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Characteristics of an Outpatient Cohort with HBeAg-Negative Chronic Hepatitis B

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Keywords: Chronic hepatitis B; Inactive carriers; ALT; HBeAg; HBV DNA; Quantitative HBsAg

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Abstract

BACKGROUND: Patients with hepatitis Be antigen-negative chronic hepatitis B (HBeAg-negative CHB), and patients' inactive carriers (IC) have similar laboratory and serologic characteristics and are not always easy to distinguish.

AIM: To characterise hepatitis Be antigen (HBeAg) negative chronic hepatitis B cohort based on their laboratory and virology evaluations at one point of time.

METHODS: A prospective non-randomized study was conducted on 109 patients with HBeAg negative chronic hepatitis B treated as outpatients at the Clinic for Infectious Diseases and Febrile Conditions. All patients underwent laboratory and serology testing, quantification of HBV DNA and HBs antigen (qHBsAg).

RESULTS: A group of 56 patients were inactive carriers (IC), and 53 patients had HBeAg-negative CHB (AH). The mean values of ALT, HBV DNA and qHBsAg in IC were 29.13 U/L; 727.95 IU/ml and 2753.73 IU/ml respectively. In the AH group, the mean values of ALT, HBV DNA and quantitative HBsAg were 50.45 U/L; 7237363.98 IU/ml and 12556.06 IU/ml respectively. The serum value of ALT was more influenced by qHBsAg than HBV DNA in both IC and AH groups ($R = 0.22$ vs $R = 0.15$) ($p > 0.05$).

CONCLUSION: patients with inactive and active HBeAg-negative CHB have similar laboratory and serology profile. It is necessary to combine analysis of ALT, HBV DNA and qHBsAg for better discrimination between patient's IC and patient with HBeAg-negative CHB.

Introduction

Infection with hepatitis B virus (HBV) remains the leading cause of liver damage and represents one of the major health problems worldwide. Nowadays, it is considered that approximately 30% of the world population has serologic evidence of current or past infection with HBV and 248 to 257 million people are chronic HBV carriers on a global level [1]. Chronic hepatitis B virus infection is associated with serious complications such as cirrhosis, hepatocellular carcinoma (HCC) end-stage liver disease and death [2], [3], [4]. The natural history of chronic hepatitis B (CHB) is characterised by different phases of infection, and patients may evolve from one phase to another or may revert to a previous phase and not necessarily in sequential order. The phases of the natural history of chronic HBV infection have been schematically divided into five phases, taking into account the presence of HBeAg, HBV DNA (hepatitis B virus deoxyribonucleic acid) levels, alanine transaminase (ALT) values and the presence or absence of liver inflammation [5]. The hepatitis B-e

antigen (HBeAg) positive phase is characterized by high serum HBV DNA levels, and HBeAg negative phase is characterised with HBeAg loss and seroconversion with the occurrence of anti HBe antibodies, which is usually associated with the decline of HBV DNA levels, and normalisation of ALT values [5], [6]. In some patients, this process of seroconversion to HBeAg negative phase is associated with the selection of HBV variants that express little or no HBeAg at all and is usually characterised with continuing HBV DNA replication and progression of liver damage [5], [6], [7]. Usually, most of the chronically infected HBV patients experience the inactive phase with normal ALT levels, low viraemia and negative HBeAg after HBeAg seroconversion. However, up to 10-30% of chronic HBV infected adults subjects may suffer from HBeAg-negative hepatitis flare after HBeAg seroconversion, especially in those who experience late HBeAg seroconversion, and are associated with increased life-long risk of liver cirrhosis and HCC [8], [9]. It has been estimated that the median prevalence of HBe antigen-negative chronic hepatitis B infection is around 33% in the Mediterranean, 15% in the Asia Pacific, and 14% in the USA and Northern Europe [10]. Patients with HBeAg-negative CHB represent a heterogeneous group characterised with a different range of viral replication and liver disease severity, seen by fluctuating levels of HBV DNA and transaminases with temporary remissions during the disease [11]. Therefore, it is necessary to make a distinction among those with active hepatic necrotic inflammation and persistent viraemia as they have higher rates of complications (patients with chronic HBeAg negative hepatitis B) in contrast to HBeAg-negative CHB patients who are inactive carriers. Both forms of CHB, patients with HBeAg negative chronic hepatitis and patients' inactive carriers, have similar laboratory and serologic characteristics and are not always easy to distinguish [8]. In an inactive carrier, ALT usually remains normal on serial monitoring with undetectable to low levels (i.e., < 2000 IU/ml) of HBV DNA. However, the same may also occur in a patient with HBeAg-negative CHB. It is known that maintained high levels of HBV DNA are associated with progressive liver disease. Serum DNA levels are a prognostic factor, and contribute to defining the phases of CHB infection, the treatment indication, and allow an assessment of the efficacy of antiviral therapy [11], [12].

