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Co-Infection of Hepatitis B Virus and Hepatitis Delta Virus in Yaounde-Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. Author JNT contributed in study design, offering facilities in the laboratory and editing the article. Author GCM wrote the protocol, collected data, performed the laboratory and statistical analysis and wrote the first draft of the manuscript. Author MPK contributed in study design, collection of data and editing the article. Authors DT, GBP and JF contributed in the laboratory analysis. Author ON designed the study, edited the article and supervised all the activities. All authors read and approved the final manuscript.

Case Study

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ABSTRACT

Aims: To determine the seroprevalence of HDV as well as the virological and clinical characteristics of HBV mono-infected and HBV/HDV co-infected patients.
Study Design: The few studies on HDV in Cameroon have reported a high prevalence of this viral infection. This is a first step in describing the virological and clinical profile of HBV mono-infected and of HBV/HDV co-infected patients.
Place and Duration of Study: Blood collection was carried out in the Gastroenterology Unit of the Yaounde University Hospital Centre, Yaounde General Hospital and "Centre Médical la Cathédrale", from August 2012 to May 2013.
Methodology: We included into this study treatment-naïve HBV-infected patients from Yaounde irrespective of age and gender free of HIV and HCV infection. Blood samples were collected from each patient for laboratory analysis. Detection of HDV antibodies

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(Diasorin, Germany) was performed by ELISA and viral load for HBV and HDV was determined using real-time PCR (Abbott Molecular Diagnostics). Patients were classified clinically into low replicative hepatitis, immune tolerance and chronic active hepatitis. Moreover, ultrasound and liver histological data were collected.

Results: The population comprised 128 chronic HBV-infected patients of which 77 (60.16%) were male and 51 (39.84%) were female. We found 29 HDV-positive patients representing 22.66% of the population. In the HBV/HDV co-infected group, the mean viral load for HBV was significantly low compared to patients with HBV mono-infection (P = .01). These patients also presented with higher liver cytolysis compared to HBV mono-infected patients (P<.001). Chronic active hepatitis was significantly more prevalent in HBV/HDV co-infected patients (68.96%) compared to HBV mono-infected patients (20.20%).

Conclusion: We found that HBV/HDV co-infection results in suppression of HBV replication and such patients show broader sequelae of liver disease. The prevalence of HBV and HDV co-infection is high in this population. Routine screening of HBV-positive individuals for HDV should be implemented in the health services nationwide.

Keywords: Hepatitis delta virus; hepatitis B virus; co-infection; Yaounde.

1. INTRODUCTION

Hepatitis B virus (HBV) is a major global public health problem with more than 2 billion people infected worldwide and a mortality rate of over 1 million people annually [1,2]. It is the tenth leading cause of death worldwide [3]. More than 350 million individuals are chronic HBV carriers and at least 5% of these are thought to be co-infected or super-infected with hepatitis delta virus (HDV) [4,5]. Hepatitis delta infection is considered to be the most severe form of viral hepatitis and propagates only in the presence of HBV [5]. HBV transmission may occur vertically at parturition, horizontally among children <5 years of age, or later in life either sexually or iatrogenically [6].

In a cohort of students in the Central African Republic, a prevalence of hepatitis B surface antigen (HBsAg) of between 3 and 20% was reported with markers of past exposure ranging from 60-99% [7]. Recent studies conducted in Cameroon, a region of high endemicity of HBV-related disease showed 11.8% and 10.5% prevalence of HBV infection among adults in 2010 and 2011, respectively, although an efficacious vaccine against HBV has been available for two decades [8,9].

After a decrease in the spread of HDV, largely because of the HBV vaccination campaigns and increased awareness of bloodborne infections, HDV still infects a steady proportion of HBV carriers worldwide [10]. The few studies conducted on HDV infection in Cameroon have reported the prevalence of antibodies against HDV in 6.5%, 27.3% and 17.6% in 1986, 1991 and 2011, respectively, [11,12,13] pointing to its high endemicity in the Cameroonian population. HDV infection in this region is characterized by a wide genetic diversity evidenced by the circulation of four different genotypes 1, 5, 6, and 7 with HDV-1 being predominant [13].

