

Prevalence of Hepatitis D Virus among Hepatitis B positive Blood Donors in Port Harcourt, Nigeria

ABSTRACT

INTRODUCTION: The Prevalence of Hepatitis D Virus Among Blood Donors in Port Harcourt, Nigeria is a maiden epidemiological study of Hepatitis D or delta hepatitis among the donors' population in Port Harcourt, Rivers State. Hepatitis D (Hepatitis Delta) is a disease caused by the hepatitis D virus (HDV). It is considered to be a sub viral satellite because it can propagate only in the presence of the hepatitis B virus. The prevalence of HDV in Port Harcourt has not been reported; hence this study, bridge that knowledge gap.

METHODS: Using a qualitative cross sectional study design, a general serological screening test was performed on a total of 300 blood donors recruited; 222(74.00%) males and 78 (26.00%) females, all within 20-59years. Of this number, 86(28.70%) were positive for HBV while 214(71.30%) Hepatitis B negative served as control. Samples collected were analysed at blood bank unit of the Haematology Department of the Rivers State University Teaching Hospital, Port Harcourt (RSUTH). Hepatitis B surface antigen (HBsAg), and anti-hepatitis D antibodies (anti-HDV) for the presence of HBV and HDV infections were detected by one step Hepatitis B surface antigen (HBsAg) in serum, and enzyme linked immunosorbent assay for the detection of HDV as described by the www.elabScience.com (16).

RESULTS:

Of the 300 subjects, 86(28.6%) were positive for Hepatitis B surface antigen (HBsAg). Of these HBV positive subject, 9(10.4%) were positive for Hepatitis D virus (HDV). Age and gender of the study participants were not found to be risk factors for its prevalence ($p > 0.05$). There was no statistically significant difference in the PCV of those infected when compared with the non infected group. Using Pearson correlation analysis, HDV was not found to associate significantly with PCV ($r = 0.2849$, $p > 0.05$). This study recorded HDV prevalence rate of 10.4% among the HBsAg positive blood donors.

CONCLUSION AND IMPLICATIONS FOR TRANSLATION: There is a 10.4% prevalence of HDV among the HBsAg positive blood donors. To increase the safety level of blood products, the screening process should therefore be extended to the HDV.

KEY WORDS: Hepatitis B surface antigen (HBsAg), Hepatitis D virus (HDV), Prevalence, Co-infection, Super infection, Genome,

1. INTRODUCTION

1.1 Background of the Study

The study, Prevalence of Hepatitis D Virus Among Blood Donors in Port Harcourt, Nigeria is a maiden epidemiological study of Hepatitis D or delta hepatitis suspected to exist among blood donors' population in Port Harcourt, Rivers State. This study was conducted in the Blood bank of the haematology department of the Rivers State University Teaching Hospital, Port Harcourt. Hepatitis D is a liver disease which is transmitted through percutaneous or mucosal contact with infectious blood (1). It occurs in both acute and chronic forms. Although there are also other viruses such as, (i) cytomegalovirus (ii) Epstein-Barr virus (iii) Adenovirus (iv) Herpes simplex virus, which though do not infect the liver, yet cause hepatitis (2). Hepatitis D is caused by hepatitis delta virus (HDV), a defective RNA virus that requires the help of other viruses like hepatitis B virus (HBV) for its own replication (3). It is therefore tagged Delta antigen, having been taken to be a new protein enclosed by HBV (4). Subsequently, experiment with chimpanzees showed Hepatitis Delta antigen to be a structural part of a pathogen required for the replication of HBV infection (5). It was then placed into the genus – Deltavirus. Although some recent studies have suggested an ancient African radiation, the origins of this virus remain unknown (6).

The HD virion is composed of an outer lipoprotein envelope made of the surface antigen of the HBV (HBsAg) and an inner ribonucleoprotein structure in which the HDV genome resides. The HDV genome consists of a single stranded RNA which is folded as a rod-like structure through internal base-pairing. It is complexed with the only HDV-encoded. The HDAg can elicit a specific immune response in the infected host, consisting of antibodies of the IgM and IgG class (anti-HDV). In HDV infected individuals, the timing of appearance and level of HDV RNA,

HDAg, and anti-HDV in serum allow the three HDV-related clinical entities to be discriminated (7).