Eradication of HBV should be useful both for the patients and the society. There are consensus guidelines that help the clinicians to make decisions about whether or not to treat a patient. The viral load cannot be considered as the only treatment criterion. HBV DNA persists even in persons who have serological recovery from acute HBV infection [13] Areas of uncertainty whether and when to treat patients with HBeAg negative chronic hepatitis still exist, and clinicians, patients, and public health authorities must, therefore, continue to make choices on the basis of the evolving evidence [11], [14], [15]. The identification of patients with chronic HBeAg negative infection (inactive carriers-IC) versus patients with chronic HBeAg-negative chronic hepatitis B (AH) is a complex issue due to the dynamic character of hepatitis B infection. Proper and timely assessment of patients with HBeAg negative chronic hepatitis B is important for early treatment decision and consecutive prevention of disease progression and development of chronic hepatitis B virus-associated complications. In this study, we evaluated the laboratory, serological and virological characteristics of an outpatient cohort of HBeAg negative chronic hepatitis B.

Material and Methods

A prospective non-randomized study was carried out on 109 patients with HBeAg negative CHB, treated at the Clinic for Infectious Diseases and Febrile Conditions in Skopje, the Republic of Macedonia from the period of November 2016 till January 2018. All patients who were HBsAg-positive for at least six months, but HBeAg-negative, anti-HBe-positive, and had detectable HBV DNA in the serum were included in the study. Patients under the age of 18 years, all patients who tested positive for hepatitis A, hepatitis C and HIV were not included in the study. Other excluding criteria were previous or current exposure to antiviral hepatitis B treatment, alcoholic and autoimmune liver diseases, incomplete serum profile and a follow-up period of fewer than six months. Patients with hepatocellular carcinoma (HCC), decompensated liver disease and pregnant patients were excluded from the study.

The following data were obtained for all the patients: age, sex, alcohol consumption, complete blood count, bilirubin levels, transaminase, AFP, serology, quantification of HBV DNA, quantification of hepatitis B surface antigen (qHBsAg), abdominal ultrasound, total protein electrophoresis and presence of clinical signs and symptoms for cirrhosis. Cirrhosis was determined with the presence of ascites, encephalopathy, palmar erythema, telangiectasia, jaundice, hypoalbuminemia and ultrasound finding of cirrhosis. Complete serology profile was performed with ELISA (enzyme-linked immune assay) tests. The normal upper limit of serum transaminase both for alanine aminotransferase (ALT) and aspartate aminotransferase (ALT) was 40 U/L, according to the traditional cut-off values. Quantification of HBV DNA levels in the plasma was performed in-house, by real-time polymerase chain reaction (RT-PCR) on COBAS AmpliPrep COBAS TaqMan HBV test and Abbott m 2000 sp/m 2000 rt with a lower detection limit of 10 IU/mL. The serum level of HBsAg (qHBsAg) was quantified with Architect HBsAg assay (Abbott Laboratories) in-house, according to the

manufacturers' protocol. The detection level of HBsAg varies from 0.05 to 250 IU/ml. HBsAg levels above 250 IU/ml were further diluted in a ratio of 1:500.

We evaluated the serum values of alanine transaminase (ALT) aspartate transaminase (AST), qHBsAg and HBV DNA. A multiple regression analysis was performed to establish the correlation between the serum levels of ALT with qHBsAg and HBV DNA.

