Predictive Value of Quantitative Estimation of Hepatitis B Surface Antigen and DNA load in serum of Chronic Hepatitis B Patients

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Abstract: Objectives: Quantitative estimation of serum levels of hepatitis B surface antigen (HBsAg) and HB viral DNA load (HB VDL) in chronic hepatitis B (CHB) patients and their applicability for differentiating between disease phases and to predict the outcome of liver biopsy. **Patients & Methods**: The study included 113 patients; 67 males and 46 females; with mean age of 42.6±10.8 years and mean disease duration of 5.6±1.1 years. All patients underwent clinical examination, and blind liver biopsy was taken for necrosis and fibrosis histopathological scoring. Fasting venous blood samples were collected for estimation of serum AST and ALT, estimation of hepatitis B serological markers by ELISA and quantitative estimation of serum HBsAg by Roche Cobas e 411 analyzer and estimation of HB VDL by real time PCR. **Results**: Fifty-three patients were HB e antigen (HBeAg)-positive, while 60 patients were HBeAg-negative. Mean total serum HB VDL was 2907.2±1060 IU/ml; 32 patients had low and 81 patients had high HB VDL. Mean total serum HBsAg level was $24.7\pm5.9x10^3$ IU/ml. The ratio of the median $\log_{10}$ of serum HB VDL/ serum HBsAg level was 0.42 in low VDL patients and 0.4 in high VDL patients. Regression analysis defined high $\log_{10}$ of serum HBsAg level as the persistently significant determinant of cases with immune tolerance (IT) and/or immune reactive (IR), liver necrosis score, high $\log_{10}$ of serum VDL, the ratio of $\log_{10}$ values of serum VDL to serum HBsAg and male gender. ROC curve analysis defined high $\log_{10}$ of serum HBsAg level as a significant specific and the ratio of $\log_{10}$ of serum HB VDL to serum HBsAg as a significant sensitive predictor for IT cases and high $\log_{10}$ of serum HB VDL and positive HBeAg as significant predictors for presence of fibrosis. **Conclusion**: Quantitative estimation of serum level of HBsAg and viral load could differentiate between phases of CHB disease and predict histopathological status of the liver, so could spare liver biopsy with its inherent complications.

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Key words: Chronic hepatitis B, Serum viral DNA load, HBsAg, Quantitative PCR, Liver Biopsy.

1. Introduction

Chronic hepatitis B is a major global problem, affecting more than 350 million chronic Hepatitis B worldwide, and leading to 1 million deaths each year **(Wright, 2006).** Infection with the hepatitis B virus (HBV) may lead to an acute or chronic infection. It is generally accepted that the clinical outcome of infection depends on the balance between host immunity and viral survival strategies. In order to persist, the virus needs to have a high rate of replication and some immune-escape capabilities. Hence, HBVs lacking these properties are likely to be eliminated more rapidly by the host, leading to a lower rate of chronicity **(Kuo & Gish, 2012**; **Chook *et al.*, 2013)**.

Various findings concerning the clinical significance of quantitative changes in hepatitis B surface antigen (HBsAg) during the acute and chronic phase of HBV infection have been reported. In addition to being a biomarker of HBV-replication activity, it has been reported that HBsAg could contribute to the immunopathogenesis of HBV persistent infection. Moreover, HBsAg could become an attractive target for immune therapy, since the cellular and humeral immune response against HBsAg might be able to control the HBV replication and life cycle **(Buti *et al.*, 2012; Janssen *et al.*, 2012; Kondo *et al.*, 2013)**.

European Association for the Study of the Liver defined the following phases for natural history of CHB disease: Immune tolerant (IT) phase in which patients were HBeAg-positive with high serum HB VDL (Hepatitis B virus DNA Load) and normal or low serum ALT; Immune reactive (IR) patients were HBeAg-positive with lower serum HB VDL than IT cases and high serum ALT; inactive HBV carrier state or low replicative (LR) phase where in patients were HBeAg-negative with very low serum HBV load and

normal or low serum ALT and HBeAg-negative hepatitis with fluctuating serum HBV DNA, serum ALT and active hepatitis **(McMahon, 2004; Hadziyannis & Papatheodoridis, 2006; Hoofnagle *et al.,* 2007; Fattovich *et al.,* 2008)**.

