

Molecular Detection of Parvovirus B19 in Positive Hepatitis B Haemodialysis Patients in Khartoum State, Sudan

Hadeel H E Lmahal^{1*}, Khalid A Enan², Abdel Rahim M. El Hussein², Isam M Elkhidir⁴,
Aymen Abdelhaleem^{1,3}

¹Department of Microbiology, Faculty of Medical Laboratory Sciences El Nellin University, Sudan

²Department of Virology, Central Laboratory, The Ministry of Higher Education and Scientific Research, Khartoum, Sudan

³Medical research center, Jazan University, Jazan Saudi Arabia

⁴Department of Microbiology and Parasitology, Faculty of Medicine, University of Khartoum, P.O. Box: 8067, Khartoum, Sudan

*Corresponding Author: Hadeel H ELMahal, Department of Microbiology, Faculty of Medical Laboratory Sciences El Nellin University, E-mail: betmlookalneelps@gmail.com

ABSTRACT

Background: Human parvovirus B19 has been identified in the serum of hepatitis B virus infected patient. It mostly cause erythema infectiosum in children but can also cause hydrops fetalis, arthropathies and transient aplastic crisis in adults.

Methods: The aim of this study was to determine the prevalence of B19-DNA in positive hepatitis B haemodialysis infected Sudanese patients. Serum samples from 50 haemodialysis HBV infected patients and 34 healthy donations were collected and analyzed for the presence of B19-DNA by nested PCR.

Result: 34 (11%) of healthy donors showed B19-DNA in nested PCR while 50 HBV haemodialysis patient showed the infection.

Conclusion: Our findings suggest that human parvovirus B19 had higher prevalence among hepatitis B virus infected patients. Further investigation is needed to clarify if such infections can seriously affect patient health.

Keywords: Parvovirus B19, PCR, Sudan

I. INTRODUCTION

Parvovirus B19 was discovered in 1979 as the first human pathogen in the family parvoviridae, genus Erythro parvovirus. The virus was discovered by Cossart and colleagues during screening of normal blood bank donors' sera for hepatitis antigen. ⁽¹⁾⁽²⁾

B19 is a single stranded DNA virus that measures about 23-26 nm in diameter ⁽³⁾ the virus is non enveloped with an icosahedral capsid. The genome routes comprises a unique region that contain all coding sequence and flanked by two repeated inverted terminal region. The presence of self complementation in terminal regions allow the DNA molecules to adopt terminal loops. ⁽⁴⁾

The B19 virus causes fifth disease which is featured by high fever, malaise, headache, nausea, diarrhea and rash (slapped cheek syndrome). Once the characteristic rash appears the patient are usually no longer infectious ⁽⁵⁾

The virus can cause chronic anemia in HIV patient ⁽⁶⁾ also the infection is more serious in patient with pre existing bone marrow stress. ⁽⁷⁾⁽⁸⁾ and during pregnancy the infection with B19 virus may cause hydrops fetalis. ⁽⁹⁾ Furthermore the infection of B19 can be associated with anti double stranded DNA antibody, anticardiolipin, antineutrophil cytoplasmic antigen, ⁽¹⁰⁾ rheumatoid arthritis ⁽¹¹⁾ and systemic lupus erythematosus. ⁽¹²⁾

The B19 is also implicated in causing different type of acute hepatitis. (18) (19) fulminate liver failure of unknown etiology, (20) (21) Co-infection between B19 and hepatitis B and C (22) can lead to more severe HBV associated liver disease.(23)

Transmission of virus is usually by respiratory routes⁽¹³⁾ vertical transmission from mother to fetus, blood transfusion⁽¹⁴⁾⁽¹⁵⁾ and organ transplantation.⁽¹⁶⁾ B19 infection can be diagnosed by using enzyme linked immune assay⁽¹⁷⁾ radioimmune assay or immune fluorescence test and polymerase chain reaction (PCR).

II. METHODS AND MATERIAL

Ethical Approval

Ethical approval was obtained from Alneelain University and informed consent were obtained regarding data and blood samples collection only from patient who agreed to participate in this study.

SAMPLE Processing

A total of 84 blood samples was included in this study, fifty samples were collected from hemodialysis patients in Khartoum state hospitals (Dr.Salma Center for transplantation and hemodialysis, Bahri teaching hospital, and Bshaier teaching hospital) confirmed positive by ELISA for hepatitis B surface antigen (HBsAg). In addition,34 samples were collected from healthy blood donors at central blood bank in Khartoum. Collected blood samples were centrifuged at 5000 r.p.m for 5 min , separated serum was stored at -80 °c until tested by PCR.

DNA Extraction

DNA was extracted from 100 ul of serum by using viral DNA extraction kit (Roch diagnostic Germany) according to the manufactures' protocol.

