

Evaluation of Plasma Protein Alpha 2 and Beta in the Patients of Hepatitis B and C Virus by Radial Immunodiffusion Technique (RID)

Aamer. M. Ali¹, Hayder H. Abed², Ahmed Ghdban Al-Ziaydi³, Eman Mobder Nayif⁴

¹Assist lecturer/ Department of Pathological Analysis Techniques, Al-Mustaqbal University College, Iraq, ²Assist prof. Al-Muthanna university, Veterinary medicine college-physiology and chemistry department, ³Assist lecture: Department of Medical Chemistry, College of Medicine, University of Al-Qadisiyah, Iraq, ⁴Lecturer: Department of Pathological Analysis Techniques, Al-Mustaqbal University College, Iraq

Abstract

Proteins usually found in all body fluids, the proteins it is only of the blood plasma that are examined utmost frequently for diagnostic purposes. Over 100 proteins individual have a physiological mission in the plasma, plasma protein Mostly synthesize by liver, usually exclusion of protein hormones and immunoglobulins. In this study include the twenty four patients suffering from infection hepatitis C virus and twenty four patients suffering from infection hepatitis B virus. The estimation of plasma proteins alpha 2 kind are ceruloplasmin and haptoglobin and beta type is transferrin concentration by used radial immunodiffusion technique (RID). The transferrin and haptoglobin are affected, the transferrin is decline below the normal value while the haptoglobin contain the normal value. The twenty patients from each of the HCV and HBV transferrin concentration were (105 - 109) mg/dl, while ceruloplasmin (17.4 - 49.4) mg/dl and haptoglobin (71.2 - 100) mg/dl. The concentration of the last two were the normal value haptoglobin and ceruloplasmin levels that as measured in this study, Thus, it could possibly be assumed that the level of ceruloplasmin was not affected to an extent that is clinically significant.

Consequently, it is justifiable to conclude that reporting blood levels of ceruloplasmin is not of clinical value to HBV and HCV patients. Furthermore, this study showed the transferrin and haptoglobin level were the marker (out of the three) that were affected in patients with HBV and HCV when its level was compared with levels in healthy individuals, it was significant and the transferrin P (0.001), haptoglobin P (0.003). The reduction in transferrin level and increase in haptoglobin found by this study may be well linked with increased serum iron levels reported in HBV and HCV patients and the level of the fibrosis liver.

Keyword: Ceruloplasmin, Radial immunodiffusion, Haptoglobin, Proteins

Introduction

Hepatitis B is an infectious disease case through the hepatitis B virus (HBV), an covered virus have a partially double stranded, circular DNA genome, and classify within the family hepadnavirus, which affects the liver⁽¹⁾. It can cause together chronic and acute infection. Exact people have no symptoms by method

of the primary infection. Some improve a quick onset of sickness with yellowish skin, tiredness, vomiting abdominal pain and dark urine⁽²⁾. The severe pathological consequences of persistent HBV infections involve the expansion of chronic hepatic failure, cirrhosis, and hepatocellular carcinoma (HCC)⁽¹⁾.

Hepatitis C: It is an infectious illness caused through the hepatitis C virus (HCV), very small, positive sense RNA virus, single stranded, enveloped,⁽³⁾. It is a member of the Hepacivirus genus in the family Flaviviridae Chronic (HCV), Improvements in Treatment,⁽⁴⁾. that

Corresponding author:

Aamer. M. Ali

Aamer.M.Ali@mustaqbal-college.edu.iq

primarily affects the liver. Through the firstly impurity people often need mild or no symptoms. Occasionally a fever, yellow tinged skin occurs, abdominal pain, and dark urine. It could similarly be feast from an infected mother to her baby throughout birth. It is not feast by superficial contact. Diagnosis is through blood testing to look for as well anti-bodies to the virus or it's RNA. Testing is suggested in all people who are at risk. They no vaccine against hepatitis C⁽⁵⁾.

Ceruloplasmin: a ferry-oxidase enzyme that in humans is encoded through the CP gene⁽⁶⁾. It is the chief copper-carrying protein in the blood, and in adding plays a part in iron metabolism. Ceruloplasmin is an enzyme synthesized in the liver having six atoms of copper in its structure⁽⁷⁾. Ceruloplasmin transfers further than 95% percentage total of the copper in healthy human plasma⁽⁸⁾. Ceruloplasmin exhibitions a copper-reliant oxidase activity, which is related with promising oxidation of (ferrous iron) Fe^{2+} in to (ferric iron) Fe^{3+} , therefore helping in its carrying in the plasma in association with transferrin, which can transport iron that only the ferric state⁽⁹⁾.

