ПОВЫШЕНИЕ УРОВНЯ BSF-2 В СЫВОРОТКЕ КРОВИ БОЛЬНЫХ С ВИРУСОМ ГЕПАТИТА В

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Резюме. Справочная информация: Гепатит В - основная инфекция пораженной печени человека. Воспаление печени, вызванное вирусами гепатита, может привести к циррозу и гепатоцеллюлярной карциноме. Фактор стимуляции В-клеток 2 (BSF-2) является одним из цитокинов, влияющих на регуляцию и дифференциацию иммунного ответа человека. Цель: этот отчет направлен на оценку уровней BSF-2, GPT и GOT в сыворотке крови пациентов с различными стадиями гепатита В по сравнению со здоровым контролем. Методы. В этом исследовании участвовало 52 пациента, предположительно с острыми и хроническими заболеваниями, у которых обнаружен HBsAg. BSF-2 был обнаружен с помощью анализа ELISA. Биохимические показатели определяли с помощью комплектов автоматического анализатора. Для статистического анализа использовалось программное обеспечение SPSS версии 16. Результаты. У пациентов с острым гепатитом В уровень BSF-2 повышен больше, чем при хроническом гепатите В. Уровни GPT и GOT повышены в группе острого гепатита больше, чем в группе хронического гепатита. Мы сообщили о значительном значении между уровнями BSF-2, GOT и GPT. Мы не оценивали связь между возрастом пациента и группами случаев гепатита. Заключение: наши данные подтверждают повышение уровня BSF-2 при повышении уровня GOT больше, чем уровень GPT при остром гепатите В. Уровни BSF-2, GPT и GOT варьируются в зависимости от течения острого и хронического HBV. Мы предположили, что повышение уровня BSF-2 указывает на повреждение печени у пациентов с острым HBV.

Ключевые слова: ELISA, GPT, GOT, BSF-2, HBsAg

ELEVATION OF BSF-2 LEVEL IN SERUM OF PATIENTS WITH HEPATITIS B VIRUS

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Abstract. Background: Hepatitis B is the main infection of the injured liver for humans. Inflammation of the liver is caused by hepatitis viruses may lead to cirrhosis and hepatocellular carcinoma. The B-cell stimulatory factor 2 (BSF-2) is one of the cytokines that affect the regulation and differentiation of the human immune response. Objective: this report aims to estimate the BSF-2, GPT, and GOT levels in patients' serum with different stages of hepatitis B compared with healthy control. Methods: This study assessed 52 patients

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presumably with acute and chronic cases who have HBsAg positive. BSF-2 was detected using ELISA assay. Biochemical parameters were determined using kits of an automated analyzer. SPSS version-16 software was used for statistical analysis. Results: Acute hepatitis B patients had shown elevation in BSF-2 level more than of chronic hepatitis B. GPT and GOT levels elevated in the acute hepatitis group more than of the chronic hepatitis group. We reported a significant value between BSF-2, GOT, and GPT levels. We didn't score an association between patient's age and cases groups of hepatitis. Conclusion: our data confirmed increasing of BSF-2 levels with the increase of GOT level more than GPT level with acute hepatitis B. BSF-2, GPT and GOT levels are varied in different courses of acute and chronic HBV. We surmised that the elevation of BSF-2 levels designates liver injury of patients with acute HBV.

Keywords: ELISA, GPT, GOT, BSF-2, HBsAg

Introduction

Infection of hepatitis B (HBV) is more infective than the human immunodeficiency virus, a public health criminal (HIV). About four hundred million people are vulnerable to hepatitis disease annually [1]. The hepatocellular cells damaged by HBV are usually targeted by the immune system. The T- and B-cell immune response extracted. HBsAg is the primary and the first marker for the identification of other antibody HBV infections (anti-HBc, anti-HBe, and anti-HBs). These anticorps are aimed at their unique antigens [2]. The synthesis and secretion of immune cells, such as IL-6, IL-10, IL-13, IL-21, and Anti-inflammatory chemical mediators such as cytokines.

