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EUROMEDITERRANEAN BIOMEDICAL JOURNAL
2020,15 (20) 85–89
(*FORMERLY: CAPSULA EBURNEA*)

Original Article

SERUM HSP 70 LEVELS OF CHRONIC HEPATITIS B PATIENTS ARE SUBSTANTIALLY CORRELATED WITH HBsAg

Awaz A. Saadi [1], Ahmed M. Salih [2], Muayed A. Merza [3]

1. Biology Department, University of Duhok, Kurdistan, Iraq,
2. Duhok Medical Research Centre, College of Medicine, University Of Duhok, Kurdistan, Iraq
3. Azadi Teaching Hospital, Department of internal medicine, University of Duhok, Kurdistan, Iraq

ARTICLE INFO

Article history:
Received 22 January 2020
Revised 05 May 2020
Accepted 23 May 2020

Keywords:
HBsAg, HBeAg, anti HBe Ab, and HBV DNA.

ABSTRACT

Heat-shock protein 70 (HSP70) is a stress-induced protein that demonstrates the anti-apoptosis response that ensures the survival of cells. The aim of this study is to investigate the significant correlation of serum HSP70 levels in chronic hepatitis B viral infection (HBV) patients. The study was performed on fifty-three chronic HBV patients from June–December 2015. HBV-DNA, HBsAg, HBeAg, and anti HBeAb, ALT, AST, ALP, and AFP levels were determined. Serum HSP70 level was estimated for positive HBV patients. The results showed a significant correlation between HSP70 [mean of 41.325±29.7206], HBsAg [mean of 12074.93±5681.619] at p=0.006 [P=or <0.01], and also between HBV DNA [3.2E+10±2.4E+11] and HBeAg [96454.29321±300533.25] at P=0.002 [P= or <0.05]. The expression of HSP70 is increased significantly with the increase of HBsAg titre, which together may work as indicators of chronic HBV infection.

1. Introduction

HSPs were first discovered accidentally in 1962 [1]. These proteins are an extremely preserved cluster of super molecule product generated as results of natural stressors, like fever and active commensal gut microflora, or non-natural stressors [2], such as hyperthermia, non-steroidal anti-inflammatory drugs (NSAIDS), aspirin, nutrient withdrawal, reactive oxygen species (ROS), proteasome inhibition, UV radiation and chemotherapy-induced deoxyribonucleic acid harm [1, 2].

In spite of the fact that heat-shock proteins (HSPs) are intracellular, they can be released from the cells and made distinguishable within the blood of healthy people as dissolvable HSPs [3, 4], so estimation of HSP concentration may provide clinically critical information in many infections. HSPs have multiple roles, including: membrane translocation, macromolecule degradation, folding, and repair of misfolded proteins, and regulation of macromolecule equilibrium in traditional and stressed cells [5 6].

One of the recent findings has discovered the effect of heat-shock proteins as mediators of the immune-mediated process via tumour peptide presentation [7].

Kampinga *et al*. 2009 proposed guidelines for the nomenclature of the human HSP families, they classified, them into HSPH (HSP110), HSPC (HSP90), HSPA (HSP70), DNAJ (HSP40), and HSPB (small HSP), and as similarly for the human chaperonin families HSPD/E (HSP60/HSP10) and CCT (TRiC). In their nomenclature guidelines, the HSP70 superfamily is comprised of HSPA(HSP70) family including 13 protein members (HSPA1A, HSPA1B, HSPA1L, HSPA2, HSPA5, HSPA6, HSPA7, HSPA8, HSPA9, HSPA12A, HSPA12B, HSPA13 and HSPA14) and HSPH (HSP110) family including 4 protein members (HSPH1, HSPH2, HSPH3 and HSPH4) [8].

