Iranian Journal of Clinical Infectious Diseases
2011;6(1):5-17

ORIGINAL ARTICLE

Hepatitis B virus DNA level as predictor of response to therapy with interferon-alpha-2b (PDferon) in chronic hepatitis B infection

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ABSTRACT

Background: To evaluate the strength of association and to determine the best prediction of response in terms of sensitivity and specificity among quantitative baseline HBV-DNA levels in blood serum in patients with chronic hepatitis B (CHB) infection who treated with interferon-alpha-2b.

Patients and methods: Totally, 78 CHB patients with serum HBV-DNA>10^5 copies/mL were treated with interferon-alpha-2b (PDferon: Pooyesh Darou, Tehran, Iran) for 52 weeks as 5 MU Sc. for 24 weeks in HBeAg(+) and 48 weeks for HBeAg(-) at baseline of study in Tehran, Iran. Serum HBV-DNA level using Cobas Amplicor HBV Monitor test and HBeAg status were assessed at baseline and end of 6-months follow-up. Sustained response (SR) (n=42, 56%) was defined by HBeAg seroconversion (n=12), or with a decrease in HBV-DNA >10^5 copies/mL to undetectable value (n=33), or chemical response (n=20).

Results: Higher pretreatment HBV-DNA levels have a significant relationship with better response to treatment in HBeAg (+) (R=0.7, p=0.04). Positivity of HBeAg in SR was a better predictor of chemical response in our patients, when compared to HBeAg negative (SR: 85% vs. 15%, respectively). At end of follow up, HBeAg (-) patients revealed more decrease in HBV-DNA levels than HBeAg (+) (412 vs. 290 ×10^5 copies/ml, p<0.05). Sensitivity of HBV-DNA in HBeAg (+) was more than HBeAg(-) (75% vs. 62%), but specificity was less in HBeAg(+) (58% vs. 45%). Area under ROC was 0.63 in HBeAg (-).

Conclusion: Higher pretreatment HBV-DNA levels have a significant relationship with better response to treatment in HBeAg positive patients of CHB. Although HBV-DNA in HBeAg negative was decreased significantly from baseline to end of follow-up, monitoring with sensitive quantitative baseline HBV-DNA measurement in these patients was not a better predictor of SR than HBeAg positive.

Keywords: *Hepatitis B; Interferon-alpha-2b; Hepatitis B antigens.*
(Iranian Journal of Clinical Infectious Diseases 2011;6(1):5-17).

INTRODUCTION

The serological markers of hepatitis B virus (HBV) infection are observed in over two billion people of the world. More than 75% of them live in Asia (1). According to the WHO estimates, HBV infection causes more than 2 million deaths every year (1). Prevalence of HBV infection have been reported from various part of the world including 1.5% in Poland (2), 4% in Brazil, 3.5% in Palestine, 8% in India (3), and approximately 10% in China (2). In Iran, the percentage of chronic HBV carriers was between 2.5-7% (4). More than

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3% of Iranian populations are infected with HBV (5). Compared with other developing Asian countries, Iran belongs to the countries with relatively higher, but gradually decreasing, incidence of hepatitis B (6). Only 9% of adults infected with HBV and almost 90% of younger children are at risk of progressing to chronic hepatitis B (7). This clinical entity is associated with increased risk of serious liver diseases, especially liver cirrhosis (1.3-5.9% of patients every year) and/or primary liver cancer. It has been estimated that 25-40% of patients chronically infected with HBV will die prematurely of these HBV-related complications (8).

Keep in mind the pathogenetic of HBV and profile of activity, up to now, interferon alpha (IFN-2a, IFN-2b, lymphoblastoid IFN, IFN-consensus alfacon-1) has been the only available treatment for chronic hepatitis B (CHB). All these agents, including IFN-alpha, have limited and variable therapeutic efficacy, and evidences of disease elimination have been observed in less than 40% of treated patients (2). IFN-alpha treatment increases the spontaneous response rate and leads to loss of hepatitis B e antigen (HBeAg) in 15–40% of patients (9). For CHB, the accepted duration of treatment with IFN-alpha is 16–24 weeks (10). The most important predictive factors known for response to IFN-alpha are baseline HBV-DNA levels and elevated alanine aminotransferase (ALT) levels (9), however, little information is available about factors predicting response to this therapy in the patients.

We have performed a univariate analysis to assess the strength of association between quantitative HBV-DNA level value in blood serum at baseline and response to the interferon-alpha-2b (PDFeron: Pooyesh Darou, Tehran, Iran), and a multivariate analysis to determine the best prediction of response in terms of sensitivity and specificity. Generally, low initial replication activity of HBV, without detailed assessment of HBV-DNA level, has been reported by some of the prior investigators as particularly important predisposing factor of good response to IFN-alpha therapy (11,12).