One of the multifunctional cytokines that occur in the differentiation, modulation, and maturation of the immune response is the human B-cell stimulatory factor 2 (BSF-2) [4, 5, 6]. The BSF-2 gene consists of four introns and five exons on the 7th chromosome. Serum BSF-2 levels are related to seriousness of illness. It may be useful for the medicinal success and predictor of most diseases [5, 7]. Including the calculation of serum levels of alanine and aspartate aminotransferases, biological tests for liver function enzymes are used respectively to estimate initial liver damage. Levels of AST and ALT are used mostly to help detect hepatic conditions [8].

The present trial will use BSF-2 to discriminate between specific acute and chronically recurring hepatitis B infections. In addition, in the last 3 decades in our region, the number and request for early detection and treatment of hepatitis infections has increased [9].

The aim of the present study was to demonstrate the BSF-2, AST and ALT serum levels and assess the correlation coefficient of these parameters with the case groups under study.

Materials and methods

Study design: the number of HBV infected patients gradually increased in the city of IN Mosul, so that our study focussed its attention on the case with specific, more sensitive kits, including selected interleukins.

In the municipality of Mosul (Iraq), we conducted our research among communities who were infected or suspected of having HBV. From October 2019 to January 2020, samples have been obtained. 43 samples were obtained at the Ibn-Alatheer Teaching Hospital, Mosul City, Iraq by the central blood bank and 30 samples at the hemodialysis centre. They were aged 9 and 66 years of age, aged 33.4 years. Our research aimed to quantify the magnitude of human cytokine BSF-2 in patients with acute and chronic HBV.

Inclusion and Exclusion Criteria: according to Table 1, for the high frequency of the tests, our analysis only selected acute and chronic HBV. This research includes: the vaccinated, non-infected, past-registered illnesses, treatment, and active carrier and incubation patients. All categories of cases identified in this study were omitted.

HBV assay: study samples were three: health control (HC), acute HBV (AHBV) and chronic HBV. Samples (CHBV). The positive HBsAg findings have been approved for all patients with HBV infection. Five minutes of blood centrifuged. Multiple labelled pure tubes at (-20 °C) store serum samples. One step multi HBV test device is a rapid, qualitative immune chromatography assay used to determine the HBV markers HBsAg, HBeAg, anti-HBs, anti-HBc, and anti-HBe in a covalent one-step test format in serum (Plasmatek, UK). This rapid test is used to detect all cases of hepatitis in 15 min (Figure 1). Because of the rapid qualitative immune chromatography test, the accuracy reaches 80%.

Enzyme-linked immunosorbent assay (ELISA) was used to confirm the positive results of HBsAg with the commercial kit from (DIALAB, Austria) purchased and tested according to the manufacturer's instruction. The specificity and sensitivity of the kit is 99%.

According to the manufacturer's guidance, the AST and ALT biochemical hepatitis testing was evaluated using an autonomous automated chemical analyser package (BIOLABO, France). The velocity system using the broad biochemistry automated analyzer calculated the AST and ALT serum quantities (Olympus

2700, Japan). Biochemical measurements have a precision and sensitivity of 99 percent.

ELISA with high sensitivity was used to determine the concentration of BSF-2 (pg/ml) level in serum using (Awareness-USA) reader. The test was done duplicate using a commercial kit from (Komabioteh) with OD450 nm. Based on the manufacturer's information, the sensitivity and specificity of the kit is 98.4%.

Ethics statement: collection of samples was carried out in compliance with standards guidelines accepted by the Medicine University Authority Committee, Mosul University. According to ethical authorities, approval forms is obtained from patients and health inspectors.

Statistical Analysis

SPSS Inc. was used to evaluate results, quantify mean values, SD defects and SE error for serology and biochemistry parameters. Software version 16.0 is available in Chicago, IL, USA. For homogeneity and similarity between parameters with a relevant P value of up to 0.05, one-way ANOVA test was used. The graphical description of values was used for Microsoft Office Excels version 2013.

Results

We evaluated 82 different cases of hepatitis include, inactive carrier (n = 8), acute (n = 24), chronic (n = 28), incubation (n = 4), recovery (n = 5), none infected (n = 6) and vaccinated (n = 7) (Table 1). We can guess that most cases of hepatitis were acute and chronic cases. According to the clinical manifestation of all cases, acute and chronic cases are dominant so that we have selected acute and chronic cases. We designated acute and chronic samples for confirmation using the ELISA assay.

