Elevation of B-Type Natriuretic Peptide Level in Asymptomatic Hepatitis B Virus-Positive Patients Is Not Associated with Abnormalities of Cardiac Function

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Key Words

Hepatitis B virus • B-type natriuretic peptide •
High-sensitivity C-reactive protein • Inflammation

Abstract

Objectives: To compare B-type natriuretic peptide (BNP) and echocardiographic parameters in patients with hepatitis B virus (HBV) and healthy control subjects. **Subjects and Methods:** 52 consecutive patients with HBV and 47 healthy controls were examined. All subjects underwent transthoracic echocardiography after a complete medical history and laboratory examination including BNP, C-reactive protein (CRP) and high-sensitivity CRP (hsCRP). **Results:** Demographic characteristics were similar in patients with HBV and the control group. No significant difference was found in conventional Doppler and tissue Doppler parameters between the two groups. BNP levels were significantly higher in patients with HBV [6.5 ng/l (range 0.5–85.2)] than controls [4.3 ng/l (range 0.5–18.3)], $p = 0.039$. hsCRP [3.25 mg/l (0.02–40.2) vs. 0.5 mg/l (0.02–8.0)] levels were significantly higher in patients with HBV than control subjects ($p < 0.001$). **Conclusion:** Patients with HBV had higher BNP, CRP, and hsCRP

levels than controls. Echocardiographic findings were similar in both groups. This slight BNP elevation in HBV patients may be related to chronic inflammation due to HBV.

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Introduction

Several extrahepatic manifestations are associated with chronic hepatitis B virus (HBV) infection, many with significant morbidity and mortality. The cause of these extrahepatic manifestations is generally believed to be immune mediated. Polyarteritis nodosa, HBV-associated glomerulonephritis, arthritis, and dermatitis are some known systemic complications of chronic HBV infection [1, 2]. In recent years, progress has been achieved in the treatment of HBV [3].

The cardiac manifestation of the HBV infection is not well known. There are only several studies investigating the relationship between HBV infection and the heart. However, the results of these studies are controversial [4–6].

B-type natriuretic peptide (BNP) is a cardiac neuro-hormone which is secreted from the ventricles in response to ventricular volume expansion and pressure overload [7–9]. The BNP test can be used to detect pre-clinical heart disease or to confirm the cardiac etiology in symptomatic patients. Follow-up of BNP levels is useful in patients with heart failure [10]. Recent studies suggest that BNP may be a diagnostic tool for diastolic heart failure [11]. BNP had been investigated in several conditions such as rheumatic and pulmonary diseases [12, 13]. However, BNP levels have never been studied in patients with HBV. Therefore, the aim of this study was to compare the BNP levels and echocardiographic parameters in patients with HBV and healthy control subjects.

Subjects and Methods

52 HBV-positive patients and 47 healthy controls were enrolled in the study. The controls were chosen from healthy people admitted for routine checkup. All the subjects underwent transthoracic echocardiography after taking a complete medical history and laboratory investigations.

Inclusion criteria were HBV patients who have normal liver function tests, normal hepatobiliary ultrasonography, are hepatitis B e antigen (HBeAg) negative, anti-hepatitis B e (anti-HBe) antibody positive, and anti-hepatitis B c (anti-HBc) IgM negative, have normal bilirubin and albumin levels, and positive HBV DNA. The effect of hepatitis B viral load on BNP levels in patients without liver damage was studied. Exclusion criteria were presence of diabetes, hypertension, coronary artery disease, pulmonary disease, symptomatic heart failure, chronic renal disease (creatinine >13.6 mmol/l), inflammatory bowel diseases, and systemic autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and scleroderma, liver cirrhosis, and alcohol intake above 30 g per day. The study protocol was in accordance with the Declaration of Helsinki. All patients provided informed consent.

Serum Sample

Venous fasting blood sample was collected into EDTA-containing Vacuette tubes (Greiner Bio-One) and centrifuged at 3,000 g for 15 min at 4°C. Plasma samples were separated and stored at –70°C for not more than 3 months until used.