PATIENTS and METHODS

Between 2004-2007, 78 consecutive chronic hepatitis B infected subjects (CHB) (60 males and 18 females, with a mean age of 34.5 years, a range: 18-65 years) with a median duration of HBV infection of 6 years (a range, 4-21 years) referred to Tehran Hepatitis Center, Baqiyatallah University of Medical Sciences, Tehran, Iran, were studied. Consecutive patients were included into this study if they fulfilled the following baseline criteria: (I) serum HBV-DNA more than 100000 (5 log) copies per milliliter with histopathological features in liver biopsy consistent with CHB (more than of 3) in the Knodell score; (II) elevation of serum ALT level of more than 1.5 times above the upper limit of normal (normal range: 13–31 U/L for females and 13–53 U/L for males) for at least 6 months before commencement of therapy (13); and (III) the patient's written consent to the therapy and monitoring. The following exclusion criteria were applied at baseline: (I) not cooperating with medical staff; (II) IV drug abusers, alcoholics less than 6 months before beginning the therapy; (III) been treated with immunomodulators in the 24 weeks prior to commencement of PDFeron; (IV) decompensated liver disease; (V) co-existing psychiatric disease; (VI) with leucopenia less than 3000 cells/mm^3; thrombocytopenia less than 60000 cells/mm^3 or (VII) co-infection with either hepatitis C virus (HCV) or human immunodeficiency virus (10,13).

Virologic measurements: HBV-DNA level was assessed before and after the trial. If a baseline sample was not available for assessment of HBV DNA, a sample of week 4^{th} or 8^{th} was used. Quantification of HBV-DNA was performed according to the manufacturer’s instructions by the Cobas Amplicor HBV Monitor test (Roche

Diagnostic Systems, Branchburg, NJ, USA) (14). The HBV-DNA TaqMan assay, calibrated according to EUROHEP HBV-DNA standards, was used for the quantitative measurement of HBV-DNA in serum (9). All liver samples obtained in our center by percutaneous biopsy with Menghini needle have been revised histologically by the author and were reported based on Knodell score. HBsAg, HBeAg, and hepatitis B e antibody (anti-HBe) (Abbott Laboratories, North Chicago, IL, USA) were assayed with the second-generation enzyme-linked immunosorbent assay. All patients underwent a physical examination and blood testing for liver biochemistry (alanine aminotransferase, aspartate aminotransaminase, alkaline phosphatase, albumin and bilirubin), complete blood count, prothrombin time, activated partial thromboplastin time and renal biochemistry before commencement of therapy (10).

Treatment and follow-up: According to HBeAg status, we divided patients into two groups (positive and negative) that were under treatment with interferon-alpha-2b (PDFeron: Pooyesh Darou, Tehran, Iran) 5 MU Sc. as 3 injections per week for 24 weeks in HBeAg positive and for 48 weeks in HBeAg negative patients. Moreover, 6 months after cessation of PDFeron therapy, all patients underwent control virological and chemical tests. Basic hematological tests and liver function tests were performed and recorded at baseline, 1^{st}, 3^{rd}, 6^{th} (end of treatment for HBeAg positive patients), 9^{th}, 12^{th} (end of treatment for HBeAg negative patients and also 6 months follow-up for HBeAg positive patients) and 18^{th} months after treatment (6 months follow-up for HBeAg negative patients) during everyone's visit. Of 78 patients, three patients discontinued IFN therapy, because of drug complications in 2 patients (one with itching, hair loss, and the other with neurological and psychological side effects) and tendency to pregnancy in the third patient.

Response to treatment: For the definition of sustained response (SR) at the end of treatment, we used the definition of "Response" recommended by the National Institute of Health Workshop on Chronic Hepatitis B (15). Total sustained virological response in 42 patients (56%) is defined as a loss of HBeAg with the development of anti-HBe for three consecutive readings 8 weeks apart (HBe seroconversion in 19 patients), together with a decrease in HBV-DNA $>10^5$ copies/mL to undetectable value (<300 copies/mL) (n=33); or chemical response which defined as normal ALT values for patients (n=20). Patients not fulfilling these criteria were considered non-responders (NR) (33 patients, 44%) (figure 1). The early virologic on-treatment responses were evaluated to determine their ability to predict response or non-response at the end of follow-up (9, 10, 12, 16-18).

Statistical analysis: The study protocol was approved by BRCGL institutional review board and local ethics committee; hence, informed written consent was obtained from all patients. Initial data including demographic data, history of transfusion and previous medical history were obtained by reviewing medical records and interviews (19). Results are expressed as mean± standard deviation (SD). The Mann–Whitney U-test was used for continuous variables with skewed distribution and the chi-squared with Yates' correction for continuity or Fisher's exact test for categorical variables. The Pearson correlation coefficient was calculated for log values of HBV-DNA at baseline and end of follow-up. The primary endpoint was total SR. A secondary analysis was performed to identify factors that were associated with SR at the end of follow-up. Factors that were significant in the univariate analysis were subsequently incorporated into a stepwise backward logistic regression analysis to identify the most important factors associated with SR at the end of follow-up. A p-value<0.05 was considered statistically significant. All statistical analyses were performed using SPSS software for Windows (version 15.0; SPSS Inc. Chicago, IL, USA). For each test (HBeAg status and

quantitative HBV-DNA at baseline in all patients and HBV-DNA in two groups of HBeAg status), we calculated the positive predictive value (% SR if the test is normal), its negative predictive value (% NR if test is abnormal), its sensitivity (% SR identified by test) and its specificity (% NR identified by test) using the 2×2 method which can be used for calculation of sensitivity and specificity. Because of its clinical relevance, we also calculated the reverse forms of the sensitivity and specificity i.e. the fraction of all SR not identified by the test and the fraction of NR not identified by the test. For all tests the areas under the receiver operating characteristic (ROC) curves were calculated and compared according to the method described by Delong et al. (20).