Three of the genome, (GI, GII and GIII) genotypes have so far been identified, although research has shown that there exist at least 8 genotypes of the Hepatitis D – Viral pathogen. Although the actual quantification of its infectious rate is yet to be generally agreed on, **hepatitis D** viral infection among hepatitis B carriers remain a global disease (3). Nevertheless, a recent systematic review proposes about 60 million infected of this disease globally (4). In 2015, the global prevalence of HDV among HBV carriers has Genotypes 2 and 4 in documentation in East Asia. Genotype 3 is documented to have occurred in South America and Genotypes 5, 6, 7, 8 have been found only in Africa (5). The Amazon basin and especially the low income regions of Asia and Africa has been found to have a very high endemicity eruptions of fulminant hepatitis D. while the North America, North Europe and Australia are noted for its low endemicity(8). Due to immigrants from these endemic HDV areas accounting for the larger proportion of cases from 2009 – 2011, the outstanding occurrence of chronic hepatitis D in HBV liver diseases in Western Europe is reported to fall between 4.5% and 10% (8). HDV prevalence is endemic about 9.2% positive cases is noted among 15.6% of HBV carriers (7). In Nigeria, there is record of 4.3% of HDV prevalence in patients with acute hepatitis and about 15% prevalence in those with chronic hepatitis, hepatocellular carcinoma and liver cirrhosis (7,9). The prevalence of HDV in Port Harcourt has not been reported amidst a prevailing percentage of HB infection in the state - a predisposing factor for HD infection, hence this study, to bridge this knowledge gap. HDV infection has become a global menace affecting about 15 – 20 million HBV positive individuals. It is the major cause of hepatitis in older children and adult and common in adults with its maximum prevalence geographically distributed in Africa, especially in Gabon (7, 23).

Although variable, the clinical course of HDV is typically more severe than that of the other hepatitis viruses. After an incubation period of 3-7 weeks, nonspecific clinical symptoms, including fatigue, lethargy, nausea, and anorexia, begin and last for about 3-7 days. Viral replication is usually diminished during this phase. Jaundice occurs in the next phase of

symptoms. Fatigue and nausea usually continue, and the serum bilirubin level becomes abnormal. At the same time, the infected person may have clay-colored stool and dark urine. This is evidence of the liver's diminished ability to excrete bilirubin (6).

Co-infection occurs when both HDV and HBV are contracted simultaneously and causes chronic HDV infections in less than 5% of co-infected patients. Although clinical symptoms disappear, fatigue and lethargy may persist for weeks or months. Superinfection occurs when chronic HBV carriers are infected with HDV. This leads to severe acute hepatitis and chronic Hepatitis D infection in 80% of the cases. Superinfection is associated with the fulminant form of viral hepatitis which is the most severe form of acute disease, characterized by hepatic encephalopathy that is manifested by changes in personality, disturbances in sleep, confusion, difficulty concentrating, and sometimes abnormal behavior and coma (7).

Clinically, it is the smallest virus affecting humans, being the major cause of liver cirrhosis and fulminant hepatitis (2). When compared with other hepatitis infections, HDV appears to have the greatest fatality rate, with a very high tendency to degenerate or progress into hepatocellular carcinoma and liver cirrhosis, (9). Super-infection with HDV likely causes more clinical impediment than a single infection with only HBV. HDV entry into the hepatic cells follows the same pattern as HBV. This is done by accessing this cell and recognizing its receptor through the N-terminal domain of the large HBsAg, having gained entrance into the hepatic cells through the NTCP bile transporter (9,10). This receptor binding site is of the amino acid residue 9-15 as shown by mutagenic mapping. The virus on gaining full access into the liver cells and through a signal in HDAg, shed off its coat and transfer its nucleocapsid to the nucleus (11). The virus then, through the aid of cellular RNA polymerases, replicates itself. This is because the nucleocapsid lacks an RNA polymerase used in viral genomic replication. Therefore, RNA polymerase II, I and III are utilized in the process of HDV replication (3). The cellular damage caused by HDV affects the liver mainly (12). A cell culture experiment has also revealed that, in acute HDV infection, there occur a degenerative change in the effected liver cells. This is evident through traces of inflammatory cells in the parenchyma of the liver, which continues as long as the hepatocellular damage persists. This degenerative change is also proved by the evidence of a shrunken eosinophilic cytoplasm and pyknotic nuclei (13). In the pathogenesis of

HDV infection, two expressions are noted: (i) Expression of a trace of the delta antigen by the infected liver cell is thought to be the cause of the direct cytoplasmic effect of the HDV, while (ii) the expression of considerable delta antigen, which is also said to confer no cytotoxic effect, is thought to be responsible for the HDV chronicity and undermined hepatocellular immunity, thus making it vulnerable to immune mediated damages (14).

