PREVALENCE OF HEPATITIS B,C AND HIV AMONG SICKLE CELL DISEASE PATIENTS IN HODEIDAH CITY, YEMEN.

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(Received on Date: 16 December 2017  Date of Acceptance: 20 January 2018)

ABSTRACT

The purpose of this study is to assess the knowledge level of Hepatitis B virus (HBV) and Hepatitis C virus (HCV) and human immunodeficiency virus antibodies (anti-HIV) infection in sickle cell anemia patients (SCA) receiving multiple blood transfusions in Hodeidah city, Republic Yemen. A total of 121 blood samples were collected from patients attending to the medical department at Yemeni association for patients with sickle cell anemia and heredity blood, at AL-Salkana hospital in Al-Hodeidah city, between March to September 2016. Screening was carried out by rapid qualitative serologic (RTDs) and enzyme linking immunoassay tests (ELISA). Rapid qualitative serologic tests are used for detected viral hepatitis B surface antigen (HBsAg), Anti-hepatitis C virus (anti-HCV). Enzyme linking immunoassay tests (ELISA) for detected viral hepatitis B surface antigen (HBsAg), Anti-hepatitis C virus (anti-HCV) and human immunodeficiency virus antibodies (anti-HIV). Of the 121 patients participants tested by rapid qualitative serologic tests 1 (0.8%) male and 3 (2.5%) female was positive by Hepatitis B virus surface antigen (HBsAg) and 1 (1%) male was positive by HCV (anti-HCV). Screening by used ELISA test all specimen for Hepatitis B virus (HBV) and Hepatitis C virus (HCV) and human immunodeficiency virus antibodies (anti-HIV) was negative (0%). In conclusion: the prevalence of HBV, HCV and HIV infection in sickle cell anemia patients is significantly lower than that observed in normal individuals. The results of our study highlights the need for contours regular screening for HBV, HCV and HIV among sickle cell disease patients. Keywords: HBV, HCV and HIV, Sickle Cell Disease, Incidence, Prevalence

No: of Tables: 3  No: of Figures: 1  No: of References: 37
INTRODUCTION
Sickle cell disease (SCD) is one of the most common inherited blood anaemias [1]. It occurs when a person inherits two abnormal genes for haemoglobin β, one of which codes for haemoglobin S (HbS) that is poorly soluble when deoxygenated [2]. The polymerization of deoxy HbS is essential to vasoocclusive phenomena. Fetal hemoglobin (HbF) is a major modulator of polymerization in that the higher the HbF levels, the more benign the clinical and hematologic features of sickle cell anemia [3]. Subsequent changes in red cell membrane structure and function, disordered cell volume control, and increased adherence to vascular endothelium also play an important role [4].

Transfusion is usually performed when haemoglobin is less than five g/dL. Patients with SCD will have haemoglobin levels as low as six g/dL in their steady state. The sickle cell disease is a genetic disorder that is characterized by a chronic Anaemia occurring almost exclusively in individuals of African descent [5]. Because patients with Sickle Cell disease lack enough red blood cells for life sustenance they require frequent blood donation. They are therefore susceptible to blood borne infections such as hepatitis B, hepatitis C and HIV [6&7]. Viral hepatitis occurs at a variable prevalence in different countries of the world [8]. The tropical countries are regarded as ranging between the middle and high prevalence areas. Viral hepatitis in sickle cell haemoglobinopathy has been found recently to be an important observation. Although SCD patients are frequently transfused, and therefore represent a high-risk population for hepatitis C and B virus infection (HCV), the prevalence of HCV, has rarely been reported [9-12]. HCV is known to be an important cause of liver disease in SCD patients [13]. In addition, HCV has been recently suspected to be a cardiovascular risk factor for the development of carotid atherosclerosis, heart failure and stroke [14&15]. The prevalence of chronic viral hepatitis is increased in patients with SCD compared with the general population. In addition, patients with SCD may be more susceptible to developing severe disease when acutely infected [16]. The devastating effect of the HIV on the immune system has led to the successful establishment of other opportunistic infections and there are several diseases which are generally not harmful but have been able to cause fatality due to the destruction of the immune system by HIV [17]. Also the effect of HIV on people with genetic defect condition such as Sickle Cell Anaemia has been documented [18]. However, studies of the prevalence and the effect of HIV on Sickle Cell Anemia subjects have not been well elucidated. A few studies conducted suggested that HIV infection progresses slowly in patients with sickle cell disease (SCD) [19]. Although some studies had been carried out on Sickle Cell diseases and the relationship with hepatitis in development countries but documentation on the prevalence of HCV, HBV and HIV among Sickle Cell patients in Yemen country not far taken. This study aims to determine Prevalence of hepatitis B, C and HIV among sickle cell
disease (SCD) patients in Hodeidah city, Republic of Yemen.

