

Evaluation of Red Cell Membrane Fragility in Patients with Hepatitis B Virus Infection

Chukwurah Ejike Felix^{1*}, Ohaneme Lydia Uchechi², Nworie Amos³, Chukwurah Felix Chinedum⁴

¹Department of Haematology & Immunology, Faculty of Clinical Medicine, College of Health Sciences, Ebonyi State University, Abakaliki, Nigeria

²Hamatology Unit, Department of Medical Laboratory Sciences, Faculty of Health Science, Ebonyi State University, Abakaliki, Nigeria

³Department of Medical laboratory Sciences, Faculty of Health Science & Technology, Ebonyi State University, Abakaliki, Nigeria

⁴Shenyang Medical College, Shenyang Liaoning P.R. China/Department of Public Health, Enugu State University Teaching Hospital, Enugu, Nigeria

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Abstract

Background: Hepatitis B virus infection is a major global health problem and of immense clinical importance. Hepatitis B virus infection can lead to hepatocellular carcinoma (HCC) and is found to be associated with macrocytic anemia. The mechanism of anaemia largely depends on red cell membrane integrity. Osmotic fragility (OF) of red cells was therefore evaluated in patients with HBV infections to access the diagnostic/prognostic utility. **Methods:** A total of 1744 patients from surgical and medical wards/clinics of Alex Ekwueme University Teaching Hospital Abakaliki were screening serologically for HBV using immunochromatographic strip method and positive samples were confirmed by the use of One Step Hepatitis B Multi-5 Test. A total of 100 subjects (50 HBV positive and 50 HBV negative) were evaluated for osmotic fragility of red blood cells. **Results:** HBV prevalence rate of 3.5% was found among the patients. The osmotic fragility of red blood cells were significantly ($p < 0.05$) decreased in Hepatitis B virus positive individuals compared to Hepatitis B virus negative individuals. Significant ($p < 0.05$) differences were observed in the osmotic fragility of the two groups at 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2% and 0.1% saline. The mean corpuscular fragility (MCF) which is the sodium chloride concentration causing 50% hemolysis for Hepatitis B positive individuals was 0.33% saline while that of Hepatitis B negative individuals was 0.41% saline. **Conclusion:** The HBV positive patients showed decreased OF compared with HBV negative control population. This study was on small population of HBV positive subjects without disease staging. Further studies using more sample size and clinical staging is recommended to clearly associate OF as a diagnostic or prognostic marker in HBV infection.

Keywords: Osmotic fragility, HBV infection, Diagnostic utility.

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INTRODUCTION

Background

Globally, HBV infection is a major health problem with potentially life-threatening liver disease [1]. It can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer. Worldwide, two billion people have been infected with hepatitis B virus (HBV), 360 million have chronic infection, and 600,000 die each year from HBV-related liver disease or hepatocellular carcinoma [2]. It is hyper endemic (i.e. >8% of the population infected) in Sub-Saharan Africa and a major cause of chronic liver disease. An estimated that 44% of cirrhotic liver disease and 47% of hepatocellular carcinoma cases in Sub-Saharan Africa are attributed to HBV. Though a highly effective and inexpensive recombinant DNA vaccine for hepatitis B has been available since 1982 and debuted in Nigeria in 1995 [3]; this vaccination programs in Nigeria have not received adequate

attention or funding by the government. Prevention and early detection through screening still remain the best way of combating the disease.

In Nigeria; community misconceptions have hindered the success of vaccination coverage rates. The United Nations Children's Fund (UNICEF) and the World Health Organization (WHO) estimated that only 41% of Nigerians were vaccinated against HBV in 2013[4-6]. The risk of contracting HBV in Nigeria is substantial, not only due to low vaccination rates but also given that as many as 75% of the population will be exposed [7].

Studies indicate varying national and risk group-specific prevalence in different parts of Nigeria. High prevalence of 10-15%, 25% and 23.4% were reported in the average risk Nigerian population [8], surgeons [9] and blood donors [10] respectively. High

prevalence among pregnant women was also reported [11]. Evidence of co-infection with HIV was equally observed [12-15]. Hepatitis B is the commonest cause of chronic liver disease in Nigeria [16]. There is need therefore to look for the diagnostic and/or prognostic markers that may be of clinical utility in the management of HBV.