Amount of HBsAg in circulation basically serve as key to assess duration of therapy, such HDV markers as IgM and IgG are scarcely found soon after therapy. The stage of liver cirrhosis however, is achieved using liver biopsy, especially in patients already screened positive for HDV RNA. Diagnosis of HDV is best done alongside with anti HBV antibodies, especially of the acute HBV infection which is detectable following an incubation period of 6weeks but no specific symptom (21). Important markers such as HBsAg and anti-HBV core antibodies (anti-HBc-IgM) are useful, in that, (i) negative HBsAg rule out the presence of acute HBV infection (ii) positive HBsAg shows an indication of HBV infection and (iii) anti HBc are often used when HBsAg are negative, hence a marker mostly used to monitor early HBV convalescence. Positive anti-HBc is therefore an indication for either current or resolved HBV infection, while negative anti-HBc is an indication for total absence of current HBV infection (14). Therefore, anti-HBc-IgM which shows positive for HBV infection is not often necessary for the diagnosis of active HBV-infection, since chronic HBV-carrier shows positive anti-HBc- IgM for years following convalescence (14,16). In the diagnosis to monitor ongoing HDV-infection, the use of reverse transcriptase-polymerase chain reaction (RT-PCR) is a better approach. This is because RT-PCR is capable of detecting and identifying up to 10 to 100 copies of the HDV genome in any HDV infected serum (14).

1.2 Specific Aims and Hypothesis

The study aimed at finding out if there is an existence of Hepatitis D viral infection among hepatitis B positive blood donors in Port Harcourt, and then, to determine the prevalence.

1.3 Justification

It is very conspicuous that the screening for HDV infection is omitted in the routine serological screening of blood donors in Port Harcourt. Moreover there is not also any research documentation to this effect among blood donors in Port Harcourt. This seems to justify the claim of Uchenna *et al.*, (2022), that though thought to exceed the global prevalence of 5% base on studies done in some regions of the country, the prevalence of HDV infection in Nigeria is not known (15, 22). Hence, this could be a novel research in the region of Port Harcourt, Nigeria. Also, as HBV is a predisposing factor for HDV, it is likely that those already diagnosed positive for HBV could be co-infected with HDV. This can render treatment for hepatitis in such donors ineffective if not detected, and such donors may also go down with liver cirrhosis even while under treatment for hepatitis. Consequently, this study serve as better tool for HBV treatment and prevention of liver cirrhosis, since HDV most easily leads to liver cirrhosis.

1.4 Objectives of the Study

This research conducted a rapid test for Hepatitis B surface antigens for the presence of HBV, enzymatically determined the presence of acute (current) Hepatitis B virus infection and tested for the presence of anti- Hepatitis D Virus for co-infection of HBV and HDV among blood donors in Port Harcourt.

2. METHODS

2.1 Study Variables

A qualitative cross sectional study design was employed in this research to achieve general serological screening test on 300 sampled males and females donors of 18 – 59 years age bracket between August 1st to December 31st 2022. The collected samples were analyzed for HbsAg and Anti-Hepatitis D Virus to detect HBV Infection using the HBsAg Dry Chemistry Technology and Immunoenzymatic method using ELISA **as described by the www.elabScience.com (16)**. The process of this research included all donors, all HBV infected donors while intended donors less than 20 years and above 59 years were excluded. Using the non-anticoagulated vacuum system, 4mls each of venous blood of the subjects were collected by the venipuncture method of sample collection and transported to the RSUTH's laboratory within 4 hours for analysis.

