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SERUM HSP 90 LEVELS OF CHRONIC HEPATITIS B PATIENTS ARE SUBSTANTIALLY CORRELATED WITH HBV DNA VIRAL LOAD

Awaz A. Saadi

The University Of Duhok, Kurdistan Region, Iraq

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ABSTRACT

The highly conserved molecules that make up the mammalian HSP90 family of proteins are engaged in a wide range of cellular functions. HSP90 and its co-chaperones regulate vital physiological processes as apoptosis, hormone signaling, and cell cycle regulation. The aim of this research are to examine the critical function that HSP90 plays in chronic HBV patients and to determine the association between this protein type and other immunological and biochemical markers as well as the HBV DNA factor. 88 chronic HBV patients were included in the trial, which ran from June to December 2015. The levels of ALT, AST, AFP, HBsAg, HBeAg, and anti-HBsAb were measured. The results showed a significant correlation between HSP90 [mean of 4.909±10.4839] with the titer of HBV DNA in the serum [190943784.21±180568331.64] in the chronic HBV patients at (P=0.002) involved in the study [P=or <0.01]. the relationship between HSP90 and other serological and biochemical parameters are shown no significant correlation.

1. Introduction

Heat Shock Proteins HSPs are a group of highly conserved molecules that are involved in a wide range of biological functions in the mammalian. They play crucial functions in maintaining cellular homeostasis, as evidenced by their distribution in different cellular compartments. It has been discovered that overexpressing HSPs helps cells survive and guards against protein deterioration or aggregation. In addition to heat shock, a number of factors, including as environmental and chemical factors, physiological causes unrelated to stress, and illness states, promote the expression of HSPs [1].

Furthermore, studies revealed that multiple HSP families and related antigens can induce more potent polyclonal immunity than a single HSP, as has been demonstrated in the realm of cancer and some of infectious diseases [2,3].

Members of the Heat Shock Protein 90 (HSP90) family are ATP-dependent molecular chaperones that contains a homodimer biologically functional unit divided of three distinct regions linked by flexible bind. Each promoter contains a highly-conserved N-terminal domain (NTD) responsible for nucleotide binding, a middle domain (MD) important for client recognition and ATP hydrolysis and a C-terminal domain (CTD), which is the primary site responsible for dimerization [4].

HSP90 chaperones control the stability and performance of client proteins implicated in cancer cell proliferation, survival, and cellular stress adaptation. Also, cell cycle regulation, hormone signaling, and apoptosis are important physiological processes that are controlled by HSP90 and its co-chaperones. In addition to being a crucial part of various signal transduction pathways, the HSP90 is now recognized as a crucial host factor for hepatitis B virus replication. Furthermore, HSPs as general and specially HSP90 and their cofactors have been shown to block both necrotic and apoptotic pathways through comparable methods. In order to cure neoplasms and other disorders. The experimental validation of these proteins may result in the expansion of existing protein interaction networks and the discovery of new processes. Diagnostic proteomic techniques may be used to therapeutically screen people who are at high risk of developing cancer as example of devastating disease and other serious chronic diseases because the creation of these big stable chaperone species appears to be cancer-specific, targeting such species is most likely to be advantageous [5].

The steroids nuclear receptors pathway is another route by which HSP90 may be implicated in cell survival. The interactions between HSP70, HSP40, and HSP90 stabilize the complex in the absence of stimulation to stop it from activating. These receptors' maturation, intracellular trafficking, and regulation are all affected by HSP90.

When a particular ligand connects with its receptor, an ATP-dependent conformational change happens, causing transcription factors to go to the nucleus and activate target genes [6].

* Corresponding author*:* Awaz A. Saadi, *awaz.arshad@uod.ac*
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The main aim of this study to investigate the crucial role of HSP type 90 in chronic HBV patients and to find out the relationship between this type of protein and other immunological and biochemical parameters and the correlation with the HBV DNA factor. The experimental validation of these proteins may result in the expansion of existing protein interaction networks and the discovery of new processes.

