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Real-Time PCR Contribution in The Treatment of Patients Infected with Viral Hepatitis B in Bobo-Dioulasso

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ABSTRACT

Background: *Quantitative detection of hepatitis B virus (HBV) in serum or plasma has become the most direct and reliable method for monitoring chronic hepatitis B. Here, we report the contribution of real-time PCR hepatitis B DNA quantitative assay, the COBAS TaqMan HBV test (Roche Diagnostics, Meylan, France) in the treatment of patients infected with viral hepatitis B in Bobo-Dioulasso.*

Methods: *This is a prospective study conducted over a period of ten months (December 2014 to October 2015). Samples come from patients with viral hepatitis B infectious who are being monitored at hospital from Bobo Dioulasso. The serums were from whole blood collected by venipuncture in subjects of study. After centrifugation, all serum obtained was aliquoted into two cryotubes; one used to screen for HBs Ag and total Anti-HBc and the other stored at -80°C until use for this study. Serologically positive samples were sent to the Cerba laboratory in France for the determination of viral DNA by real-time PCR.*

Results: *A total of 75 patients tested positive for HBs Ag. The average age was 40 ± 12 years, the great majority (41.33%) between 31-45 years of age. Real-time PCR confirmed the serological status of 62.66% of patients by the presence of viral DNA. The viral load was greater than 8log in 20% of patients. Male subjects had higher HBV viral loads than female subjects (p = 0.002).*

Conclusion: *Detection of HBs antigen is not sufficient to initiate antiviral therapy. We have coupled the viral load with the detection of HBs antigen. This has led to better management of patients suffering from viral hepatitis. Unfortunately, the inaccessibility of this technique in terms of its cost is a major obstacle in monitoring the evolution of the disease in low income setting.*

Keywords

HBV, PCR, DNA, Bobo-Dioulasso.

Introduction

Infection with the hepatitis B virus is a major public health problem in Burkina Faso; the prevalence varies from 7.9% to 17% [1,2]. Also, according to French Speaking Alliance of Health Actors against HIV and Chronic Viral Infections [3] 2016 in Burkina Faso the prevalence of Hepatitis B virus (HBV) is 8.8% nationally. There is no statistically significant difference between regions,

whereas the prevalence of Hepatitis D virus (HDV) appeared low. In contrast, the prevalence of Hepatitis C virus (HCV) in Burkina Faso is 3.5% and differs from one health region to another. If it is less than 4% in 11 of the 13 regions, it reaches 6% in the Cascades regions Burkina Faso and 12.7% in South-West Burkina Faso. Seroprevalence of HCV tends to increase with age in both sexes and to be lower among people living in urban areas 1.7% [4]. In sub-Saharan Africa, the prevalence is between 8% and 18% and constitutes an area of high endemicity.

Thus, the prevalence is 14.7%; 9%; 25% respectively in Mali, Ivory Coast and Mauritania [5]. Chronic HBV infection can lead to liver cirrhosis and hepatocellular carcinoma [6]. Detection and quantification of HBV DNA in serum or plasma are now considered important indicators for managing disease in HBV infected patients and predicting and monitoring the efficiency of antiviral treatment as well as identifying the emergence of drug resistance by detecting HBV DNA breakthrough [7,8]. Viral hepatitis B and C are endemic in Burkina Faso and require management with accessible molecular biology tools [1,2]. Also molecular diagnosis of HVB is expensive in Burkina Faso. The purpose of our study is to establish the contribution of real time PCR in the treatment of patients infected with viral hepatitis B in low income setting.

Methods and Patient

This is a prospective study conducted over a period of ten months (December 2014 to October 2015) at the CHU Souro Sanou / Bobo Dioulasso (CHUSS). We included 75 HBs antigen positive patients. Our study population comes from patients with viral hepatitis B infectious who are being monitored at hospital from Bobo Dioulasso. In these patients, we diagnosed HVB.

Peripheral whole blood was collected on a tube without anticoagulant. Serum obtained was used to performe the detection of HBs antigen. Positives samples were aliquoted and stored at -80°C for analysis at the Cerba laboratory in France for the determination of viral DNA use real-time PCR. When the viral load is < log 20, the test is negative. It is positive when it is > log 20. Architect CI4100® (Abbott, Wiesbaden, Germany.) has been used for the detection of HBs antigen. Its principle is based on a particulate micro immunoassay technique by chemiluminescence thus allowing the detection of the desired markers.

