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A Comparative Study between HBV Viral DNA Detection and Conventional Serological Methods of Diagnosis

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Abstract: This work was directed to get a comparison between the conventional methods ELISA commonly used in lab study for detection of viral hepatitis B and viral DNA by PCR. Thirty plasma samples were collected from patients referred to the Babylon GIT Center in Merjan Teaching Hospital. Those patients were already suffering from viral hepatitis B, or newly diagnosed as being infected with HBV and diagnosed after blood donation and a screening of their family members. Patients were segregated according to age, gender, disease duration. Viral antigenæmia (serum HBs Ag.) was estimated by ELISA technique, while HBV viral DNA was detected after extraction of plasma DNA by Real-Time PCR. Detection of viral revealed 6 (20%) cases with undetectable viral load in their plasma, indicating either, the virus load was below the detectable level of the PCR Kit, or false positive by ELISA, assuming that detection of viral DNA carries a high rate of specificity and sensitivity. Comparison between DNA copies and the level of HBs Ag. Revealed that most instances of high HBs Ag. Demonstrate a high viral load of DNA copies.
Keyword: HBV, Real-time PCR, ELISA, HBs Ag.

Introduction

Viral hepatitis is a rising problem in Iraq. Since the initiation of viral screening to find different types of blood born viral infections, especially viral hepatitis B and C, an increasing number of new cases is observed (1). There are five main viruses that cause liver disease, they make up two groups of hepatitis: enteric (Hepatitis A virus and Hepatitis E virus) and parenteral (Hepatitis B, Hepatitis C and Hepatitis D) [2,3]. Enteric hepatitis is transmitted by a facial-oral way and cause only acute hepatitis and possess high infectivity and stability [4]. Viruses of hepatitis type B, C and D are enveloped viruses, are transmitted by parenteral way and are able to encourage not only acute but chronic hepatitis. Viruses of HBV and HCV have a main role in the progression of chronic hepatitis, they are cause for development of 60-70 % of hepatic cirrhosis and up to 70-80 % of primary cancers of liver [5].Because a high occurrence of hepatitis viruses, one of the main assignments is the development of highly sensitive and reproducible ways of diagnostics permitting detection of the causative agent at all stages of the disease as well as monitoring of antiviral therapy effectiveness [6].

Hepatitis B virus (HBV) cause, acute hepatitis, chronic hepatitis, fulminate hepatitis with enormous liver necrosis, and the backdrop for hepatitis D infection. Chronic hepatitis patients account carriers of vigorously replicating virus and then are a source of infection to other persons. HBV also plays a critical role in the progression of hepatocellular carcinoma [7]. Hepatitis caused by HBV is a huge dilemma in the world, with an evaluate worldwide about 350 million carriers [8].

The HBV vaccine has been effective reduction in the prevalence of HBV in endemic regions, therefore also it reduce the risk of malignancy and hepatocellular carcinoma, in these regions. [9].

Materials and Methods:

Sample collection:

Plasma samples were collected from patients referred to the Babylon GIT Center in Merjan Teaching Hospital. Those patients were already suffering from viral hepatitis B, or newly diagnosed as being infected with HBV. Most patients of the second group were diagnosed after blood donation and a screening of their family members. Patients were segregated according to age, sex, disease duration, antiviral administration and viral antigenæmia (serum HBs Ag.) as estimated by ELISA technique.

ELISA Test:

This test was performed in central public health laboratory as a routine work after blood donation for detection any Hepatitis B, C and HIV infected subjects. The test was performed according to restriction manual of the manufacturing company.

Real-time PCR:

This test was accomplished after plasma DNA extraction to detect HBV viral DNA, with the use of kit materials and equipments provided by **Sacace™,** Italy.

Results:

Thirty plasma samples from patients with HBV infection, all were diagnosed by detection of HBs Ag. By ELISA (Enzyme-Linked Immunosorbant Assay). Patients were of different age groups and were 7 females and 23 males. Most patients were discovered incidentally either after blood donation or preoperative screening (23 patients), while the rest (7 patients) was complaining of chronic viral hepatitis B on specific therapy. The following table shows the age and sex distribution of patients with HBV infection.

Table (2) showing the age and sex distribution of our patient sample.

Age	Males	Females	Total	Percent
0-9	1	Zero	1	3.3
10-19	4	Zero	4	13.3
20-29	9	4	13	43.3
30-39	5	2	7	23.3
40-49	2	1	3	10
50-59	1	Zero	1	3.3
>60	1	Zero	1	3.3
Total%	76.6	23.3	30	100

HBs Ag. Determination involved indexing the serum samples with a control negative, the net result revealed indexes ranging from 0.4 to 3.0 or more. Most cases revealed an index of > 3.0 as shown in the following table. This table also illustrates the number of false positive results as compared with results obtained by PCR.

