and platelets are activated by proteins synthesized by liver. These serum markers cannot be used in predicting first three sages of fibrosis except ALT which has a fair predictive value only in in 3rd stage of fibrosis. these serum markers can be used in in predicting 4th stage of fibrosis in which ALT, ALBUMIN, and bilirubin have fair predictive value and AST has good predictive value. If they are used with other markers like AST to ALT ratio, hyaluronic acid, AST to platelet ratio index and other non-invasive markers they can replace invasive techniques

CONCLUSION:

ALT, AST, albumin and bilirubin can be used for predicting fourth stage of fibrosis but cannot be used for other stages of fibrosis, only ALT has fair value in assessing 3rd stage of fibrosis.

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