Relevant clinical variables were gender, age, platelet count, ALT, AST, HBsAg, hepatitis B e antigen, HBV DNA. The value of ALT and AST are expressed in units per litre (U/L), and those of qHBsAg and HBV DNA were expressed in international units per millilitre (IU/ml).

Adopted Definitions

An inactive carrier was considered when HBeAg nonreactive with normal transaminase levels, HBV-DNA < 2000 IU/ml [11].

Active chronic HBeAg negative hepatitis was considered when HBeAg nonreactive, and if ALT was elevated above the upper normal limit and HBV-DNA was more than 2,000UI/mL [11].

The study was approved by the Ethics Committee of the Medical Faculty in Skopje.

Statistical Analysis

All data were processed using a statistical computer program Statistica 7.1 for Windows and SPSS Statistics 17.0. Series with attributive variables were analysed with percentages of structure. For numerical variables descriptive statistics ((Mean; Std. Deviation; ± 95.00% CI; Minimum; Maximum) was used, where frequencies and percentages were used for the description of the categorical variables. Distribution of the data was tested with Kolmogorov-Smirnov tests; Lilliefors test; Shapiro-Wilks test(p). The differences between the groups were analysed with Pearson Chi-square (p) and Fisher's exact test (p). T-test for independent variables (t/p) and Mann-Whitney U test (Z/p) were used depending on the distribution of the data. Multiple Regression (R/p) was used to determine the correlation between ALT, qHBsAg and HBV DNA. For all analyses P values of <, 0.05 were considered significant.

Results

Out of 109 patients included in the study, 80 (73.39 %) were male, and 29 (26.61%) were female. If in the analysis of the presentation forms at one point of time we considered only the baseline values of ALT and HBV DNA according to definitions used, 56 patients (51.37%) had chronic hepatitis B infection, or as previously defined inactive carriers (IC), and 53 (48.62%) had HBeAg-negative CHB (AH). In the group of inactive carries, the mean age of the patients was 37.50 ± 10.84 years, while in the group of AH was 43.91 ± 11.72 years. For Pearson Chi-square = 0.002 and p > 0.05 (p = 0.97) there was no statistically significant difference between both groups of patients in terms of gender. Patients with AH for t = -2.96 and p < 0.01 (p = 0.004) were significantly older than patient's IC (Table 1)

Table 1: Demographic and descriptive statistics in patients' inactive carriers and patients with HBeAg-negative chronic hepatitis B

Characteristics					n (%)			
		IC			56 (51.37%)			
		AH			53 (48.62%)			
	Sex			Male	80 (73.39 %)			
				Female	29 (26,61%)			
Parameter	Group	N	Mean	Confidence -95,00%	Confidence +95,00%	Minimum	Maximum	Std.dev.
Age	IC	56	37,50	34,60	40,40	19	67	10,84
(years)	AH	53	43,91	40,67	47,14	22	74	11,72
ALT U/L	IC	56	29,13	24,43	33,82	10	89	17,53
AST U/L		56	22,20	20,10	24,29	14	57	7,81
ALT U/L	AH	53	50,45	39,83	61,07	10	173	38,53
AST U/L		53	34,74	29,37	40,10	12	101	19,46

Abbreviations: IC-inactive carriers; AH- HBeAg-negative chronic hepatitis B; ALT-alanine transaminase; AST-aspartate transaminase.

The mean value of ALT and AST in IC and AH patients was 29.13 ± 17.53 U/L; 22.20 ± 7.81 U/L; 50.45 ± 38.53U/L and 37.74 ± 19.46 U/L, respectively (Table 1). When the levels of transaminases were compared, patients with AH had significantly higher ALT values compared to IC for Z = -3.18 and p > 0.01 (p = 0.001), as well as for AST (Z = -4.06 и p < 0.001 (p = 0.000)) (Table 2).

Table 2: Differences in transaminases levels in patients' inactive carriers and patients with HBeAg-negative chronic hepatitis B

Parameter	Rank Sum IC	Rank Sum AH	U	Z adjusted	p-level	N IC	N AH
ALT U/L	2556.50	3438.50	960.50	-3.18	0.001	56	53
AST U/L	2410.50	3584.50	814.50	-4.06	0.000	56	53
alkaline phosphatase U/L	3028.00	2967.00	1432.00	-0.32	0.75	56	53
Gamma GT U/L	2649.50	3345.50	1053.50	-2.61	0.009	56	53

Abbreviations: IC-inactive carriers; AH- HBeAg-negative chronic hepatitis B; ALT-alanine transaminase; AST-aspartate transaminase; Gamma GT-Gamma-glutamyl transferase.