The proposed diagnostic criteria for differentiation between different phases of CHB disease relied mainly on the positivity of serum for HBeAg as the main diagnostic criterion for differentiating cases as two main categories; among each category differentiating parameters provided gray zone of diagnosis. Liver biopsy has long been essential to evaluate the degree of liver damage and to decide therapeutic plan in these subjects. Besides establishing the diagnosis, the biopsy is often used to assess the severity of the disease in terms of both grade and stage. The stage relates to the degree of scarring with the end stage being cirrhosis with its clinical complications. The grade relates to the severity of the underlying disease process, with features that vary with the pathogenetic mechanisms **(Lee *et al.,* 2001, Goodman, 2007, Ganji *et al.,* 2011).** So that the current study was designed to evaluate the diagnostic yield of quantitative estimation of serum HBsAg level and DNA viral load in differentiation between the main categories of CHB disease and their subtypes and to define their applicability for differentiating between phases of CHB disease and to predict the outcome of liver biopsy.

2.Patients & Methods

Patients:

The current prospective study was conducted at Departments of Hepatology, Infectious Diseases and Medical microbiology, At King Fahad and Al-Ansar Hospitals, KSA since June 2012 till April 2013. After approval of the study protocol by the Local Ethical Committee and obtaining written fully informed patients' consent, all patients with CHB disease irrespective of being on treatment or not were enrolled in the study. Patients with undetectable HBV DNA levels, patients with co-infection with HCV, Human Immunodeficiency Virus (HIV) or Hepatitis D virus (HDV), chronic renal failure patients with serum creatinine >4 mg/dl and patients with autoimmune liver disease were not enrolled in the study.

All patients underwent clinical examination for determination of the current clinical status and their files were revised for their constitutional data, disease duration, preliminary lab data and clinical status and to judge if they were on treatment or not and its type and duration. Blind liver biopsies were done by means of the Biopsy gun (Biopter) MBD-Multiple Biopsy Device, US, Biopsy, Franklin.

Histopathology:

Quality Control of Histopathologic Assessment

The maximum aggregate length of each needle core biopsy specimen was measured to be at least 1-1.5 cm, containing a minimum of three portobiliary spaces and evaluated by a pathologist unaware of the clinical and virological results. Each biopsy sample was scored after examination of at least three histological H&E-stained sections and corresponding special stains. Biopsy samples in which the liver capsule was identified were graded and staged using all but a 1-mm subcapsular rim of tissue**.** The implicit subjective component was clearly diminished by using a complex numerical system; Ishak Modified Histology Activity Index (HAI) (Table 1) which is appropriate for evaluation of large cohorts of patients when statistical analysis is required **(Goodman, 2007, Skripenova *et al.,* 2007, Stănculeț *et al.,* 2012).**

Histopathologic Scoring

The fragments of hepatic tissue were fixed in formalin and then processed according to the standard protocol of the Pathology Laboratory. Slides were stained with hematoxylin and eosin (H&E), reticulin, trichrome and Periodic Acid–Schiff (PAS). The histopathological grading and staging were performed using Ishak Modified HAI system which contains a necroinflammatory score from 1–18 (The necroinflammatory score is the sum of four scores graded as: None=0; minimal=1-4; mild=5-8; moderate=9-12; severe= 13-18), and a fibrosis score from 0–6 **(Ishak *et al.,* 1995).**

Laboratory investigations

Sampling: A 15-ml fasting venous blood sample was collected under complete aseptic conditions from a large antecubital vein. Blood sample were equally divided in two parts:

1. The first was allowed to clot in water bath at 37°C for 20 minutes and centrifuged, then serum was separated and divided into 2 aliquots:
   a- The first aliquot was used for measurement of serum AST, ALT, total and direct bilirubin using the Hitachi 7600 DDP modular chemistry analyzer (Hitachi High-Technologies, Tokyo, Japan).
   b- The second aliquot was used for ELISA estimation of hepatitis B serological markers (HBsAg, HBeAg, anti-HBe, anti-HBc total/IgM) using commercially available kits (Bio-Rad, France) with PEB III (Dade Behring)
2. The second part was collected in dry plain tube and centrifuged and serum was stored at -80°C to be used for quantitative estimation of HBsAg.
3. The third part was collected in EDTA tube and centrifuged in the PCR unit and plasma was stored at -80°C to be used for and HBV quantitation by PCR.

Table (1): The Ishak Modified HAI for grading and staging chronic hepatitis

Modified HAI Grading: Necroinflammatory Scores		
A. Periportal or periseptal interface hepatitis (piecemeal necrosis)	Absent	0
	Mild (focal, few portal areas)	1
	Mild/moderate (focal, most portal areas)	2
	Moderate (continuous around 50% or less of tracts or septa)	3
	Severe (continuous around more than 50% of tracts or septa)	4
B. Confluent necrosis	Absent	0
	Focal confluent necrosis	1
	Zone 3 necrosis in some areas	2
	Zone 3 necrosis in most areas	3
	Zone 3 necrosis plus occasional portal-central (P-C)bridging	4
	Zone 3 necrosis plus multiple P-C bridging	5
	Panacinar or multiacinar necrosis	6
C. Focal (spotty) lytic necrosis, apoptosis and focal inflammation	Absent	0
	One focus or less per 10x objective	1
	Two to four foci per 10x objective	2
	Five to ten foci per 10x objective	3
	More than ten foci per 10x objective	4
D. Portal inflammation	None	0
	Mild, some or all portal areas	1
	Moderate, some or all portal areas	2
	Moderate/marked, all portal areas	3
	Marked, all portal areas	4
Modified HAI Staging: Architectural Changes, Fibrosis, and Cirrhosis		
No fibrosis		0
Fibrous expansion of some portal areas, with or without short fibrous septa		1
Fibrous expansion of most portal areas, with or without short fibrous septa		2
Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging		3
Fibrous expansion of portal areas with marked bridging (P-P) as well as portal-central (P-C)		4
Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)		5
Cirrhosis, probable or definite		6

Investigations

1. **HBsAg quantitation**: HBsAg levels were measured by Roche Cobas e 411 analyzer with Elecsys HBsAg II Quant reagent kits (Roche Diagnostics, Indianapolis, IN). This test uses the electrochemiluminescence immunoassay with a sandwich complex formed from the 2 biotinylated monoclonal anti-HBsAg antibodies and a mixture of monoclonal and polyclonal anti-HBsAg antibodies labeled with a ruthenium complex as a chemiluminescence molecule. The resulting chemiluminescence reactions were measured and converted to HBsAg concentrations in the specimens using the calibration curve generated by 2-point calibrators. The analyzer provided an onboard dilution function, which prediluted the samples automatically after they were loaded; the analytic measurement range (AMR) suggested by the manufacturer is between 5 and 13,000 IU/mL when the samples are 100-fold diluted using the automated dilution function. Diluted samples with HBsAg levels less than 5 IU/mL were retested without predilution, and the specimens were manually diluted by 20-fold before being loaded on the analyzer (total dilution, 2,000-fold) when the HBsAg concentration was greater than 13,000 IU/ml.
2. **HBV DNA quantitation**: a plasma aliquot (1050 µl) was used for HBV DNA quantitation using Cobas AmpliPrep/Cobas TaqMan test (Roche Diagnostics, Mannheim, Germany) and TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. This is an automated real-time PCR test based on dual labeled hybridization probe targeting the pre-core and core regions. An internal quantitation standard (QS) is added to each sample during the processing step. Two targets are amplified: HBV DNA and the internal QS. The QS is a noninfectious construct containing fragments of HBV sequences with primer binding regions identical to those of the HBV target sequence but with a detection probe different from that for HBV. The results were expressed as international units per milliliter (IU/ml) with a 5.82 copies per IU

conversion factor. Lower limit of detection for the assay was 6 IU/ml and samples were categorized according to viral DNA load (VDL) into high HB VDL if serum DNA level was ≥2000 IU/ml and were considered as low HB VDL if serum DNA level was <2000 IU/ml **(Andersson & Chung,2009)**.