Using the immunoenzymatic method, and on fixing the plate containing micro wells coated with anti-HDV antibodies onto the cartridge. A test mode for ant-HDV was selected. On addition of 50ul of the test serum and following its 60 minutes incubation, the plate was immediately washed to remove all unbound serum before an additional 50ul of an anti- HDV conjugate. A second incubation for 15 minutes at 37⁰ C was done to allow the conjugate attach to the HDV in the serum and a second washing to remove all unbound conjugates. The TMB Substrate (as chromogenic substance) was added to cause a colour change with a target viral antigen. The plate was then incubated again to allow the chromogenic substance undergo a color change if the target antibodies were present. The result (intended) was finally displayed on a visual screen for the presence or absence of Hepatitis B or D viral infections.

Statistical Analysis

The percentage/frequency distribution, standard error of mean, Pearson correlation models were used at significance level of $P > 0.05$ using the SPSS statistical package.

3. RESULTS

3.1 Percentage Frequency Distribution of the Demographic Details (Number of Subjects, Gender and Age) of Hepatitis B Positive and Control Subjects of the Study Population

Table 1. show that a total of 300 blood donors were recruited into this research out of which 86 (28.67%) cases were diagnosed Hepatitis B positive and 214 (71.33%) cases were free of Hepatitis B infection. Among the 86 Hepatitis B positive individuals 72(24%) were males and 14(4.7%) were females. From the 214 Control Subjects 150(50 %) were males while 64(21.33%) were females. The age range of the participants in this study was 20-59years; among the HBV positive subjects there were 19 (22.10%), 46 (53.50%), 19 (22.10%) and 2 (2.30%) of 20-29, 30-39, 40-49 and 50-59 years age ranges respectively. Then among the Control subjects 62 (28.97%), 94 (43.93%), 52 (24.30%) and 6 (2.80%) of 20-29, 30-39, 40-49 and 50-59 years age ranges respectively.

**Table 1. Percentage Frequency Distribution of the Demographic Details
(Number of Subjects, Gender and Age) of Hepatitis B Positive and
Control Subjects of the Study Population**

Parameters	Hepatitis B Positive Subjects	Hepatitis B Negative Subjects
Number of Subjects	86 (28.67%)	214 (71.33%)
Gender		
Males	72 (24%)	150 (50%)
Females	14 (4.7%)	64 (21.33%)
Age (years)		
20-29	19 (22.10%)	62 (28.97%)
30-39	46 (53.50%)	94 (43.93%)
40-49	19 (22.10%)	52 (24.30%)
50-59	2 (2.33%)	6 (2.80%)

3.2 Percentage Frequency Distribution of Demographic Details ((Number of Subjects, Gender and Age) of Hepatitis D Positive Subjects among the Hepatitis B Positive Subjects of the Study Population

Table 2. show that, among the 86 Hepatitis B positive subjects 77 (89.53%) were negative for Hepatitis D While 9(10.47%) were detected and confirmed Hepatitis D positive; from the later population 8(88.89%) were males while 1(11.11%) was a female. The percentage frequency distribution of age ranges (20-29, 30-39, 40-49 and 50-59 years) among the Hepatitis D positive subjects were 1 (11.11%), 6 (66.67%), 2 (22.22%) and 0 (0.00%) respectively

Table 2. Demographic Association (Number of Subjects, Gender and Age) of Hepatitis D Status of the Study Population

Parameters	Hepatitis D Negative Subjects	Hepatitis D Positive Subjects
Number of Subjects	77(89.53%)	9 (10.47%)
Gender		
Males	64 (74.42%)	8 (9.30 %)
Females	13 (15.12%)	1 (1.17%)
Age (years)		
20-29	18 (23.38%)	1 (11.11%)
30-39	40 (51.95%)	6 (66.67%)
40-49	17 (22.08%)	2 (22.22%)
50-59	2 (2.60%)	0 (0.00%)

3.3 Association of Sex and Age Group, and HBsAg Status

Table 3 shows the association of sex and age Group, and HBsAg status. With a P-Value of $P = 0.940$, there was no statistical significance of age and sex on the infection and spread of hepatitis B viral infection.

Table 3. Association of Sex and Age Group, and HBsAg Status

Characteristic	HBsAg		Test Statistics	
	Negative	Positive	X ² (df)	P-Value
Sex				
<i>Female</i>	64 (29.91)	14 (16.28)		
<i>Male</i>	150 (70.09)	72 (83.72)	0.011 (1)	0.917 ^{ns}
Age Group (Years)				
<i>20-29</i>	62 (28.97)	19 (22.1)		
<i>30-39</i>	94 (43.93)	46 (53.50)		
<i>40-49</i>	52 (24.30)	19 (22.10)		
<i>50+</i>	6 (2.80)	2 (2.30)	0.415 (3)	0.940 ^{ns}

Note: Percentages may not add up to 100 due to rounding up; Frequency for each variable may vary due to nonresponses or missing values. ns=Not Significant at $P > 0.05$.