In the human genome, many HSP70 genes have been reported to encode the HSP70 proteins family. These genes are identified as 47 HSP70 sequences, 17 genes and 30 pseudogenes, they are distributed into seven evolutionarily distinct groups with distinguishable subgroups according to phylogenetic and other data, such as exon-intron and protein features [9]. Additionally, the main families of HSP comprise tiny chaperones and ubiquitin, HSP60, HSP70, HSP90 and HSP100 [8, 10]. HSP70 is an extremely preserved ~70 Kilo Dalton [KD] enzyme that uses energy of ATP-hydrolysis to alter the structure and, consequently, it operates

* *Corresponding author*: Awaz A. Saadi, *awaz.arshad@uod.ac*
DOI: 10.3269/1970-5492.2020.15.20
 ISSN: 2279-7165 - Available on-line at www.embj.org

specific native proteins, and unfolds, solubilizes, and thereby cuts back the cellular concentration of harmful misfolded proteins [9, 11].

Furthermore, HSP70 is a stress-induced protein that modulates cell necrobiosis and proliferation and demonstrates anti-apoptosis response which ensures the survival of cells and promotes tumour cell proliferation [10, 12]. Researchers found that heat stress cognate seventy (HSc70) could be a host macromolecule related to hepatitis B virus (HBV) replication [11, 13]. It has been shown in a study [12, 14] that both the messenger RNA and protein range of HSP70 increase significantly more in advanced hepatocellular carcinoma (HCC) than in early HCC, and serum HSP70 is adjusted up in both cirrhotic liver and HCC patients. HSP70 is also a potent activator of the human complement system in an antibody-independent fashion [13-16].

HBV is associated with liver disease (cirrhosis) and is complicated with liver pathology (Fibrosis) that is typically progressive, irreversible, and the only choice for the treatment is liver transplantation in designated patients [15, 17]. In Iraq, it has been reported that the prevalence of HBV infection is estimated to be 1.6% as a country and 1.79% in Duhok as a province [16-19]. The relation between the expression of HSP70 in chronic hepatitis B patients and the disease progression is controversial. We propose that the HSP70 might be expressed differentially based on the status of the disease, so this study aimed to explore the HSP70 level of expression in chronic HBV patients, to investigate the correlations with other immunological and biochemical parameters in HBV infection, and to investigate whether HSP70 works as a prognostic or diagnostic parameter in the disease.

2. Methods

The present study included a total of 53 Chronic Hepatitis B patients (CHB), who were referred to the infectious diseases unit at Azadi Teaching Hospital in Duhok/Kurdistan region in Iraq from June–December 2015. A descriptive analysis was applied for this study. Informed consent was obtained from all patients and the study was approved by the ethics committee of the health directorate in Duhok city. The study protocol conforms to the ethical guidelines of the 1975 declaration of Helsinki [20]. A serum sample was taken from each patient. All practical work in this study was performed in Duhok Medical Research Centre (DMRC) based on the immunological and biochemical analysis laboratory techniques and protocols. Rapid test for each of HBsAg, anti HBsAb, HBeAg, antiHBeAb, and total HBcore Ab was measured by using ONE STEP Multi-HBV TEST DEVICE (PLASMATEC Laboratory Products). Quantitative HBsAg was measured by ELISA using HBsAg ELISA Test Kit, (PLASMATEC Laboratory Products), while quantitative HBe Ag and anti HBeAb were estimated by using DiaSorin LIAISON® HBeAg and LIAISON® Anti-HBe Kit using Chemiluminescent Immunoassay (CLIA) techniques. Both ALT and AST were estimated using Boeki Prestige 24i - Biolis 24i Kit, ALP levels were measured by spectrophotometer (JENWAY-6300) by using BIOLABCompany Colorimetric kit, and AFP level was measured using ELISA (bioactive products, Human Alpha-Fetoprotein (AFP) ELISA Kit, PRB-5058 96 assays). HSP-70/HSPA9 level (formerly named as GRP75; HSPA9B; MOT; PBP74; mot-2) [8], was estimated by using ELISA [antibodies company, Germany, (ABIN1115353 kit)], with a detection range from (0.781-50 ng/mL) according to the manufacturer guidelines. The DNA from each sample was extracted using QIAgene (DNA Extraction company, Germany), QIAamp (virus DNA Blood Mini250 Kit, CatNo 955 134) according to the manufacturer guidelines.

Then amplification for HBV DNA was determined by applying QIAGEN TQ for DNA Extracting and using artus® HBV RG PCR Kit 96V1, Roter Q Gene for Real Time PCR.