RESULTS

Of 75 patients with CHB which were included and treated with PDferon (Interferon-alpha-2b), 59 (78.7%) were males and 16 (21.3%) were females by mean±SD age of 35.9±11 years vs. 32±7 years, respectively (NS). HBeAg-negative patients had significantly higher values in age, height and weight when compared with HBeAg-positive patients ($p<0.05$).

The study population consisted of 32% HBeAg-negative and 68% HBeAg-positive cases. Route of diagnosis (clinical symptoms, family case, blood donation, and screening) and probable route of transmission (tattoo, surgery, war injuries, etc.) were similar in both groups of HBeAg +/- (NS); however, the most important route of transmission among these patients was intra-familial in 22 of 75 patients (30%) (table 1).

There were no significant differences between paraclinic findings (such as: AST, ALT, ALKP, bilirubin, prothrombin, INR, albumin, WBC, Hb) when comparing HBeAg-negative or -positive patients (NS), likewise, pathological findings (grade, stage, and total Knodell scoring) in these two groups were non-significant. Among all side effects of PDferon administration at end of follow-up in our patients, only HBeAg-positive patients had predominant gastrointestinal side effects (90%), whilst among patients with HBeAg negative status, 10% complained of such side effects ($p< 0.05$) (table 1).

Of 51 HBeAg-positive patients at baseline, 14 (27%) had HBeAg loss or seroconversion, of these, 12 patients (23%) became HBeAg negative and 2 patients (4%) seroconverted to HBeAb positive at the end of follow-up. HBV-DNA at end of follow-up was undetectable in 33 patients (44%). According to ALT decrease as criterion for chemical response, we had chemical response in 20 patients (27%), most of which (n=17) were HBeAg positive at baseline ($p=0.04$). Totally, we reported both chemical and virological response to treatment in 14 patients (20%) and total response (any of them) in 42 patients (56%) at the end of follow-up. HBeAg-positive patients showed better response to treatment than HBeAg-negative (32 vs. 10 patients, $p=0.02$) (figure 1, table 2).

In all SR-patients (n=29), the mean±SD of HBV-DNA level was significantly decreased from $(353.28\pm726)\times10^5$ copies/ml at baseline to $(29.31\pm59)\times10^5$ copies/ml ($r=0.8$, $p=0.02$), however, this variable was significantly increased in patients who were NR [$(130.07\pm357)\times10^5$ copies/ml at baseline vs. $(286.34\pm420)\times10^5$ copies/ml at the end of follow up, respectively, $r=0.6$, $p=0.01$)]. Among HBeAg-negative and –positive patients with SR HBV-DNA levels decreased significantly from baseline to end of follow-up [$(420.8\pm761)\times10^5$ copies/ml to $(8.57\pm16)\times10^5$ copies/ml in HBeAg negative, $r=0.2$, $p=0.01$ and $(327.82\pm730)\times10^5$ copies/ml to $(37.21\pm67)\times10^5$ copies/ml in HBeAg positive, $r=0.7$, $p=0.04$)]. The mean of HBV-DNA was significantly increased from baseline ($38.05\pm73)\times10^5$ copies/ml to end of follow-up ($81.6\pm104)\times10^5$ copies/ml, in HBeAg negative patients who had not responded to treatment ($r=0.6$, $p=0.06$) (table 3).

Table 1. Baseline characteristics of 75 chronic hepatitis B patients according to HBeAg at the time of admission