Material and methods

Study area:

Hodeidah Governorate is the fourth largest Governorate in Yemen in the term of population which are (2157552), male: female ratio is 1:1, and children below 15 years are 50% of population. It is in a tropical zone, and the weather is typically hot and humid, summer months of April to November are very hot with temperatures sometimes exceeding 38 to 40° C, during the rest of the year temperature range between 27-35° C. It is located on western a flat and narrow coastal plain between the foothills of the highlands and the Red Sea. Most of Hodeidah population is underline of poverty, almost 22% of people living in urban areas. It was estimated that 38% of adults were illiterate, 40% of households had no access to sanitary services.

Study disgenand data collection:

This is a cross-sectional study was conducted from March 2014 to August 2016, performed on patients with sickle cell anemia (age range, 1 to 31 years) attending the medical department at Yemeni association for patients with sickle cell anemia and heredity blood at AL-salkana hospital in Al-Hodeidah city, Yemen.A structured questionnaire was designed to collect Information included age, gender, living conditions (number of members in the family, number of rooms), parental educational level and occupation. Blood transfusion, unsafe injections and surgery were ascertained. Most the questions questionnaire was the yes/no questions which offer a dichotomous choice. The questionnaire was first developed in English and translated into Arabic language.

Sample collection:

Five-ml blood sample was collected from 121 patients with sickle cell anemia (the 62 males and 59 females), age 1≥31 years by venipuncture, using sterile disposable syringes, under strict aseptic technique, and drained into sterile anticoagulant-free test tube, and allowed to clot. The clotted blood sample was centrifuged (3000 rpm, 5 min), and the serum (the supernatant) was transferred into cryovials and stored at -20° C until required for use.

Serologic testing:

Samples of serum were tested for surface antigen (HBsAg) Anti-hepatitis C virus (anti-HCV) and human immunodeficiency virus antibodies (anti-HIV) using the Rapid HBV and HCV ((INTEC) which is a newly developed qualitative by using 1-Immune chromatographic test ICT(INTEC) to detect HBsAg and specific antibodies for HCV in blood.

2- Enzyme-linked immunosorbent assay (ELISA) DRG kit used to screen for surface antigen (HBsAg) , Anti-hepatitis C virus (anti-HCV) and human immunodeficiency virus antibodies (anti-HIV). All assay protocols, cut-offs and interpretation were according to the manufacturer's instructions.
All the samples, reagents and calibrators were brought to room temperature an hour before the test according to the manufacturer’s instruction.

**Ethical approval:**

Approval for this study was obtained from the Ethical Review Committee in the laboratory Department, Faculty of medicine and health sciences, Hodeidah University, Yemen.

**Statistical analysis:**

Statistical analysis were done using SPSS version 15.0 software for data compilation and calculations and the chi-squared test was used to determine the significance of difference between categorical variables. P-values < 0.05 were taken as significant.

**Results:**

Table 1: Distribution of the frequency of HBsAg and anti-HCV positive and negative infections according to Rapid among patients with sickle cell anemia in Hodeida city, Yemen.

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Rapid test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Result / Viral</td>
</tr>
<tr>
<td>HBsAg</td>
<td></td>
</tr>
<tr>
<td>Anti-HCV</td>
<td></td>
</tr>
</tbody>
</table>

A total of 121 blood specimens were collected from patients during the period from December 2016 to May 2017, for Prevalence of hepatitis B, C and HIV among sickle cell disease (SCD) anemia patients, (62/121), (51.2%) were males, and (59/121), (48.8%) females Table 1. The age ranged from 1 to above 31 years old; where all patients were Yemeni living in Hodeida. In the present study and by using rapid test found that 5 samples (4.1%) were seropositive and 116 cases (95.9%) were seronegative for HBsAg and 1 samples (0.8%) were seropositive and 120 samples (99.2%) were seronegative for Anti-HCV as shown in Table 1 and Figure 1. In present investigation and by using Elisa test found that there were not seropositive result for HBsAg and Anti-HCV as shown in Table 2. Regarding to HIV all cases was negative by Elisa and rapid tests Table 2.
Figure 1: Distribution of the frequency of HBsAg and anti-HCV positive and negative infections according to Rapid among patients with sickle cell anemia.