The mean value of HBV DNA in IC and AH group were 727.95 ± 584.24 IU/ml and 7237363.98 ± 46513427.91 IU/ml respectively. The mean value of quantitative HBsAg in IC was 2753.73 IU/ml and in the AH group 12556.06 ± 27188.85 IU/ml (Table 3).

Table 3: Quantitative HBsAg and HBV DNA levels in patients' inactive carriers and patients with HBeAg-negative chronic hepatitis B

	Parameter	N	Average	Confi-dence -95,00%	Confi-dence +95,00%	Min	Max	Std.dev
IC patients	qHBsAg IU/ml	56	2753.73	1494.72	4012.74	0.05	19636.84	4701.29
	HBV DNA IU/ml	56	727.95	571.49	884.41	10	1997	584.24
AH patients	qHBsAg IU/ml	53	12556.06	5062	20050	12.95	155311.00	27188.85
	HBV DNA IU/ml	53	7237363.98	-5583325	20058053	2061	338999252	46513427.91

Abbreviations: IC-inactive carriers; AH- HBeAg-negative chronic hepatitis B; HBV DNA-hepatitis B virus deoxyribonucleic acid; qHBsAg-quantitative hepatitis Bs antigen.

In the group of patients IC, 29 (51.79%) had qHBsAg < 1000 IU/ml, and 27 (48.21%) had qHBsAg > 1000 IU/ml, while in the group of patients with active hepatitis (AH) 6 (11.32%) had qHBsAg < 1000 IU/ml, and 47 (88.68%) had qHBsAg > 1000 IU/ml. When the levels of HBV DNA in AH group were stratified, 27 (50.94%) had HBV DNA > 2000 ≤ 20 000 IU/ml and 26 (49.06%) had HBV DNA > 20000 IU/ml.

Obviously, all patients' inactive carriers had HBV DNA level < 2000 IU/ml (Table 4). For Pearson Chi-square = 20,45 and p < 0.001 (p = 0.000) in the AH group of patients qHBsAg > 1000 IU/ml is significantly more represented.

Table 4: Distribution of quantitative HBsAg and HBV DNA in patients' inactive carriers and patients with HBeAg-negative chronic hepatitis B

	IC					AH			
qHBsAg	No.	Cumulative No.	%	Cumulative %	qHBsAg	No	Cumulative No.	%	Cumulative %
< 1000 IU/ml	29	29	51.79	51.79	< 1000 IU/ml	6	6	11.32	11.32
> 1000 IU/ml	27	56	48.21	100.00	> 1000 IU/ml	47	53	88.68	100.00
Missing	0	56	0,00	100.00	Missing	0	53	0.00	100.00
HBV DNA	No.	Cumulative No.	%	Cumulative %	HBV DNA	No.	Cumulative No.	%	Cumulative %
< 2000 IU/ml	56	56	100.00	100.00	≥ 2000 ≤ 20000 IU/ml	27	27	50.94	50.94
					> 20000 IU/ml	26	53	49.06	100.00
Missing	0	56	0.00	100.00	Missing	0	53	0.00	100.00

Abbreviations: IC-inactive carriers; AH- HBeAg-negative chronic hepatitis B; HBV DNA-hepatitis B virus deoxyribonucleic acid; qHBsAg-quantitative hepatitis B s antigen.

Patients with AH for Z = -5.10 and p < 0.001 (p = 0.0000) had statistically significant higher values of qHBsAg compared to patients' inactive carriers. Likewise, for Z = -8.99 and p < 0.001 (p = 0.000) patients with active hepatitis have significantly higher values of quantitative HBV DNA than patient's IC (Table 5).

