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Detection of Hepatitis B virus (HBV) by using the RT- PCR and Serological Assay for Detecting Surface Antigen HBsAg

Hanan Waleed Muhamed Al-Sammarraie [1], Marwan Q.AL-Samarraie[2], Ashwak I. Al-Obaedi[3]

[1,2]*Department of Pathological Analysis, College of Applied Science, University of Samarra, Samarra, Iraq. Email: marwan.walady@yahoo.com*

[3]*Researches and Islamic Studies Center, Al- Iraqia University, Baghdad, Iraq*

**Corresponding author's E-mail: hanan.waleed.m@uosamarra.edu.iq*

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Abstract

The diagnosis of hepatitis B virus (HBV) in Iraq depend on the clinical symptoms and the positively serological assay and within which direct examination of the surface antigen (HBsAg) in serum. CDC and WHO recently considered the detection viral DNA interaction by real time-polymerase chain reaction (RT-PCR) as a standards laboratory for the diagnosis of HBV, therefore the aim of study is to evaluate this method in the diagnosis of HBV and a feasibility of this test as compared with the traditional way serological assay for direct detection of surface antigen (HbsAg) and recognize the ability of each test to find a relationship between the infection frequency and the gender to know the capacity of each scan in determining the extent of the disease . were taken samples from patients (33male and27female) that clinically 60 bloods suspected to be infected with HBV and compared the direct serological assay with RT-PCR ratios. The result showed that were 100%, 63.3% and chi square values were 1.279 , 1.865 respectively, F-test showed significant difference among females 10.22 and male 9.36 when examined by RT - PCR , followed by test Duncan which determined the different viral loads in both sexes . Our findings showed that RT –PCR offers a way sensitive, efficient and accurate for the diagnosis and determination the extent of HBV infection.

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Keywords: *Hepatitis B, RT- PCR , Surface Antigen HBsAg*

1. Introduction

Hepatitis B virus was major global problem, as the nearly two billion people have been exposed to infect with the virus worldwide (1,2).While the disease caused by viral hepatitis type B is the reason why the tenth president of the deaths in the world (3), and more than 350 million people develop them injury, which leads eventually to death all over the world (4).and because it has developed a lot of tests for the diagnosis of this disease at all stages. The incidence of primary HPV in susceptible individuals as possible to be symptoms of a phenomenon or without asymptomatic, called the incidence of acute infection, which is rare to turn to the injury peek fulminant and evolve during this phase sharp to be fatal for the individual.

The Hepatitis B surface antigen (HBsAg) is a brand diagnostic commonly used in the diagnosis of infection through a blood test of infected people, as the individual who gives positive test for HBsAg is infected with viral hepatitis type B or by direct examination (5). As for the HBcAg basic pulp antigen is an antigen within the cell and which expresses get infection inside liver cells and cannot be detected in blood or blood products (6). Since he HBsAg disappear when the symmetry of healing, we

need to mark a long-term diagnosis for the detection of infection during which type B hepatitis in all stages of acute and chronic (7).

Recently, have been developed the using of Real Time PCR technology, which supports the basis of its work on quantitative measurements of DNA in viruses with increased advantages of this process in terms of high sensitivity, low risk of contamination and high accuracy in the diagnosis of the DNA has been used a number of especially designed for this purpose (8).

The aim of the study to use the RT- PCR technique for the diagnosis of HBV infection, by comparing with the direct examination of surface antigen HBsAg and demonstrate the ability of each test to determine the extent of HPV infection.

2. Materials And Methods

Samples Collection

The study was included 60 patients (33 males and 27 females) their aged ranged between 10-60 years, they diagnosed by physiology valuable hospitals as well as clinics scattered throughout the province of Erbil in the period between September to October2022, as has been collecting blood samples were taken and kept in a cooler box containing ice cubes, and was expelled centrally speed 4500 rpm / 10 minutes, then took the blood serum and stored in the freezer at -20c^0 until testing (9,10,11,12).

Serological Assay for Detecting Surface Antigen Hbsag in Serum by using Direct Examination:

This method had done by using a kit prepared by Diaspot America company(USA) (13), as added 75 Micro-liter of the sample to the hole prepared in the slide, as the inner membrane will be absorbed by the sample to be with her combination of interaction note that the membrane may be covered in advance HBs anti- area line test, and here the membrane will vaporize ascending on chromatographic membrane by capillary action to interact with HBs anti- area line test, generates the font color, the appearance of the red colored in the line examination will indicate the result is positive examination, but in the absence of antigen reaction will not get the font and will not appear stained and this refers to the negative result of the test.