2. Material and methods

The present study included a total of 88 Chronic Hepatitis B patients (CHB), who were referred to the infectious diseases unit at Azadi Teaching Hospital in Duhok/Kurdistan region in Iraq in summer 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 [7]. A serum sample was taken from each patient. Most practical work in this study was performed in Duhok Medical Research Centre (DMRC) based on the immunological and biochemical analysis laboratory techniques and protocols, however, the rapid test for each of HBsAg, anti HBsAb, HBeAg, antiHBe Ag(HBeAb), were measured by using ONE STEP Multi-HBV TEST DEVICE (PLASMATEC Laboratory Products) was applied in virology department in central laboratory in Azadi teaching hospital. Alternatively, 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. Human Alpha-Fetoprotein (AFP) ELISA Kit, PRB-5058 96 assays). AFP level was measured using ELISA (bioactive products, HSP-90/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 ranges from (0.625ng/mL-40ng/mL) according to the manufacturer guidelines. The DNA from each sample was extracted using QIA gene (DNA Extraction company, Germany), QIA amp (virus DNA Blood Mini250 Kit, Cat No 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

In Table 1, it showed the age range was 14-67 years among the 90 chronic HBV samples and revealed a mean of 31.09± 11.505, The highest rate of HBV infection was found among the group 25-44 years old [51 (56. 7%)]. Males had a greater rate compared with females: males [(53/58.7% and females 37/41.1%)] respectively.

The serum HSP 90 level mean was 4.909±10.4839 its level was significantly increased with the titre of HBV DNA in the serum (190943784.21±180568331.64) in the chronic HBV patients at (P=0.002) involved in the study, correlations with other parameters all are shown in Table 3.

	Frequency	Valid (percent)
1-14	5	5.6
15-24	21	23.3
25-44	51	56.7
45-64	11	12.2
65 and more	2	2.2
	90	100

Table 1. Age groups and categories.

Characteristics	Mean
HSP 90 ng/mL	4.909±10.4839
HBs Ag IU/mL	11657.74±6221.92
HBe Ag IU/mL	68168.811±253649.49
Anti HBe Ab IU/mL	48912.90±184425.48
HBV DNA	1.9E+10±1.8E+12

Table 2. Mean of HSP90, serological and HBV-DNA parameters.

Parameters			Mean	Patients correlations of parameters[n=90]
HSP90ng/mL	Mean 4.909±10.4839	HBV DNA IU/mL	1.9E+10±1.8E+12	P value=0.002, = 0.330*** Significant Correlation
HSP90ng/mL		HBS Ag IU/mL	11657.74±6221.92	0.65, P value = 0.540, No Significance
HSP90ng/mL		HBeAg IU/mL	68168.811±253649.49	0.109, P value = 0.308, No Significance
HSP90ng/mL		HBeAb IU/mL	48912.90±184425.48	0.109, P value = 0.308, No Significance

Table 3. Correlation of HSP90 with HBV DNA, and the serological parameters

The mean of HBV DNA viral load was 3.2E+10±2.4E+11. Mean of HBsAg was (11657.7486), and mean each of HBeAg and HBeAb were (681688.11±253649.49) and (48912.90 ±184425.48) respectively. The biochemical parameters of the patients are shown in Table 4. HSP 90 levels in the 88 samples patients and controls all together are shown in Table 3 according to the manufacturer guidelines and the reference range of HSP 90 Normal (0-40 ng/mL) and abnormal (>40 ng/ml). The concentration of HSP90 was above the reference range in only 3 (3.3%), while 87 (96.7%) of the patients had the normal concentration of HSP90. In HBV viral load, most patients showed a high replicative DNA 47 (52.2%) over the limit of >2000IUI/mL, while 21 (23.3%) were replicative and under < 2000IU/mL, but above 500 IU/mL.