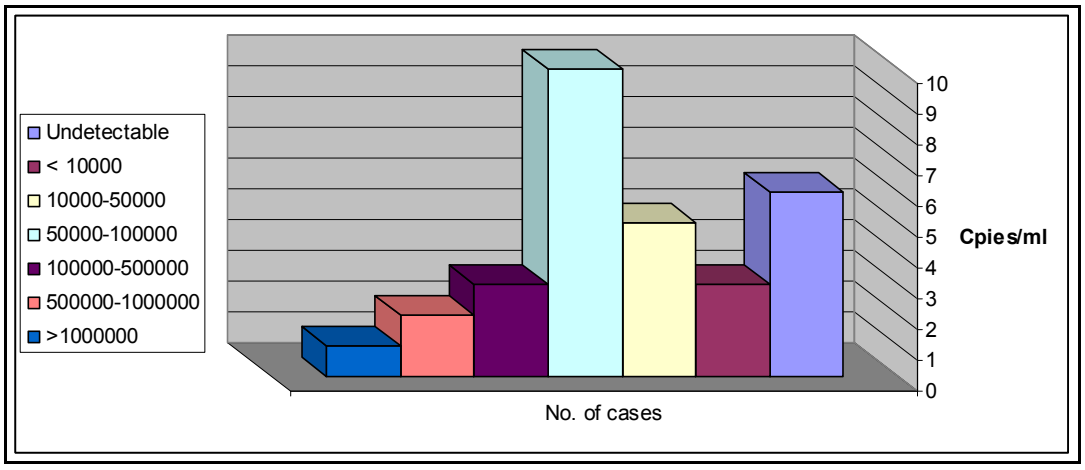
Table (3) showing the results of the ELISA technique in comparison with PCR viral DNA

ELISA Index	No. of cases	Percentage	No. of Negative PCR Result	Percentage
< 0.3	3	10	3	10
0.3-0.6	6	20	2	6.66
0.6-1.2	6	20	1	3.33
1.2-3.0	Zero	Zero	Zero	Zero
>3.0	15	50	Zero	Zero
Total	30	100	6	20

Detection of viral DNA revealed 6 (20%) cases with undetectable viral load in their plasma, indicating either, the virus load was below the detectable level of the PCR Kit, or false positive by ELISA, assuming that detection of viral DNA carries a high rate of specificity and sensitivity.

The following graph indicates the distribution of cases according to their viral load levels.

Table (4): Bar chart showing the distribution of cases, according to the viral load result.



Comparison between DNA copies and the level of HBs Ag. Revealed that most cases of high HBs Ag. Show a high viral load of DNA copies. All the fifteen cases (50%) of HBs Ag index higher than 3.0 showed a viral load higher than 10^5 copies. The following table illustrates the relationship between HBs Ag and viral load. The p value was calculated using SPSS software and Z test.

Table (5): Showing the significance of HBs Ag by ELISA in relation to viral load, assuming $5x10^5$ viral load level as the comparative value.

% Viral load > $5x10^4$	% HBs Ag. (1.2-3.0)	P value	% HBs Ag > 3.0	P value
53.3	Zero	1.02	15	0.002

Discussion:

In this study, all cases that were collected showed a positive result for HBs Ag. in their sera. Not all patients revealed HBV viral DNA in their sera (20% negative results)[10]. This might indicate either non-specific reaction by ELISA due either to inherent errors by this method, especially when we notice that nearly all negative PCR results in HBs Ag[11,12]. Seropositive patients were in those showing a mildly increased index, or due to technical errors during handling of samples in laboratory [13,14]. It seems that the first possibility is more acceptable, bearing in mind that the sensitivity of PCR in detecting minimal levels of DNA copies is very high (a minimum of 400 copies)[15]. This fact also raises another explanation for this phenomenon, those patients being possibly carriers of HBV without any evidence of viral replication yielded by PCR results. This has to be clarified by follow up on those cases [16].

There was a strong correlation between viral load and the index of HBs Ag detection index done by ELISA technique, as all 15 cases with index > 3.0 showed a viral load above $5x10^{4.}$ Despite this, it is reported that many cases with low titers of HBs Ag show active viral replication and high viral load. It has been concluded that detection of viral DNA by PCR is more sensitive and accurate than the ELISA(20% false positive results in ELISA), besides its value in following patients receiving specific therapy.

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