The positive outcome for HBsAg with the ELISA test was obtained in samples with acute and chronic events. HBsAg has a negative effect on the balanced test community. According to the case forms, all parameters were revealed in Table 2 with mean, standard deviations and standard errors.

According to the age groups, mean, slandered deviations, and standard errors for all parameters were analyzed in the Table 3. Depending on the case groups, the current study showed a significant elevation of AST in AHBV and CHBV compared with the health control (HC). AHBV assortment is elevated more than CHBV although some samples showed normal range (Figure 2). On the other hand, according to age groups, AST levels showed no significant value with $P > 0.05$ (Figure 3).

Elevation of serum ALT level for most samples depending on AHBV and CHBV samples were more than that of the health control (HC) with no significant value $P < 0.05$ (Figure 4). Moreover, there is no significant value $P > 0.05$ according to the age groups (Figure 5). AHBV and CHBV cases demonstrated elevation of BSF-2 serum level though some control samples exhibited elevation of BSF-2 with high significant value $P < 0.05$ (Figure 6). BSF-2 serum levels were presented with no significant value at $P > 0.05$ according to the age groups (Figure 7).

The current study assumed most numbers of cases scattered in all ages with different ranges of AHBV cases recorded elevation in numbers with age between 25-44 years old. CHBV records different numbers with all age groups. According to Pearson 1-tailed correlation analysis, the present study was revealed no significant value between age groups and case groups with $P = 0.425$ (i.e.) here is no relative between age groups and case groups (Figure 8). The current study recorded a highly significant correlation coefficient at Pearson 1-tailed analysis at $P < 0.01$ (Figure 9). Moreover, our data doesn't detect association between age and any type of hepatitis B case.

TABLE 1. TYPES OF HEPATITIS B CASES ACCORDING TO THE POSITIVE AND NEGATIVE NBV IMMUNE PARAMETERS

Case group	%	Male	Female	HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc
Inactive Carrier	9.7	6	2	+	-	-	+	+
Acute	29.26	10	14	+	-	+	-	+
Chronic	34.14	15	13	+	-	-	-	+
Incubation	4.87	2	2	+	-	-	-	-
Past infection, recovery	6.19	4	1	-	-	-	-	+
				-	-	-	+	+
Non-infected	7.31	6	0	-	-	-	-	-
Vaccinated	8.53	3	4	-	+	-	-	-
Total	100%	46	36					

TABLE 2. NUMBER, MEAN, STANDARD DEVIATIONS, STANDARD ERRORSAND P VALUE FOR ALL PARAMETERS WITH CASE GROUPS. HC: HEALTH CONTROL, AHBV: ACUTE HBV, CHBV: CHRONIC HBV. *: SIGNIFICANT VALUE AT P < 0.01

		N	Mean	Std. Deviation	Std. Error	P value
BSF-2 pg/ml	**HC**	21	112.0924	152.67685	33.31682	.023
	AHBV	24	604.1833	122.93831	25.09468	.212
	CHBV	28	279.1536	166.14833	31.39908	.072
	Total	73	337.9540	248.00120	29.02634	
AST U/L	**HC**	21	26.7143	16.67976	3.63982	.153
	AHBV	24	63.7917	15.86897	3.23924	.283*
	CHBV	28	43.9286	13.28025	2.50973	.015
	Total	73	45.5068	20.95904	2.45307	
ALT U/L	**HC**	21	23.0952	14.89263	3.24984	.021*
	AHBV	24	50.8333	16.27125	3.32136	.212
	CHBV	28	34.7857	14.11424	2.66734	.071
	Total	73	36.6986	18.51702	2.16725	
Age	**HC**	21	1.95	.740	.161	.091
	AHBV	24	2.08	.717	.146	.023
	CHBV	28	2.00	.609	.115	.849
	Total	73	2.01	.677	.079	