Patients' categorization

Patients were categorized according to HBeAg into HBeAg-positive and HBeAg-negative. Then patients were further differentiated according to serum ALT into normal level (less than upper limit of normal), ≤2 ULN or >2ULN. The ULN of serum ALT was defined as 30 U/L for men and 19 U/L for women **(Prati *et al.*, 2002)**.

Statistical analyses

Obtained data were presented as mean±SD, ranges, numbers and ratios. Results were analyzed using Wilcoxon; ranked test for unrelated data (Z-test) and Chi-square test (X^2 test). Sensitivity & specificity of estimated parameters as predictors were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) compared versus the null hypothesis that AUC=0.05. Regression analysis (Stepwise method) was used for stratification of studied parameters as specific predictors as models for identifying the persistently significant predictors. Statistical analysis was conducted using the SPSS (Version 15, 2006) for Windows statistical package. *P* value <0.05 was considered statistically significant.

3.Results

The study included 113 patients; 67 males and 46 females; with mean age of 42.6±10.8; range: 15-53 years. Mean disease duration was 5.6±1.1; range: 3-8 years. Histopathological examination of liver biopsy revealed that each specimen had between 3 and 12 portobiliary spaces, with an average of 6–8 spaces. No or minimal necroinflammatory lesions were evident in 44 biopsies (38.9%), whilst, mild and moderate activity were seen in 54.9% and 6.2% of biopsies respectively. None of biopsies showed severe activity. Stage 0 and 1 fibrosis were detected in 40 biopsies (35.4%), stage 2/3 were seen in 65 (57.5%) biopsies and a small percentage had stages 4/5 fibrosis. Cirrhosis was not evident in any of the specimens. Thus the mean score of necroinflammatory activity was 0.66±0.59; range: none-moderate (score 0-12) and that of fibrosis was 0.71±0.58; range: 0-5, (Table 2).

Table (2): Patients' enrolment data

Data				Findings	
Age (years)	Strata		<20	8 (7.1%)	17.4±1.8 (15-19)
			20-30	12 (10.6%)	24.2±3.8 (20-29)
			>30-40	9 (8%)	37.6±2.6 (32-40)
			>40-50	64 (56.6%)	47±2.5 (41-50)
			>50	20 (17.7%)	51.8±0.8 (51-53)
	Total			113 (100%)	42.6±10.8 (15-53)
Gender	Males			67 (59.3%)	
	Females			46 (40.7%)	
Body mass data	Weight (kg)			80.5±4.7 (71-90)	
	Height (cm)			168±4.5 (157-180)	
	BMI	Strata	<25	4 (3.5%)	24.4±0.6 (23.5-24.9)
			25-30	82 (72.6%)	27.8±1.3 (28-34.3)
			>30-35	27 (23.9%)	31.7±1.2 (30.1-34.3)
		Total		113 (100%)	28.6±2.3 (23.5-34.3)
Duration of disease (years)	Strata	≤5		55 (48.7%)	4.7±0.6 (3-5)
		>5		58 (51.3%)	6.4±0.6 (6-8)
	Total			113 (100%)	5.6±1.1 (3-8)
Liver biopsy	Necro-inflammatory grade		None/minimal	44 (38.9%)	0.66±0.59
			Mild	62 (54.9%)	
			Moderate	7 (6.2%)	
	Fibrosis stage		0/1	40 (35.4%)	0.71±0.58
			2/3	65 (57.5%)	
			4/5	8 (7.1%)	

Data are presented as means±SD & Numbers; ranges & percentages are in parenthesis