Detection of viral DNA by using the RT- PCR:

The Real Time PCR technology of the modern techniques used to detect DNA by cells multiplied using specialized primers Specific Primers and enzymes Taq Polymerase chain specializes in building DNA (14). This technique is used for the diagnosis of Viral Load in the blood of patients with multiple extraction and amplification for viral DNA samples examined. Aztm the conduct all tests detect viral DNA in Pharma in the city of Erbil, the working methods and diagnosis as follows: The extraction process was by using several PromegaReliaprep Blood gDNAMiniprep System U.S. based on the work of this kit is the Binding Column link in the centrifuge tubes precise high-speed microcentrifuge tubes, 200 microliter were drawn from a sufficient sample purification processes (8), either way amplified DNA was using several HBV Real-TM Quant which several specialized to amplify the virus HBV DNA and produced by the company SacaceBiotech-nologies-Italian, as in (15). It has been prepared kit the Home Master Kit according to the manufacturer's instructions, was to add a leaky 12.5 mL of a DNA sample to 12.5 mL of the main solution kit and put in the pipeline Smart cycle RT instrument. Thena pipe was transferred to a RT PCR instrument, which can detect the DNA of 200 copies / mL and upwards. Less than this level is undetectable for the device, and was reading the mix according to the software installed in the instrument, after fully reading instrument gave two results for reading are FAM and Cyanine 3 (CY3), Where the value was divided CY3 the FAM and beat the result in the coefficient of the device to give the final result of the viral load found in the sample and be units (IU / mL).

Statistical Analysis4

two types of statistical analysis, were used the first Chi-Squared (16), to study the existence of differences in real and substantial for ways studied, which the second use the analysis of variance (ANOVA) specifically for using F test to prove the existence of differences in real among the injured male and female by Duncan test (17).

3. Results and Discussion

The Table (1)numbers and percentages of HBsAg in sera for patients with viral hepatitis B pattern using direct serological examination.

	HBsAg _ve		HBsAg +ve		
Groups study	**Percentage %**	**No.**	**Percentage %**	**No.**	**Total No.**
Females group	0	0	100	27	27
Males group	0	0	100	33	33
Total	0	0	100	60	60

Chi-Square test=1.279 $^{n.s}$

Table (2) the distribution of the results of examination of Real Time PCR for the presence of viral DNA in sera of patients with viral hepatitis B HBV style.

Groups study	**HBsAg _ve**		**HBsAg +ve**		
	Percentage %	**No.**	**Percentage %**	**No.**	**Total No.**
Females group	44.4	12	55.5	15	27
Males group	30.3	10	69.6	23	33
Total	36.6	22	63.3	38	60

Chi-Square test=1.865 $^{n.s}$

Table (3) the difference viral load to the presence of HBV DNA in sera samples for the study patients (female) received a positive result byRT- PCR.

	Viral Load difference for HBV copy / ml						
Groups study	**1ml/10^4**	**1ml/10^5**	**1ml/10^6**	**1ml/10^7**	**1ml/10^8**	**1ml/10^9**	**Total No.**
Females group	0	3	4	2	4	2	15
Males group	0	18.000	176.000	1.320.000	21.200.000	193.000.000	
		21.781	192.000	1.658.690	59.500.000	458.000.000	
		43.200	197.600		15.100.000		
mean			307.340		76.773.000		
± std		27.660	218.230	1.490.000	43.140.000	325.500.000	
		±13.59	±60.10	±0.24	±29.91	±187.38	
Duncan test		c	b	d	c	a	

Viral Load of Real Time above (200 copy /1ml). F test=10.22**

Table (4) Viral Load difference in the presence of HBV DNA in sera samples for the study patients (female) received a positive result byRT- PCR.

	Viral Load difference for HBV copy / ml						
Groups study	**1ml/10^4**	**1ml/10^5**	**1ml/10^6**	**1ml/10^7**	**1ml/10^8**	**1ml/10^9**	**Total No.**
Females group	2	3	6	2	6	4	23
Males group	8900	45.400	827.170	6.960.000	64.100.000	263.000.000	
	2550	67.730	138.000	2.407.360	55.000.000	123.000.000	
		13.400	589.000		22.220.310	123.000.000	
			637.980		26.500.600	188.000.000	
			144.000		94.800.000		
			150.000		20.228.000		
mean	5725	45.000	414.000	5.000.000	47.000.000	174.000.000	
± std	±4490	±32	±307	±3	±30	±67	
Duncan test	a	d	b	e	d	C	

Viral Load of Real Time PCR above (200 copy /1ml). F test=9.36**

The results showed that have been obtained from testing the direct examination antigen of HBsAg that HBV was present 100% in both sexes, which gave positive for the presence of HBV for 60 patients (Table 1) the test didn't give any values being depends on the color change just so used Chi Square to know the moral serological test results and gave values of 1.279 ns a non - moral and non - statistically significant .For the second test the result was revealed DNA for HBV assay RT-PCR) in

the 15/27 sample of females (55%) (12 were direct positive test to examine antigen HBsAg) and 23/33 males (69.6 %) which 10 of them were direct positive test to examine antigen HBsAg was revealed 60 / 38 (63.3 %) using (RT - PCR) (table 3).