	HBe antigen at the time of admission				
	Negative (n=24)	**Positive (n=49)**	**Total**	**P-Value**	**OR (95% CI)**
Gender					
Male	22 (37.3%)	37 (62.7%)	59 (100%)	0.088	3.56(0.73- 17.44)
Female	4 (25%)	12 (75%)	16 (100%)		
Age (years)	40±11.6	33.3±7.9	34.5±10.8	0.02	
Height (m)	1.7±0.07	1.6±0.1	1.69±.12	0.019	
weight	82.95±13	68.02±14	73.2±15	0.000	
Route of diagnosis					
Clinical Symptom	1 (20%)	4 (80%)	5 (100%)	0.088	
Family Case	3 (13.6%)	19 (86.4%)	22 (100%)		
Blood donation	14 (45.2%)	17 (54.8%)	31 (100%)		
Screening or Check-Up	6 (40%)	9 (60%)	15 (100%)		
Probable Route of Transmission					
Sexual contacts	0	0	0		
IV drug abuse	0	0	0		
Blood transfusion	1 (100%)	0	1 (100%)	0.329	
Tattoo	1 (33.3%)	2 (66.7%)	3 (100%)	0.704	0.97 (0.08- 11.3)
Hejamat	2 (33.3%)	4 (66.7%)	6 (100%)	0.649	0.97 (0.16- 5.75)
Surgery(even minor or major)	20 (37%)	34 (63%)	54 (100%)	0.161	0.45 (0.13- 1.55)
History of war injury	2 (100%)	0	2 (100%)	0.105	
Familial history of hepatitis B	14 (26.4%)	39 (73.6%)	53 (100%)	0.053	2.78 (0.95- 8.1)
Paraclinic findings at baseline					
AST (IU/L)	87.9±108	72.5±50	76.6±72	0.413	
ALT (IU/L)	142.39±128	118.3±100	124.32±108	0.391	
Alkaline phosphatase (IU/L)	241.47±108	282.52±262	264.9±221	0.476	
Total bilirubin (mg/dL)	1.12±1.06	0.78±0.2	.91±.6	0.052	
Direct bilirubin (mg/dL)	0.27±0.2	0.25±0.1	.26±.2	0.733	
Prothrombin time(PT) (second)	13.4±1.3	13.01±0.5	13.14±.9	0.079	
Prothrombin time (PT) percentile (%)	93.9±12.3	97.08±6.1	96.14±8.6	0.157	
INR	1.08±0.1	1.02±0.07	1.04±.1	0.086	
Serum Albumin (g/L)	4.34±0.43	4.17±0.8	4.22±.7	0.401	
Serum Albumin (%)	55.65±8.7	54.03±5.9	54.55±6.9	0.419	
WBC	5740.86±1404	7996.25±17151	7187.1±1392	0.533	
PMN	54.08±11.7	53.05±9.5	53.32±10	0.694	
Lymphocytes	36.78±12.3	40.14±9.5	39.17±10	0.214	
Hemoglobin (g/dL)	15.37±1.3	14.8±1.7	14.93±1.6	0.168	
Platelet ($\times 10^9$ /L)	198.82±42	201.68±43	200.83±42	0.797	
Side effects of PDferon at end of treatment	17 (32.7%)	35 (67.35)	52 (100%)	0.583	1.02 (.35- 3.02)
Skin disorders	3 (23.1%)	10 (76.9%)	13 (100%)	0.301	1.84 (.45- 7.44)
Fever and chills	6 (37.5%)	10 (62.5%)	16 (100%)	0.435	.76 (.24- 2.44)
Neurological side effects	7 (41.2%)	10 (58.8%)	17 (100%)	0.29	.62 (.2- 1.9)
Musculoskeletal side effects	5 (29.4%)	12 (70.6%)	17 (100%)	0.48	1.2 (.37- 4.01)
Respiratory side effects	5 (45.5%)	6 (54.5%)	11 (100%)	0.264	.53 (.14- 1.95)
Gastrointestinal side effects	2 (10.5%)	17 (89.5%)	19 (100%)	0.013	5.8 (1.2- 27.87)
General symptoms like fatigue and constitutional symptoms	8 (29.6%)	19 (70.4%)	27 (100%)	0.42	1.26 (.45- 3.53)
Genitourinary side effects	1 (33%)	2 (67%)	3 (100%)	0.7	.97 (.08- 11.3)
Pathological Findings					
Grade	5.04±3.2	5.177±2.7	5.1±2.9	0.860	
Stage	2.08±1.2	1.8±1.6	1.8±1.5	0.462	
Total Knodell scoring	7.13±3.5	6.97±3.6	7.02±3	0.869	

Among 14 HBeAg-negative patients, mean of HBV-DNA decreased from $(256.79\pm594)\times10^5$ copies/mL at baseline to $(39.87\pm75.9)\times10^5$ copies/mL at the end of follow-up, with a decrease of $(216.92\pm 603.4)\times10^5$ copies/mL (r=0.01, NS), on the other hand, among 27 HBeAg-positive patients, HBV-DNA decreased from $(304.32\pm679)\times10^5$ copies/mL at baseline to $(138.07\pm306.6)\times10^5$ copies/mL at the end of follow-up with a decrease of $(166.24\pm 715)\times10^5$ copies/mL (r=0.1, NS). Among 24 HBeAg-negative patients HBV-DNA level was correlated inversely with the decrease in viral load between baseline and end of follow-up (r=-0.99; p <0.001). Furthermore, this correlation was as follow for 51 HBeAg-positive patients (r=-0.88; p<0.001).

Table 2. Definition of sustained response (SR) in patients with chronic hepatitis B treated with PDferon

		HBeAg at Baseline			
		Negative	**Positive**	**Total**	**P-value**
Virological response					
HBV DNA decrease	Responder	9 (27.3%)	24 (72.7%)	33 (100%)	0.3
	Non-Responder	15 (35.7%)	27 (64.3%)	42 (100%)	
HBeAg seroconversion	Responder	0	14 (100%)	14 (100%)	0.002
	Non-Responder	24 (39.3%)	37 (60.7%)	61 (100%)	
Chemical response (ALT)*	Responder	3 (15%)	17 (85%)	20 (100%)	0.04
	Non-Responder	21 (38.2%)	34 (61.8%)	55 (100%)	
Both (chemical & virological response)	Responder	2 (14.3%)	12 (85.7%)	14 (100%)	0.1
	Non-Responder	22 (36.1%)	39 (63.9%)	61 (100%)	
Total response	Responder	10 (23.8%)	32 (76.2%)	42 (100%)	0.02
	Non-Responder	14 (42.4%)	19 (57.6%)	33 (100%)	