BNP Determination

The concentrations of BNP were determined with a 2-site sandwich chemiluminescence immunoassay (Lot No.: 22161145, reference No.: 02816634) on the ADVIA® Centaur® platform (Siemens Healthcare Diagnostic, Ill., USA). The ADVIA Centaur BNP assay is a fully automated 2-site sandwich immunoassay using direct chemiluminescent technology, which applies constant amounts of two monoclonal antibodies. The first antibody, in the Lite reagent, is an acridinium ester labeled monoclonal mouse anti-human BNP F(ab')* fragment specific to the ring structure of BNP. The second antibody, in the solid phase, is a biotinylated monoclonal mouse anti-human antibody specific to the C-termi-

nal portion of BNP, which is coupled to streptavidin magnetic particles. The limit of detection for this assay was 0.5 ng/l and showed that the within-run and total imprecision coefficient of variation was 3.5%. Values less than 0.5 ng/l were regarded as non-detectable [14].

C-Reactive Protein and High-Sensitivity C-Reactive Protein Determination

C-reactive protein (CRP) and high-sensitivity CRP (hsCRP) concentrations were measured with the IMMAGE® immunochemistry system (Beckman Coulter). The normal value for CRP as measured by this method is <0.08 mg/l. The lower limit of detection of hsCRP assay was 0.02 mg/l. Interassay coefficients of variation for controls at 0.84 and 13.8 mg/l were 4.2 and 3.5%, respectively.

Hepatitis B Surface Antigen and Anti-Hepatitis B Surface Determination

Serum hepatitis B surface antigen (HBsAg) and anti-hepatitis B surface (anti-HBs) concentrations were measured with an automatic diagnostic system (Liaison® diagnostic system, DiaSorin S.p.A., Saluggia, Italy). The Liaison anti-HBs (Lot No.: 040042) and HBsAg assay (Lot No.: 042038) uses chemiluminescence immunoassay technology for the quantitative determination of HBsAg and anti-HBs in human serum or plasma.

Anti-HBe Antigen, Anti-HBc IgM and Anti-HBe Antibody Determination

Serum HBeAg, anti-HBe and anti-HBc were measured with a commercial chemiluminescence immunoassay kit (DiaSorin S.p.A., Italy) by Liaison automated analyzer (Liaison, Italy).

HBV DNA Determination

DNA was extracted from 200 µl of serum using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA was eluted into 100 µl nuclease-free water and 5 µl were added to a 25-µl PCR mixture.

The reaction was carried out using a commercial SYBR Green reaction mix (Qiagen). The kit contained HotStarTaq polymerase which is included to avoid false positives in the quantitative PCR. The primary sequences were 5'-GTG TCT GCG GCG TTT TAT CA (sense) and 5'-GAC AAA CGG GCA ACA TAC CTT (antisense) designed to amplify a 98-base pair product from positions 379 to 476 of the HBV genome. Thermal cycling was performed in an ABI 5700 sequence detection system (PE Applied Biosystems, Warrington, UK). Reaction conditions were: 95°C for 15 min followed by 40 cycles of 94°C for 15 s, 55°C for 30 s and 72°C for 30 s. A four-point standard curve (1.5×10^8 copies per milliliter, 1.5×10^6 copies per milliliter, 1.5×10^4 copies per milliliter, and 1.5×10^2 copies per milliliter) was generated from a high-titer plasma donation quantified by end point dilution PCR. The calibration of this standard was confirmed by comparison with an international HBV DNA standard (97/746; NIBSC, Potters Bar, UK). Test samples falling above the top of the standard curve were reassayed at a dilution of 1:100. Each test run included positive and negative controls. The performance of the assay was evaluated by comparison with a commercial assay (HBV Monitor, Roche Molecular Systems, Inc., Branchburg, USA) performed according to the manufacturer's instructions.