Statistical Package for Social Science (SPSS) version 26 computer software was used for data analysis. The means and standard deviations of variables were calculated (significant level was set at $p < 0.01$).

3. Results

The serum HSP 70 level mean was 41.325±29.7206. Its level was significantly increased with the titre of HBsAg in the serum (mean 12074.93±5681.619) in all of the 53 chronic HBV patients (P=0.006) involved in the study. The mean of HBV DNA viral load was 3.2E+10±2.4E+11, which was significantly associated with HBeAg level (mean 96454.29±300533.3; P = 0.002). Age category showed a mean of 29.62±11.573, and the age range was 12-67 years. The highest rate of HBV infection was found among the group 25-44 years old [28 (52. 8%)]. Males had a greater rate compared with females: males 28/53 52.8% and females 25/53 47.2%, respectively. The serological and the biochemical parameters of the patients are shown in Table 1 and Table 2. HSP 70 levels in the 53 patients are shown in Table 3 according to the manufacturer guidelines and the reference range of HSP70. The concentration was above the reference range in 19 (35.8%), while 34 (64.2%) of the patients had the normal concentration of HSP70 (Table 3). In HBV viral load, most patients showed a high replicative DNA 25 (47.2%) over the limit of >2000IUI/mL, while only 4 (7.5%) were replicative and under < 2000IU/mL, but above 500IU/mL. Non-replicative were 24 (45.3%) as shown in Table 4. The majority of the involved patients were HBsAg positive at detectable titres of 51 (96.2%), but only 2 (3.8%) were negative for HBsAg as shown in Table 5. HBeAg/anti HBeAb results [frequency and percentages] are shown in Table 6.

Characteristics	Mean	Patients correlations of parameters [n=53]
HSP 70ng/mL	41.325 ± 29.7206	P value=0.006
HBsAg IU/mL	12074.92528 ± 5681.618531	
HBeAg IU/mL	96454.29321 ± 300533.25	
Anti HBeAb IU/mL	70509.57025 ± 215480.75	
HBV DNAIU/mL	3.2E+10±2.4E+11	P value=0.002

Table 1. Correlation of HSP 70 results with serological parameters and HBV DNA

Characteristics	Mean	Correlation
HSP 70 ng/mL	41.325 ± 29.7206	*No significant correlation*
ALT IU/L	30.731 ± 46.6912	
AST IU/L	26.941 ± 18.7875	
ALP IU/L	70.2679 ± 42.34598	*No significant correlation*
AFP ng/L	21.5070 ± 57.46240	

Table 2. Correlation of HSP 70 with biochemical parameters.

HSP 70	Frequency [*n.*]	[%]
Highly increased [> 50 ng/mL]	19	35.8
Normal [0.781-50 ng/mL]	34	64.2
Total	53	100.0

Table 3. HSP 70 levels in the HBV infected patients enrolled.

HBV DNA	Frequency [*n.*]	[%]
Highly Replicative	25	47.2
Replicative	4	7.5
Non Replicative	24	45.3
Total	53	100.0

Table 4. HBV-DNA status among the patients enrolled.

HBs Ag	Frequency [*n.*]	[%]
Positive	51	96.2
Negative	2	3.8
Total	53	100.0

Table 5. HBsAg in HBV patients enrolled.

		Frequency [n]	[%]
HBe Ag	Positive	37	69.8
	Negative	16	30.2
	Total	53	100.0
Anti HBeAb	Positive	18	34.0
	Negative	35	66.0
	Total	53	100.0

Table 6. HBeAg and anti HBeAb status in HBV patients enrolled.

