Research Article

85

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Circulating Glucose-Regulated Protein 78 Levels in Patients with Chronic Hepatitis B Infection

Kronik Hepatit B Enfeksiyonlu Hastaların Dolaşımında GRP78 Seviyesi

Selma CIRRIK[1], Yeliz ÇETİNKOL[2], Arzu ALTUNÇEKİÇ YILDIRIM[3], Mustafa Kerem ÇALGIN[2], Tevfik NOYAN[4]

[1]*Ordu University Faculty of Medicine, Department of Physiology, Ordu,Turkey*
[2]*Ordu University Faculty of Medicine, Department of Medical Microbiology, Ordu,Turkey*
[3]*Ordu University Faculty of Medicine, Department of Infectious Diseases, Ordu, Turkey*
[4]*Ordu University Faculty of Medicine, Department of Medical Biochemistry, Ordu,Turkey*

ABSTRACT

Objectives: The role of endoplasmic reticulum (ER) stress in the pathogenesis of hepatitis B virus (HBV) has been reported. However, serum levels of glucose-regulated protein (GRP) 78 which is an ER stress marker both in tissue and circulation have not been reported yet. This study aimed to evaluate serum GRP78 levels in patients with chronic HBV infection.

Materials and Methods: A total of 60 patients with HBV infection and 50 control subjects were included in this study. According to HBV-DNA levels, patients with HBV infection were subdivided into low, mild and high HBV-DNA subgroups (n=20, in each). Serum GRP78 level was measured by ELISA and then correlation analysis was performed between GRP78 and alanine aminotransferase (ALT), aspartate aminotransferase (AST), HBV-DNA or hepatitis B surface antigen (HBsAg).

Results: HBsAg levels were significantly higher in each HBV subgroup compared to controls. ALT and AST levels were significantly increased in the high HBV-DNA subgroup. There was no significant difference in serum GRP78 level between controls and HBV subgroups and no correlation between serum GRP78 levels andother variables.

Conclusion: As a result of our study, there was no relationship between the serum level of GRP78 and the HBV infection parameters. Since ER stress is an important mechanism in HBV-related liver injury, other ER stress markers, such as GRP94, might be examined in future studies.

Keywords: Hepatitis B, glucose-regulated protein 78, endoplasmic reticulum stress

ÖZ

Amaç: Hepatit B virüsünün (HBV) patogenezinde, endoplazmik retikulum (ER) stresinin rolü daha önceden gösterilmiştir. Ancak, hem dokuda hem de dolaşımda bir ER stres belirteci olan glukozla düzenlenen protein (GRP) 78'in serumdaki seviyesi bugüne kadar çalışılmamıştır. Bu çalışmada, kronik HBV enfeksiyonlu hastaların serum GRP78 seviyelerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışma kontrol (n=50) ve HBV (n=60) olmak üzere iki grup ile yapılmıştır. HBV hastaları, HBV-DNA miktarına göre düşük, orta ve yüksek HBV-DNA alt gruplarına (her birinde n=20) bölünmüştür. Serum GRP78 seviyesi ELISA ile ölçülmüş ve arkasından GRP78 ile alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), HBV-DNA veya hepatit B yüzey antijeni (HBsAg) arasında korelasyon analizi yapılmıştır.

Bulgular: Kontrol ile kıyaslandığında, her bir HBV alt grubunda HBsAg seviyesi önemli yüksek bulunmuştur. ALT ve AST seviyesindeki artış, yüksek HBV-DNA alt grubunda önemli bulunmuştur. Serum GRP78, hem kontrol hem de HBV alt gruplarında benzer düzeylerde olup, serumdaki değişkenlerle bir korelasyon göstermemiştir.

Sonuç: Çalışmamızın sonuçlarına göre, HBV hastalarının parametreleri ile serum GRP78 seviyesi arasında bir ilişki yoktur. ER stresi, HBV ile ilişkili karaciğer hasarında önemli bir mekanizma olduğundan, daha sonraki çalışmalarda GRP94 gibi diğer ER stres belirteçleri incelenebilir.

