Analysis of hepatitis C virus infection among sickle cell anemia patients by an antigen-antibody combination assay

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Abstract

Hepatitis C virus (HCV) has a major impact on public health. In spite of the progress made in the prevention of transfusion-transmitted infections over the last years, these still occur, especially in multi-transfused patients such as sickle cell anemia patients. Sickle cell disease (SCD) is highly prevalent in Eastern Saudi Arabia. Little is known about the prevalence of HCV in Saudi sickle cell disease patients. The present study aimed to assess HCV and HBV antigens, antibodies and viral genome among sickle cell anemia patients in a tertiary hospital in Eastern Saudi Arabia. Methods used included measurement of HCV antigen and antibodies using the novel HCV antigen/antibody combination assay, assessment of HCV core antigen and measurement of viral genome using standard commercial kits. Of the 138 sickle cell disease samples tested, 5 (3.6%) samples gave positive results. Their hemoglobin ranged between 7.8 and 10.1 g/dL, their erythrocyte count ranged between 3.1×10^6 and 3.9×10^6 . Out of these 5 samples, 4 were also positive by the HCV Core Ag assay and by the HCV RNA PCR test (80%). None of the control group was positive. Seven patients were positive for HBsAg, and this indicates chronic carrier state. Improving the testing for blood-borne infections such as HCV and HBV will result in better control of these infections in sickle cell disease patients which will inevitably lead to lower mortality and morbidity in this group of patients.

Introduction

Hepatitis C virus (HCV) is a small enveloped positive-strand RNA flavivirus. With an estimated 170 million infected individuals, HCV has a major impact on public health.¹ HCV is primarily transmitted parenterally. Recreational injection drug use (IDU) has been and continues to be the dominant mode of HCV

acquisition.² Risk of HCV transmission through needle stick injury is in the order of 1-3%, compared with 30% for hepatitis B virus and 0.3% for HIV.²⁻⁴

The initial laboratory test is usually an enzyme immunoassay (EIA) for HCV antibodies (anti-HCV). The 3rd generation anti-HCV EIA has a sensitivity of 95-99% and can detect HCV antibodies 6-8 weeks after exposure.¹ Polymerase chain reaction (PCR) methods detect the presence of HCV RNA much earlier, at 1-3 weeks after exposure.

More recently, HCV antigen assays have become available which demonstrate that HCV core antigen detection can significantly reduce the window period prior to detection of antibody.⁵⁻⁷ Studies have shown that the average time from the first viremic bleed to the first HCV antigen (Ag) positive bleed is estimated at 2.0 days and the average time to the first HCV antibody (Ab) positive bleed at 50.8 days.³

One of the newest assays, the HCV Ag/Ab combination assay, combines the detection of anti-HCV antibodies with the detection of core antigen in a single assay, significantly reducing the window period from infection to detection compared with conventional serological HCV antibody screening assays.⁸

In spite of the progress made in the prevention of transfusion-transmitted infections over the last years, these still occur especially in multi-transfused patients such as sickle cell anemia patients.⁹⁻¹¹ Many patients require chronic transfusions and as a result, substantial proportions of sickle cell patients are at high risk for infection with blood-borne diseases, such as HCV and hepatitis B virus (HBV) infections.¹²⁻¹⁵

The sickle cell gene is common in certain countries. Sickle cell disease (SCD) is highly prevalent in Eastern Saudi Arabia. People who are homozygous (HbSS) have severe disease and life-long anemia.⁹ The Hb S gene frequency range was reported to be 0.005-0.145 in various areas of Saudi Arabia.¹⁶ A prevalence of sickle trait of 5.7% was previously reported.¹⁷

Little is known about the prevalence of HCV in Saudi sickle cell disease patients.¹⁸⁻¹⁹ The present study was aimed to assess HCV and HBV antigens, antibodies and viral genome among sickle cell anemia patients in a tertiary hospital in Eastern Saudi Arabia.