Fifty-three patients (46.9%) were HBeAg-positive, while the remaining 60 patients (53.1%) were HBeAg-negative. Only 3 females (2.7%) had serum ALT<ULN, 47 patients (41.6%); 14 males and 33 females had serum ALT of ≤2ULN and 63 patients (55.7%); 53 males and 10 females had serum ALT >2ULN. Mean serum HB VDL of the total studied patients was 2907.2±1060; range: 1100-5200 IU/ml. Thirty-two patients (28.3%) had low HB VDL with median level of 1560 IU/ml and 81 patients (71.7%) had high HB VDL with median level of 3200 IU/ml. The median log_{10} of total serum HB VDL was 3.47, of patients had low HB VDL was 3.19 and of patients had high HB VDL was 3.52. Mean total serum HBsAg level was 24.7±5.9; range: 12-39x10^3 IU/ml; in patients had low HB VDL mean serum HBsAg level was 23±5.1; range: 12-37x10^3 IU/ml and that of patients had high HB VDL was 25.3±6.1; range: 13-39x10^3 IU/ml. The median log_{10} of total serum HBsAg level was 1.36, that of patients had low HB VDL was 1.36 and of patients had high HB VDL was 1.38, (Fig. 1). The ratio of the median log_{10} of serum HB VDL/ median log_{10} of serum HBsAg level was 0.42 in patients had low HB VDL and was 0.4 in patients had high HB VDL, (Table 3).

Table (3): Patients' laboratory data

Data			Number (%)	Level
HBeAg	Positive		53 (46.9%)	
	Negative		60 (53.1%)	
Serum ALT (U/L)	<ULN	Males	0	0
		Females	3 (2.7%)	18±1 (17-19)
	≤2ULN	Males	14 (12.4%)	52.9±3.2 (46-58)
		Females	33 (29.2%)	29.5±6.1 (21-38)
	>2ULN	Males	53 (46.9%)	85.9±15.4 (67-136)
		Females	10 (8.8%)	43.4±2.5 (39-46)
HB VDL (IU/ml)	Low		32 (28.3%)	
	High		81 (71.7%)	
	Low VDL (<2000)	Level	1530±255.7 (1100-1980)	
		Median of Log_{10}	3.19	
	High VDL (≥2000)	Level	3451.1±700 (2300-5200)	
		Median of Log_{10}	3.52	
HBsAg (10^3 IU/ml)	Low VDL (<2000)	Level	23±5.1 (12-37)	
		Median of Log_{10}	1.36	
	High VDL (≥2000)	Level	25.3±6.1 (13-39)	
		Median of Log_{10}	1.38	
HB VDL/HBsAg ratio (Log_{10}/Log_{10})	Low VDL (<2000)		0.42	
	High VDL (≥2000)		0.40	

Data are presented as numbers, mean±SD & median; percentages & ranges are in parenthesis; HBeAg: Hepatitis B e antigen; HB: Hepatitis B virus; VDL: viral DNA load; HBsAg: Hepatitis B surface antigen.

