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Assessment of the alteration in the level of total protein, albumin and globulin in serum and saliva of Hepatitis B virus Iraqi patients

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Abstract

Chronic hepatitis B virus infection is a major global health concern, accounting for substantial liver-related illness and death worldwide. The complex protein profile present in both blood serum and saliva is composed of numerous individual proteins, which collectively contribute to the overall protein composition of these bodily fluids. Valuable diagnostic insights can be gleaned from assessing alterations in total serum protein concentrations or variations within distinct protein fractions. Current study aimed to investigate the influence of hepatitis B disease on the matter of total protein concentration, as well as the levels of albumin and globulins, exploring the feasibility of utilizing saliva as a diagnostic fluid to monitor fluctuations in these parameters is of paramount importance. This line of research shows potential for replacing serum with saliva for such diagnostic purposes. This study about the individuals infected with chronic hepatitis B virus was conducted where a total of 50 patients with hepatitis B and 50 with the control group. Concentrations of serum and saliva total proteins and albumin were measured, meantime the concentration of globulin and the ratio of Albumin/Globulin were calculated in both saliva and serum samples of the patients and the healthy groups. Agarose gel electrophoresis was employed to detect the changes in the protein profile in these samples. The findings from total protein measurements indicated a slight non-significant decrease in serum samples, whereas a highly significant decrease ($P=0.002$) was observed in the saliva samples of patients with hepatitis B in contrast to the healthy control group. Furthermore, a significant decrease ($P<0.001$) in albumin concentration was noted in the serum samples of these patients, while no significant variation was observed in the saliva samples compared to the respective control groups. However, no significant differences were observed in the concentration of globulins in serum samples, nor the [albumin] /[globulin] ratio. In patients with hepatitis B, the total protein and albumin concentration were found to decrease in both serum and saliva samples. Age and gender were found not significantly affect total protein and albumin levels in the patient group. Albumin concentration decreased in patients compared to the control group when their total protein concentration ranged from 6.1 to 8.4 g/dl. Based on the results of this study, it can be concluded that chronic hepatitis B influences the composition and concentration of serum and salivary proteins

Keywords: Agarose gel electrophoresis, Albumin, Globulin, Hepatitis B virus, Total protein.

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تقييم التغير في مستوى البروتين الكلي والألبومين والجلوبيولين في مصل ولعاب المرضى العراقيين بفيروس التهاب الكبد من نوع ب.

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الخلاصة

تعد العدوى المزمنة بفيروس التهاب الكبد ب مصدر قلق صحي عالمي كبير، حيث تسبب أمراضًا ووفيات كبيرة مرتبطة بالكبد في جميع أنحاء العالم. يشتمل محتوى البروتين الكلي الموجود في كل من مصل الدم واللعاب من العديد من البروتينات الفردية، والتي تساهم بشكل جماعي في تكوين البروتين العام لهذه سوائل الجسم. يمكن استخلاص رؤى تشخيصية قيمة من تقييم التعديلات في إجمالي تركيزات بروتين المصل أو الاختلافات داخل اجزاء البروتين المتميزة. تهدف الدراسة الحالية إلى دراسة تأثير مرض التهاب الكبد ب على مسألة تركيز البروتين الكلي، وكذلك مستويات الألبومين والجلوبيولين، واستكشاف جدوى استخدام اللعاب كسائل تشخيصي لرصد التقلبات في هذه المعلمات له أهمية قصوى. يُظهر هذا الخط من الأبحاث إمكانية استبدال المصل باللعاب لمثل هذه الأغراض التشخيصية. أجريت هذه الدراسة على الأفراد المصابين بفيروس التهاب الكبد الوبائي المزمن حيث بلغ عدد المرضى 50 مريضاً بالتهاب الكبد الوبائي ب و 50 مع المجموعة الضابطة. تم قياس تراكيز البروتينات الكلية والألبومين في مصل الدم واللعاب، وفي الوقت نفسه تم حساب تركيز الجلوبيولين ونسبة الألبومين إلى الجلوبيولين في عينات اللعاب والمصل للمرضى والمجموعات الضابطة. تم استخدام الترحيل الكهربي لهلام الاغاروز للكشف عن التغيرات في صورة البروتين في هذه العينات. اشارت نتائج قياسات البروتين الكلي إلى انخفاض طفيف غير معنوي في عينات المصل، في حين لوحظ انخفاض كبير للغاية ($P=0.002$) في عينات اللعاب لمرضى التهاب الكبد ب على عكس المجموعة الضابطة وعلاوة على ذلك، لوحظ انخفاض معنوي ($P<0.001$) في تركيز الألبومين في عينات مصل هؤلاء المرضى، في حين لم يلاحظ أي اختلاف كبير في عينات اللعاب مقارنة بالمجموعات الضابطة. ومع ذلك، لم تلاحظ فروق ذات دلالة إحصائية في تركيز الجلوبيولين في عينات المصل، ولا في نسبة [الألبومين] / [الجلوبيولين]. في المرضى الذين يعانون من التهاب الكبد ب، وجد أن تركيز البروتين الكلي والألبومين ينخفض في عينات المصل واللعاب. تم العثور على أن العمر والجنس لا يؤثران بشكل كبير على مستويات البروتين الكلي والألبومين في مجموعة المرضى. انخفض تركيز الألبومين لدى المرضى مقارنة بالمجموعة الضابطة عندما تراوح تركيز البروتين الكلي لديهم من 6.1 إلى 8.4 جم/ديسيلتر. وبناء على نتائج هذه الدراسة يمكن استنتاج أن التهاب الكبد المزمن ب يؤثر على تكوين وتركيز بروتينات المصل واللعاب.