In present investigation and by using Elisa test found that there were not seropositive result for HBsAg and Anti-HCV as shown in table 2. Regarding to HIV all cases was negative by Elisa and rapid tests table 2.
Table 2: Distribution of the frequency of HBsAg, anti-HCV and HIV positive and negative infection depending on ELISA tests among 121 patients with Yemeni association for patients with sickle cell anemia.

<table>
<thead>
<tr>
<th>General characters</th>
<th>NO (=121)</th>
<th>%</th>
<th>Rapid test</th>
<th>ELISA TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HBV</td>
<td>HCV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive (%)</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N= 4</td>
<td>N=1</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5y</td>
<td>28</td>
<td>23.1</td>
<td>2(1.7)</td>
<td>.903</td>
</tr>
<tr>
<td>6-10</td>
<td>41</td>
<td>33.9</td>
<td>1(0.8)</td>
<td>0(0)</td>
</tr>
<tr>
<td>11-15y</td>
<td>29</td>
<td>24.0</td>
<td>1(0.8)</td>
<td>0(0)</td>
</tr>
<tr>
<td>16-20</td>
<td>14</td>
<td>23.1</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>21-25</td>
<td>3</td>
<td>2.5</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>26-30</td>
<td>1</td>
<td>8.</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>&gt;31</td>
<td>5</td>
<td>4.1</td>
<td>1(0.8)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>62</td>
<td>51.2</td>
<td>1(0.8)</td>
<td>.286</td>
</tr>
<tr>
<td>Female</td>
<td>59</td>
<td>48.8</td>
<td>3(2.5)</td>
<td>.999</td>
</tr>
<tr>
<td>Place of birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alhodidah</td>
<td>116</td>
<td>95.9</td>
<td>4(3.3)</td>
<td>.999</td>
</tr>
<tr>
<td>Taiz</td>
<td>1</td>
<td>0.8</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>1</td>
<td>0.8</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Rimah</td>
<td>1</td>
<td>0.8</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Aden</td>
<td>1</td>
<td>0.8</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Almahwite</td>
<td>1</td>
<td>0.8</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>ABO-grouping</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>26</td>
<td>21.5</td>
<td>0(0)</td>
<td>.832</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>5</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>AB</td>
<td>3</td>
<td>2.5</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>O</td>
<td>81</td>
<td>66.9</td>
<td>4(3.3)</td>
<td>0(0)</td>
</tr>
<tr>
<td>UNKNOWN</td>
<td>5</td>
<td>4.1</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>
All positive study patients were found in the 1 to 15 years age group by rapid test and above 31 by Elisa test (Table 3). The distribution of positive serum samples among the different age groups for HBsAg and anti-HCV by using rapid test showed that patients of the age group 1-5 years had the highest percentage positive results with (1.7%), followed by the age group 6-10 and 11-15 years which have same percentage with (0.8%) while anti-HCV by rapid test showed that patient of the age group above 31 years only one patient has positive result with percentage (0.8%) as shown in table 3. In present study found that total 62 (51.2%) were male, which 0.8% positive for HBsAg and 0.8% for anti-HCV by using rapid test, while total 59 (48.8%) were female which 3 (2.5%) positive for HBsAg as shown in table 3. In present investigation found that both male and female shown snot positive result for HBsAg, anti-HCV and HIV by using rapid and Elisa test (table 3). In current investigation found that total 116 (95.9%) who borne in Hodeidah city whichone patient with 0.8% showed positive for anti-HCV and 4 (3.3%) for HBsAg by rapid test and showed not positive result for HBsAg, anti-HCV and HIV by Elisa test while patients who borne out of hodeidah city showed no positive for all tests (table 3). In our study found that among 72 (59.5) who has +O group there was 1 (0.8%) seropositive for anti-HCV and 1 (0.8%) seropositive for HBsAg but total 9 (7.4%) patients who has -O blood group there was 1 (0.8%) seropositive for HBsAg. Total 24 (19.8%) patients who has +A blood group there was 1 (0.8%) seropositive for HBsAg while number patient with -A was 2 (1.7%) in which 1 (0.8%) was positive for HBsAg and 1 (0.8%) negative. Sickle cell anemia patient with blood grouping +B,+AB showed not positive result for HBsAg, anti-HCV and HIV (table 3).