HDV is an insufficiently characterized virus. Individuals having HBV/HDV co-infection may have more severe acute disease and higher risks of fulminant hepatitis, cirrhosis and hepatocellular carcinoma (HCC) than those having HBV infection alone [4]. However, reports on its virology show a repression of HBV DNA in the presence of this virus [5,14]. Different

factors can be linked to this manifestation which can be viral, host, environmental factors as well as the presence of other underlying infections such as HIV and HCV.

There is no published data on the impact of HDV in patients with HBV infection from Cameroon. We expect that data collected from this study will provide the rationale for distinguishing HBV/HDV co-infected patients whose prognosis is severe from HBV mono-infected patients. In Cameroon where mainly two genotypes of HBV (A and E) circulate, co-infection with HDV may show different rates of disease induction and disease progression. We report here virological and clinical characteristics of HBV mono-infected and HBV/HDV co-infected patients in three tertiary care centres in Yaounde.

2. MATERIALS AND METHODS

2.1 Study Population and Sampling

The study population comprised of all chronic HBV-infected patients attending three hospitals in Yaounde (Yaounde University Hospital Centre and Yaounde General Hospital and "Centre Médical la Cathédrale"), the capital city of Cameroon from August 2012 to May 2013. Furthermore, all treatment-naive chronic HBV-infected patients with a viral load over at most 6 months were included in the study. None of these was HIV or HCV-infected. A written consent was provided by each patient before specimen or data collection.

Blood was collected into dry tubes then centrifuged at 2250 rpm for 8 minutes. Separated serum was put in cryogenic vials and stored at -20ºC for subsequent laboratory analysis. Relevant data were collected from patients' medical records such as socio-demographic data (age, gender, and alcohol consumption), virus serological characteristics (for HDV, HBeAg, HIV and HCV), virological characteristics (HBV DNA and HDV RNA levels), biochemistry record (alanine aminotransferase and aspartate aminotransferase levels), stage of liver disease and morphological characteristics of the liver (ultrasound, biopsy and fibrotest®-actitest®).

2.2 Laboratory Analysis

For patients without record of virus serology, detection of HDV antibodies (Diasorin, Germany), HBeAg and anti-HBe antibodies (Chicago, IL, USA) was performed by ELISA, HBV and HDV viral load by real-time PCR (Abbott Molecular Diagnostics) and HIV antibodies by rapid assays (Alere Determine, Immunochromatographic and ImmunoComb II EIA) as well as HCV antibodies (Healthmate HCV EIA).

2.3 Clinical Classification of Study Subjects

Patients were classified clinically into three groups based on the following characteristics:

2.3.1 Low replicative hepatitis group

These were patients with HBV DNA level below 20 000 IU/mL, and who had normal ALT levels but tested negative for HBeAg.

2.3.2 Immune tolerance group

These were patients who were HBeAg-negative or positive, had normal ALT levels, with HBV DNA levels above 20 000 IU/mL and normal liver on histology.

2.3.3 Chronic active hepatitis group

These patients were HBeAg-negative or positive associated with increased ALT or HBV DNA above 20 000 IU/ml and signs of active inflammation on liver ultrasound.

2.4 Classification of Study Subjects using Morphological Data

Liver biopsy or fibrotest®-actitest® results were classified based on the METAVIR scoring system. A grade was assigned for the degree of liver inflammation (A0 to A3) and staging of fibrosis was done for predicting disease progression (F0 to F4). Fibrosis was scored as F0 (absent), F1 (mild), F2 (moderate), F3 (severe) and F4 (cirrhosis) whereas necro-inflammatory activity was graded as A0 (absent), A1 (mild), A2 (moderate) and A3 (severe).