The liver plays a key role in hematopoiesis and synthesis of coagulation proteins, therefore liver disease is associated with a broad spectrum of hematological abnormalities [17]. It has been demonstrated that macrocytic anemia, defined as anemia in which the RBCs are larger than their normal volume, is associated with the severity of liver impairment in patients with HBV-related decompensated cirrhosis. There are several potential pathological mechanisms that explain why macrocytic anemia is associated with the severity of liver impairment. Patients with advanced liver damage are more likely to have vitamin B or folate deficiencies [18], which directly result in macrocytic anemia. Vitamin B and folate coenzymes are required for thymidylate and purine synthesis, thus, their deficiencies result in retarded DNA synthesis and eventually will develop into macrocytic anemia [19]. Macrocytic anemia in liver disease may also be due to an increased deposition of cholesterol on the membranes of circulating RBCs [20]. This deposition effectively increases the surface area of the erythrocyte. Moreover, erythrocyte morphology is affected by various factors in liver disease; degree of liver damage, and drugs used. Complicated mechanisms, which allow the synchronized performance of their independent or collaborative functions, determine the shape of RBCs.

Erythrocyte fragility refers to the propensity of erythrocytes (red blood cells) to haemolyse (rupture) under stress [21]. It can be thought of as the degree or proportion of hemolysis that occurs when a sample of red blood cells are subjected to stress (typically physical stress, and most commonly osmotic and/or mechanical stress). Osmotic fragility is affected by various factors, including membrane composition and integrity as well as the cells' sizes or surface-area-to-volume ratios [22].

The osmotic fragility test is common in hematology, and is often performed to aid diagnosis of diseases associated with RBC membrane abnormalities [23]. Since HBV is implicated in liver diseases and may possibly lead to impaired function of the liver, it is of interest to study the red cell membrane integrity of such patients especially in our environment with rising trends in HBV infection and possibly assess the diagnostic/prognostic utility in management of patients.

MATERIALS AND METHODS

Study Area

This study was carried out in Alex Ekwueme University Teaching Hospital, Abakaliki between

February, 2018 and March, 2019. Abakaliki is one of the major cities in the south eastern part of Nigeria and the capital of Ebonyi state. It lies between 6.2°49'N and 80.6°1'E and is located at the lower belt of the Niger [9]. The common climate is tropical and vegetation characterized by predominantly semi-tropical rainforest with an average annual atmospheric temperature of 30°C. The hospital is located within Abakaliki metropolis. The patients attending clinics comprises of 80% Abakaliki urban residents and 20% of rural dwellers from various L.G.A that constitute Ebonyi State.

Sampling and Sample Size

This is a prospective study on 1744 patients attending medical and surgical clinics/wards at Alex Ekwueme University Teaching Hospital, Abakaliki – Nigeria. Ethical approval duly obtained from the hospital's Ethics and Research Committee and informed written consent obtained from the participants. Patients with sickle cell disease and other haemoglobinopathies were excluded. All those who met the set inclusion criteria were recruited by self-selection. Minimum sample size was calculated using the known prevalence rate of 8.1% as previously reported [12] among road accident victims in the same environment and the method of Lwanga and Lemshaw [24] was used in calculations.

Initial 2mls of blood was collected from the patient's group for screening and confirmatory test for HBV. Those that were confirmed positive for HBV infection were further bled aseptically into lithium heparin container for OF test along side with age/sex matched apparently healthy control subjects and analyzed immediately.

Assay Methods

HBV screening was performed using the HBsAg rapid immunochromatographic strip method (kit was sourced from Lindex, UK, Batch no 20180122) while the confirmatory test was done by competitive binding immunoassay using One Step Hepatitis B Multi-5 method and kit sourced from Cortez Diagnostics, Inc. (One Step HBV Combo RapiCard™ InstaTest, Lot 177471-1-44) detecting the 5 markers associated with HVB.

The test kit was removed from the pouch and placed horizontally on a desk. The test device was used as soon as possible. Four (4) drops of serum was pipette into each sample well. The results were read after 15 minutes.

For the evaluation of the osmotic fragility test; gradient hypotonic buffered saline solution method as reported by Ochei and Kolhatker [25] was adopted. The test determines the resistance of red cells to hemolysis in various concentrations of hypotonic saline solutions. The ability of the erythrocytes to absorb water without

lysis depends on the ratio of volume to surface area of the cells. In the normal red cells the volume may increase up to 70% before lysis may occur.

Statistical Analysis

Data generated from the study was analyzed using Statistical Package for Social Sciences (SPSS), version 20. Descriptive and inferential statistic were used and values were expressed as mean ± SD. Degree of association were determined as appropriate using chi-square test and *p* < 0.05 considered statistically significant.