Haptoglobin: the haptoglobin in clinical settings confirms is used to screen for and monitor intravascular hemolytic anemia, this causes a decline in haptoglobin levels. Hemoglobin free has released into circulation and hence haptoglobin will bind the hemoglobin. in reverse, in extravascular hemolysis the reticuloendothelial method, expressly hemoglobin is comparatively not released into circulation and splenic monocytes, inversely, causing haptoglobin levels to be decreased to extra hemolysis can release some hemoglobin therefore haptoglobin is not .adding, the haptoglobin gene is expressed in murine and human adipose tissue⁽¹⁰⁾. Mutations in this gene or its regulatory territory cause a hypohaptoglobinemia or haptoglobinemia. This gene has also linked to diabetic nephropathy⁽¹¹⁾. Transferrin an iron - binding blood plasma glycoproteins that take control the level of free iron in biological fluids⁽¹²⁾. Human transferrin is encoded through the gene TF⁽¹³⁾. Transmitting glycoproteins bind iron tightly, but then reversible. Though iron bound to Transmitting is fewer than (4 mg) 0.1% of over-all body iron. In humans, transferrin involves of a polypeptide chain having 679 two carbohydrate chains, amino acids. Protein the composed of beta sheets and alpha helices that form two domains⁽¹⁴⁾. Because the iron overload

protein and diseases malnutrition can happen decreased plasma transferrin increasing plasma transferrin level is often realized in patients suffering from iron deficiency anemia, throughout pregnancy. known atransferrinemia, a by means of the results from a rare genetic disorder absence of transferrin , an best characterized as a result of hemosiderosis and anemia in the liver and heart that leads to heart failure and many other complications. When the receptor is using to attract anti-bodies . Furthermost recently, transferrin and its receptor have shown to diminish tumor cells⁽¹⁵⁾.

Radial immunodiffusion: Is an immunodiffusion method used in immunology to limited, the concentration or quantity of an antigen in a model,. Anti-body is incorporated into a medium such an agar gel. The antigen is then put in a well that is punched out of the medium while the medium is on a microscope slide or in an open vessel, such as a Petri dish. The slide or vessel is then wrapped or locked, to prevent vaporization. The antigen is quantitated by means of measuring the diameter of the precipitin circle and contrast, it with the diameters of precipitin circles created by known quantities or the antigen of concentrations. Antigen-antibody complexes are little, and soluble when in antigen overflow. For most antigens, the square and area of the diameter of the circle at the circle's end- point are inversely proportional to the concentration of anti-body and nonstop proportional to the quantity of antigen. Radial immunodiffusion is use expansively for the quantitative evaluation of antigens. The antigen anti-body precipitation is made extra critical through the incorporation of antiserum in the agarose. Antigen (Ag) is then allowed to diffuse from wells cut in the gel in which the antiserum is uniformly distributed. Initially, as the antigen diffuses out of the well, its concentration is rather high and soluble antigen anti-body adducts are formed. However, as Ag diffuses within from the well, the Ag - Ab complex replies with large amount of anti-body resulting in a lattice that precipitates to form a precipitin ring.

Material and method:

1.1. EASY RID KIT

It is plates with twelve wells for quantitative limitation in radial immunodiffusion of human plasma proteins in plasma and serum. EASY RID made by Liofilchem S.r.l.

1.2. Configuration:-

Table (1): EASY RID Configuration

Product	Code	Plasma protein	Gel Colour	Test
h-Transferrina	93006	Transferrin	Green	12
h-Ceruloplasmina	93016	Ceruloplasmin	Red	12
h-Aptoglobina	93018	Haptoglobin	Green	12

1.3. Composition:

EASY RID contains, in a layer of agarose gel the monospecific antiserum to human plasma protein, produced by immunizing rabbits or goats.

1.4. Preparation of Samples:

That agreement the type of plasma protein you are look for, use a sample diluted in PBS or in isotonic salty solution, or sample undiluted. in this types of parameters we were used undiluted sample. it was stored correctly at 2-8 °C, Turbid sample was clarified by centrifugation prior to the assay.

1.5. Experimental design:

Sample was used serum samples, it was collected from marjan hospital in Al- halli privacy. Samples were diagnosed by hepatitis C and B virus with healthy individual as control, this was divided to three groups

First group: (12) samples from healthy individual as a control

Second group: (24) samples from patients suffering from HBV already diagnosis

Third group: (24) samples from patients suffering from HBC already diagnosis

1.6. Test Procedure:

1- EASY RID was removed from the covering, it was opened the plate and left to stand for nearly five min at room temperature so that any condensed water in the wells can evaporate.