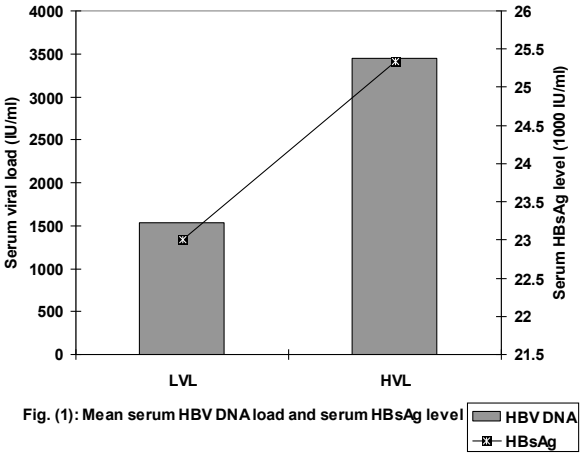

Fig. (1): Mean serum HBV DNA load and serum HBsAg level

There was positive significant correlation between HBeAg positivity and liver fibrosis score, high serum HB VDL, high serum HBsAg, the ratio between log of VDL and HBsAg level, serum ALT and duration of disease. However, HBeAg positivity showed negative significant correlation with male gender and BMI. On the other hand, HBeAg positivity showed positive non-significant correlation with presence of necrosis on liver biopsy and age of patients. Liver fibrosis score showed positive significant correlation with high serum HB VDL and high serum HBsAg, but showed positive non-significant correlation with the presence of necrosis and the ratio between log of VDL and HBsAg level, and constitutional parameters. Liver fibrosis score showed non-significant correlation with serum ALT, (Table 4).

Table (4): Correlation coefficient "r" between HBeAg positivity and fibrosis on liver biopsy and constitutional and laboratory data

	HBeAg-positivity		Liver fibrosis score	
	r	*P*	r	*P*
HBeAg-positivity			0.242	=0.010
Liver fibrosis score	0.242	=0.010		
Liver necrosis score	0.175	>0.05	0.149	>0.05
Serum ALT	0.199	=0.035	0.120	>0.05
Serum HBV DNA load	0.258	=0.006	0.223	=0.017
Serum HBsAg level	0.402	<0.001	0.258	=0.006
DNA load/HBsAg ratio	0.205	=0.029	0.101	>0.05
Age	0.099	>0.05	0.152	>0.05
Male gender	-0.268	=0.004	0.185	=0.050
BMI	-0.185	=0.049	0.120	>0.05
Duration of disease	0.240	=0.011	0.163	>0.05

"r": Pearson correlation coefficient; HBeAg: Hepatitis B e antigen; ALT: Alanine transaminase enzyme; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; BMI: Body mass index

Verification of parameters used for identification of CHB disease phases using Regression analysis defined high $\log_{10}$ of serum HBsAg level as the persistently significant determinant of cases with IT and/or IR who had HBeAg positive among studied patients in 5 models of analysis, followed by liver necrosis score in 4 models of analysis, high $\log_{10}$ of serum DNA viral load in 3 models, the ratio of $\log_{10}$ of serum DNA viral load to serum HBsAg in 2 models and male gender in one model, (Table 5).

Table (5): Regression analysis of studied parameters used for differentiation between determinants of CHB phases

Model	Parameter	Standardized coefficient	t	Sig.
Model 1	Log_{10} of serum HBsAg level	4.819	3.066	=0.003
	Liver necrosis score	0.274	3.502	=0.001
	Log_{10} of serum HB VDL	0.199	2.503	=0.014
	DVL/HBsAg $\log_{10}$ ratio	4.628	2.849	=0.005
	Male gender	2.913	2.660	=0.009
Model 2	Log_{10} of serum HBsAg level	0.650	5.148	<0.001
	Liver necrosis score	0.265	3.292	=0.001
	Log_{10} of serum HB VDL	0.208	2.549	=0.012
	DVL/HBsAg $\log_{10}$ ratio	0.318	2.504	=0.014
Model 3	Log_{10} of serum HBsAg level	0.406	4.940	<0.001
	Liver necrosis score	0.261	3.177	=0.002
	Log_{10} of serum HB VDL	0.171	2.081	=0.040
Model 4	Log_{10} of serum HBsAg level	0.403	4.829	<0.001
	Liver necrosis score	0.269	3.225	=0.002
Model 5	Log_{10} of serum HBsAg level	0.402	4.629	<0.001

The ROC curve analysis of parameters used for differentiation between IT and IR phases of CHB disease defined high $\log_{10}$ of serum HBsAg level as a significant specific predictor for IT cases and the ratio of $\log_{10}$ of serum HB VDL to serum HBsAg as a significant sensitive predictor, (Table 6, Fig. 2).