RESULTS

Out of the 1744 patients screened; a total of 61 (3.5%) patients were confirmed positive for HBV. The HBV confirmed positive samples (N=50) and HBV negative (N=50) apparently healthy age/sex –matched

control samples were evaluated for OF. The mean ± SD of the osmotic fragility test were as shown in table 1. The tube 1 mean ± SD of the osmotic fragility test of Hepatitis B positive individuals was 0.98 ± 2.62% while that of the Hepatitis B negative individuals was 7.38 ± 13.16%. There was significant (*p*<0.05) difference between the osmotic fragility for the two groups in tube 1. The OF tests also differed significantly in the different buffered saline gradients used (tubes 2-9)

The mean corpuscular fragility (MCF) which is the sodium chloride concentration causing 50% haemolysis for the two groups is as shown in Figure-1. The mean corpuscular fragility (MCF) of HBV-positive patients was 0.33% saline while that of HBV-negative individuals was 0.41% saline.

Table-1: Mean ± SD of the osmotic fragility test of HBV positive patients and control group

Tube	Hepatitis B positive (%)	Hepatitis B negative (%)	T-value	p-value
0.9% Saline	0.98 ± 2.62	7.38 ± 13.16	27.502	0.0001
0.8% Saline	1.16 ± 3.67	8.56 ± 65.96	6.816	0.0001
0.7% Saline	1.25 ± 2.26	7.94 ± 87.18	4.388	0.0001
0.6% Saline	0.78 ± 2.20	6.62 ± 16.10	21.147	0.0001
0.5% Saline	1.20 ± 14.08	10.76 ± 136.56	4.126	0.0001
0.4% Saline	12.54 ± 67.16	27.88 ± 160.83	4.597	0.0001
0.3% Saline	44.87 ± 53.12	68.96 ± 235.12	16.151	0.0001
0.2% Saline	76.89 ± 51.53	88.23 ± 97.52	20.054	0.0001
0.1% Saline	84.64 ± 52.74	92.03 ± 151.34	17.594	0.0001
0% Saline	100 ± 100	100 ± 100		0.0001

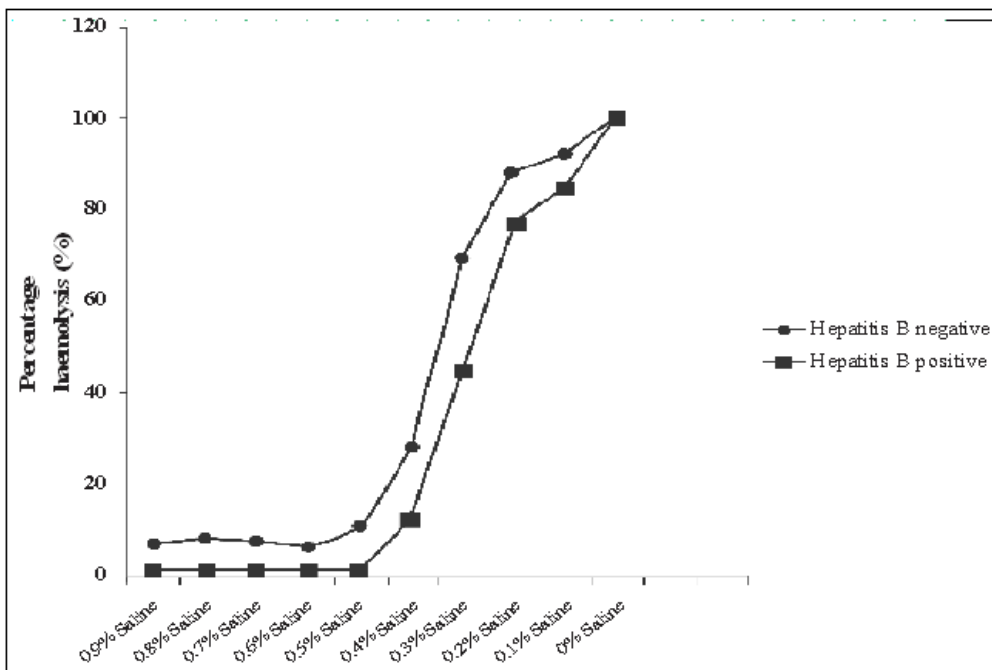


Fig-1: The mean ± SD of the osmotic fragility of Hepatitis B positive individuals with respect to that of Hepatitis B negative individuals

DISCUSSION

One of the major causes of liver cirrhosis apart from alcohol, drugs etc. is infection with microbial

agents chiefly viruses. Hepatitis B virus (HBV) causes an infectious disease known as Hepatitis B virus disease which affects the liver. It has been irrefutably shown that chronic Hepatitis B virus infection can lead to hepatocellular carcinoma (HCC) and macrocytic anaemia. There is need therefore to study the effect of some of these clinically important viruses on red cell membrane integrity.