Table 3. Changes in quantitative HBV-DNA from baseline to end of follow-up in patients according to response to treatment in CHB treated with Interferon-alpha-2b (PDferon)

		5 log HBV-DNA (copies/mL)[¶]				
		N	**Baseline Mean± SD**	**End of follow-up Mean± SD**	**R***	**Sig.[¥]**
All patients	Responder	29	353.28± 726	29.31± 59	0.8	0.023
	Non-responder	12	130.07± 357	286.34± 420	0.6	0.015
HBe-Ag negative	Responder	8	420.8± 761	8.57± 16	0.2	0.01
	Non-responder	6	38.05± 73	81. 6± 104	0.6	0.06
	Total	14	256.79± 594	39.87± 75.9	0.1	0.202
HBe-Ag positive	Responder	21	327.82± 730	37.21± 67	0.7	0.045
	Non-responder	6	222.08± 504	491.09± 527	0.6	0.199
	Total	27	304.32± 679	138.07± 306	0.1	0.602

[¶] 5 Log HBV DNA copies/mL , means 100000 copies/mL
[*] R is calculated via paired samples correlations and is an abbreviation for Pearson correlation coefficient.
[¥] P-value is calculated from paired samples t-test and less than 0.05 is significant.

Table 4. Sensitivity, specificity and predictive values of testing HBV-DNA at baseline for early discrimination between eventual sustained responders and non-sustained responders in 75 CHB patients treated with PDferon

Test Result Variable(s)	**Area under the Curve**	**Std. Error***	**Asymptotic Sig.[¶]**	**Cutoff point[¥]**	**Sensitivity**	**1 - Specificity**
HBV DNA at Baseline						
All Patients	.634	.076	.091	32.17	68%	78%
HBeAg negative	.626	.150	.362	344	62%	42%
HBeAg positive	.557	.113	.594	32.7	75%	55%

The test result variable(s): HBeAg at baseline, HBV-DNA at baseline has at least one tie between the positive actual state group and the negative actual state group.
Statistics may be biased. [*] Under the nonparametric assumption, [¶] Null hypothesis: true area=0.5, [¥] The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Moreover, we evaluated the predictive value of precise quantitative HBV-DNA measurement for the response and non-response of PDferon treated patients. The question was whether, as in CHB, quantitative measurement of HBV-DNA could predict outcome of response in all patients according to pretreatment HBeAg status. Stepwise logistic regression analysis identified HBV-DNA at baseline as independent predictor of response in all patients. A receiver operating characteristic (ROC) curve is a graph of the pairs of true positive (=sensitivity) and false positive rates (=1-specificity) that correspond to each possible cut-off for the diagnostic test result. We therefore selected the cut-off that maximized the true positive rate (=sensitivity of 100%) and used the corresponding

HBV-DNA value and HBeAg at baseline to calculate the positive and the negative predictive values. Figure 2 and table 4 show the ROC curves for HBV-DNA testing at baseline in all patients (n=75, figure 2-1), and also HBeAg-negative and –positive patients (n=24, figure 2-2; n=51, figure 2-3) at baseline. The area under the ROC curve was higher for HBV-DNA testing at baseline in HBeAg-negative patients (0.63) as compared to HBeAg-positive patients (0.56), but the areas under the ROC curves were not significantly different between the two tests (NS). According to this curves, we estimated cut-off points of these variables as 344×10^5 copies/ml and 32.7×10^5 copies/ml for HBV-DNA at baseline in HBeAg-negative and –positive patients, respectively. In 75 patients a complete set of HBV-DNA results at baseline as well as HBeAg status at baseline were available. We calculated the positive and negative predictive value, using the values of HBeAg level and HBV-DNA level at baseline, which corresponded with the cut-off that maximized the true positive rate (= sensitivity of 100%) at baseline (table 5). In all patients, if we use the limit of HBV-DNA level at baseline as a stopping criterion, only 33% SR undergoing treatment would have been missed, whereas it was 55% in case of HBeAg status at baseline.

For all patients, the most relevant test characteristics were comparable for HBV-DNA and HBeAg at baseline (72% versus 65% prediction of non-response, 33% versus 55% misidentification of response), while the overall test performance was better for HBV-DNA at baseline, due to a better prediction of sustained response (57% versus 53%) and lower misidentification of non-response (22% versus 38%). For HBeAg-negative patients, HBV-DNA at baseline was not a better predictor of response to treatment compared to HBeAg-positive patients in all respects (sensitivity: 75% vs. 61%; specificity: 55% vs. 42%; positive predictive value: 75% vs. 66%; and negative predictive value: 54% vs. 37%, respectively for HBeAg-negative vs. HBeAg-positive patients, NS) (table 5).

Having performed the univariate analysis, quantitative HBV-DNA at baseline were subsequently incorporated into a logistic regression analysis to identify the best predictor group among three categories of quantitative HBV-DNA at baseline of study which was associated with sustained response (dependent variable) at the baseline of study. Stepwise logistic regression analysis revealed that sustained virologic response was not associated neither three categories of HBV DNA at baseline (NS) nor HBeAg status (table 6).