TABLE 3. NUMBER, MEAN, STANDARD DEVIATIONS, STANDARD ERRORS AND P VALUE FOR ALL PARAMETERS WITH AGE GROUPS

		N	Mean	Std. Deviation	Std. Error	P value
BSF-2 pg/ml	**9-24y**	16	308.2437	238.52489	59.63122	.030
	25-44y	40	352.7925	252.53443	39.92920	.804
	45-66y	17	331.0024	257.87534	62.54396	.102
	Total	73	337.9540	248.00120	29.02634	
AST U/L	**9-24y**	16	42.6250	16.41493	4.10373	.067
	25-44y	40	46.1000	21.39111	3.38223	.573
	45-66y	17	46.8235	24.44953	5.92988	.221
	Total	73	45.5068	20.95904	2.45307	
ALT U/L	**9-24y**	16	39.8125	17.08496	4.27124	-.142
	25-44y	40	37.4000	19.14292	3.02676	.232
	45-66y	17	32.1176	18.51649	4.49091	.065
	Total	73	36.6986	18.51702	2.16725	

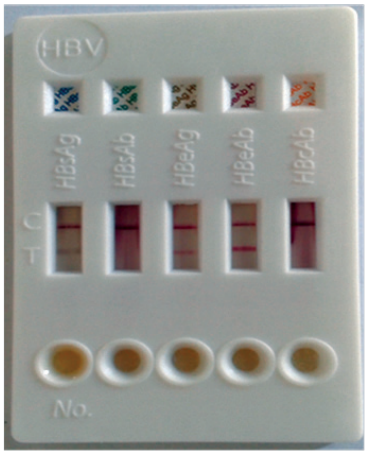

Figure 1. HBV Markers of positive infected patient with acute phase

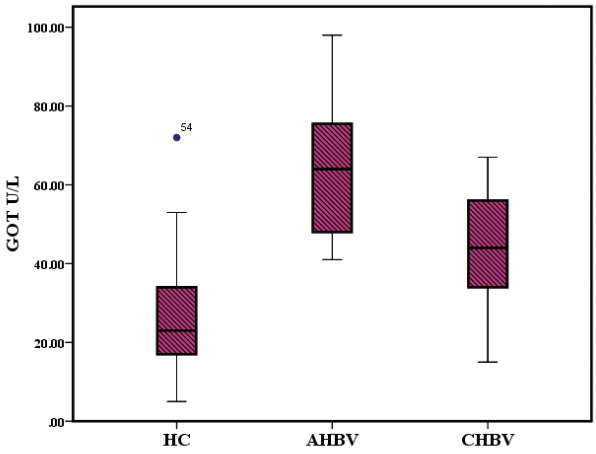

Figure 2. GOT levels with the cases groups

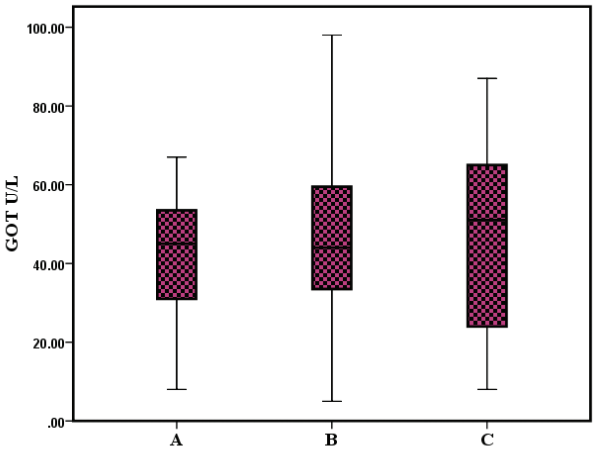

Figure 3. GOT levels with the age groups: A (9-24y), B (25-44), and C (45-66)