Ultrasound assessment was grouped into normal ultrasound, liver cirrhosis without tumour characterized by a nodular surface, firm consistency, blunt liver edge, altered echo texture, dilated portal vein and splenomegaly and the third group was liver tumour whereby liver presented with focal lesions indicating the presence of tumour.

Thus, we obtained the following: of 128 patients with viral load for HBV of at most 6 months old with complete serological and clinical characteristics, 105 patients had data on alcohol consumption. Alanine aminotransferase was available from 125 patients and aspartate aminotransferase from 105. Liver ultrasound data were available for 88 patients and liver histology from 60.

2.5 Statistical Analysis

Results were analysed using Statistical Package for the Social Sciences software (SPSS version 18.0, Inc., Chicago, IL.). Data was summarized as the means with standard deviations (for continuous variables), and as frequency and proportions (for categorical variables). Chi-square test and Fisher's exact test were used to compare proportions wherever appropriate while the student t test was used to compare means. A p-value of below 0.05 was considered statistically significant.

3. RESULTS

Overall, 128 patients or patients' case files were finally retained into this study based on the selection criteria. The male gender was predominant at 60.2%. The mean age of the population was 33.02±8.56 years (8 to 55 years).

Data concerning alcohol consumption was available for 105 subjects. Heavy alcohol consumption was noticed in 7 (6.67%) patients while the rest consumed little or no alcohol.
There was no association between the quantities of alcohol consumed with biochemical, clinical or morphological characteristics (P>.05).

3.1 Serological Characteristics of the Entire Population

i. Among the entire HBV population 29 (22.66%) were positive for antibodies against HDV. The distribution of age and gender was similar in HDV-positive and HDV-negative populations with a *P*>.05.

HBeAg a marker of viral replication was positive in 10.94% of the entire population. HBeAg status strongly correlated with the quantity of HBV DNA level, stage of HBV disease and liver enzymes activity.

All HBeAg-positive patients had HBV DNA levels above 20 000 IU/ml whereas 7 (6.14%) had HBV DNA levels above 20 000 IU/ml among HBeAg-negative patients (Table 1).

In HBeAg-positive patients 9/14 (64.28%) had chronic active hepatitis while in HBeAg-negative patients this stage was present at 27.19%.

Overall, HBeAg-positive patients had raised levels of ALT and AST compared to patients with HBeAg-negative infection (*P* < .05).

ii. HBeAg-positive status was observed in 2/29 HDV-infected patients (6.90%) and in 12/99 HBV mono-infected patients (12.12%). We noticed that HDV co-infection has no effect on HBeAg status (*P* = .43).

Table 1. Characteristics of patients according to HBeAg status

	HBeAg +N = 14	**HBeAg -N = 114**	***P***
Mean HBV DNA (IU/ml)	48 304 198	1 502 919	.01
HBV DNA (IU/ml)			
0 - 20 000	0	107 (93.86%)	
> 20 000	14 (100%)	7 (6.14%)	
HBV stage			<.001
Low replicative hepatitis	0	79 (69.30%)	
Immune tolerance	5 (35.71%)	4 (3.51%)	
Chronic active hepatitis	9 (64.29%)	31 (27.19%)	
ALT			.02
Raised ALT	8 (61.50%)	34 (30.40%)	
AST			.009
Raised AST	6 (60%)	21 (22.10%)	

3.2 Biochemical Characteristics of the Entire Population

i. A majority of the patients had normal ALT (67.20%) and AST levels (74.29%).
ii. There were more patients with raised transaminases in HBV/HDV co-infected patients compared to HBV mono-infected patients (Table 2).