Table 5: Differences between quantitative HBsAg and HBV DNA in patients' inactive carriers and patients with HBeAg-negative chronic hepatitis B

Parameter	Rank Sum IC	Rank Sum AH	U	Z adjusted	p-level	N IC	N AH
qHBsAg IU/ml	2238.00	3757.00	642.00	-5.10	0.000	56	53
HBV DNA IU/ml	1596.00	4399.00	0.00	-8.99	0.000	56	53

Abbreviations: IC-inactive carriers; AH- HBeAg-negative chronic hepatitis B; HBV DNA-hepatitis B virus deoxyribonucleic acid; qHBsAg-quantitative hepatitis Bs antigen.

Individualized analysis of the serum profile and the measurements of HBV DNA/ALT/qHBs antigen showed that the increase of the level of HBV DNA is followed with a non-significant decrease of ALT, both in IC and in patients with AH, while the increase of the level of quantitative HBsAg is followed with the increase of the level of ALT in both groups of patients. The influence of qHBsAg is significantly stronger than that of HBV DNA.

For each single increase of serum HBsAg, serum ALT increases for 0.0008 IU/ml p > 0.05 (p = 0.11) in IC group of patients, while in AH group of patients, the serum level of ALT increases for 0.0002 IU/ml p > 0.05 (p = 0.33) (Table 6).

Table 6: Multiple regression analysis of ALT/HBV DNA/qHBsAg in patients' inactive carriers and patients with HBeAg- negative chronic hepatitis B

	IC						AH					
	Dependent Variable: ALT; R= 0.22; F(2.53)=1.39 and p<0.26						Dependent Variable: ALT; R= 0.15; F(2.50)=0.59 and p<0.66					
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(53)	p-level	Beta	Std.Err. of Beta	B	Std.Err. of B	t(53)	p-level
Intercept			28.29	3.89	7.27	0.000			47.64	5.93	8.03	0.000
qHBsAg	0.22	0.13	0.0008	0.0005	1.64	0.11	0.17	0.17	0.0002	0.000	0.99	0.33
HBV DNA	-0.07	0.13	-0.002	0.004	-0.49	0.63	-0.04	0.17	-0.000	0.000	-0.22	0.83

Abbreviations: IC-inactive carriers; AH- HBeAg-negative chronic hepatitis B; HBV DNA-hepatitis B virus deoxyribonucleic acid; qHBsAg-quantitative hepatitis Bs antigen.

Discussion

HBeAg negative, antique positive chronic hepatitis B is a capricious disease characterised with a dynamic and complex interaction between the virus, the hepatocytes and the host's immune system [6], [7], [8], [16], [17]. The Republic of Macedonia has an estimated HBsAg prevalence around 1-4% [18] and having in mind that chronic HBV infection is the major pathogen causing chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) in the world, this imposes serious burden both on the individual and society as well [3], [5], [19]. It is very important to distinguish chronic inactive HBsAg carriers from HBeAg-negative CHB patients because the progression of the liver damage occurs primarily during the active hepatitis phase, and this group of patients has the potential of developing marked viral reactivation and has less chance of response to antiviral medications [20]. In the currently available guidelines, the recommendation is that HBV DNA, ALT, and HBeAg be analysed together and with great care for therapy decision making and the indication of the need for biopsy [11], [14], [15].

The results from the patients included in our study showed that there is no statistical significance between inactive carrier group and patients with HBeAg-negative CHB in terms of gender, although in the literature HBeAg-negative CHB is more expressed in males [21], [22], [23]. At the same time, the patients in our cohort with HBeAg-negative CHB are older than the patient's inactive carriers, which is consistent with the findings described in the literature [5], [23]. Chu et al., [24] followed 1.965 inactive HBV carriers' patients during 11.5 years and found out that 314 patients had reactivation of HBV. The risk for reactivation had a positive correlation with older age (p < 0.0001) and male sex (p < 0.0001). At the same time, the risk for developing cirrhosis also had a statistically significant correlation with advanced age and HBV reactivation (p = 0.004) and (p < 0.0001), respectively. The study showed that male sex (p = 0.037) and advanced age (p = 0.006) were two independent factors for HBV reactivation. The REVEAL-B study carried out by Chen et al., [23], besides HBeAg positivity and high HBV viraemia identifies male gender, older patients,

alcoholism and high BMI (body mass index), as factors associated with HBV disease progression similar to the findings of Fattovich et al., [5].