Materials and Methods

Patients

The study used the samples from a previous study.²⁰ The study was conducted in a tertiary hospital in Eastern Saudi Arabia over a period of one year (2009-2010). All sickle cell anemia

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patients attending the hematology clinics of the hospital were included in the study (n=138). Sickle cell disease was confirmed by high performance liquid chromatography (HPLC). Age of the patients included in the study (n=138) ranged from six months to 61 years (mean 28 years). Both sexes were included (77 males and 61 females). Blood donors were used as control group (n=56). They included 50 males and 6 females with a mean age of 29 years.

Clinical assessment (history and physical examination) was made for all patients. Sickle cell anemia patients were chosen for laboratory assessment of HCV and HBV antigen, antibody and viral genome. Erythrocyte count, reticulocyte count, sickling test and hemoglobin electrophoresis were also performed. Written informed consent was obtained from all individuals included in this study or their parents.

Techniques

Detection of anti-HCV antibodies

Anti-HCV antibodies were assayed by a 3rd generation enzyme-linked immunosorbent assay [AxSYM HCV version 3.0 (Abbott Diagnostics, Chicago, IL, USA)] and HCV 3.0 ELISA test system (Ortho-Clinical Diagnostics, Raritan, NJ, USA). In addition, the recombinant immunoblot assay (RIBA HCV 3.0; Ortho-Clinical Diagnostics) was used as previously described and in accordance with the manufacturer's instructions.⁶⁻⁷

Detection of HCV core Ag HCV

HCV core Ag assay (Ortho-Clinical Diagnostics) was used (including the neutralization protocol) according to the manufacturer's recommendations and as described previously.⁶⁻⁷

Simultaneous detection of HCV antibodies and antigen (HCV Ag/Ab combination assay)

Simultaneous detection of HCV antigen and HCV antibodies was performed by Murex HCVAg/Ab combination assay (Abbott Murex). Briefly, test specimens and control sera were placed in the wells and incubated for 60 min to allow the binding of HCV core antigen and HCV antibodies. After washing, peroxidase labeled conjugate was added and the wells were incubated for 60 min. Unbound conjugate was washed and the substrate was incubated for 30 min. Thereafter, the reaction was stopped and the plate was read at 450 nm.⁸

HCV RNA assays (qualitative)

To detect HCV RNA, the COBAS AMPLICOR HCV test version 2.0 (Roche Molecular Systems, Branchburg, NJ, USA) was used as described previously and according to the manufacturer's instructions. This assay has a limit of detection of 2.0 log 10 IU of HCV genotype 1 RNA per mL.

HBsAg, HBs antibodies and HB core antibody total were carried out on an Abbott AxSym machine, using microparticle enzyme immunoassays (MEIA).

Results

The age of the patients included in the study (n=138) ranged from six months to 61 years (mean 28 years). The patients were seen in the hematology clinic for routine follow up. CBC and sickling test positive was performed for all patients. All patients included in the study had sickle cell disease, as confirmed by HPLC.

Analysis of HCV antigen and antibody among our study group (n=138) was made by antigen-antibody (Ag-Ab) combination assay. Of the 138 sickle cell disease samples tested, 5 (3.6%) samples gave positive results (named thereafter samples 1, 2, 3, 4 and 5). Their hemoglobin ranged between 7.8 and 10.1 g/dL, their erythrocyte count ranged between 3.1×10^6 and 3.9×10^6 (Table 1). Out of these 5 samples, 4 were also positive by the HCV Core Ag assay (samples 1-4). All 5 samples positive by HCV Ag-Ab combination assay (samples 1-5) were tested for HCV RNA. These samples were found to be positive by the HCV RNA assay (80%; none of the control group was positive) (Table 2).

Of the 138 sickle cell patients, 7 patients were positive for HBs antibodies. One sample was positive for HBsAg (0.7%).

Discussion

Simultaneous detection of antigen and antibody in the same sample helps to narrow the window period. This is defined as the period from acquiring the infection until the detection of the organism. Narrowing the window period will help accelerate patient management, and promote safer blood transfusions and better control of the disease.

Four of the 5 samples positive by the HCV Ag-Ab assay were found to be positive by the HCV Ag assay and the HCV RNA assay. One sample was positive for HCV Ag Ab and has no antigenemia or viremia. This patient may have cleared the virus from the circulation and the only remaining evidence for the HCV infection is the HCV antibodies. The good correlation between the HCV combination assay and the antigen testing and the nucleic acids testing (NAT) may lead to the replacement of the NAT by HCV Ag assay or the new HCV Ag-Ab combination assay. NAT is expensive, needs specialized set up within the hospital laboratories and requires experienced molecular technologists.²¹ On the other hand, the HCV combination assay is based on serological techniques that are easy to perform, less expensive, and do not require a special location or specialized technologist.