3.4 Association of Sex and Age Group, and Anti-HD Status

Table 4 shows the association of sex and age group, and anti-HD status. A P-Value of $P = 0.675$, showed no statistical significance of age and sex on the infection and spread of hepatitis D viral infection.

Table 4. Association of Sex and Age Group, and Anti-HD Status

Characteristic	Anti-HD		Test Statistics	
	Negative	Positive	X ² (df)	P-Value
Sex				
<i>Female</i>	13 (15.12)	1 (1.17)	0.259	0.611 ^{ns}
<i>Male</i>	64 (74.42)	8 (9.30)		
Age Group (Years)				
	18 (23.38)	1 (11.11)	1.530	0.675 ^{ns}
20-29	40 (51.95)	6 (66.67)		
30-39	17 (22.08)	2 (22.22)		
40-49	2 (2.60)	0 (0.00)		
50+				

Note: Percentages may not add up to 100 due to rounding up; Frequency for each variable may vary due to nonresponses or missing values. ns=Not Significant at $P > 0.05$.

3.5. Comparison of Mean \pm SEM of Weight and Packed Cells Volume(PCV)

Table 5 show a Comparison of Mean \pm SEM of Weight and Packed Cells Volume (PCV) of the Study population. With a P-Value of $P = 0.0001$ & $P = 0.01$, there is an observed specific statistical significance in the PCV status of the male subjects and the weight of subjects above the 50 years age group respectively, but there is no significance in the weight and PCV of both positive and negative subjects in the study population of both hepatitis B and D viral infection.

Table 5. Comparison of Mean \pm SEM of Weight and Packed Cells Volume(PCV)

Characteristic	N	Weight		PCV	
		Mean \pm SEM	p-value	Mean \pm SEM	p-value
Sex					
<i>Female</i>	78	69.67 \pm 1.24		39.74 \pm 0.18	
<i>Male</i>	222	74.00 \pm 0.80	0.005	44.71 \pm 0.20	0.0001
Age Group (Years)					
<i>20-29</i>	79	70.44 \pm 1.22		42.92 \pm 0.39	
<i>30-39</i>	140	73.21 \pm 1.01		43.09 \pm 0.31	
<i>40-49</i>	73	73.49 \pm 1.34		44.47 \pm 0.34	
<i>50+</i>	8	85.25 \pm 5.42	0.006	44.75 \pm 0.73	0.011
HBsAg					
<i>Negative</i>	214	73.24 \pm 0.80		43.45 \pm 0.23	
<i>Positive</i>	86	71.97 \pm 1.03	0.399 ^{ns}	43.35 \pm 0.37	0.812 ^{ns}
Anti-HD					
<i>Negative</i>	291	72.99 \pm 0.70		43.45 \pm 0.20	
<i>Positive</i>	9	69.00 \pm 3.07	0.318 ^{ns}	42.67 \pm 1.35	0.502 ^{ns}

Note: Percentages may not add up to 100 due to rounding up; Frequency for each variable may vary due to nonresponses or missing values. ns=Not Significant at $P > 0.05$.

3. 6 Correlation Analysis of Measured Parameters by HBsAg and anti-HDV Status

Table 6 shows a positive Pearson correlation of hepatitis B surface negative and blood donors with a non-significant correlation for weight, $r = 0.070$ & $r = 0.489$ and PCV, $r = 0.1205$ & $r = 0.2408$ respectively. Also, a positive Pearson correlation for hepatitis D virus negative and positive blood donors with a non-significant correlation for weight, $r = 0.1613$ & $r = 0.4937$ and PCV, $r = 0.0228$ & $r = 0.1849$ respectively.