2- The wells were filled with 5µL of undiluted patient's serum as indicated.

3- The plate was closed with the lid, after the samples had diffused into the gel and left to stand, it was incubated into the envelope, at room temperature for 48 hr.

4- The zone was measured by scale.

Results

Ceruloplasmin levels were within normal level range in all subjects of the study. However, in contrast to ceruloplasmin results, there were fluctuations in the blood level of ceruloplasmin in all patient groups (control, HBC and HCV). In HCV patient group, the fluctuation in the ceruloplasmin blood level is sharper than in the other two groups (control and HBV) with peak level of 50 mg/dl and trough level of around 17.2 mg/dl patient group. Although, all blood level measurements in the three different groups were in the normal level range.

The measurement of transferrin blood levels which were below the normal level range. 50% of transferring measurements in HBV patient group and 67% of the measurements in HCV patient group were lower than the normal level range. In the control group, all readings of transferrin were in the normal level range. Ten out of 24 values were around the lower end of the normal level range (about 200 mg/dl).

Table (2): Compare results of ceruloplasmin blood level in control, HBV and HCV patients groups

The groups		Mean ± SD U/1	SE	95% Confidence Interval		P-value
				Lower	Upper	
The group	Control	24.875± 1.08	1.8652	20.770	28.980	0.105*
	HBV	21.958± 1.06	1.6014	18.646	25.271	0.217*
	HCV	20.550± 0.92	1.4015	17.422	23.678	0.513*

Otherwise the reported levels of transferrin in the control patient group were approximately in the middle of the normal level range (about 300 mg/dl). Transferrin measurements in HBV patients group can be divided into two halves; one half of the values in the lower half of the normal level range (200-300 mg/dl) and the other half of transferrin values were below the normal level range (approximately 100mg/dl). The most characteristic feature of the results in this study was regarding the blood level of transferrin in HCV patients groups. In this patient group, a notable decline in the transferrin blood level that was below the normal range can be clearly observed (67% of readings were in a reported range of 100-160mg/dl). This variation in transferrin level is most likely of clinical significance to report in HCV patient due to its impact on the iron status of the patient.

The reported results of haptoglobin were generally within the normal level range in all subjects of this study. In control patients' group, haptoglobin results were approximately free of significant fluctuations (values were generally around 70 mg/dl). In HBV patients' group, the measured haptoglobin had approximately ups' and downs' level within the range of 70-230 mg/dl. HCV patient group reported relatively wider level measure, however all readings of haptoglobin were in the normal level range but its clinical significant compare with healthy individual control the P (0.007).

Table (3): Compare results of transferrin blood level in control, HBV and HCV patients groups

The groups		Mean ± SD U/1	SE	95% Confidence Interval		P-value
				Lower	Upper	
The group	Control	259.667± 1.5	1.8652	238.689	280.644	0.01*
	HBV	186.000± 1.04	1.6014	150.006	221.994	0.001*
	HCV	157.000± 1.92	1.4015	133.921	180.079	0.00*

Table (4): Compare results of haptoglobin blood level in control, HBV and HCV patients groups

The groups		Mean ± SD U/I	SE	95% Confidence		P-value
				Interval		
				Lower	Upper	
The group	Control	71.11± 1.08	0.230	70.609	71.62	0.000*
	HBV	85.60± 1.06	3.002	79.83	91.82	0.000*
	HCV	137.37± 0.92	11.370	133.84	160.88	0.000*

1.7. List of Abbreviations:

HBV = Hepatitis B Virus, HCV = Hepatitis C Virus, CP = Ceruloplasmin, HP = Haptoglobin, TF = Transferrin, RID = Radial Immunodiffusion, Hb = Hemoglobin and HCC = Hepatocellular Carcinoma

Discussion

Proteins are present in all body fluids, but it is the proteins of the blood plasma that are examined most frequently for diagnostic purposes. Over 100 individual proteins have a physiological function in the plasma, most plasma protein synthesized by liver, exception of immunoglobulins and protein hormones. The damage of liver by hepatitis B and C virus is affected on the plasma protein synthesis, in this study the beta type is more affected than alpha 2 type. We are choose the parameters transferrin, haptoglobin and ceruloplasmin evaluate by radial immunodiffusion technique (RID) and invasive method to diagnosis the liver damage. This study included twenty four patients suffering from infection hepatitis B virus and twenty four patients suffering from infection hepatitis C virus. The transferrin and haptoglobin are affected, the transferrin is decline below the normal value while the haptoglobin includes the normal value. The twenty four patients from each of the HCV and HBV transferrin concentration were (105 - 109) mg/dl, while ceruloplasmin (17.4 - 49.4) mg/dl and haptoglobin (71.2 - 100) mg/dl.