Patients who should be tested for HCV RNA include those whose anti-HCV EIA result was inconclusive: immunocompromised patients (e.g. those with HIV or who are undergoing hemodialysis) who may not generate antibodies to infection; patients who are thought to be in a period of acute infection, when the PCR

test result will be positive but antibodies have not yet developed; and patients with a positive anti-HCV test result but persistently normal alanine transferase levels. A significant minority of patients who have HCV antibodies and persistently normal liver chemistry test results (especially if alanine transferase values are in the lower half of the normal range) have spontaneously cleared their acute HCV infection but will continue to have detectable HCV antibodies for an indefinite period. The Murex Ag/Ab HCV Combination Assay is an enzyme immunoassay. Wells are coated with anti-core monoclonal antibody, and with recombinant antigen and peptides representing the immunodominant regions of NS3 and core viral antigens. Hence both HCV core Ag and HCV antibodies are detected simultaneously.⁸

The HCV antibody positivity is directly related to the number of transfusions given, and on average the prevalence rate in transfused patients is more than 10%. The prevalence of HCV in our samples was lower than the worldwide average of 10%. This could well be due to the better screening of blood donors in Saudi Arabia. It is known that the combination of iron overload and HCV can lead to a more rapidly progressive liver disease. The treatment of HCV in sickle cell patients poses a challenge to clinicians. A novel approach described by some is the pre-treatment of these patients with hydroxyurea to increase the fetal hemoglobin, therefore decreasing the severity of ribavirin-related hemolysis. Treatment with Peg-interferon alone has not been used to treat HCV in sickle cell patients, but in the setting of controlled clinical trials it would be feasible.

Little is known about the prevalence of HCV

Table 1. Hematologic data for the 5 HCV-infected sickle-cell anemia patient (n=5).

Patient	Gender/age	Hemoglobin concentrations g/dL (normal value)	Erythrocyte count millions/ul (normal value)	Reticulocyte % (normal value)
1	F/35 Y	9.7 (13-18)	3.7 (4.5-6.5)	2.5 (0.5-2)
2	F/11 Y	8.4 (11-15)	3.5 (4-5.6)	3 (0.5-2)
3	F/37	8.5 (13-18)	3.59 (4.5-6.5)	0.9 (0.7-2.8)
4	M/26 Y	7.8 (11.5-16.5)	3.1 (4.5-6.5)	0.5 (0.7-2.8)
5	M/22 Y	10.1 (11.5-16.5)	3.9 (4.5-6.5)	0.4 (0.7-2.8)

Table 2. Pattern of reactivity sickle cell disease patients with HCV antigen, antibody and viral RNA.

Group tested	HCV Ag-Ab combination N.(%) positive	HCV Ag N. (%) positive	HCV Viral RNA N. (%) positive
Sickle cell anemia N=138	4(2.89%)	4 (80)	4 (80)
Control N=56	0	0	0

in Saudi sickle cell disease. Al-Fawaz *et al.*²² found that HCVAb positivity was significantly higher in patients with sickle cell anemia (16.0%) and in thalassemics (57.1%) than in the respective control groups. Bahakim *et al.*¹⁸ reported that HCV antibodies were detected in 33.3% of patients with thalassemia and sickle cell disease and in 5.3% of controls. None of these studies assessed the presence of HCV antigenemia in Saudi sickle cell disease patients.

Seven patients were positive for HBs antibodies, which may indicate past infection or immunization. One sample was positive for HBsAg, and this indicates chronic carrier state. The low prevalence of HBV among Saudi sickle cell disease patients is likely to be due to the routine vaccination program for HBV. The HBV recombinant vaccine is given to all children in Saudi Arabia.

Improving the testing for blood-borne infections such as HCV and HBV will result in better control of these infections in sickle cell disease patients which will inevitably lead to lower mortality and morbidity in this group of patients.

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