Table 5. Predictive value, sensitivity and specificity of HBeAg test versus HBV-DNA at baseline in 75 patients with chronic HBV

	% SR if test is normal*	%NR if test is abnormal ¶	%SR not identified by test #	%NR not identified by test Ƃ	Odds ratio¥
All Patients:					
HBV DNA at baseline	57	72	33	22	3.3$
HBeAg negative:					
HBV DNA at baseline	75	54	25	45	2.5^
HBeAg positive:					
HBV DNA at baseline	66	37	39	58	3.2$

* Predictive value of a normal test for a sustained response (SR) (Positive predictive value); ¶ Predictive value of an abnormal test for NR (Treatment failure) (Negative predictive value); # 100% minus sensitivity (= %SR identified by test); Ƃ 100% minus specificity (=%NR identified by test); ¥ Odds ratio: p<0.05$;p>0.05^

Table 6. Multivariate analysis* of 3 categories of quantitative HBV-DNA at baseline for patients with chronic hepatitis B according to HBeAg level

	HBeAg at baseline					
	Negative			Positive		
	B	Sig.	OR (95.0% C.I.)	B	Sig.	OR (95.0% C.I.)
HBV DNA categorical						
< 10 (6) copies/ml		0.399			0.750	
10 (6)- 10(7) copies/ml	0.539	0.682	1.71 (0.1- 22.5)	-0.523	0.590	0.59 (0.08- 3.9)
> 10 (7) copies/ml	-1.253	0.274	0.28 (0.03- 2.6)	-0.724	0.451	0.48 (0.07- 3.19)

* Backward logistic regression (Dependent variable: response to treatment.)

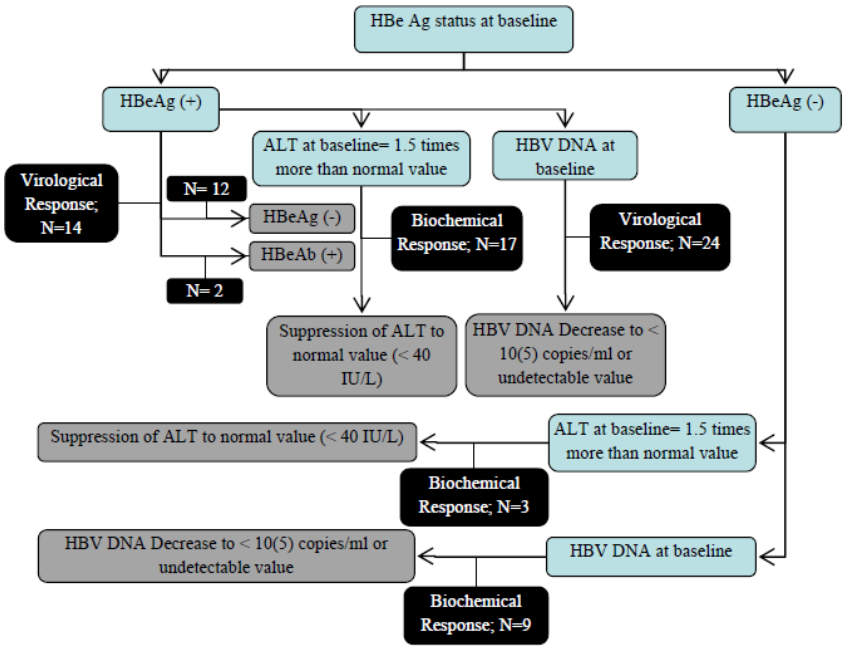

Figure 1. Response to treatment in patients with CHB treated with PDferon according to HBeAg status at baseline of study. There was sustained response in 42 patients, of whom 32 were HBeAg positive and 10 were HBeAg negative