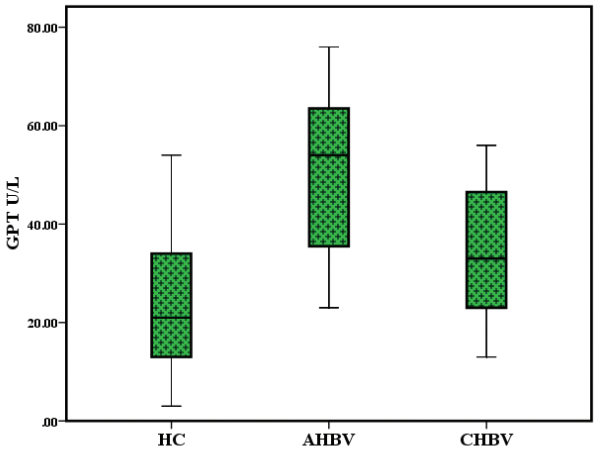

Figure 4. GPT levels with the cases groups

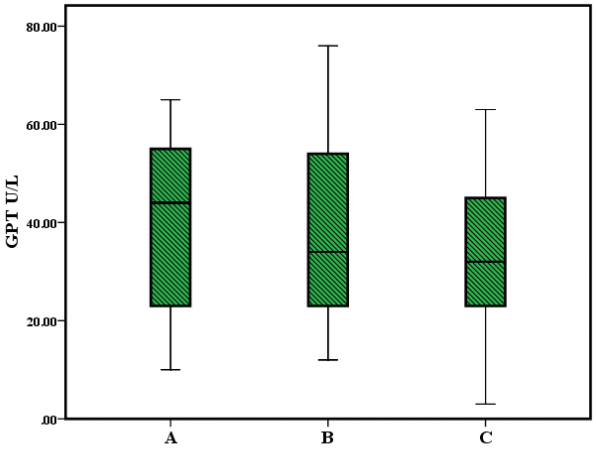

Figure 5. GPT levels with the age groups: A (9-24y), B (25-44), and C (45-66)

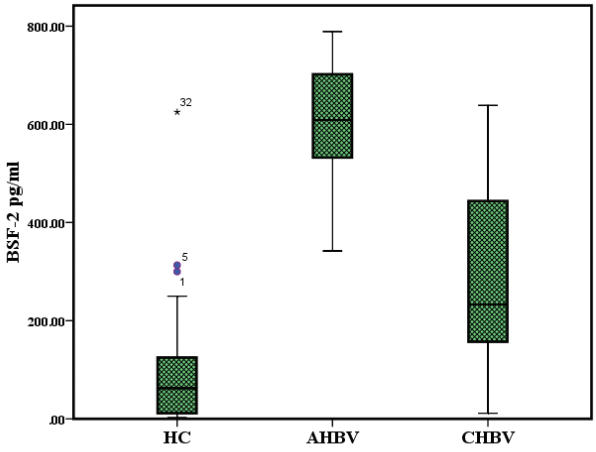

Figure 6. BSF-2 levels with the cases groups

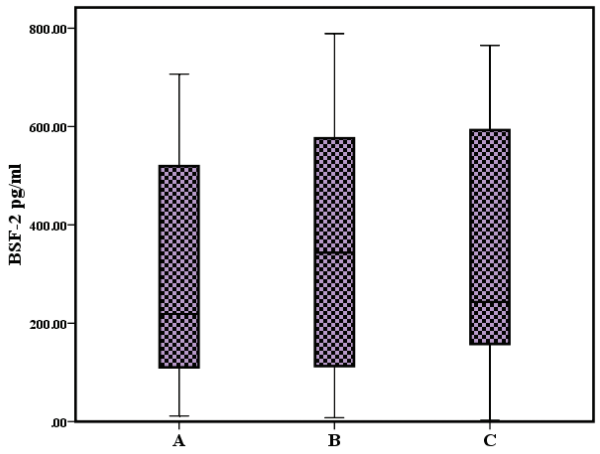

Figure 7. BSF-2 levels with the age groups: A (9-24y), B (25-44), and C (45-66)