1.Introduction

Chronic hepatitis B virus (HBV) infection poses a formidable global health challenge. It significantly elevates the likelihood of developing liver cirrhosis and hepatocellular carcinoma (HCC). Remarkably, even in the absence of cirrhosis, individuals afflicted with chronic hepatitis B (CHB) are at heightened risk of progressing to HCC [1]. Valuable diagnostic insights can be obtained by evaluating alterations in the total protein [TP], which, its content in serum is made up of a large number of individual proteins [2]. Since serum total proteins are represented by albumin [A] and globulin [G], it is more significant to determine which specific protein fraction has undergone alteration [3]. Albumin is one of the oldest recognized proteins within the plasma, where human albumin represents 60% of the normal total protein. The normal total protein concentration typically ranges from 6.0 to 8.0 g/dl. In plasma, albumin maintains a normal concentration between 3.5 to 5.0 g/dl, making it the most

abundant protein therein. Remarkably, albumin exhibits a wide range of physiological functions that are crucial for bodily homeostasis [4].

The globulin fraction contains hundreds of serum proteins, including carrier proteins, enzymes, complements, and immunoglobulins. Many of them are synthesized in the liver while the immunoglobulins are synthesized by plasma cells [5]. Due to their size, shape and charges therefore they can be separated by the electrophoresis technique [6]. This technique has been widely used in clinical medicine for aiding in diagnosis of various clinical conditions like acute and chronic inflammations, monoclonal gammopathies, nephropathy and liver diseases [6, 7].

Saliva is indeed a colourless fluid that is produced by the salivary glands in the human mouth. It serves various functions including aiding in digestion, lubricating the mouth, protecting against tooth decay, and facilitating speech. Saliva is composed primarily of water, with around 99% of its volume. The remaining 1% comprises a mixture of organic and inorganic molecules [8]. Saliva exhibits promising potential as a substitute for serum in community-based seroprevalence studies. Its components are from oral derived proteins, in addition it reflects the plasma components, thus it is considered as a mirror of the body [9] and thus it may be used for monitoring the systemic and oral health. Whole saliva is most frequently studied because its collection is easy, non-invasive and rapid to obtain without the need for specialized equipment. The identification of salivary biomarkers, combined with recent advancements in diagnostic technologies, has substantially improved the diagnostic potential of saliva for various clinical applications, making it a valuable tool in the field of medicine [10]. Human saliva proteomics, in particular, has emerged as a pioneering approach in the quest for identifying protein biomarkers used to detect different diseases [11]. The primary objective of this study is to investigate alterations in total protein ([TP]), albumin ([A]), globulin ([G]) levels, and the [A]/[G] ratio in both serum and saliva samples obtained from patients with hepatitis. Furthermore, the study aims to explore the feasibility of utilizing saliva as an alternative specimen for monitoring these changes in the a forementioned patient cohort. Agarose electrophoresis was employed as the method of choice to discern variations in the different protein fractions.