Table 6. Correlation Analysis of Measured Parameters by HBsAg and anti-HDV Status

Characteristic	N	Weight		PCV	
		R	p-value	r	p-value
HBsAg					
<i>Negative</i>	214	0.070		0.1205	
<i>Positive</i>	86	0.489	0.399 ^{ns}	0.2408	0.812 ^{ns}
Anti-HD					
<i>Negative</i>	291	0.1613		0.0228	
<i>Positive</i>	9	0.4937	0.318 ^{ns}	0.1849	0.502 ^{ns}

4. DISCUSSION

Blood is the body fluid considered to be most vital to the survival of human and the proper functioning of all the body cells, organs, tissues, and systems. Therefore, Clinical remedy for shortage of blood diagnosed by its low Hemoglobin (Hb), or packed cell volume (PCV) requires a transfusion of blood from healthy donors. Such donors are considered healthy if they are screened free of blood transmissible infections such as HIV, Hepatitis strains (Hepatitis A, B, C, D, & E), etc. However, for donors' safety, this study has observed a gap - the prevalence of HDV - in the screening of donors. Hence, this cross sectional study.

The association of sex with HBV and HDV infection with P-Value of $P = 0.611$ and 0.917 respectively, shows that sex is not a determinant of these infections. The 72%, a far greater percentage of the male sex, infected by this HBV than the females of just 26% is no guaranteed that male are prone to the disease than female. Rather, that male show more readiness to blood donation than female. Again, most of the female are simply differed from blood donation either been found to be in the peak, just starting or ending their menstrual cycles, while others are either pregnant or in lactation. This conclusion agrees with panelLeila *et al.*, (2021), which in their research on Reasons of under-representation of Iranian women in blood donation argued that, although women considered blood donation as a positive act, but only about 18.7% of women are willing to donate blood (17).

Although the results of this study showed the highest percentage participation of 53.50% by donors of 30-39 years age bracket, the association of age with HBV and HDV infection with P-Value of $P = 0.675$ and 0.940 respectively shows no effect of age on the infection and prevalence of the hepatitis B and D viral infections. This conclusion validates the claims of [Malewe Kolou *et al.*, 2017](#) that, the HBV and HDV affect all women and men irrespective of age, though with high Prevalence among those of 20-39 age range (18). This could be as a result of the youth active age of responsibility and self determination to donate blood without parents or peer pressure.

With a p-value of $P < 0.05$, the result shows a statistical significance in the weight and PCV of the study subjects, a p-value of 0.318 and 0.502 for weight and PCV of the study subjects showed neither weight nor PCV of the participants to be of any significance on their infection with HBV and HDV infection. Since this infections affects the blood cells, the weight and blood level of the individuals appears to have no influence on its infection, so long as the individuals have evidence of living tissues to host the virus.

This current study shows a low percentage (28.67%) diagnosed Hepatitis B positive cases of the study population with far higher (71.33%) cases of the study subjects free of Hepatitis B infection. This percentage of detected positive cases might be as a result of insensitive rapid screening methods that failed to capture this 28.67% as positive cases. On the other hands, it could be as a result of new infection of this population due to their contacts with the infected population. This percentage was compared with the World Health Organization study to this effect, which affirmed that HBV infection affects more than 5% of the local population in sub-Saharan Africa, with more than 8% in West Africa and reaching up to 15% in some areas (19).

A comparison of this study with the global prevalence of HDV showed that out of 86 Hepatitis B positive subjects, only 10.47% showed Hepatitis D positive. While similar study on the global estimation an anti-HDV prevalence by Alexander *et al.*, (2020) showed a 4.5% positive among HBsAg-positive people. This differences may be traced to the differences in the geographical distribution of the study subjects (20).

5. CONCLUSION AND IMPLICATIONS FOR TRANSLATION

A 10.47% Hepatitis D positive from the study population of this research showed a low prevalence of Hepatitis D Viral infection in Port Harcourt, an tend to manifest adult than teenagers.

Ethical Approval

Ethical approval for of this research was obtained from the Rivers State Research and Ethics Committee for the full analysis in the blood bank unit of the Haematology Department of the Rivers State University Teaching Hospital (RSUTH), Port Harcourt.

Limitations

This study was greatly challenged with financial constraints.

Recommendation for further studies

Individual who are infected with Hepatitis B could also be co-infected with HDV therefore, all donors positive for HBV should be screened for HDV (ii) Since the disease seems to be asymptomatic from its onset, government and NGOs should embark on massive screening for Hepatitis D to advert its development, spread and chronicity. (iii) This study should be extended to other citizens outside the donors group.

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