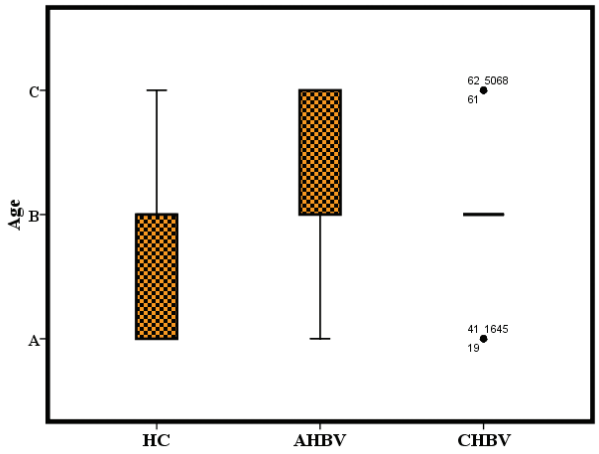

Figure 8. Correlation coefficient between age and cases groups

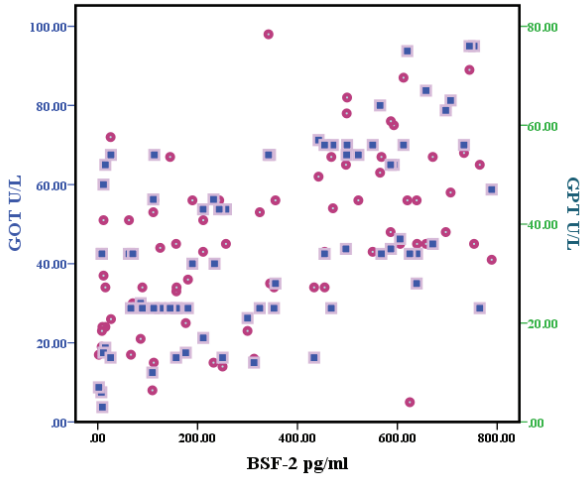

Figure 9. Correlation coefficient between GOT, GPT and BSF-2 at p < 0.01

Discussion

The major injuries of the human liver are caused by HBV and hepatitis C virus (HCV) worldwide annually [10]. BSF-2 may be a marker for patients with hepatitis virus and other viral infections. It has been verified that BSF-2 blocks the replication of the viral DNA through the reduction of viral transcripts include TGF-β1, IL-1, and IL-4. It is important to take in mind that the neutralization of BSF-2 may contemporary risks for patients with viral infections [11, 12, 13, 14]. Most studies revealed that cytokines such as (IL-1, IL-4, IL-6, IL-10, IL-22, and IL-32) grasped the highest expression in the acute HBV group [15, 16]. Over activation of the BSF-2 gene effect trigger of initial immune cell that leads to oncogenic transformation. It was stated that this activation may lead to chronic disease progression [17, 18, 19]. Several studies confirmed that elevated IL-10 levels correlate with the increased level of the viral DNA [20, 21]. Other studies exposed that IL-33 and ST2 serum levels elevated with the increase of ALT levels in patients with chronic hepatitis B [22]. This verdict is consistent with our findings for liver biochemical parameters.

The current study presented elevation of biochemical parameters (AST and ALT) in the AHBV group more than the CHBV group which was closed with the health control (HC) group. The coefficient significant value with the AST level was P = 0.08 in Pearson analysis with case groups that more significant than in ALT level P = 0036. By the way, we found that the parameters assumed no significant correlation with age groups P = 0.286 and P = 0.116 respectively. On the contrary, we found a high correlation coefficient between AST level and ALT level P = 0.004 as well as with BSF-2 P = 0.006. Our findings do not agree with a previous study that enrolled the low concentration of serum BSF-2, lack of association with histopathological and biochemical strictures of the chronic hepatitis patients [23, 24].

Our findings enrolled elevation of BSF-2 serum level with ACHBV more than the CHBV group compared with the health control group in a high concentration absorption. Statically, a positive correlation between case groups and BSF-2 level was shown P = 0.036. This result is settled with a previous study established that the patients in different stages of chronic HBV infections exhibited various levels of BSF-2 in serum [25].

Our data analysis provided evidence that the correlation coefficient between BSF-2 serum level and biochemical parameters (AST and ALT) is disclosed as an increase of acute hepatitis patients compared with chronic cases.

By contrast, our study publicized a negative association between types of hepatitis infection and the age with P = 0.425. A negative correlation was found between BSF-2 level and age groups P = 0.402.

Conclusion

Our data confirmed that BSF-2 serum levels rise with the elevation of GOT and GPT levels in patients with acute hepatitis B more than with chronic cases. BSF-2, GOT and GPT serum levels are varied in different courses of acute and chronic hepatitis B infections. We surmised that BSF-2 serum level may indicate liver injury of hepatitis B patients' specifically acute cases. In addition to that, negative associations emerged between chemical parameters (GOT and GOT), BSF-2, and hepatitis cases according to age serial groups.

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