2. Material and Methods

Blood and saliva samples were collected from the patients which amounted to 50 samples of patient with hepatitis B while attending the Gastroenterology Hospital in the Medical City, Baghdad/ Iraq. Infected individuals were diagnosed by rapid test (a new rapid immunochromatographic test for the qualitative detection of HBsAg that usually is performed manually). To detect viral RNA in the blood, PCR device was used. The patients group comprised both males and females, with ages ranging from 18 years to 77 years with a mean value of 44 years. Age and gender matched healthy individual (n= 50) were included to be used as control group. Blood samples of patients and healthy individual were subjected to a centrifuge to obtain serum and saliva to conduct laboratory tests to measure the concentration levels of total protein and albumin, as well as globulin and the [Albumin/Globulin] ratio were calculated. Determination of Total protein concentration in serum samples using. Meanwhile, the modified Lowry method, as developed by Hartree, was employed to ascertain the protein content in saliva samples, with some modification and as mentioned in [12]. Bovin Serum albumin (BSA) served as the standard reference for protein concentration determination. The protein concentrations of both serum and saliva were expressed in grams per deciliter (g/dl). The albumin concentration was determined using biosystem kit. Calculation of globulin concentration: The concentration of globulin in the serum and saliva samples of the healthy

and hepatitis B patients was calculated using the following equation [TP] g/dl-[A]= [G] g/dl as well as the ratio of [A]/[G] was calculated in serum and saliva of all individuals including in the current study [13]. A volume of 20 µl serum, which was diluted five times, was used for Agarose gel electrophoresis, and the samples were applied to a commercial protein electrophoresis system (Hellabio (MPE) kit). After conducting the agarose gel electrophoresis, the gel was stained for protein detection with acetic acid free concentrated amido black solution and as described in the Hellabio (MPE) kit (Cat No.: J66501.18 Greece).

3. Statistical Analysis

The GraphPad Prism 9.5.1 (733) program (t-test, One-Way ANOVA, and Pearson correlation) were used to analyze the obtained results and to perform the correlation relationships, respectively. Through-out this work, the obtained results were reported as a mean value ± standard deviation. The differences were considered highly significant if (P< (0.001) ***), and significant where (P= 0.002^{**}) and (P<0.05) *) (Prism 9.5.1 (733) program).

4. Results

The patient's group in this study consisted of 50 patients with chronic hepatitis B, with a mean age of 43.170 ± 15.090 years and ages ranging from 18 to 77 years. The control group included 50 healthy individuals with a mean age of 41.800 ± 14.400 years and ranged age from 18 to 77 years. The main characteristics of the study groups are presented in Table 1. The diagnosis of hepatitis B infection disease was confirmed by measurement the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as polymerase chain reaction which were carried out by the laboratory staff of the Gastroenterology Hospital in the Medical City, Baghdad/ Iraq hospital.

Table 1: **The main characteristic of the participants and the used diagnostic parameters.**

		Control group	Patients group	P value
Number		50	50	
Age	Total range (18-77) year	41.800 ± 14.400	44.000 ± 15.307	<0.999
	range (18-50) years	33.733 ± 6.313 (n=30)	34.867 ± 8.476 (n=30)	0.559
	range (51-77) years	63.615 ± 7.206 (n=9)	65.111 ± 6.900 (n=9)	0.632
Male	Percentage (number)	34% (n=17)	79.5% (n=31)	
	Age range	44.180 ± 9.665	48.118 ± 14.115	0.351
Female	Percentage (number)	66% (n=33)	20.5% (n=8)	
	Age range	49.125 ± 19.838	43.000 ± 18.134	0.530
alanine aminotransferase activity (U/L)		19.940 ± 7.230	46.350 ± 15.850	<0.001
aspartate aminotransferase activity (U/L)		23.130 ± 5.127	36.550 ± 11.320	0.008
alkaline phosphatase activity (U/L)		195.820 ± 19.800	129.228 ±10.590	<0.001
Polymerase Chain Reaction Test (PCR)		-	+	

The age difference between the patient and control groups was not statistically significant, according to the data (P < 0.999). In comparison to the control group, the patients' ALT, ALP, and AST level activities were statistically significant (P< 0.001), (P<0.001) and (P = 0.008). These changes in enzyme activity were indicators of a Hepatitis B infection, and this was founded on [14]. Throughout the study, the concentrations of total protein [TP] and

albumin [A] in both serum and saliva were measured according to the methodologies detailed in the Materials and Methods section. Additionally, the levels of globulin [G] were assessed, and the [A] / [G] ratio was calculated for both the healthy control and the patients. The number of patients used here to compare with the control were 39 patients, since this number among the total patients (n=50) enrolled in the present study, did not receive any type of treatment. The obtained results are presented in Table 2

Table 2: Comparison of TP, A, and G concentrations levels and [A]/[G] between the healthy control group and hepatitis B patients.