In this study 3.5% of the surgical and medical patients were confirmed positive for HBV. This shows a changing paradigm from the previous reports [12,13,26,27] from the same author in the same environment and could be attributed to increasing awareness and vaccination coverage. Though the WHO reported poor vaccination coverage rates in different parts of Nigeria; this is more pronounced in the northern part of the country where misconceptions have hindered the success of vaccination coverage. The key challenges include not reaching most infants and children with routine immunization services [28] and lack of governmental funding. Monitoring data at subnational levels is critical to helping countries prioritize and tailor vaccination strategies and operational plans to address immunization gaps and reach every person with lifesaving vaccines. The risk of contracting HBV in Nigeria is substantial, not only due to low vaccination rates and awareness [29] but also given that as many as 75% of the population will be exposed [7]. Though measures are being put in place for the management of Hepatitis B virus (HBV) infection in Nigeria, children remain the most vulnerable to develop chronic hepatitis. Routine screening in children is therefore necessary for effective control [28].

Liver diseases have been associated with production of target cells; red blood cells that have the appearance of a shooting target with a bulls eye [17]. These cells are characterized by a disproportional increase in the ratio of surface membrane area to volume (a characteristic of red cells also seen in macrocytic anemia). This is also described as a "relative membrane excess." It is due to either increased red cell surface area, or a decreased intracellular hemoglobin content; which may cause an abnormal decrease in cell volume without affecting the amount of membrane area. The increase in the surface area to volume ratio also gives the cell decreased osmotic fragility, as it allows it to take up more water for a given amount of osmotic stress. Lecithin-cholesterol acyltransferase (LCAT, also called phosphatidylcholine-sterol O-acyltransferase) is an enzyme that converts free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol), which is then sequestered into the core of a lipoprotein particle. This eventually makes the newly synthesized HDL spherical and forcing the reaction to become unidirectional since the particles are removed from the surface. In patients with liver disease, lecithin cholesterol acetyltransferase activity is

depressed. Decreased enzymatic activity of LCAT increases the cholesterol to phospholipid ratio, producing an absolute increase in surface area of the red blood cell membranes or may be increased red cell membrane fluidity.

Depression of lecithin cholesterol acetyltransferase activity in patients with liver disease which increases the cholesterol-to-phospholipid ratio and produces an absolute increase in the surface area of the red cell membrane could lead to increased resistance of the red cells to hypotonic stress. This will therefore lead to a decreased osmotic fragility of the red blood cells. Elevations in target cells are the result of a shift in the exchange equilibrium between the red blood cells and the cholesterol [30]. In contrast, membrane excess is only relative in patients with iron-deficiency anemia, macrocytic anemia and thalassemia because of the reduced quantity of intracellular hemoglobin. When a cell membrane collapses it becomes static and stops pulsating. Target cell formation decreases the amount of oxygen that is circulated through the blood and unable to deliver it to all areas of the body.

CONCLUSION

A total of 100 subjects (50 Hepatitis B positive and 50 Hepatitis B negative) were evaluated for osmotic fragility of red blood cells. The osmotic fragility of red blood cells were significantly ($p < 0.05$) decreased in Hepatitis B virus positive individuals compared to Hepatitis B virus negative individuals. Significant ($p < 0.05$) differences were observed in the osmotic fragility of the two groups at 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2% and 0.1% saline. The mean corpuscular fragility (MCF) which is the sodium chloride concentration causing 50% haemolysis for Hepatitis B positive individuals was 0.33% saline while that of Hepatitis B negative individuals was 0.41% saline.

RECOMMENDATIONS

This work was carried out in a small population of Hepatitis B virus positive subjects (50 subjects) with some limitations including non staging of the infections with respect to acute or chronic disease state. We therefore recommend that further work on it using higher population of infected persons be carried out to establish the correlation existing between osmotic fragility of red cells and disease progression in Hepatitis B virus infection. This may establish possible prognostic utility.

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