Table (6): ROC curve analysis of parameters differentiating IT from IR cases

	AUC	Std. Error	Sig.	CI	
				Lower	Upper
Log_{10} of serum HBsAg level	0.755	0.079	=0.017	0.601	0.910
Liver necrosis score	0.312	0.089	>0.05	0.137	0.487
Serum ALT	0.682	0.075	>0.05	0.535	0.829
DVL/HBsAg $\log_{10}$ ratio	0.191	0.058	=0.004	0.077	0.304

Analysis of laboratory markers for prediction of fibrosis score using ROC curve analysis defined high log_{10} of serum HB VDL and positive HBeAg as a significant predictors for presence of fibrosis even if undetectable histopathologically (Table 7, Fig. 3). Regression analysis, defined high log_{10} of serum HBsAg level as the significant predictor for presence of fibrosis, irrespective of the histopathological result. Considering the ratio of log_{10} of HB VDL and serum HBsAg, so high ratio could be considered as determinant of presence of fibrosis even if it is minimal, (Table 8).

Table (7): ROC curve analysis of parameters for diagnosis of liver fibrosis, irrespective of histopathological examination of liver biopsy

	AUC	Std. Error	Sig.	CI	
				Lower	Upper
Positive HBeAg	0.369	0.054	=0.022	0.262	0.476
Serum ALT	0.582	0.059	>0.05	0.467	0.698
Log_{10} of serum HB VDL	0.355	0.056	=0.011	0.246	0.464
Log_{10} of serum HBsAg level	0.411	0.059	>0.05	0.297	0.526

Table (8): Regression analysis of laboratory parameters used for diagnosis of liver fibrosis, irrespective of histopathological examination of liver biopsy

Parameter	Standardized coefficient	t	Sig.
Log_{10} of serum HBsAg level	0.258	2.815	=0.006
Positive HBeAg	0.165	1.658	>0.05
Serum ALT	0.141	1.537	>0.05
Log_{10} of serum HB VDL	0.161	1.686	>0.05

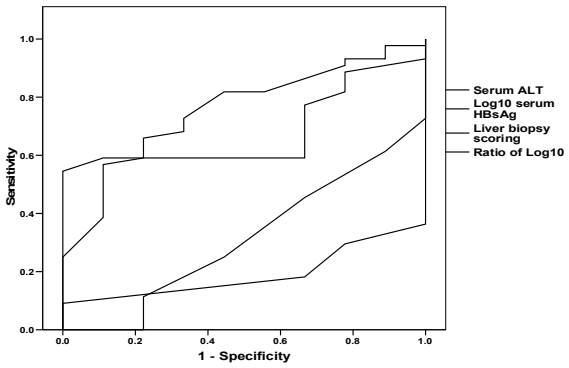

Fig. (2): ROC curve analysis of parameters differentiating between IT and IR cases

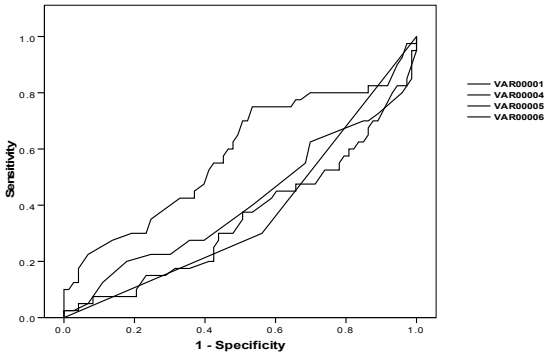

Fig. (3): ROC curve analysis of laboratory parameters for prediction of liver fibrosis irrespective of liver biopsy