		Groups	N	Mean ± SD	P value
Serum	[TP] (gm/dl)	control	50	6.728± 0.910	0.259
		patient	39	6.480±1.700	
	[A] (gm/dl)	control	50	3.819± 0.854	< 0.001***
		patient	39	2.985± 0.891	
	[G] (gm/dl)	control	50	2.908± 0.952	0.254
		patient	39	3.480±0.920	
	A/G ratio (g/dl)	Control	50	1.570± 0.813	0.882
		patient	39	1.345± 0.117	
saliva	[TP] (gm/dl)	Control	23	3.050± 0.691	0.002**
		Patient	23	2.885±0.559	
	[A] (gm/dl)	Control	23	2.055±0.519	0.436
		Patient	23	1.901±0.727	
	[G] (gm/dl)	Control	23	1.067±0.314	0.017*
		Patient	23	0.984±0.112	
	A/G ratio (g/dl)	Control	23	1.435±0.496	0.267
		patient	23	1.466± 0.415	

Values are expressed as mean value ±S.D.
***** Refers to a highly significant difference at P value<0.001**
**** Refers to significant difference at P value= 0.002**
***Refers to significant difference at P value<0.05**

It can be observed from these results that there were no significant differences in serum concentration of TP, G and [A] / [G] ratio between the control and the patient groups, as well as in saliva albumin and [A] / [G] ratio. While the level of [A] in the serum samples was a highly statistically significant reduced (P<0.001) in the patients as compared with the control. In saliva the level of both TP and G decreased significantly (P=0.002, p=0.017 respectively. These findings are consistent with those of [15] who studied Nigerian patients with hepatitis c and [16] in their study on Iraqi patients with alcoholic and non-alcoholic liver disease. In this study, the healthy people and the patients were divided into two groups based on their ages. The first group consisted of 30 people aged between 18 and 50 years, and the second group consisted of 9 people aged between 51 and 77 years .and the results were as shown in

Table *3*

Table 3: Comparison of TP, A, and G concentrations levels between the healthy control and hepatitis B groups according to their age.

		groups	NO.	(18-50) year groups	P value	(51-77) year groups	NO.	P value
serum	[TP](g/dl)	Control	30	6.920±0.915	0.194	6.314±0.742	9	0.625
		Patient	30	6.418±1.591		6.687±2.119	9	
	[A](g/dl)	Control	30	3.562±0.792	$< 0.001^{***}$	3.899±0.901	9	0.657
		Patient	30	2.786±0.572		3.649±0.415	9	
	[G] (g/dl)	Control	30	2.860±0.971	0.325	2.415±0.651	9	0.408
		patient	30	3.612±0.879		3.038±0.100	9	
	A/G ratio (g/dl)	Control	30	1.346±0.463	0.043^{*}	1.810±0.873	9	0.015^{*}
		patient	30	1.058±0.709		0.773±0.268	9	
saliva	[TP](g/dl)	Control	15	3.600±0.776	0.019^{*}	2.813±0.882	8	0.137
		Patient	15	2.995±0.526		2.679±0.594	8	
	[A](g/dl)	Control	15	2.107±0.679	0.688	1.936±0.466	8	0.413
		Patient	15	2.002±0.742		1.711± 0.706	8	
	[G](g/dl)	Control	15	1.455±0.115	0.058	0.969±0.187	8	0.172
		patient	15	0.993±0.426		0.968±0.170	8	
	A/G ratio (g/dl)	Control	15	1.645±0.262	0.866	1.498±0.695	8	0.734
		patient	15	2.001±0.154		1.326±0.515	8	

Values are expressed as mean value ±S.D.
***** Refers to highly significant difference at P value <0.001**
*** Refers to significant difference at P value <0.05**
**** Refers to significant difference at P value =0.002**

In the first patient group aged 18-50 years, the level of serum albumin concentration was statistically significantly decreased (P<0.001) as compared with the control. While in saliva, only the concentration of total protein was significantly decreased (P=0.019). Meantime there were no observed significant variations in all other measurement parameters in the second age group.

The changes in the measured biochemical parameters according to the gender of the patient individuals were presented in Table 4.

Table 4: Comparison of TP, A and G concentrations and A/G ratio levels in male and female between patient with hepatitis B and control group.