4. Discussion

In the current study, the higher rate of chronic hepatitis B infection appeared in the age group between 25-44 years, 28 (52.8%), which indicates that the transmission mostly occurred perinatally. This finding is also supported by another study [19] [21], which revealed that age at acquisition affects the likelihood of chronicity and the development of liver complications, as the disease is usually self-limiting when exposure to HBV occurs during adolescence or young adulthood. In regard to HBV DNA viral load, the current study revealed a highly significant correlation with quantitative HBeAg (P 0.002 P=or<0.05), the HBeAg status revealed a higher positive rate among HBeAg than positive anti-HBeAb rates. Chronic hepatitis B infection has two phases, early replicative phase with active liver disease, and non- or low replicative phase with normal liver disease [20] [22]. During the initial phase of infection with high levels of HBV DNA in the blood and positive HBeAg, those patients are prevalently identified as having prenatally acquired the infection, which has been also shown in our results in the present study [20]. These patients are most likely in a stage (Immune tolerance phase IT) with a positive HBeAg with their normal biochemical liver test, especially ALT levels, but high HBV DNA with minimal changes in liver biopsy [21] [23]. Patients with negative HBeAg usually undergo seroconversion and the anti HBeAb start to reveal those patients who have low or undetected HBV DNA in their serum [20] [22]. Accordingly, determining the HBV DNA marker with HBeAg and anti HBeAb are crucial steps to investigate the prognosis and development of the disease. HBeAg correlates with high infectivity and can be selectively used to help follow the evolution of chronic HBV. While the presence of anti HBeAb in chronic carriers usually appears with the HBeAg and antibody together [21] [23], this cannot be confirmed unless investigating the presence of HBV DNA. Depending on the viral load of the patients, which is directly associated to their infectivity, the tests can be used to analyse the prognosis and follow the antiviral therapy [21] [23]. To the best of our knowledge, this is the first study done to assess the significance of HSP70 expression in the HBV infection. In our study, HSP70 results showed a significant increase with HBsAg titre (P 0.006) [P=or<0.01]. This could be attributed to the immunological concept that HBsAg might be controlled by the innate immunity and complement system, since it has been found that the innate immunity is activated and stimulated in particular in the presence of HSP70. The results in the current study are inconsistent with those found in another study, which showed that HSP, especially type 70, stimulates the complement system and, as a result, triggers the innate immunity [13] [15]. Furthermore, a study performed [22] [24] revealed that the relationship between HSP70 over expression and chemo resistance in different tumor types is likely due to the ability of HSPs, especially type 70, to inhibit apoptosis.

As a result, the pharmacological manipulation of HSP levels could be used to distribute tumor cells susceptible to the induction of apoptosis by chemotherapeutics and/or UV irradiation or, alternatively, to directly and selectively interrupt their survival. In the case of CHB infection, the increase in HSP70 expression might lead to the progression towards hepatocellular carcinoma. In contrast, other studies [23] [25] displayed that HSP70 has a major role in anti-tumor immunity, and it is an accessory to tumor-associated antigens. As a result, it may stimulate specific tumor cell killing by cytotoxic T cells. Consequently, it is provoking to believe that highly soluble HSP70 levels in the serum may inhibit cellular anti-tumor immunity. Thus, high levels of HSP70 found in our study could be a prognostic factor for hepatocellular tumorigenesis and a risk marker for the hepatocellular carcinoma. These findings are supported by the data reported by Gehrmann *et al*. 2014, however, they studied the HSP70 levels in hepatitis C virus infection. They indicated that that serum HSP70 levels are consecutively increased in patients with chronic hepatitis, liver cirrhosis and liver carcinomas, thus they considered it to have a potential prognostic value [26]. Other biochemical parameters that are included in this study showed no significant correlations among HSP70 and no association among other immunological markers, indicating most of the patients had a normal range of the liver enzymes such as ALT, AST and ALP and other parameters like AFP.

5. Conclusion

It has been found that the soluble HSP70 could be reliable as a marker for the chronicity of the hepatitis B infection when linked with HBsAg level. It works as a risk marker for the development of hepatocellular carcinoma, however, further studies are recommended with a larger sample size to confirm the significance of HSP 70 in HBV infection.

6. Acknowledgements

We are grateful to all of the team of the Duhok Medical Research Centre (DMRC) at the College of Medicine, University of Duhok. Thanks to the technicians, scientists and doctors of the Central Lab in Azadi Teaching Hospital. We express our profound gratitude to Dr. Jasim M. Abdo for his kind assistance in Molecular Applications and Amplification in using RT PCR and for (Dr. Alaa Noori Sarkees) for his assistance in the statistical analysis.

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