4.Discussion

Although, the experts still rely on liver biopsy not necessarily for the confirmation of the clinic diagnosis but rather for the estimation of the prognosis and the choice of the antiviral therapy **(Stănculeţ *et al.*, 2011).** The current study detected positive significant correlation between HBeAg positivity and liver fibrosis score, high serum HBV DNA load, high serum HBsAg, the ratio between log of viral load and HBsAg level, serum ALT and duration of disease with negative significant correlation with male gender and BMI. These correlations coincided with that reported by **Chen *et al.* (2004)** who found HBsAg levels were correlated with HBV DNA on a log scale and concluded that quantitative measurement of HBsAg titres may be an easy and economical reference for HBV replication in HBV carriers. **Li *et al.* (2007)** reported that serum levels of HBV DNA of the patients in IT stage were high, the stage of fibrosis was higher in the non-active status group than in the IT stage group and the fibrosis stages of the livers of patients of a higher but within normal ALT level were markedly higher than those of a lower but within normal ALT level patients. **Madan *et al.* (2008)** also reported a strong positive correlation of HBV DNA load with inflammatory grade, fibrosis stage and ALT levels. Recently, **Chen *et al.* (2013)** found that the positivity of HBeAg was related to age, region, and sex and testing HBeAg and serum ALT levels were effective ways to assess HBV infectiousness in community-level hospitals in China.

Regression analysis of parameters used for identification of CHB disease phases showed that high $\log_{10}$ of serum HBsAg level, high liver necrosis score, high $\log_{10}$ of serum DNA viral load and the ratio of $\log_{10}$ of serum DNA viral load to serum HBsAg are persistently significant determinant of cases with IT and/or IR, but in decreasing order of significance. Thus, the reliance on estimation of serum viral load and HBsAg level could differentiate HBeAg-positive (IT and IR) cases from HBeAg-negative (LR and Hepatitis) cases. Moreover, ROC curve analysis showed that high $\log_{10}$ of serum HBsAg level is a significant specific predictor and the ratio of $\log_{10}$ of serum HB VDL to serum HBsAg is a significant sensitive predictor for IT cases among HBeAg-positive cases.

In line with these findings, **Jang *et al.* (2011)** found the HBsAg levels were significantly lower in the HBeAg-negative stage, with the lowest levels in inactive carriers and age and HBV DNA were independently associated with HBsAg levels, also the ratios of HBsAg/HBV-DNA were highest, but steadily decreased with age in inactive carriers. **Viganò & Lampertico (2012)** reported that median HBsAg levels differ significantly during the natural history of HBV infection, progressively declining from IT to inactive phase and the combination of an HBsAg <1000 IU/ml and HBV DNA <2000 IU/ml at a single time point accurately identifies true inactive carriers. **Seto *et al.* (2013)**, found low baseline HBsAg levels and greater rate of HBsAg reduction achieved high predictive values for predicting HBsAg seroclearance, strengthening the prognostic role of HBsAg measurements during nucleoside analogue therapy. **Liu *et al.* (2013)**, reported that low serum levels of both HBsAg and HBV DNA were the strongest predictors of spontaneous HBsAg seroclearance, the predictive ability of HBsAg levels was modified by HBV viral load and the inclusion of serum HBsAg levels greatly improved predicting HBsAg seroclearance.

Statistical analyses of laboratory markers for prediction of fibrosis score showed that using ROC curve, high $\log_{10}$ of serum HB VDL and positive HBeAg are significant predictors for presence of fibrosis even if undetectable histopathologically and Regression analysis showed that high $\log_{10}$ of serum HBsAg level is the significant predictor for presence of fibrosis, irrespective of the histopathological result.

These data go in hand with **Liaw (2011)** who reported that the combined use of HBsAg and HBV DNA levels might help in the identification of true inactive carriers with high accuracy. **Zeng *et al.* (2012)** reported that HBsAg level is correlated with liver inflammation and fibrosis stages for patients with CHB and might represent a useful noninvasive marker of the degree of hepatic fibrosis. **Martinot-Peignoux *et al.* (2013)** found serum HBsAg and HBV DNA levels in HBeAg[+] patients showed strong correlation, as did serum HBsAg levels and fibrosis severity and modeling analysis suggested a serum HBsAg cut-off of 3.85 log IU/ml would provide a theoretical sensitivity of 100%, theoretical specificity of 86%, and a negative predictive value of 100% in HBeAg(+) patients infected with HBV genotype B or C.

Conclusion

Quantitative estimation of serum level of HBsAg and HBV DNA load could differentiate between phases of CHB disease and predict histopathological status of the liver, so could spare liver biopsy with its inherent complications.

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6/2/2013