Table 1. Clinical and laboratory characteristics of the subjects

Characteristics	HBV patients (n = 52)	Controls (n = 47)
Age, years	46 ± 14	42 ± 11
Female/male	20/32 (39/61)	24/23 (51/49)
Body mass index, kg/m ²	22.2 ± 3.1	23.1 ± 3.8
Smoking, %	34	38
Systolic blood pressure, mm Hg	123 ± 11	121 ± 12
Diastolic blood pressure, mm Hg	79 ± 8	78 ± 10
High-density lipoprotein, mmol/l	1.11 ± 0.23	1.22 ± 0.26
Low-density lipoprotein, mmol/l	2.67 ± 0.80	2.95 ± 0.73
Triglyceride, mmol/l	1.50 ± 0.26	1.50 ± 0.24
Creatinine, µmol/l	9.5 ± 1.81	9.4 ± 1.58
Alanine aminotransferase, IU/l	34 ± 6	25 ± 4
Aspartate aminotransferase, IU/l	27 ± 7	23 ± 3
Albumin, g/l	43 ± 3	43 ± 2
Total bilirubin, µmol/l	9.92 ± 2.74	10.26 ± 2.74

All p values were nonsignificant between the groups. Figures in parentheses indicate percentages.

Routine Biochemical Analyses

Fasting serum total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, uric acid (Lot No.: B302, Konelab), γ -glutamyltransferase (Lot No.: C331, Konelab), alanine aminotransferase (Lot No.: C239, Konelab) and aspartate aminotransferase (Lot No.: C372, Konelab) concentrations were measured enzymatically with an automatic analyzer (Konelab 60i, Thermo Scientific, Finland). Total cholesterol (Lot No.: B540, Konelab) was measured by the cholesterol oxidase method and triglycerides (Lot No.: C186, Konelab) were measured by the glycerol blank method. Low-density lipoprotein cholesterol (Lot No.: C435, Konelab) and high-density lipoprotein cholesterol (Lot No.: C136, Konelab) were measured with the homogeneous enzymatic colorimetric test.

Transthoracic Echocardiography

Transthoracic echocardiographic examination was performed in all subjects using a System Three (GE Vingmed Ultrasound, Horten, Norway) cardiac ultrasound scanner and 2.5- to 3.5-MHz transducers.

Echocardiography of the Left Ventricle

Left ventricular and left atrial dimensions were measured in the parasternal long-axis view. Left ventricular end diastolic and end systolic dimensions were measured using M mode echocardiography. Aortic root diameter was taken in the parasternal long-axis view. Left ventricular ejection fraction was obtained by means of the Teichholz equation.

Doppler Echocardiography

Flow velocity indices were obtained using pulsed and continuous-wave Doppler from apical projections, and measurements were made utilizing the software of the ultrasound equipment. Mitral diastolic flow was obtained after the pulsed Doppler sam-

ple volume was positioned perpendicular to the tips of the mitral valve leaflets. The Doppler cursor was then moved toward the left ventricular outflow position, and the sample volume was placed approximately 1 cm proximal to the aortic valve so that it would come in contact with the anterior mitral valve leaflet. Isovolumic relaxation time (ms) was measured as the interval between the end of the aortic click artifact and the onset of mitral inflow waveform.

The following indices were measured from the mitral and tricuspid valve diastolic waveform: peak early (E) and atrial (A) flow velocities (m/s), and E/A ratio of the left ventricular diastolic filling. Heart rate (beats/min) was measured from simultaneous electrocardiogram recordings.

Pulsed Doppler Tissue Echocardiography

The myocardial velocities of the left ventricle were measured by sampling the mitral annulus excursion at lateral sites in the four-chamber view. Care was taken to keep the ultrasound beam perpendicular to the plane of the annulus in order to minimize the angle between the beam and the direction of annular motion. The width of the sample volume was 3–5 mm. Measurements were focused on the systolic myocardial wave, the early-diastolic and end-diastolic myocardial waves. Usually, several cardiac cycles were acquired, and the best two consecutive ones were analyzed and averaged [15].