			Groups	NO	Mean ± S.D.	P value
Male	Serum	[TP](g/dl)	Control	17	6.990±0.720	0.867
			Patient	17	6.914±1.793	
		[A] (g/dl)	Control	17	3.979±0.958	0.026*
			patient	17	3.214±0.958	
		[G] (g/dl)	control	17	3.070±0.890	0.254
			Patient	17	3.665±0.990	
		A/G ratio (g/dl)	Control	17	1.411 ±0.867	0.815
			patient	17	1.458±0.877	
	saliva	[TP] (g/dl)	Control	14	3.493±0.846	0.055
			Patient	14	2.960±0.519	
		[A] (g/dl)	Control	14	1.957±0.698	0.757
			patient	14	2.040±0.707	
		[G] (g/dl)	control	14	1.536±0.666	0.009**
			Patient	14	0.919±0.101	
		A/G ratio (g/dl)	Control	14	1.445±0.595	0.400
			patient	14	1.960±0.175	
female	Serum	[TP] (g/dl)	Control	8	6.899±0.715	0.921
			Patient	8	6.818±0.175	
		[A] (g/dl)	Control	8	4.449±0.713	< 0.001***
			Patient	8	2.368±0.830	
		[G] (g/dl)	Control	8	2.450±0.785	0.038*
			patient	8	4.450±0.951	
		A/G ratio (g/dl)	Control	8	2.050±0.872	0.124
			patient	8	1.090±0.315	
	saliva	[TP] (g/dl)	Control	8	3.455±0.857	0.032*
			Patient	8	2.790±0.669	
		[A] (g/dl)	Control	8	2.122±0.598	0.385
			Patient	8	1.763±0.758	
		[G] (g/dl)	Control	8	1.255±0.301	0.274
			patient	8	1.028±0.351	
		A/G ratio (g/dl)	Control	8	1.603±0.579	0.713
			patient	8	1.402±0.276	

Values are expressed as mean value ±S.D.
***** Refers to highly significant difference at P value <0.001**
*** Refers to significant difference at P value <0.05**
**** Refers to significant difference at P value =0.002**

In both male and female serum, albumin levels were lower in the patients as compared with the control group and the difference between them was statistically significant (p=0.026) and (p<0.001), respectively in the female saliva, the levels of total protein decreased in the patients and the difference was statistically significant (p=0.032). These findings are consistent with results from other studies, such as [17] of their study on vitiligo in Iraqi patient and the result of [9] in their study on oral tumors in Iraqi patients.

To look out if the variation in the concentration of TP was affected by the variation in [A]. The groups (healthy and patient with hepatitis B) were divided based on the total protein concentration into three groups. In the first group, the total protein concentration was less than 6 g/ dl with a number of 12 for healthy and patients with hepatitis B, in the second group the protein concentration ranged from 6.1-8.4 g/dl with a number of 12, and for the third group, it ranged from 8.5-11 g/dl with number of 9 as shown in

Table 5.

Table 5: Comparison of TP, A, and G concentrations and A/G ratio levels in patient without treatment compared with control in three groups according to their protein concentration.

	[TP] (g/dl) < 6			[TP] (g/dl) 6.1-8.4			[TP] >8.4
	control	patient	p value	control	patient	p value	Patient
age	54.000±17.770	45.611±8.131	0.239	40.583±11.920	42.5±15.462	0.744	43.3±16.866
no.	12	18		12	12		9
[A] (g/dl)	3.408±0.634	3.058±0.918	0.443	4.1±0.694	2.664±0.698	$< 0.001^{***}$	2.572±0.811
[G] (g/dl)	2.217±0.653	1.924±0.689	0.335	2.837±0.844	4.219±0.782	$< 0.001^{***}$	6.817±1.390
A/G ratio (g/dl)	1.787±0.982	1.650±0.992	0.470	0.657±0.953	0.672±0.259	0.002^{**}	0.418±0.015

Values are expressed as mean value ±S.D.
***** refers to highly significant difference at P value <0.001**
**** Refers to significant difference at P value =0.002**

When protein concentration was <6 g/dl, globulin levels in patients was lower than control group and the reduction was statistically significant (P=0.038). In the second group, where protein concentration was ranged from (6.1 to 8.4) g/dl, and the level of albumin, globulin and Albumin/Globulin ratio changed where albumin decreased while the globulin and [Albumin]/[Globulin] ratio increased.

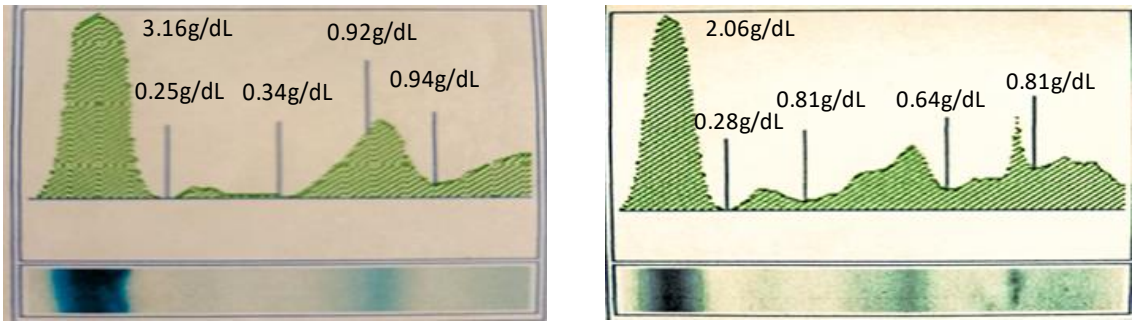
Among the 50 collected patients 22% received treatment with Entecavir drug at dose of 0.5 mg once a day orally, the effect of entecavir drug in the treated group as compared with the patients without treatment showed non-significant variations in the level of total protein, albumin and globulin and A/G ratio Table 6.