Quantitative detection of hepatitis B virus

For the extraction of HBV viral DNA in patient's sera, we used an automated technique on the COBA ® AmpliPrep (Roche Diagnostics, Meylan, France). We used TaqMan quantification with ROCHE COBAS TaqMan 96 analyzer (Roche).

Ethic statement

Samples analyzed in our study derived from patient with HBV infection that went to laboratory for viral load control. The results of the various examinations were given to the participants of the study. The study was approved by our National Ethic Committee for health research in Burkina Faso Ouagadougou. Patients participating in this study gave written informed consent.

Statistical analysis of data

Data were analyzed using Wilcoxon test to compare rates. We used Fischer (t) test for the percentages. Non parametric tests (Kruskal Wallis and Mann-Witney) were used when normality of distribution wasn't verified. Results were considered statistically significant when $p < 0.05$. Analysis was performed in SPSS version 23 (SPSS Inc., Chicago, IL).

Results

General characteristics of the population

In total, 75 patients were included. Average age of patients was 40 ± 12 years. Patients with 31-45 years (41.33%) are the most represented ($p = 0.001$). Overall, the majority of the study participants were males 64% compared to 36% for female. The sex-ratio was 1.77. Viral load was undetectable in 37.32% and > log 20 in 62.66% of patients. Among patients with detectable viral load, 20% have a viral load > 8log. Table I shows patients distribution according age and viral load.

Age (years)	Percent of patients having undetectable viral loads (%)	Percent of patients having detectable viral loads (%)
16 - 30	6.66	13.33
31 - 45	25.33	41.33
46 - 60	5.33	4
> 60	0	4
Total	37.32	62.66

Table 1: Patients distribution according age and viral load.

Viral load distribution according age and sex

The mean viral load observed in male patients is 16.09 log IU / mL and 15.82 log UI / ml in female patients ($p = 0.06$). Table 2 shows average viral load distribution according age and sex.

Age (years)	Average viral load (log UI/ml)		p value
	Male	Female	
16-30	5.68	3.75	
31-45	3.38	5.61	
46-60	5.49	3.63	0.06
>60	1.54	2.83	
Total	15.82	16.09	

Table 2: Average viral load distribution according age and sex.

The cost of real-time PCR

The number of our patients is reduced because of the cost of real-time PCR. Indeed, the cost of Real time PCR in our study is 47500F or 85.58 US $ fully supported by the patient himself. This cost is repeated as many times as it is necessary to perform a Real time PCR as part of the follow-up.

Discussion

In our study, patients' average age is 40 ± 12 years. Patients with 31-45 years (41.33%) are the most represented ($p = 0.001$). In addition, these patients have the highest average viral load. Patients with 16-30 years have lower mean HBV viral load. The majority

of the study participants were males 64% compared to 36% for female. In a similar study conducted in Canada, Payne et al., in 2014, made the same observation for patients with 25-39 years.

Other previous studies conducted by Pietra et al., 2008 in Burkina Faso found a higher prevalence of HBV in men than women. In French, Pivert indicate that hepatitis B affects five times more men than women unlike hepatitis C [9]. But the mechanism that explains the fact that men are more affected than women remains poorly understood.

Of the 75 patients selected, only 62.66% reveal the presence of viral DNA. The calculation of sensitivity and specificity would have allowed us to assess the effectiveness of the serological test. But the data collected does not lend itself to this calculation.

The benefits of PCR in the management of patient infected with HBV are well established: for managing disease in HBV infected patients and predicting and monitoring the efficiency of antiviral treatment as well as identifying the emergence of drug resistance by detecting HBV DNA [10].

There is treatment for chronic hepatitis B and C. This treatment has been shown to reduce the risk of chronic liver disease, liver cancer and death. However, treatment is expensive. Indeed, the average cost of HBV PCR is 47,500 CFA or US $ 85.58. This test is expensive for developing countries like Burkina Faso. We suggest that a sensitive and specific viral load generic test be developed to improve the management of HBV in resource-limited settings.

Conclusion

Indeed, if the serological detection tests of antigens and antibodies for hepatitis B using 3rd generation ELISA are sensitive and specific, the detection of the viral genome is essential at the time diagnosis and especially therapeutic treatment. Hence the need to develop a sensitive and specific viral load generic test to improve the management of HBV in low incomes settings.

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