Statistics

Statistical Package for Social Sciences (SPSS) version 10.0 was used for the analysis. The distribution of data was assessed by using the one-sample Kolmogorov-Smirnov test. Continuous variables with a normal distribution are expressed as mean $\pm$ SD, variables with a skewed distribution are expressed as median (range), and categorical variables are expressed as a percentage. For comparison of categorical variables or percentages, we used Fisher's exact and χ^2 tests. Differences between numeric variables were tested with Student's t test or Mann-Whitney U test. Correlation was tested with Spearman's rank order or Pearson correlation coefficient. A p value <0.05 was considered as statistically significant.

Results

Demographic characteristics of HBV and control groups were similar. There were no significant differences with respect to sex, age, systolic and diastolic blood pressure, and lipid parameters (table 1). There was no significant difference in alanine aminotransferase (34 ± 6 vs. 25 ± 4 ; $p = \text{NS}$) and aspartate aminotransferase (27 ± 7 vs. 23 ± 3 ; $p = \text{NS}$) between patients and controls. HBsAg was positive, whereas anti-HBs antibody was negative in all patients. HBeAg and anti-HBc IgM were negative in all patients. Anti-HBe antibody was positive in all patients. All subjects had normal bilirubin and albumin levels. No patients had liver cirrhosis.

All patients and control subjects had a normal ejection fraction (64 ± 5 vs. 65 ± 7 ; $p = \text{NS}$). The diastolic param-

Fig. 1. Distributions of BNP levels in controls and patients with HBV [6.5 ng/l (0.5–85.2) vs. 4.3 ng/l (0.5–18.3), $p = 0.039$]. The black lines within the boxes indicate the median, the edges of the boxes are the 25th and 75th percentiles, and the lines extend to the maximum and minimum values.

Fig. 2. Distributions of hsCRP levels in controls and patients with HBV [3.25 mg/l (0.02–40.2) vs. 0.5 mg/l (0.02–8.0), $p < 0.001$]. The black lines within the boxes indicate the median, the edges of the boxes are the 25th and 75th percentiles, and the lines extend to the maximum and minimum values.

Fig. 3. Distributions of CRP levels in controls and patients with HBV [0.09 mg/l (0.01–0.70) vs. 0.03 mg/l (0.01–0.11), $p < 0.001$]. The black lines within the boxes indicate the median, the edges of the boxes are the 25th and 75th percentiles, and the lines extend to the maximum and minimum values.

eters of both groups are presented in table 2. No significant difference was found in conventional Doppler and tissue Doppler parameters between the two groups. There was no association between the measured echocardiographic indices and the presence of any of the serological markers of HBV.

BNP levels were significantly higher in patients with HBV [6.5 ng/l (0.5–85.2)] than in controls [4.3 ng/l (0.5–18.3), $p = 0.039$] (fig. 1). Three subjects had a nondetectable BNP level. There was no correlation between BNP levels and any serological markers of HBV correlation factor. There was also no correlation between BNP levels and age, gender or liver function tests.

hsCRP [3.25 mg/l (0.02–40.2) vs. 0.5 mg/l (0.02–8.0); $p < 0.001$] and CRP [0.09 mg/l (0.01–0.70) vs. 0.03 mg/l (0.01–0.11); $p < 0.001$] levels were significantly higher in patients with HBV than in control subjects (fig. 2, 3). There was no correlation between BNP and hsCRP ($r = 0.033$, $p = 0.744$) or CRP ($r = 0.015$, $p = 0.886$). In multivariate analysis, neither CRP nor hsCRP levels are independent parameters on BNP levels.

HBV DNA levels were 1,310 IU/ml ($345 - 17 \times 10^6$). HBV DNA levels were correlated with hsCRP levels ($r = 0.410$; $p = 0.003$). There was no significant correlation between HBV DNA levels and any markers such as BNP ($r = 0.075$, $p = 0.599$) and biochemical parameters.