Table 6: Comparison of TP, A, and G concentrations and A/G ratio levels in patient without treatment compared with control in three groups according to their protein concentration.

		Control group no.=11	Patient without treatment group no.=11	Patient with treatment no.=11			
serum	[TP] (g/dl)	6.725±0.700	5.798±1.351	6.927±1.084	0.057^{a}	0.834^{c}	0.280^{b}
	[A] (g/dl)	3.837±0.553	2.571±0.618	2.772±0.497	0.010^{a**}	$< 0.001^{c***}$	0.873^{b}
	[G] (g/dl)	2.888±0.896	3.263±0.986	4.155±1.103	0.889^{a}	0.208^{c}	0.280^{b}
	A/G ratio (g/dl)	1.550±0.854	0.899±0.407	1.390±0.100	0.412^{a}	0.589^{c}	0.474^{b}

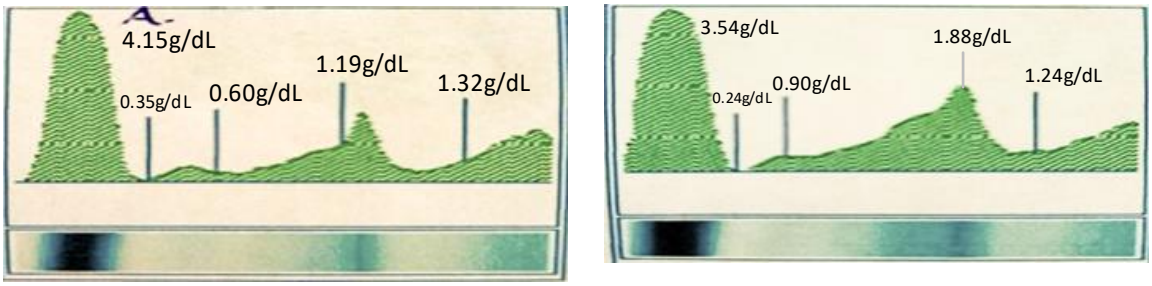
a: Refers to the comparison between the control and the patient without treatment.
c: refers to the comparison between the control and patient with treatment.
b: refers to the comparison between patient without treatment and those with treatment.
**** Refers to a significant difference P……**
***** Refers to a highly significant difference P….**

Hellabio Agarose Gel electrophoresis is a specialized diagnostic tool that enables the simultaneous quantitative and qualitative analysis of proteins in serum samples, providing a comprehensive assessment of protein profiles. The clinical application of electrophoresis in protein analysis relies on the straightforward electrophoretic separation of proteins, categorizing them into distinct fractions based on their relative mobility and molecular weight, namely α1-, α2-, β-, and γ-globulins (Hellabio agarose gel electrophoresis kit). Agarose gel electrophoresis of serum proteins for control and patients with hepatitis b was done to detect any alterations in the protein profile, one may correlate changes with variations in the concentration of total protein, When the protein concentration was less than 6 g/dl, protein concentration from 6.1-8.4 d/dl and protein concentration >8.5 g/dl in patients and control the electrophoresis protein was shown in Figure 1 and Figure 2.

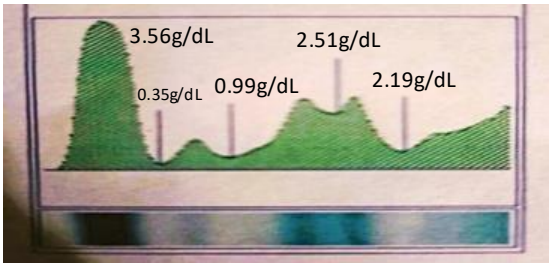


(a) **(b)**

Figure 1: Serum protein electrophoresis on agarose gel using non-barbital buffer (a) serum control group with [TP]<6 g/dl (b) serum patient group with [TP] <6 g/dl