Alanine transaminase (ALT) levels have traditionally been used for treatment decisions in chronic hepatitis B virus-infected patients. In the study of Ijaz et al., [25] 567 patients with HBeAg negative CHB were investigated, and 228 were classified as chronic inactive carriers, and 339 with chronic active hepatitis B. The serum enzyme levels of ALT, AST showed significant and high AUROC in differentiation between HBeAg negative IC and HBeAg negative patients with chronic hepatitis. The AUROC for ALT and AST was 0.997 and 0.969, respectively. Similar to Ijaz's study, when the levels of transaminases were compared in our cohort, patients with HBeAg-negative CHB had significantly higher ALT and AST values compared to IC patients. The serum level of ALT is a factor to consider in the treatment of CHB patients, and a high ALT level helps to distinguish between the inactive carrier state and asymptomatic HBeAg-negative CHB patients with normal ALT [26]. The findings of low serum levels of transaminases in the patients inactive carriers included in our study is compatible with the observation that low and normal levels are expected in both patients inactive carriers and patient with HBeAg negative chronic hepatitis B, [5], [27], and it is prudent to emphasize the need for serial monitoring of the levels of transaminases over time and that the sole monitoring of ALT is not strong enough criteria for evaluation of hepatic injury as described in the study of Hadziyannis et al., [7].

It is known that maintained high levels of HBV DNA are associated with progressive liver disease. Serum DNA levels are a prognostic factor, and contribute to defining the phases of CHB infection, the treatment indication, and allow an assessment of the efficacy of antiviral therapy. Kumar et al., [26] in a recent, large prospective study have shown clearly that baseline ALT, and DNA level is good predictors of histologically significant fibrosis. The ten years long retrospective study conducted by Madan et al., [28] showed that the mean level of HBV DNA is lowest in patients' inactive carriers, and it is highest in patients with chronic active hepatitis B. Patients inactive carriers had significantly lower serum values of HBV DNA than patients with HBeAg negative chronic hepatitis B. The cut-off values of 3,5 log 10 cp/ml had sensitivity and specificity of 83 and 58% respectively in differentiation of patient's IC from patients with HBeAg negative CHB. The study of Zacharakis et al., [29] investigated 263 patients with chronic hepatitis B, genotype D, patients who were treatment naïve, HBeAg negative, antique positive, and all patients inactive carriers had low or almost undetectable levels of HBV DNA < 2000 IU/ml, and only 2% had HBV DNA reactivation with the level of HBV DNA > 2000 IU/ml. Similar to these studies, from the patients included in our study, patients inactive carriers had significantly lower values of serum HBV DNA compared to patients with HBeAg-negative CHB. An interesting finding in our study was that when the levels of HBV DNA were stratified in patients with HBeAg-negative CHB, 50.94% had HBV DNA > 2000 ≤ 20 000 IU/ml and 49.06% had HBV DNA > 20 000 IU/ml. The levels of HBV DNA have to be monitored on a close and regular basis in order, not to mistaken patients with active hepatitis for inactive carriers, especially in patients with HBeAg negative chronic hepatitis. In an inactive carrier, ALT usually remains normal on serial monitoring with undetectable to low levels (i.e., < 2000 IU/ml) of HBV DNA but the same can occur in a patient with HBeAg-negative CHB [20]. As the understanding of the complex problem that HBeAg negative chronic hepatitis B represents grows, it is understood that HBV DNA is not always a useful indicator for treatment decision. A Chinese study, involving 165 patients, reported that a single HBV DNA measurement misdiagnosis 45% HBeAg-negative CHB as chronic inactive HBsAg carriers. The study further revealed that even HBV DNA measurement on 3 separate occasions also misdiagnoses 30% cases [30]. Moreover, a study of 196 CHB patients revealed that 10.5% HBeAg-negative CHB patients had HBV DNA < 30,000 copies/mL [31].

Evaluation of the level of quantitative hepatitis B surface antigen (qHBsAg) reflects the amount of transcriptional activity of cccDNA (covalently closed circular DNA) and the integrated DNA in the hepatocytes representing one of the main serologic markers in chronic HBV infection; accurately monitoring both disease progression and prognosis as well as response to antiviral therapy [32], [33] At the same time, the correlation between the serum levels of HBsAg and HBV DNA improves and helps in better understanding and following the phases and outcome of CHB during its natural history and treatment as well [34].