Non detective patients were only in 2 (2.2%) and in controls results there were 22.2% in the 20 participants as shown in (Table 4). The majority of the involved patients were HBsAg positive at detectable titres of 88 (97.8%) but only 2 (2.2%) were negative for HBsAg that have been neglected from the study. HBeAg/antiHBeAb results [frequency and percentages] are shown in (Table 7).

Characteristics	Mean	Correlation
HSP 90 ng/mL	4.909±10.4839	
ALT IU/L	33.1470 ± 45.03790	No significant correlation
AST IU/L	35.4232 ± 27.87736	No significant correlation
AFP ng/L	17.0318 ± 51.88208	No significant correlation

Table 4. Correlation of HSP90 with biochemical parameters

HBV DNA	Frequency	[%]
Highly Replicative	47	52.2
Replicative	21	23.3
Non detected	2	2.2
Control	20	22.3
Total	90	100

Table 5. HBV-DNA status

HSP 90 Median (IQR Range)	Frequency [*n.*]	Patients [N=68] [%]	Controls (N=20)
Normal Mean at (4.909±10.4839)	85	96.6	1.25 (1.61)
Highly increased [{>40ng/mL] [{0.625ng/mL-40ng/mL	3	3.4	
Total	90	100	

Table 6. HSP90 levels in the HBV patients and controls enrolled

		Frequency [n]	[%]
HBe Ag	Positive	9	10.0
	Negative	81	90
	Total	90	100
Anti HBe Ab	Positive	57	63.3
	Negative	33	36.7
	Total	90	100

Table 7. HBe Ag and HBe Ab status among patients enrolled

4. Discussion

The HSPs' anti-apoptotic characteristics are largely responsible for their cytoprotective effects. The carefully controlled programmed cell death machinery's many proteins can directly interact with HSP specially type 90, which can then stop the apoptotic process at certain sites [6]. Therefore, targeting HSP90 is a promising approach for the management for cancer diseases and other disorders.

This fact leads to study, investigate, and find out precisely of the HSP90 prospective roles that they may play within pathophysiology process.

From this study it is shown that the highest incident in the group between 25-44 age group in 56.7% of the samples have been used, which indicate that is mostly the transmission is perinatal 8. According to the relationship of HSP 90 and HBV DNA viral load, it has been found that a highly significant correlation between the two markers (HSP 90 and HBV DNA) P value =0.002 (P ⩽ 0.05_ and this finding has been supported with other study that showed the same correlation with different types of heat shock proteins such as HSP 70 [11]. Quantification of serum HBV DNA has become a pivotal marker to guide the management of the HBV carriers in order to identify different risks in terms of disease progression to select candidates for antiviral therapy and treatment5. The findings demonstrated that HSP70 expression was significantly higher in CHB than in healthy controls, and this was positively linked with HBV DNA copy counts 11. Also, HSP70 and HSP90 are both down-regulate by small interfering RNAs which can significantly inhibit HBV production as it has been found by Liu et al. [8, 12]

Furthermore, as it is known that HBV mRNA, which contains the HBV genome and serves as a template for HBV replication, and HBV total mRNA levels both rise in response to HSP90 overexpression, showing that HSP90 overexpression may encourage replication. This is demonstrating that HSP90 overexpression can facilitate replication. [13,14]

The anti HBeAg status revealed a higher positive rate (57 63.3%) among anti HBeAg (HBe Ab) than positive HBeAg rates (9 10%). Chronic hepatitis B infection has two phases, early replicative phase with active liver disease, and non- or low replicative phase with normal liver disease. However, as is shown that most HBV cases are within the stage of transition of Immune-control phase and inactive carrier state which is an outcome of the immune-active phase that usually marked by seroconversion from HBeAg to anti-HBe positivity and characterized by low (< 2000 IU/mL) or undetectable serum of the parameter HBV DNA, normal ALT levels, and disappearance of liver necro inflammation. [15]