(a) **(b)**

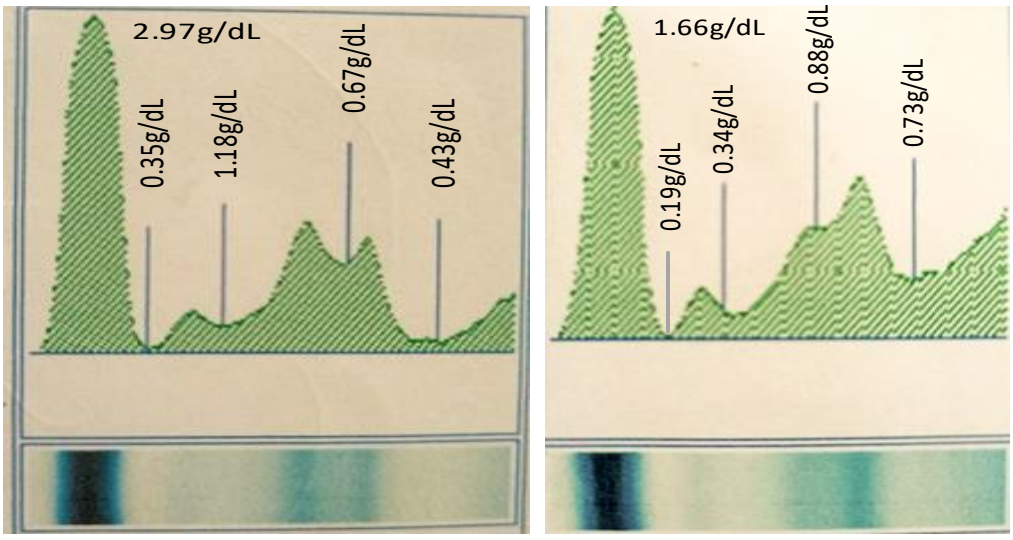


(c)

Figure 2: Serum protein electrozymograms on agarose gel using non-barbital buffer (a) serum control with [TP] 6.1-8.4 g/dl (b) in serum patient with [TP] 6.1-8.4 g/dl (a) serum patient with [TP] >8.5 g/dl

Upon analysis of total protein in serum using Hellabio scan and software, researchers observed that the proteins were separated into five distinct bands: albumin, α1- and α2-globulins, β-globulins, and γ-globulins Figure 1. When TP concentration was less than 6 g/dl, the level of A decreased, [β and ɣ-globulin] were slightly decreased while [α1 and α2-globulin] The observed protein levels were found to be slightly elevated in the patient samples when compared to the control group. When TP concentration ranged from 6.1 g/dl to 8.4 g/dl [A] level was decreased, and the [α1, ɣ- globulin] were slightly decreased while [α2 and β globulin] was slightly increased in patients as compared with control group.

Agarose gel electrophoresis was performed on serum samples from two groups of patients to identify differences in serum proteins before and after treatment. The results were presented in Figure 3a & 3b. It is obvious from these electro zymograms, that there was a slightly increase in [β- globulin] and [ɣ- globulin] in patients' serum. while the level of A in the patients decreased. The results as well showed presence of a decreased level of α1and α2 globulin in serum patients' samples.



(a) **(b)**

Figure 3: Serum protein electrophoresis on agarose gel using non-barbital buffer (a) serum patients without treatment (b) serum patients with treatment

5. Discussion

Globally, approximately 350 million people are chronic carriers of the hepatitis B virus (HBV). The infection can cause acute and chronic liver disease including cirrhosis and hepatocellular carcinoma (HCC) [18]. In this investigation, both serum and saliva samples were utilized to monitor fluctuations in the levels of total protein (TP), albumin (A), globulin (G), and the [A/G] ratio among Iraqi patients diagnosed with chronic hepatitis B. The results indicated that the serum total protein in patients decreased, but such a decrease was statistically non-significant as shown in Table 2. This finding is consistent with several studies that have demonstrated the absence of differences in serum total proteins, levels between patients and healthy controls, this result agrees with result of Iraqi patients with

hepatitis B [19] and in Iraqi patients with alcoholic and nonalcoholic liver disease [20]. Typically, the total protein concentration in the blood falls within the range of 6-8.3 g/dl, with albumin constituting the majority (approximately 60%) of the total serum protein and globulins. The result in