Table 2. Characteristics of patients according to HDV status

	HDV+ N (%)	HDV- N (%)	*P*
Mean HBV DNA (IU/ml)	608 813	8 383 192	.01
HBeAg			.43
Positive	2 (6.90%)	12 (12.12%)	
Negative	27 (93.10%)	87 (87.88%)	
Total	29	99	
ALT			<.001
Normal ALT	10 (37.04%)	74 (75.51%)	
Raised ALT	17 (62.96%)	24 (24.49%)	
Total	27	98	
AST			<.001
Normal AST	9 (42.86%)	69 (82.14%)	
Raised AST	12 (57.14%)	15 (17.86%)	
Total	21	84	

3.3 Virological Characteristics of the Study Population

3.3.1 HBV viral load for mono-infected patients

The mean viral load of HBV was 6 621 809±3 037 450 IU/ml (< 10 to 170 000 000 IU/ml). Population distribution according to HBV DNA levels showed that a high proportion of patients had an HBV DNA level of < 20 000 IU/ml while HBV DNA levels > 20 000 IU/ml was present only in 17.19% of the entire population (Fig. 1).

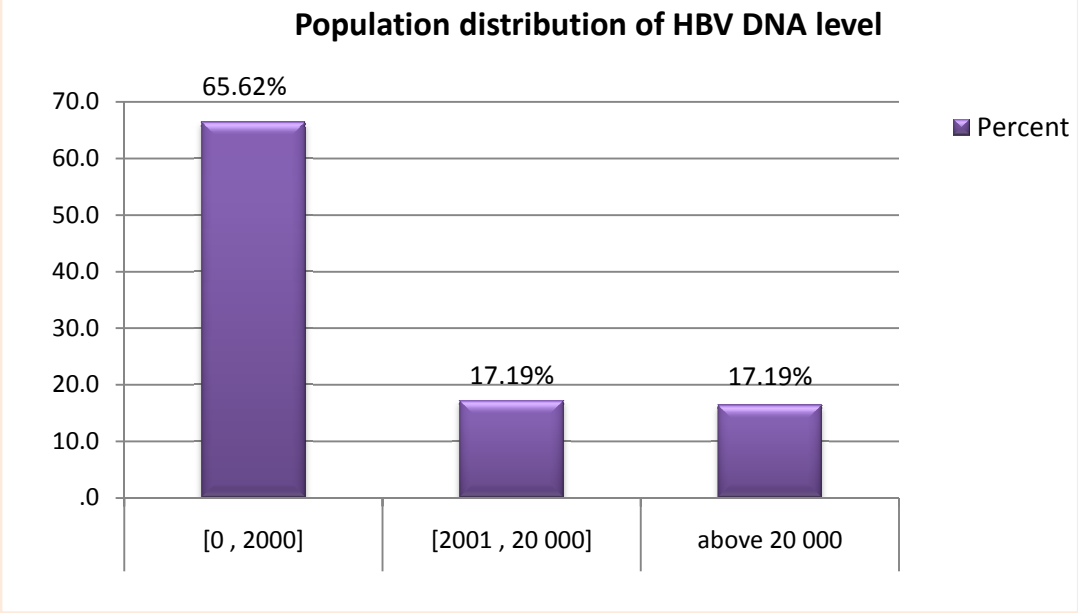

Fig. 1. HBV DNA levels in HBV-infected patients

3.3.2 HBV viral load for HBV/HDV co-infected patients

Hepatitis B viral load was significantly higher (8 383 192 IU/ml) in HBV mono-infected patients compared to 608 813 IU/ml in HBV/HDV co-infected patients (Table 2).

3.3.3 HDV viral load

Of the 29 HDV antibody-positive cases, RNA viral load was determined in 10 and 7 had detectable levels (>100 copies/mL). Two patients were HBeAg-positive as well as HDV RNA-positive (Table 3). Six patients had raised liver transaminases all of whom had detectable HDV RNA levels (*P*<.05).