The concentration of the last two were the normal value haptoglobin and ceruloplasmin levels that as measured in this study, Thus, it could possibly be assumed that the level of ceruloplasmin was not affected to an extent that is clinically significant. Consequently,

it is justifiable to conclude that reporting blood levels of ceruloplasmin is not of clinical value to HBV and HCV patients. Furthermore, this study showed the transferrin and haptoglobin level were the marker (out of the three) that were affected in patients with HBV and HCV when its level was compared with levels in healthy individuals, it was significant and the transferrin P(0.001) , haptoglobin P(0.003).The reduction in transferrin level and increase in haptoglobin found by this study may be well linked with increased serum iron levels reported in HBV and HCV patients and the level of the liver fibrosis and cirrhosis. The HBV is more effected in this parameters may be will linked with types of fibrosis stages.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1. Mahoney FJ, Kane M. Hepatitis B vaccine. In: Plotkin SA and Orenstein WA, eds. Vaccines, 3rd ed. Philadelphia, W.B. Saunders Company, 1999:158-182.
2. Hepatitis B Fact sheet N°204. Who.int. July 2014.

Retrieved 4 November 2014.

3. Rosen, HR “Clinical practice. Chronic hepatitis C infection”. *The New England Journal of Medicine* 364 2011: (25): 2429–38. doi:10.1056/NEJMcp1006613. PMID 21696309.
4. *Chronic Hepatitis C Virus Advances in Treatment, Promise for the Future*. Springer Verlag. 2011. pp. 103–104. ISBN 9781461411918.
5. *Hepatitis C FAQs for Health Professionals*. CDC. January 8, 2016. Retrieved 4 February 2016.
6. Takahashi N, Ortel TL, Putnam FW. “Single-chain structure of human ceruloplasmin: the complete amino acid sequence of the whole molecule”. *Proceedings of the National Academy of Sciences of the United States of America* 1984: 81 (2): 390–4. doi:10.1073/pnas.81.2.390. PMC 344682. PMID 6582496.
7. O’Brien PJ, Bruce WR (2009). *Endogenous Toxins: Targets for Disease Treatment and Prevention*, 2 Volume Set. John Wiley & Sons. pp. 405–6. ISBN 978-3-527-32363-0.
8. Hellman NE, Gitlin JD “Ceruloplasmin metabolism and function”. *Annual Review of Nutrition* 2002; 22: 439–58. doi:10.1146/annurev.nutr.22.012502.114457. PMID 12055353.
9. Song D, Dunaief JL (2013). “Retinal iron homeostasis in health and disease”. *Frontiers in Aging Neuroscience* 5: 24. doi:10.3389/fnagi.2013.00024. PMID 23825457.
10. Trayhurn P, Wood IS “Adipokines: inflammation and the pleiotropic role of white adipose tissue”. *Br. J. Nutr.* 2004: 92 (3): 347–55.
11. Asleh R, Levy AP “In vivo and in vitro studies establishing haptoglobin as a major susceptibility gene for diabetic vascular disease”. *Vasc Health Risk Manag* 2005: 1 (1): 19–28. doi:10.2147/vhrm.1.1.19.58930. PMC 1993923. PMID 17319095.
12. Crichton RR, Charlotiaux-Wauters M (May 1987). “Iron transport and storage”. *European Journal of Biochemistry / FEBS* 1987: 164 (3): 485–506.
14. *Transferrin Structure*. St. Edward’s University. 2005-07-18. Retrieved 2009-04-24.
13. Yang F, Lum JB, McGill JR, Moore CM, Naylor SL, van Bragt PH, Baldwin WD, Bowman BH (May 1984). “Human transferrin: cDNA characterization and chromosomal localization”. *Proceedings of the National Academy of Sciences of the United States of America* 1984: 81 (9): 2752–6.
15. Macedo MF, de Sousa M (Mar 2008). “Transferrin and the transferrin receptor: of magic bullets and other concerns”. *Inflammation & Allergy Drug Targets* 7 (1): 41–52. doi:10.2174/187152808784165162. PMID 18473900.