Table *3* indicated presence of a decrease in TP accompanied by low albumin levels. According to these results, albumin decreased in HBV patients compared to control. Several studies have shown that albumin decreased in cases of malnutrition [19], infection, chronic liver disease [21], Any condition that diminishes serum albumin levels will invariably result in a reduction of total serum protein, a condition known as hypoproteinemia [22]. Globulins comprise a much smaller protein components and are divided into 4 categories: α1-, α2-, β-, and ɣ-globulins, α2-globulins were present at significantly higher levels in patients compared to the control. The α2-globulins include some acute phase proteins (APPs), such as ceruloplasmin، α2-macroglobulin, which were reported to be involved in inflammation [23]. In the present study, Salivary total protein levels also decreased in the patients with hepatitis B as compared with healthy control as shown in Table 2. This decrease may be elucidated by the concept that saliva functions as a partial filtrate of blood, housing serum components transported from capillaries into saliva through mechanisms such as diffusion, active transport, or ultrafiltration via the gingival sulcus. The concentrations of salivary proteins are contingent upon the salivary flow rate, with a decrease in flow rate being associated with heightened salivary viscosity [22]. The decrease in total protein and albumin may be as a result of low immunity and malnutrition [24] and hypoproteinemia may be due to hypogammaglobulinemia [25], in contrast, true hyperproteinemia is always due to increase in serum globulins, mainly gammaglobulins. The A/G serum and saliva in patients with hepatitis B was found to be significantly increased. An increase in the A/G ratio typically signifies either an elevation in serum globulin levels with normal or reduced serum albumin levels [26]. In the current study, the observed increase in this value was attributed to low serum albumin levels and an elevated in both serum and saliva globulin concentrations Table 2. The higher incidence of HBV among younger individuals coincided with other studies carried out in Iraq such as [27] Gheorghe *et al* reported that in young individuals among the important multifunctional processes of HBV transmission routes, is the medical procedure such as sungery, acupuncture, dental treatment and injection [28]. At a young age, individuals often lack awareness and may not prioritize or complete the therapy. [28], [29]. It can be observed from the

Table ***5***, that the results of the measured parameters showed no difference between male and female. This may be due to low number of females as compared with male patients in the current study. This study results, agreed with the results obtained in a study in Nigeria about HBC [15]. This study used electrophoresis to evaluate changes in serum protein fractions, Gel electrophoresis is widely known technique used to separate and identify the different types of serum proteins which are: Albumin, α1 globulin, α2 globulin, β globulin, ɣ globulin. Each of these five protein groups move at a different rate in an electrical field and together form a specific pattern. This pattern helps to identify some diseases, The results showed that the peaks of albumin and globulin fractions were markedly different between the control and the patients with hepatitis B. When total protein concentration was less 6 g/dl

Table **5** there was no difference between control and patient. These findings were are consistent with previous research on diabetic retinopathy in Iraqi and chines patients [22], [30] respectively When total protein concentration was 6.1 to 8.4 g/dl, The observed decrease in serum albumin, recognized as a negative acute-phase reactant, was coupled with an increase in serum globulin, a known positive acute-phase reactant. [A] and [G] constitute the primary protein constituents in serum, playing crucial roles in the inflammatory response [31]. Indeed, any alterations in the concentration of these individual proteins will inevitably result in a change in total serum protein levels. The presence of distinct variations in the albumin and globulin fraction separation profile was observed among the studied groups, Figure 1 demonstrates difference in the peaks of albumin and α2, β and ɣ globulin fraction between patients and control. However, no difference was observed in peak of α1 globulin, between control and patient and this result agreed with the study on Iraqi patients with liver cirrhosis [21] and on Iraqi patients with diabetic diseases [22]. When total protein concentration from 6.1-8.4 g/dl in Figure 2 it was obvious from the comparison among the serum samples of the patient and control groups that the peak of β globulin increased in patient as compared with the control. The ɣ-globulin fraction is actively involved in the defense system against infectious agents. According to the Figure 2, overall ɣ-globulin concentrations were low in patients with hepatitis B. The reason for this reduction in the patient's group may be the shift from ɣ-globulin to α2-globulins. As such, a decrease of ɣ-globulin may confer lower immune resistance resulting in worsened clinical symptoms. This result did not agree with the study on Iraqi patients with diabetic disease [22] and agreed with the study on Iraqi patients with different kidney tumors [32]. Figure 3 showed that the peaks of α1 and α2 globulin (a known positive phase reactant) decreased while β and ɣ globulin which is a protein fraction involved in the immune response increased in the patients with treatment as compared with patient without treatment. The use of entecavir as a treatment regimen did not have an impact on any of the measured parameters. This drug in known to affect HBV replication [33]. This obtained result with this type of treatment may be due to the short-used treatment period in the included present patients.

Conclusion

Based on the findings of this study, it can be concluded that chronic hepatitis B affects the composition and concentration of serum and salivary proteins. To draw a definitive conclusion regarding the feasibility of utilizing saliva as a non-invasive and painless diagnostic alternative to blood for measuring the parameters included in this study among patients with chronic hepatitis B, further research is warranted. Specifically, future studies should involve a larger patient cohort to provide more robust evidence.

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