In HBV DNA results it showed a highly replicative HBV DNA at 47 52.2% and that is a conformation characteristic that most patients in active stage of chronicity of the infection. When these markers are tested simultaneously, a testing profile is created that can be used to distinguish between acute and chronic infections stage the disease and determine who might benefit from treatment, track the disease's progression or response to antiviral therapy, and identify people who would be eligible for HBV vaccination or re-immunization [16,17]. In other immunological factors that have been used in the study showed no significant correlation between HSP 90 and each of HBs Ag, HBe Ag, and antiHBe Ag as shown in Table 5. In fact, the capacity of exogenous antigens to penetrate the endogenous loading route of MHC Class I molecules and hence prime CD8+ T lymphocytes is the most intriguing aspect of the uptake of HSP chaperoned peptides by APCs. [18]

HBV Vaccination actually represents the best strategy to prevent infection worldwide [19, 20].

5. Conclusions

All these findings determine the reason of developing novel therapeutic drugs in HBV to improve patient outcome by investigating the indicator and the crucial role of HSPs as general and type 90 specifically via studying and having more researches in this kind protein, and the relationship between the other type specially HSP70 and HSP90 as it has been showed the association between these types of HSP in different researches in cancer.

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References

1. Hoter A, El-Sabban ME, Naim HY. The HSP90 Family: Structure, Regulation, Function, and Implications in Health and Disease. Int J Mol Sci. 2018 Aug 29;19(9):2560. doi: 10.3390/ijms19092560. PMID: 30158430; PMCID: PMC6164434.
2. Cho Y., Dobos K. M., Prenni J., YANG H., Hess A. M. R., Rosenkrands I., Andersen P. C., Ryoo S., Bai G., Brennan M. J., Izzo A., Bielefeldt-Ohmann H., Belisle J. T. Deciphering the proteome of the in vivo diagnostic reagent “purified protein derivative” from Mycobacterium tuberculosis // Proteomics. 2012. Vol. 12. No. 7. pp. 979-991.
3. Yang H, Kruh-Garcia NA, Dobos KM. Purified protein derivatives of tuberculin--past, present, and future. FEMS Immunology and Medical Microbiology. 2012 Dec;66(3):273-280. DOI: 10.1111/j.1574-695x.2012.01002. x. PMID: 22762692; PMCID: PMC3491170.
4. Joly AL, Wettstein G, Mignot G, Ghiringhelli F, Garrido C. Dual role of heat shock proteins as regulators of apoptosis and innate immunity. J Innate Immun. 2010;2(3):238-47. doi: 10.1159/000296508. Epub 2010 Mar 16. PMID: 20375559
5. Pearl LH, Prodromou C. Structure and mechanism of the Hsp90 molecular chaperone machinery. Annu Rev Biochem. 2006; 75:271-94. doi: 10.1146/annurev.biochem.75.103004.142738. PMID: 16756493.
6. .Merza M.A, Hassan WM, Muhammad AS. Frequency of HBV and HCV among patients undergoing elective surgery in a tertiary care referral hospital in Duhok, Iraqi Kurdistan. JMSCR. 2014;2(7):1810–1815.
7. Serum HSP 70 Levels OF Chronic Hepatities B Patients are substation ally correlated with HBsAg Awaz A. Saadi, Ahmed M. Salih, Muayed A. Merza.