Anahtar Kelimeler: Hepatit B, glukozla düzenlenen protein78, endoplazmik retikulum stresi

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Address for Correspondence: *Selma Cırrık, PhD, Ordu University Faculty of Medicine, Department of Physiology, Ordu,Turkey*
Phone: +90 452 226 52 14 E-mail: selmacrrk@yahoo.com ORCID ID: orcid.org/0000-0001-8474-0185 **Received:** *31.05.2018* **Accepted:** *31.10.2018*

Introduction

The endoplasmic reticulum (ER) is a membranous organelle required for folding and processing of nascent proteins. Physiological, pathological or pharmacological insults that disrupt ER function induce accumulation of unfolded or misfolded proteins in the ER lumen. This condition is defined as ER stress and triggers a conserved cellular response called unfolded protein response (UPR) (1). UPR aims to relieve ER stress via different mechanisms, including enhancement of protein folding and degradation processes as well as specific inhibition of protein synthesis in the cell. UPR-related mechanisms are mediated by three different signaling pathways: protein kinase R-like ER kinase (PERK), activated transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1) (1,2). Although UPR provides a defense mechanism for cells, ER stress can trigger apoptosis depending on the severity and duration of stress (2,3,4). It has been reported that a number of pathophysiological conditions, such as diabetes mellitus, cardiovascular diseases, obesity, cancer, neurodegenerative diseases and hepatic steatosis are associated with excessive or persistent ER stress (5,6,7,8,9,10).

Hepatocytes possess quite well-developed ER and high capacity for protein synthesis similar to other secretory cells. Therefore, ER stress and UPR play an important role in various liver diseases such as non-alcoholic steatohepatitis, alcoholic liver disease, and ischemic reperfusion injury as well as toxic liver damage (11,12,13,14,15). Moreover, different studies conducted on mammalian cells, including hepatocytes, have reported that viruses were also able to induce ER stress. Increased viral protein synthesis in the infected cells induces ER stress by disturbing protein folding mechanisms and enhancing protein aggregates in the ER lumen (16). It is known that hepatitis B virus (HBV) induces ER stress and UPR activation, which contributes to liver pathogenesis during HBV infection (16,17,18,19).

Glucose-regulated protein 78 (GRP78) is an ER resident chaperone protein that monitors ER stress and regulates UPR-induced survival or apoptosis. During ER stress, expression of GRP78 significantly increases; hence, increased expression of GRP78 indicates UPR induction (1,2,3,4). Khadir et al. (20) reported that obesity, which induces hepatic ER stress, caused an elevation in GRP78 levels both in plasma and liver. These results suggest that GRP78 can pass into blood from tissue during hepatic ER stress (20,21). In HBV-infected patients, activation of UPR and its contribution to HBV pathogenesis have been shown previously, however, no circulating marker has been reported. Therefore, alterationsin circulating GRP78 level in patients with chronic HBV infection is evaluated in the present study.

Materials and Methods

In the current study, samples collected from 60 patients with chronic HBV infection whose serum specimens were processed for HBV-DNA quantification in the molecular microbiology laboratory were evaluated for GRP78 analysis. Control serums were obtained from 50 hepatitis B surface antigen (HBsAg)-, anti-hepatitis C virus- and anti-human immunodeficiency virus-negative subjects who had no chronic disease including chronic liver disease. According to HBV-DNA levels, HBV-infected patients were subdivided into low (20-1x10^2 IU/mL, n=20), mild (1x10^3-1x10^5 IU/mL, n=20), and high HBV-DNA (1x10^6- 1.7x10^8 IU/mL, n=20). All the samples were stored at -40 °C until assayed throughan ELISA. This study was approved by the Clinical Research Ethics Committee in Ordu University (2017-157).

Real-time polymerase chain reaction: Quantification of HBV-DNA was performed via a real-time polymerase chain reactionmethod using the COBAS AmpliPrep/COBAS Taqman 48 system (Roche, Branchburg, NJ, USA). Viral nucleic acids were extracted from serum (500 µl) using Cobas AmpliPrep automatic extractor system according to the manufacturer's instructions. The assay range for HBV-DNA was 20-1.7x10^8 IU/mL.

Detection of GRP78: Levels of circulating GRP78 were determined in serum using the human GRP78 ELISA kit (Elabscience; E-EL-H5586) with a detection range of 0.63-40 ng/mL. Samples and standards were added to the appropriate micro ELISA plate wells pre-coated with an antibody specific to human GRP78 and then the manufacturer's instructions were followed. The optical density was measured spectrophotometrically at the wavelength of 450 nm (BioTek, ELx800 brand REF ELX508 SN1310149). The level of GRP78 in the samples was calculated by comparing the optical density of the samples with the standard curve.

Statistical Analysis

All data are given as mean ± standard deviation. Statistical analysis was performed with One-Way ANOVA and Tukey's test.A p value of less than 0.05 was considered statistically significant.