Table 2. Echocardiographic measurements

Measurements	HBV patients (n = 52)	Controls (n = 47)
LVEDD, cm	4.8 ± 0.5	4.7 ± 0.5
LVESD, cm	3.1 ± 0.4	3.0 ± 0.4
LVEF, %	64 ± 5	65 ± 7
LVPWT, mm	8.1 ± 0.9	8.0 ± 0.7
IVST, mm	8.2 ± 1.0	8.1 ± 0.7
Mitral E peak, m/s	0.73 ± 0.15	0.71 ± 0.16
Mitral A peak, m/s	0.61 ± 0.16	0.59 ± 0.15
Mitral E/A	1.20 ± 0.16	1.21 ± 0.16
Mitral IVRT, ms	98 ± 24	98 ± 30
Mitral E/A <1, %	8	13
Mitral early diastolic annular velocity, m/s	9.0 ± 2.7	9.7 ± 2.4
Mitral late diastolic annular velocity, m/s	8.7 ± 1.6	8.8 ± 0.91
Mitral peak systolic annular velocity, m/s	9.7 ± 2.1	8.9 ± 1.4

All p values were nonsignificant between groups. LVEDD = Left ventricle end-diastolic diameter; LVESD = left ventricle end-systolic diameter; LVEF = left ventricle ejection fraction; LVPWT = left ventricle posterior wall thickness; IVST = interventricular septum thickness; IVRT = isovolumic relaxation time.

Discussion

We showed that HBV patients had higher BNP levels than controls. Diastolic dysfunction did not occur more frequently in HBV patients than controls. We also demonstrated that HBV patients had higher hsCRP and CRP levels than controls.

We found that CRP and hsCRP levels were elevated in patients with HBV compared with controls and HBV DNA levels were correlated with hsCRP levels, but Gedik et al. [16] described a lack of correlation between CRP and hepatitis B viral load in serum of patients with chronic HBV. There are no other studies reporting quantitative comparison of HBV DNA and hsCRP values [17]. We suggest that elevation in hsCRP is related to chronic HBV infection.

There are several studies available to prove the association between HBV infection and cardiovascular system. Some studies investigated HBV and carotid atherosclerosis. Kiechl et al. [18] found no significant association between chronic hepatitis and the development of new carotid atheromatous plaques, although they did not specify the type of hepatitis virus. However, another study in Japan demonstrated an increased prevalence of carotid atherosclerosis in HBV carriers [19]. In our study, we found that increased hsCRP level is a marker of atherosclerosis. It may be related to heart disease as well as chronic liver disease.

BNP is a potent modulator of vascular tone, fluid-electrolyte balance, and cardiovascular growth. As a rule, under pathophysiological conditions such as chronic congestive heart failure, the expression and secretion of BNP are significantly augmented. In addition to this, proinflammatory cytokines have been identified as additional triggers inducing BNP secretion [20]. Therefore, it can be stated that BNP levels may increase in inflammatory processes.

BNP evaluation has some limitations. Its specificity and sensitivity depends on clinical status. BNP evaluation should be related to clinical signs and examinations of the patients. In our study, no patients had a severe clinical cardiac symptom and sign. Therefore, BNP levels may be affected in this condition.

In this study, we found that BNP levels were higher in HBV patients than in controls. A diagnosis of heart failure was highly unlikely when the BNP level was <100 ng/l [21]. Maisel et al. [22] demonstrated that BNP levels were higher than 1,000 ng/l in patients with heart failure. In our study, the median of BNP levels was 6.5 ng/l. This value is very low to say the difference is associated with heart failure. We consider that the higher BNP level in HBV patients is not due to myocarditis. We also found out that hsCRP and CRP levels were higher in patients with HBV than in controls. We suggest that this slight BNP elevation in HBV patients may be related to chronic inflammation due to HBV virus. Elevation of the inflammation markers like hsCRP and normal diastolic and systolic heart function support this hypothesis.

Conclusion

Patients with HBV had higher BNP levels than controls. However, there was no significant difference in echocardiographic findings between the two groups. We suggest that elevated BNP values did not appear to predict left ventricular dysfunction in HBV patients. This slight BNP elevation in HBV patients may be related to chronic inflammation due to HBV.

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