(Table 13) Socio-demographic characteristics and seropositive of HBsAg and anti-HCV among SCA patients

Discussion:

The overall incidence of liver disease in patients with SCD has not been well established, however, liver can be affected by a number of complications due to the disease itself and its treatment [20&21]. In addition to the vascular complications from the sickling process, patients with SCD have often received multiple transfusions, placing them at higher risk for viral hepatitis, iron overload, and the development of pigment gallstones, all of which may contribute to the development of liver disease. It is unclear whether cirrhosis was due to the sickle cell anemia itself or to concurrent liver disease acquired as a consequence.
of multiple transfusions, leading to excessive iron overload and/or chronic hepatitis B or C infection [22&23]. The present study was conducted to determine prevalence of hepatitis B, C and HIV among sickle cell disease (SCD) anemia patients which admitted in Yemeni association for patients with sickle cell anemia and heredity blood center. Frequency of hepatitis B was found to be 3.3 % (n = 4). The result matched with the observations of LweendoNchimba [24] who detected 22.17 seropositive for HBsAg. The results of this study bring out an important aspect of the evolution of the blood transfusion service in this country. Twenty two years ago, a study showed that there was a significant increase in the prevalence of hepatitis infection in SCD patients who had received multiple blood transfusions compared to those who had received nothing or few; suggesting a cumulative risk associated with exposure to donated blood [25]. Only one patient tested positive for Hepatitis C infection (0.8%). Because of the very low prevalence, it was difficult to subject this to any statistical analysis. This is a low prevalence and seem to be in agreement with other previous studies done on hepatitis C prevalence that have consistently shown low rates of infection in different subpopulations in [26]. The risk factors frequently cited as effective routes of transmission include the transfusion of blood from unscreened donors, intravenous drug abuse and the use of unsafe therapeutic injections [27]. Egypt has the highest reported seroprevalence of hepatitis C infection world-wide because of the use of contaminated glass-syringes in a nationwide campaign against schistosomiasis infection carried out from 1960 to 1987 [28]. However 2008 hepatitis screening for HCV, HBV, and HIV was included in the premarital testing program. The data from the premarital program showed, that the average HBV prevalence was 1.31% and that of HCV 0.33% [29] which is far lower than observed by Bahakim. Bahakims study revealed that HCV was endemic in the Saudi population with an overall frequency of 5.3% in healthy Saudi adults [30]. The distribution of positive serum samples among the different age groups for HBsAg and anti-HCV by using rapid test showed that patients of the age group 1-5 years had the highest percentage positive results with (1.7 %), followed by the age group 6-10 and 11-15 years which have same percentage with (0.8 %) while anti-HCV by rapid test showed that patient of the age group above 31 years only one patient has positive result with percentage (0.8%). This result differed from those obtained in 29 surveys in sub-Saharan Africa published by Madhava et al. in 2002 that showed that prevalence tended to increase with age. Thus, the specific median prevalence among subjects under 20 years was 1.3% (22 studies, range: 0% - 11%), between 20 and 40 years was 3% (29 studies, range: 0% - 28%), and among those over 40 years was 12% (29 studies, range: 0% - 55%) [31]. Blood is a lifesaving agent but its transfusion can result in life threatening infections to the recipient [32].
A blood groups are one set of antigens, which are genetically determined carbohydrate molecules carried on the surface of membranes of red blood corpuscles [33&34]. ABO blood groups have shown some association with various diseases [35]. Although there are small studies in literature about association between ABO blood groups and chronic viral hepatitis B and C. in our study found that among 72(59.5) who has +O group there was 1 (0.8%) seropositive for anti-HCV and 1 (0.8%) seropositive for HBsAg but total 9 (7.4%) patients who has −O blood group there was 1 (0.8%) seropositive for HBsAg. Total 24 (19.8%) patients who has +A blood group there was 1 (.08%) seropositive for HBsAg while number patient with −A was 2 (1.7%) in which 1(0.8%)was positive for HBsAg and 1 (0.8%) negative, the similar distribution of blood group were reported by Alireza E. Naeini, et al. (2010), and others [36]. A relation between the liability to develop hepatitis and the ABO blood groups would suggest that host factors may be of importance in the genesis of this disease [37]. However the results of this study demonstrate that a possible association between HBV & HCV infections and blood group antigens cannot be ruled out.

CONCLUSION
The lower prevalence of the HBsAg in SCD patients seen in this study could be due to better HBsAg screening techniques in blood transfusion centers. HBV infection during transfusion should not be completely excluded. There remain significant risks from infected donors who have not yet developed detectable HBsAg levels. HBV screening and vaccinations should therefore be a part of the management of SCD patients. Thus further studies are required to know the long term prognosis in such patients.

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