Results

Sixty patients with chronic HBV infection (42.1±16.0 years; 28 female, 32 male) and 50 control subjects (53.9±19.1; 21 female, 29 male) were included in this study. HBV-DNA content in the

Table 1. Hepatitis B surface antigen, alanine aminotransferaseand aspartate aminotransferaselevels in control (n=50) and hepatitis B virus subgroups (n=20, in each subgroup)

	Control	Chronic HBV infection		
		HBV-DNA low	HBV-DNA mild	HBV-DNA high
HBsAg (IU/L)	0.54±0.07	3668±3262*	3869±2397*#	2397±1576*
ALT (U/L)	11.26±5.01	18.8±8.2###	25.0±13.1###	82.6±100.1*
AST (U/L)	13.50±4.23	19.1±5.3###	21.4±5.9##	59.7±74.5*

HBV patients divided into 3 subgroups as HBV-DNA low; 20 - 1x10^2 IU/mL (n=20), HBV-DNA mild; 1x10^3- 1x10^5 IU/mL (n=20) and HBV-DNA high; 1x10^6- 1.7x10^8 IU/mL (n=20). *p<0.001 vs. control; #p<0.05, ##p<0.01, ###p<0.001 vs. HBV-DNA high
HBV: Hepatitis B virus, HBsAg: Hepatitis B surface antigen

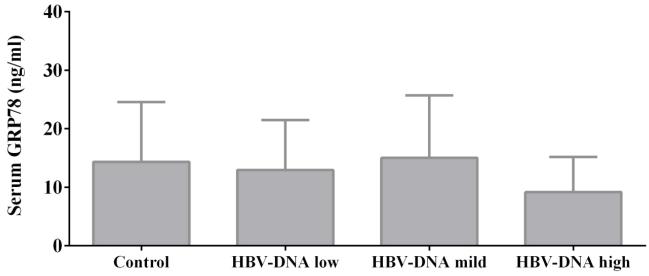

Figure 1. Serum glucose-regulated protein 78 levels in the control and hepatitis B virus subgroups; i.e. hepatitis B virus-DNA low, hepatitis B virus-DNA mild and hepatitis B virus-DNA high
HBV: Hepatitis B virus, GRP78: Glucose-regulated protein 78

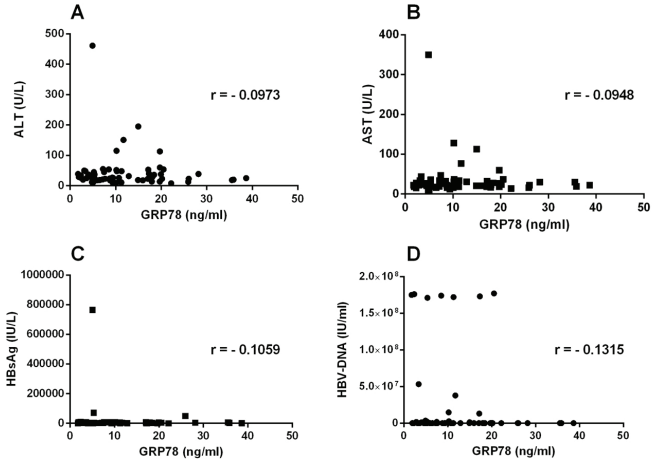

Figure 2. Correlation between serum glucose-regulated protein 78 concentration and serum levels of alanine aminotransferase (A), aspartate aminotransferase (B), hepatitis B surface antigen(C) and hepatitis B virus-DNA (D) in patients with chronic hepatitis B virusinfection. All the patients were included in the correlation analysis (n=60)
HBsAg: Hepatitis B surface antigen, GRP78: Glucose-regulated protein 78, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HBV: Hepatitis B virus

serum samples of the patients with chronic HBV infection was in three different ranges of low (20 - $1x10^2$ IU/mL, n=20), mild ($1x10^3$ - $1x10^5$ IU/mL, n=20), and high ($1x10^6$-$1.7x10^8$ IU/mL, n=20). Serum HBsAg, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in all patients are presented in Table1.

The serum GRP78 concentration was found to be 14.36±10.22 ng/mL in controls. As seen in Figure1, there was no significant difference in GRP78 concentration between the HBV subgroups (12.97±8.5, 15.01±10.7 and 9.18±6.02 ng/mL, respectively). In the control group, no correlation was found between GRP78 and serum ALT or AST levels. In each HBV subgroup, changes in serum GRP78 did not show a correlation with the serum variables such as ALT, AST and HBsAg or HBV-DNA content. When all the HBV-infected patients were included in the analysis, we did not determine a correlation between GRP78 and the other variables (Figure2).