Table 3. HDV RNA levels among HDV-infected patients with other characteristics

	HDV RNA < 100 (copies/ml) N = 3 (%)	**HDV RNA Detectable N = 7 (%)**	***P***
HBV DNA (IU/ml)			.30
0 - 20 000	3 (100%)	5 (71.43%)	
> 20 000	0	2 (28.57%)	
HBeAg			.30
HBeAg+	0	2 (28.57%)	
HBeAg-	3 (100%)	5 (71.43%)	
HBV stage			.24
Low replicative hepatitis	2 (66.67%)	1(14.28%)	
Immune tolerance	0	1 (14.28%)	
Chronic active hepatitis	1(33.33%)	5 (71.43%)	
ALT / AST			<.05
Normal	3 (100%)	1 (14.28%)	
Raised	0	6 (85.71%)	

3.4 Clinical Characteristics of the Overall Population

i. Distribution of the study population according to clinical manifestation revealed the highest proportion of patients with low replicative hepatitis (61.72%) while 31.25% of patients presented with chronic active hepatitis and 7.03% were immune tolerant.
ii. There were 20/29 (68.97%) with chronic active hepatitis among HBV/HDV co-infection compared to 20/99 (20.20%) among HBV mono-infection (*P*<.001). Most patients presenting with low replicative hepatitis B were from HBV mono-infection population (Table 4).

3.5 Morphological Characteristics of the Overall Population

i. Only 60 (46.87%) of the 128 studied patients had hepatic fibrosis evaluated by biopsy and/or by Fibrotest®-Actitest®. Of these, 3(5.0%) had cirrhosis, 28 (46.67%) had no fibrosis and the rest had different stages of fibrosis.

   Liver ultrasound results were available for 93 patients (72.65%); 78 (83.87%) had no liver abnormalities, 7 (7.53%) had liver cirrhosis and 8 (8.60%) presented lesions suggestive of liver tumour.

ii. Regarding histopathological data, of the 60 patients who had their liver biopsies performed and/or Fibrotest®-Actitest®, 15 (25%) were HDV-positive and 45 (75%) were HDV-negative. Of the 15 HDV-positive patients, 80% had fibrosis ranging from moderate liver disease to cirrhosis against 33.33% in HDV-negative patients (*P* =.027) based on the METAVIR scoring system (Table 4).

Table 4. Clinical and morphological data with respect to HDV status

	HDV+ N (%)	HDV- N (%)	*P*
HBV stage			< .001
Low replicative hepatitis	7 (24.14%)	72 (72.72%)	
Immune tolerance	2 (6.90%)	7 (7.07%)	
Chronic active hepatitis	20 (68.96%)	20 (20.20%)	
Total	29	99	
Activity			.007
A0-A1	7 (46.67%)	37 (82.22%)	
A2-A3	8 (53.33%)	8 (17.78%)	
Fibrosis			.002
F0-F1	3 (20%)	30 (66.67%)	
F2-F4	12 (80%)	15 (33.33%)	
Total	15	45	
Ultrasound			<.001
Normal	10 (50%)	68 (93.15%)	
Cirrhosis	5 (25%)	2 (2.74%)	
Liver tumour	5 (25%)	3 (4.11%)	
Total	20	73	

Histologic activity on the other hand revealed that 53.33% of HDV-positive patients had moderate to severe necro-inflammatory activity against 17.78% in HDV-negative patients (*P* = .007).

Cirrhosis and liver tumour were more frequent in HDV co-infected patients compared to HBV mono-infected patients (*P*<.001).

4. DISCUSSION

HBV/HDV co-infection-related disease may cause severe liver diseases compared to HBV infection alone [15]. The natural history of HDV is characterized by different clinical courses. Some patients have no liver disease while in others there is rapid progressive fibrosis [16]. In this study, we report a higher proportion (22.66%) of patients co-infected with HBV and HDV compared to 17.6% reported in a recent study carried out among adults in Cameroon [13]. The higher prevalence obtained in this study is probably due to a selection bias in the tertiary care centres where most patients were at an advanced stage of disease progression.

Among the 128 subjects present in this study, 89.06% were HBeAg-negative as most of these patients were long term carriers of the disease. It has been noted that differences in HBV genotypes can affect the prevalence of HBeAg and the replication level of HBV [5]. However, a correlation between HBV genotypes and HBV replication still remains largely unknown. We found no significant association between age, gender, HBeAg and HDV status which is similar to findings by Ziaee and colleague. [17].