In our cohort, the patients with HBeAg-negative CHB had significantly higher values of qHBsAg compared to patients IC. These findings correlate with the studies found in the literature. The study of Zhu et al., [35], included 124 patients with chronic hepatitis B and demonstrated that there is a correlation between HBV DNA and qHBsAg and that the serum level of HBsAg reflects the amount of HBV DNA replication. The serum levels of HBsAg were significantly higher in patients with HBV DNA > 1×10^3 cp/ml compared to patients with HBV DNA level < 1×10^3 cp/ml ($t = 5.983$, $p = 0.000 < 0.05$). Based on the HBV DNA level, the patients were divided into three groups: group A (HBV DNA level between 1×10^3 and 1×10^5 cp/ml, group B (1×10^5 cp/ml till 1×10^7 cp/ml) and group C (> 1×10^7 cp/ml). It was shown that the level of HBsAg increased with the level of HBV DNA and that it was higher in-patient group C Pearson's correlation ($r = 0.657$, $p = 0.000 < 0.05$) showed that there is a positive correlation between serum HBsAg and HBV DNA.

In many centres which do not have molecular biology testing, and in practical terms, ALT levels are used to predict the presence of viral replication and progression of liver damage. Our study showed that the increase of the serum level of HBV DNA is followed with a non – significant decrease of ALT both in IC and in patients with AH, while the increase of the level of quantitative HBsAg is followed with the increase of the level of ALT in both groups of patients. In our study, the influence of qHBsAg on values of ALT was significantly stronger than that of HBV DNA. The research done on patients with HBeAg negative chronic hepatitis have already demonstrated the weak correlation between viral load and transaminases [6]. The study of Kim et al., [36] showed that correlation of qHBsAg with ALT and HBV DNA can better predict the liver (dis)function and that at the same time this correlation can be used to discriminate between patients' inactive carriers and patients with HBeAg negative chronic hepatitis B.

As it has been said, the assessment of viral load is not a sufficient factor for treatment decision, and additional factors such as histological factors (fibrosis/cirrhosis and liver inflammation), patient age, disease evolution over time, family history of HCC, have to be taken into account for treatment decision. The most recent international guides also point out the need for multiple clinical applications, with repeated measurements of transaminases and HBV-DNA for the determination of the phases of the disease and better management of the infected patient [11], [14], [15].

Our study showed that both groups of patients, the inactive carriers and patient with active HBeAg negative chronic hepatitis have similar laboratory and identical serology profile and that it is difficult to determine who to treat based on the level of HBV DNA measured at one point of time. The presence of a positive correlation between the levels of qHBsAg and ALT may suggest the presence of more advanced liver disease and active HBV DNA replication, which can be taken into consideration for treatment decision. An especially interesting group of patients in our study are the patients who have normal ALT values and HBV DNA level above 2000 IU/ml, but less than 20 000 IU/ml, which in our cohort present 50.94% of patients with chronic HBeAg negative hepatitis. The decision of when to start antiviral treatment will be the most difficult in this set of patients.

The primary limitations of our study were the adoption of the same ALT reference value for male and female patients and the absences of the histological staging of the liver disease.

In conclusion, determination of the phases of chronic hepatitis B in patients who are HBeAg negative, anti HBeAg positive is of enormous clinical importance in order to avoid misclassification of patients inactive carriers with patients with HBeAg-negative CHB since laboratory and virology analysis were taken at one point of time can show normal transaminase activity and undetectable to low HBV DNA viraemia due to the typical intermittent profile of HBeAg negative chronic hepatitis B.

In conformity with literature, the results of our study suggest that due to the dynamism of the chronic infection by HBV, the infected patient should be continuously and carefully evaluated with a joint analysis of the clinical, serologic, biochemical, molecular biology, and sometimes histologic parameters in order to make a timely and proper decision when and who to treat. Proper and timely initiation of antiviral therapy will prevent the development of chronic HBV associated complications, and reduce the overall morbidity and mortality of these patients.

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