Discussion

Accumulation of unfolded or misfolded proteins in the ER lumen causes a condition termed ER stress (1,2,3,4). During ER stress, UPR is activated to restore cellular homeostasis, however, delayed or insufficient responses to ER stress are implicated with pathological consequences, including fat accumulation, insulin resistance, inflammation, and apoptosis, all of which play important roles in liver pathologies (11,12,13,14,15).

HBV infection is a serious health problem affecting approximately 260 million people worldwide and causing acute and chronic hepatitis, liver cirrhosis, and even hepatocellular carcinoma (22). To date, many studies have reported on the molecular mechanisms for the relationship between HBV infection and pathogenesis of hepatic diseases, but the mechanisms are still not fully understood (23-25). Recent studies indicate that ER stress may play a role in the pathogenesis of HBV infection (16,17,18,19). It has been reported that some products of HBV genome might be involved in the activation of UPR in hepatocytes. For instance, Li et al. (26) reported that regulatory X protein (HBx), which is a product of HBV genome, mediated UPR activation in Hep3B and HepG2.2.15 cells via ATF6 and IRE1-XBP1 pathways. Further studies confirmed HBx-induced ER stress in different cell lines (27,28). Additionally, Wang et al. (29,30) reported that the pre-S mutant large HBsAgs were retained in the ER lumen and induced UPR signals, leading to the increased expression of ER chaperones such as GRP78 and GRP94.

In the present study, serum GRP78 levels were evaluated as an ER stress marker in patients with chronic HBV infection whose HBV-DNA levelranged between 80 and $1.7x10^8$ IU/mL. As expected, these patients displayed higher levels of serum HBsAg compared to controls. Since the measurement of serum ALT and AST levels is a common laboratory assay to monitor liver functions, we evaluated these parameters in the subjects. In the control group, the ALT and AST levels were within the normal range. In the HBV subgroups, as the HBV-DNA content raised, serum ALT and AST levels gradually increased. However,a statistically significant elevation was observed only in the HBV subgroup with the highest HBV-DNA content. These results are consistent with the previous reports and suggest that hepatic functions declined during high viral replication (31,32,33).

GRP78 is an ER-resident molecular chaperone which also regulates ER stress and is upregulated during UPR (1,2,3,4). Since HBV induces hepatic ER stress (16,17,19,26,27,28,29,30), we hypothesized that circulating GRP78 increased in HBV-infected patients and then, measured serum GRP78 levels in controls and in patients with chronic HBV infection.Contrary to our expectation, present results showed that during chronic HBV infection, circulating GRP78 levels remained unchanged without any relationship with serum variables. These results might be attributed to a mechanism that prevents GRP78 release from hepatocytes. Li et al. (26) showed that HBx protein and GRP78 were co-localized in ER lumen and displayed a direct interaction which may results in increased GRP78 retention in ER lumen. The aforementioned HBx-GRP78 interaction or another unknown mechanism may prevent GRP78 release to the blood and be responsible for the unchanged serum GRP78 levels in HBV-infected patients. Nevertheless, GRP78 measurements are needed both in liver biopsy and serum samples to confirm this suggestion.

Study Limitations

The most important limitations of the current study are small sample size, which included only Turkish subjects, and absence of liver biopsies. It would be valuable data if we had shown the presence of ER stress in liver biopsies obtained from a larger and heterogenous subject population, despite unchanged serum GRP78 concentration.

Conclusion

Although the involvement of ER stress in HBV-induced liver damage is well-documented, the present results show that serum GRP78 remained unchanged during chronic HBV infection and there was no relationship between serum level of GRP78 and the parameters of HBV infection. Since ER stress is an important mechanism in HBV-related liver injury, other ER stress markers, such as GRP94, might be examined with liver biopsies in future studies conducted with larger patient group.

Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee in Ordu University (2017-157).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: S.C., Design: S.C., Y.Ç., Data Collection or Processing: S.C., Y.Ç., M.K.Ç., Analysis or Interpretation: S.C., A.A.Y., T.N., Literature Search: S.C., A.A.Y., Writing: S.C., Y.Ç.

Conflict of Interest: No conflict of interest was declared by the authors.

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