The suppression of HBV DNA levels by HDV is a well known phenomenon and was also noticed in this study [5,13,14,16]. There was a significant difference between the mean HBV DNA level in HDV co-infected patients compared to HBV mono-infected patients (P=.01). HBV replication is regulated by four promoters and two enhancers. HDV proteins inhibit HBV replication by trans-repressing its enhancers and by trans-activating the interferon-α inducible Myxovirus resistance protein A (MxA) [5,18]. ALT and AST are enzymes used as indicators to evaluate liver damage. The activity of both liver transaminases was higher in HDV co-infected patients than in HBV mono-infected patients (P<.001). Moreover, the majority of the HDV-positive patients showed evidence of chronic hepatitis (68.96%) suggesting that HDV is actively involved in the progression of liver disease [5,14].

A previous study carried out on HDV genotypes in Cameroon revealed that HDV genotype I was the most prevalent strain [13] and other studies showed that HDV genotype I was frequently associated with severe liver disease [19]. This could be a reason for the severe course of infection found in the HBV/HDV co-infected population. The presence of more chronic active hepatitis in the co-infected patients suggests that most of these patients acquired the infection as a superinfection on hepatitis B since the natural history of this infection shows that superinfection of HDV leads to progressive disease in approximately 80% of cases while co-infection evolves to chronicity only in a small number of patients [8,13]. This point illustrates the necessity of implementing preventive measures to increase awareness and to decrease the spread of this virus among HBV-positive patients mainly through education in order to reduce risk behaviours.

There was a significantly higher proportion of patients with severe liver damage and with high necro-inflammatory activity in HBV/HDV co-infection compared to HBV mono-infection (P<.05) based on the METAVIR scoring system as reported by Vasconcelos et al. [16]. Although the pathogenesis of HDV infection has still not been fully understood, it is considered the most severe of all hepatotropic viruses and it usually shows continuous necro-inflammatory activity that can be explained by direct cytopathic effect of the delta virus in the host cell or by an immune mediated mechanism [16]. Of the 93 patients who had liver ultrasound assessment, 50% of HDV-positive patients were found to have a normal liver ultrasound whereas in the HDV-negative population normal ultrasound was represented at 93.15%. There were significantly more patients with liver cirrhosis and liver tumour from the HBV/HDV co-infected population than in the HBV mono-infected group (P<.001).

A study carried out by Yamashiro et al. [20] showed that the level of HDV RNA was associated with the physiopathology of HDV infection but the limited number of HDV-positive patients who volunteered for RNA quantification in our study, would not allow reasonable conclusions to be drawn. The low rate of HDV quantification is due to poor accessibility of the test (carried out outside Cameroon) and its cost. However we observed more patients with chronic active hepatitis in patients with detectable HDV replication (71.43%) compared to those with undetectable levels of HDV RNA (33.33%). Moreover, raised levels of ALT and AST were noted only in those patients with detectable HDV RNA levels (P<.05).

5. CONCLUSION

We found that HBV/HDV co-infection results in suppression of HBV replication. HBV/HDV co-infected patients demonstrate a more severe sequelae of liver disease than HBV mono-infected patients. These findings illustrate the need to perform systematic screening of HDV at the onset of work-up in HBV-infected individuals for a better management of this disease. Vaccination against HBV and education to reduce risk behaviours could be put in place in

order to decrease the spread of this infection [21]. Further analysis of the distribution of different genotypes of HDV will be essential for a better characterization of the epidemiologic, virologic and pathogenic aspects of this infectious agent.

CONSENT

An informed consent was obtained from each of the study subjects for eligibility to participate in the project.

ETHICAL APPROVAL

The study protocol was reviewed for ethical considerations by the Ethics Committee of the Faculty of Medicine and Biomedical Sciences of the University of Yaounde I in Cameroon.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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