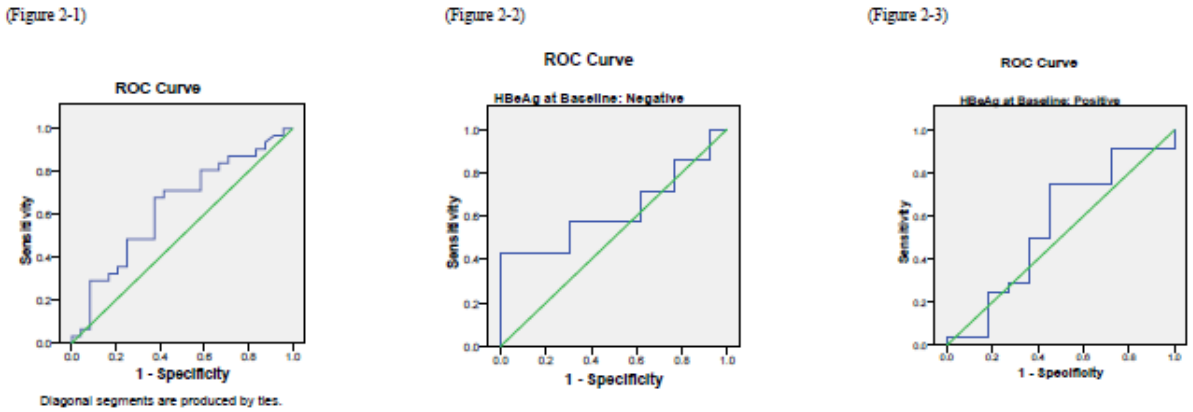

Figure 2. Value of testing HBV-DNA and HBeAg at baseline for early discrimination between eventual sustained responders and non-sustained responders. The ROC curves show the relation per test between the chances of correctly identifying an eventual sustained responder versus the chance of giving a false positive result. An optimal test would approach 100% sensitivity at 0% false positivity, while a test without discriminative value would only reach 100% sensitivity at 100% false positivity. Differences between curves are evaluated by comparing the area under the ROC curves. We studied prediction of HBV-DNA among all patients (*figure 2-1*), patients with HBeAg negative (*figure 2-2*), and patients with HBeAg positive (*figure 2-3*).

DISCUSSION

Chronic hepatitis B (CHB) is a common and often progressive liver disorder for which there is no accepted therapy. HBV is the leading cause of chronic liver disease (CLD), cirrhosis and hepatocellular carcinoma in Iran and it is evident that HBV transmission prevention can be one of the health priorities in the country (9, 21). Length of drug therapy, frequency of recurrences after primary remission, cost of drug regimen and resistance to treatment have led investigators to look for the best and most cost-effective therapeutic regimens for CHB in numerous studies throughout the world (22). The objectives of our study were to assess quantitative HBV-DNA measurements as predictor of non-response and response in PDferon treated patients.

Some predictive factors for end of treatment response in chronic HBV patients treated with IFN-alpha have been studied in the past. Results of a multinational controlled trial comprising 70 children with CHB who received IFN-alpha and 74 children who did not receive therapy, revealed that the variables that had the greatest impact on predictions for IFN-alpha response were HBV DNA pretreatment direction, baseline HBV-DNA, IFN-alpha dose and gender (23). Like our study, Eijk et al. (9) studied on quantitative HBV-DNA levels as an early predictor of non-response in chronic HBeAg positive hepatitis B patients treated with IFN-alpha. In another randomized-controlled trial of Interferon-alfa-2b, with or without prednisone priming, Perrillo et al. (21) found that baseline serum HBV-DNA level was the most important independent predictor of response ($p<0.05$). Approximately 50% of those patients with baseline HBV-DNA levels less than 100pg/mL (Solution-Hybridization Assay; Abbott Laboratories) responded to treatment with 5 MU of IFN-alpha compared to only 7% of patients with HBV-DNA levels at baseline above 200pg/mL. Moreover, in about 10% of those patients treated with IFN, HBsAg disappeared from serum (21). Furthermore, low level of HBV-DNA (<10pg/mL) at randomization was found to be the only independent predictor of response, while a low HBV-DNA level at entry tended towards significance (24). Marked reduction of viral replication in serum can be obtained with interferon in about half of patients with anti-HBe and HBV-DNA>10^5 copies/mL CHB (25).

Positivity of HBeAg in SR was a better predictor of chemical response in our patients, when compared to HBeAg negative. In study of Kurihara T. et al., they suggest that HBeAg-positive patients with higher ALT levels can be considered good candidates for lamivudine therapy, probably because lamivudine accelerates the natural seroconversion of HBeAg, accompanied by HBV-DNA loss, in these patients (26). Jordan J. Feld et al. identified factors predictive of the clinical course in 74 HBeAg negative and 32 HBeAg positive patients with CHB. For HBeAg negative patients, HBV-DNA>10^5 copies/mL are highly predictive of future ALT elevation and should prompt regular follow-up (27).

We found that SR with HBeAg positive had been treated longer with more dosage of PDferon than HBeAg negative. IFN-alpha is the primary treatment for CHB. The standard duration of IFN-alpha therapy is considered 16 weeks. Janssen et al. conducted a prospective, controlled study in order to investigate whether treatment prolongation could enhance the rate of HBeAg seroconversion. It may be possible that, like conventional interferon, extending the treatment duration of PDferon can increase the rate of HBeAg seroconversion (24). Prolonging the duration of conventional IFN-alpha from 16 to 32 weeks increased HBeAg seroconversion from 12% to 28% (10).

There was a significant decrease ($p=0.02$) in HBV-DNA from baseline to end of follow-up in patients with CHB who treated with PDferon and had sustained response to treatment. On the other hand, HBV-DNA level increased significantly from

baseline to end of study in patients with NR (p=0.01). According to pretreatment status of HBeAg, we found that a better response to treatment is acquired when a patient is HBeAg positive at baseline of therapy (p=0.04). Midtreatment HBV-DNA levels showed a significant correlation (p<0.001) with response in Chinese adults with CHB infection (28). Response was achieved in 53% of patients who had a HBV-DNA level below 0.7 Meq/mL (branched DNA assay) at midtreatment, but in only 17% of those who remained HBV-DNA positive (28). Factors influencing and predictive of seroconversion from HBeAg to anti-HBe were sought in a case-control study for early prediction of HBeAg seroconversion by HBV-DNA levels during the entire 6-year period (29). Similar to our results, HBV-DNA levels began to decrease before seroconversion in the SR, while they remained high in the NR. Seroconversion occurs in 75% of the patients with at least HBV-DNA levels <5.5 logarithmic equivalents/mL. Seroconversion occurs in 50% of those patients within 1 year, 88% within 2 years, and 93% within 5 years. A decrease in HBV-DNA levels is associated significantly and complementarily with seroconversion, and each of them or a combination thereof is predictive of seroconversion years ahead (29). HBV-DNA levels are not correlated to positivity of HBeAg status. However, HBeAg negative is more likely to develop in patients with HBV-DNA level $>10^5$ copies/mL (16).