8. Rachel E. Lackie, Maciejewsk A, Valeriy G. Ostapchenko1, Marques-Lopes J, Yiu Choy W-, Martin L. D, Vania F. P, Marco A. M. Prado. Dual Role of Heat Shock Proteins as Regulators of Apoptosis and Innate Immunity Anne-Laure Joly Guillaume Wettstein Gregoire Mignot François Ghiringhelli Carmen Garrido INSERM U866, Faculty of Medicine and Pharmacy, University of Dijon, Dijon, France, by AL Joly · 2010.
9. DECLARATION OF HELSINKI, Recommendations guiding medical doctors in biomedical research involving human subjects. Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964 and as revised by the 29th World Medical Assembly, Tokyo, Japan, October 1975.
10. Human/Mouse/Rat GRP75/HSPA9B. Antibody, Antigen Affinity-purified Polyclonal Goat IgG. Catalog Number: AF3584.
11. Lackie RE, Maciejewski A,Ostapchenko VG, Marques-Lopes J,Choy W-Y, Duennwald ML, Prado VFand Prado MAM (2017). The Hsp70/Hsp90 Chaperone Machineryin Neurodegenerative Diseases.Front. Neurosci. 11:254 Doi:10.3389/finins.2017.00254
12. Anne-Laure Joly, Guillaume Wettstein, Gregoire Mignot, François Ghiringhelli, Carmen Garrido; Dual Role of Heat Shock Proteins as Regulators of Apoptosis and Innate Immunity. J Innate Immun 1 April 2010; 2 (3): 238–247. https://doi.org/10.1159/000296508
13. Wen-ying Dai, Guo-qing Yao, Xi-chuan Deng, Guang-chao Zang, Jia Liu, Guang-yuan Zhang, Yu-meng Chen, Ming-qi Lv, Ting-ting Chen, Heat shock protein: a double-edged sword linking innate immunity and hepatitis B virus infection, Journal of Virus Eradication, Volume 9, Issue 1,2023, 100322, ISSN 2055-6640.
14. Dutta Gupta S, Pan CH. Recent update on discovery and development of Hsp90 inhibitors as senolytic agents. Int J Biol Macromol. 2020 Oct 15; 161:1086-1098. doi: 10.1016/j.ijbiomac.2020.06.115. Epub 2020 Jun 16. PMID: 32561284.
15. Ali M, Idrees M, Ali L, Hussain A, Ur Rehman I, Saleem S, Afzal S, Butt S. Hepatitis B virus in Pakistan: a systematic review of prevalence, risk factors, awareness status and genotypes. Virol J. 2011 Mar 6; 8:102. doi: 10.1186/1743-422X-8-102. PMID: 21375760; PMCID: PMC3058090
16. Dai WY, Yao GQ, Deng XC, Zang GC, Liu J, Zhang GY, Chen YM, Lv MQ, Chen TT. Heat shock protein: a double-edged sword linking innate immunity and hepatitis B virus infection. J Virus Erad. 2023 Mar 14;9(1):100322. doi: 10.1016/j.jve.2023.100322. PMID: 37128472; PMCID: PMC10148040.
17. Guidelines on hepatitis B and C testing. Global HIV, Hepatitis and STIs Programmed (HHS), Guidelines Review Committee.170. 2017. SBN: 978-92-4-154998-1
18. Bonino F, Piratvisuth T, Brunetto MR, Liaw YF. Diagnostic markers of chronic hepatitis B infection and disease. Antivir Ther. 2010;15 Suppl 3:35-44. doi: 10.3851/IMP1622. PMID: 21041902
19. Guerra A, Costantino C, Martinon-Torres F, Westerholt S, Lambeth C, Chen Z, Lumley J, Marcek T, Johnson D, Wilck M. A phase 4, open-label study to evaluate the safety and immunogenicity of DTaP5-HBV-IPV-Hib in children previously vaccinated with DTaP2-HBV-IPV-Hib or DTaP5-HBV-IPV-Hib (V419-016). Hum Vaccin Immunother. 2024 Dec 31;20(1):2310900. doi: 10.1080/21645515.2024.2310900.
20. Verso MG, Costantino C, Vitale F, Amodio E. Immunization against Hepatitis B Surface Antigen (HBsAg) in a Cohort of Nursing Students Two Decades after Vaccination: Surprising Feedback. Vaccines (Basel). 2019 Dec 19;8(1). doi: 10.3390/vaccines8010001.