The different ratios between the test direct examination antigen HBsAg and RT-PCR values of 100%, 63.3% and low ratio assay DNA RT-PCR is due to several reasons not to diagnose DNA in spite of the direct positive test to examine antigen HBsAg where it could contain the serum only on in complete virus which contains on the surface antigen without basic pulp containing DNA (18).

As well as possible be the reason for the return to low concentrations of copies of the virus in the serum that are less than the level at which can RT-PCR instrument diagnosis of viral DNA, where the instrument portability used in this research in the diagnosis up to 200 copies \ ml (5,19).

In spite of the fact that the higher the number of males were that 33and 27 females' samples injury was higher than 23 males 15 females and apply a chi square to see significant results of the assay RT – (PCR). Is gender difference relationship reluctantly injury in HBV gave values. 1.865 ^{ns} is not significant and statistically unsupported. And thus, did not show the results of our study from the foregoing that the sex or gender worker role in determining the seroprevalence of virus hepatitis B in the communities and thus did not agree with what indicated to him (20), which found that the rate of cases positive for HBsAg higher than in males than females. Also, it's not agreed with what (21). So that the number of males was more susceptible than females in Pakistan. In addition, the (22) got the same result in Nigeria so that the number of males were higher than females, no results consistent with (23) What came in the fact that most female infection than males also in Nigeria. But because this study did not agree with the results of the studies, and the cause of the difference due to the small number of samples in this study compared to the large number of samples in the above-mentioned studies, As well as the different in geographical areas in which research has worked out and the type of equipment and materials used in this research. But never the less there are several things could not test the direct examination antigen HBsAg that prescribed and outlined Comparison DNA RT-PCR and except for being easy procedure and a high cost, it did not give any values just depends on the color and did not give the different proportions depending on gender, did not give different percentages depending on the type of gender which gave full attribution of injury in both genders. As well as this study did not show the power of injury if they are sharp or weak and thus did not give any indication to see how much potions treatment prescribed by your doctor for the injured person in order to cure the disease and can also track the status of the patient after using the treatment you responded decreases the presence of Viral load and thus determine the treatment period and quantity or vice versa so the doctor take appropriate action to treat his patient.

RT-PCR could illustrate among more than that, the existence of significant differences with bacterial load and serial copy numbers (1ml /10^9.1ml /10^8.1ml /10^7.1ml /10^6.1ml /10^5 .1ml /10^4) within races. In Table (4) for females shows that ranged bacterial load of the highest value (263.000.000 / 1 ml to 2550/1 ml) and averaged mathematical one of the highest averages (174. 000.000 to below average 5725) and the value of the F-test between the averages with significant differences for females F test = 10.22 ** and male. F test = 9.36 **. Which led to the use of test Duncan which defines different bacterial load statistically gradually from the top of a difference to the least and took the symbol of a, b, c, d respectively for females were icons by sequencing (1ml / 10^9 / a. 1ml / 10^6 / b. 1ml /10^8 / c. 1ml / 10^5 c. 1ml / 10^7 (d), which showed that the load that took the code a record high variation while payloads that took symbol c both of which did not differ statistically as for the males were icons by sequencing (1ml 10^4 / a. 1ml / 10^6 / b. 1ml / 10^9 / c. / 1ml /10^8 d. 1ml / 10^5 / d and the sequence e / 1ml/10^7. The load that took **a** symbol a recorded top difference while the two loads which took the symbol **d** both of which did not differ statistically while the loads which took the symbol **b** itself in males and females. This difference sequence observed in many studies (24) and adopted the use of the treatment on the extent of the injury (specifically the number of copies of the virus), the greater the greater the injury to HBV strong and follow-up of people infected during the treatment period and compared the progress of treatment with the amount of low viral load(25), When a person is equal to the healing of the HBV HBVsAg will disappear but does not appear pulp containing the basic DNA of the virus through security detection using RT-PCR. And so is the diagnostic sign a long-term (7).

4. Conclusion

The study is concluded from the foregoing that during comparison of DNA by RT-PCR is the easy way. Precise and sequential, efficient and long-lasting in the diagnosis and determine the extent of HBV infection and its follow-up treatment viral load it down.

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