Maria Buti and her co-workers (30) performed a study to determine whether a dramatic decrease in HBV-DNA levels within the first months of lamivudine therapy can predict the emergence of YMDD variants in patients with CHB. The decline in HBV-DNA levels from baseline to month 3 was higher in 22 responders than in 13 non-responders (4.16 vs. 2.88×10^5 copies/mL; p=0.002), whereas no differences were observed in patients with and without YMDD variants at 1 year of therapy. At 3 months, HBV-DNA was undetectable in 77% of the responders, whereas, after 1 year, it was undetectable in 23% of non-responders, 40% of patients with YMDD variants, and 74% of those without variants. They concluded that quantitative HBV-DNA testing is very useful in deciding whether to continue therapy, because of the low likelihood of response in patients who remain HBV- DNA positive at month 3 of treatment (30).

To reduce unnecessary exposure to treatment, physicians must decide at an early stage whether continuation of treatment has a reasonable chance of success for the individual patient. One of the objectives of our study was to evaluate the quantitative HBV-DNA measurements for prediction of NR and SR in PDferon treated chronic HBV patients. This study showed that an HBV-DNA test performed at baseline of antiviral therapy has a high predictive value in identifying patients who have a SR with PDferon treatment regimens; in addition, use of this test criterion affects the total number of sustained responders less than criteria based on HBeAg at baseline. Furthermore, quantitative HBV-DNA measurements in patients with HBeAg negative had a higher predictive value in early identification of SR to PDferon than patients with HBeAg positive and evaluation with sensitive quantitative baseline HBV-DNA measurements in patients with HBeAg negative is superior to monitoring of this test in HBeAg positive. Hence, for HBeAg negative patients, HBV-DNA was better predictor of future SR. In order to evaluate the previously described quantitative HBeAg measurements vs. quantitative HBV-DNA measurements for prediction of non-response and response in IFN-alpha treated HBeAg positive chronic HBV patients, Van Der Eijk et al. (9) calculated the positive predictive value, negative predictive value, sensitivity and specificity of these two methods. They found a similar high predictive value for SR like our results and concluded that monitoring with quantitative HBV-DNA levels (area under ROC 0.87) was superior to monitoring with quantitative

HBeAg levels (0.76, $p<0.05$). The overall test performance of predicting non-response (predictive value 100%) was best for HBV-DNA testing at week 12 compared with testing at week 8 due to a better prediction of sustained response (46% vs. 38%) and lower misidentification of non-response (39% vs. 54%) (9).

An objection to introducing our findings into clinical practice might be the variable cut-off of the HBV-DNA test. For quantitative HBV-DNA of 32.17×10^5 copies/mL, we found the high sensitivity and specificity values for this test in all patients with CHB; however, if we divide our patients into two groups of pretreatment HBeAg status, cut-off points of the test variable (HBV-DNA) will show the value of 344×10^5 copies/mL for HBeAg negative and 32×10^5 copies/mL for HBeAg positive. Nevertheless, quantitative HBV-DNA assays are poorly standardized, and either have limited sensitivity or lack linearity in the higher ranges (28). Thus, until the standardization issue is solved and the performance of quantitative tests is established, a single qualitative HBV-DNA assay at baseline may be at least as predictive, easier in use, and more cost-effective quantitative assay.

Stepwise logistic regression did not add to the prognostic value any of three categories of quantitative HBV-DNA at baseline as independent predictor of response. This is in contrast to earlier published studies where identified HBV-DNA at baseline and a decrease in HBV-DNA levels as independent predictors of response (9). However, this study was performed using a HBV-DNA assay based on hybridization in solution (Genostics; Abbott Laboratories), which has a minimum detection limit of HBV.

The overall test performance of predicting non-response was best for quantitative HBV-DNA testing at baseline in patients with HBeAg negative, compared with quantitative HBV-DNA testing at baseline in patients with HBeAg positive. We therefore suggest that a quantitative HBV-DNA test at baseline in patients with HBeAg negative can be used as a management tool for the decision whether to continue treatment or to adjust it.

In conclusion, the present study has shown that higher pretreatment HBV-DNA levels have a significant relationship with better response to treatment in HBeAg positive patients of CHB. Positivity of HBeAg in SR was a better predictor of chemical response in our patients, when compared to HBeAg negative. Although HBV-DNA in HBeAg negative was decreased significantly from baseline to end of follow-up, monitoring with sensitive quantitative baseline HBV-DNA measurements in these patients was not a better predictor of SR than HBeAg positive.

ACKNOWLEDGEMENT

The authors thank Dr. Keivan, Head of laboratory, Virology department, for determination of the HBV-DNA levels in serum and for other paraclinic evaluations.

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