Nested PCR

B19 viral DNA PCR amplification was performed in total volume of a 25ul in the first PCR reaction, containing 5ul of DNA mixed with 2 ul (10 pmol) of each primers Outer primers: forward p1(5' AAT ACA CTG TGG TTT TAT GGG CCG 3') and reverse p6 (5'

CCA TTG CTG GTT ATA ACC ACA GGT 3') nucleotide sequences 1399-1422 and 1682-1659 respectively. 0.5 ul of 2Mm dNTPs mix, 0.75 ul of 25 Mm MgCl₂, 0.25 ul 2.5 U Taq DNA polymerase (Roche Diagnostic, Germany) , 2.5 ul 5X buffer and 14 ul DW. The amplification was done by 35 cycles of PCR reaction (denaturation at 94°C for 45 sec, annealing at 55°C for 60 sec, and extension at 70°C for 90 sec.) the second round of the nested PCR was done by 2 ul PCR product of the first round using 10 pmol of each primers Inner primers; forward p2 (5' CCA TTG CTG GTT ATA ACC ACA GGT 3') and reverse p5(5' CTA AAA ATG GCT TTT GCA GCT TCT AC 3') nucleotide sequences 1498-1525 and 1600-1576, respectively using PCR conditions (denaturation at 94°C for 45 sec, annealing at 55°C for 60 sec and extension at 72°C for 90 sec). The amplicons were resolved and screened using 1.5% agarose gel electrophoresis methods. Positive reaction was confirmed by the present of 185bp amplicons (Figure 1).

III. RESULTS

Prevalence of B19-DNA in total serum samples was 18% (N84), in positive hepatitis B hemodialysis patient was 22% (N50), while 12% in healthy control (N34) (table 1). The occurrence of B19 in hemodialysis hepatitis B patient, when compared with the control was insignificant ($p > 0.05$).

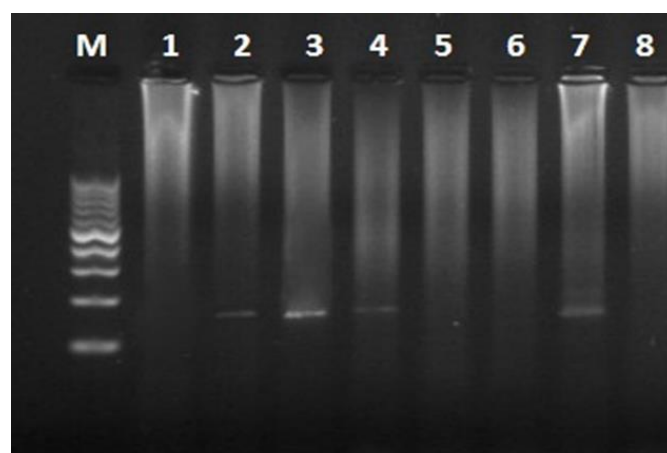


Figure 1: M: ladder 100bp, lane 1: Negative control sample, lanes: 2, 3, 4, 7: showed positive samples (185bp, lanes: 5, 6, 8 showed negative samples.

Table (1): Prevalence of B19 DNA in HBV (+ve) haemodialysis patients and in healthy blood donation in Khartoum state Sudan.

Subjects	Total number	B19+ve	B19-ve
HBV patients	50(59.5%)	11(22%)	39(1%)
Blood donations	34 (40.5%)	4(12%)	30(88%)
Total	84(100%)	15(18%)	69(82%)

IV. DISCUSSION

The human parvovirus B19 infection is now considered a serious disease and not just the mild rash experienced during childhood, because complications can occur especially among immune compromised individuals.

Monetary evidence suggests that parvovirus B19 may be a cause of hepatitis. However analyzed showed the present of active B19 infection in one patient with hepatitis among 129 cases analyzed with non A-E hepatitis. (24) but other reports suggested pathogenic role for parvovirus B19 in the development of acute hepatitis (25) (26) and fulminate liver failure of unknown etiology (27) (28). B19 DNA was detected in 37% of patient with HBV and 23.4% of HCV but not in 53 serum samples from normal patient that occurrence of liver dysfunction was not affected by B19 infection in patient with HBV and HCV($p > 0.05$) (29), In the end stage of liver tissues B19V DNA was detected in 74% and 44% from routine biopsy samples ($P < 0.05$) in healthy anti B19V positive individual 16% and patient with chronic hepatitis C 23% there is no evidence that B19 is hepatitis virus in European patient with chronic hepatitis C (30), but parvovirus B19 related hepatitis may occur in 4.1% of patient according to Mihály et al study (31)

The epidemiological profiles and association with HBV co-infection were analyzed of 1626 blood samples, 138 (8.49%) were found to human parvovirus including 3.51% with B19, 3.75% with HBV furthermore HBV was more prevalence in male 4.9% than female 1.4% there is no significant correlation between HBV and human parvovirus in serum samples from Chinese (32) also

B19V DNA was detected in 11% of patient with HBV and 8% of patient with HBV/HCV co-infection and 9% healthy control, B19 DNA was significantly higher in symptomatic HBV 20% than asymptomatic HBV 7% ($P < 0.05$) the result of study that B19 infection increase the frequency of liver disease was not supported (33)

In our study showed the presence of B19 among hepatitis B patient was insignificant this similar to study done in China (32) although high prevalence (22%) of B19 DNA was found among HBV patient this prevalence was not significantly different from that (12%) found in the healthy blood donors controls. To ever, in this study we were not concerned with the clinical parameters of our test group, clear look at such data is required to assess the impact if B19 co-infection on the severity of HB in Sudanese patients.

V. CONCLUSION

Our findings suggest that human parvovirus B19 had higher prevalence among hepatitis B virus infected patient. Further investigation is needed to clarify if such infections can seriously affect patient health

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