ISSN No. 2456-4400
Int J Med Lab Res 2023, 8(2):1-7

IJMLR International Journal of Medical Laboratory Research

RESEARCH ARTICLE

D-DIMER LEVELS IN HEPATITIS B NEGATIVE BLOOD DONORS AT THE BLOOD BANK OF UNIVERSITY COLLEGE HOSPITAL, IBADAN

Akpan Richard P [1], Ugbomoiko Daniel O [2], Ajugwo Anslem O [3], Akpan Victoria O [1]

[1] *Department of Hematology, University College Hospital, Ibadan, Nigeria – 200109.*
[2] *Department of Medical Laboratory Sciences, Igbinedion University, Okada, Benin, Nigeria – 300102.*
[3] *Department of Medical Lab Science, Madonna University Elele, Nigeria – 511101.*
Department of Medical Lab Science, Kampala International University, Western Campus Uganda.

Received: 13 April, 2023/Revision: 17 May, 2023 /Accepted: 20 June, 2023

Abstract: Background: Hepatitis B viruses (HBV) have caused chronic and acute diseases which may be asymptomatic in billions of people. Screening of blood donors is therefore important to reduce transfusion transmission. Positive Hepatitis B core antibody suggests previous infection or vaccination. HBV infections often lead to alteration in the D-dimer which may lead to complications in the donor. This study is therefore aimed at determining D-dimer levels in Hepatitis B negative blood donors. **Materials and Method:** ABON rapid test kit was used to identify 200 Hepatitis B negative donors, VITROS was used for Hepatitis B core antibodies detection, while LumiraDx D-dimer test instrument was used to determine D-dimer levels. Descriptive statistics were used for data presentation, while Chi-square was used to determine the relationships and associations between categorical variables. **Results:** 59.2% of the participants were anti-HBc negative, while 40.5% were positive. Chi-square results showed that there was no significant relationship between D-dimer levels (Ug/ml) and Anti HBc status of the participants, and age did not have significant association with D-dimer levels of negative or positive anti-HBc status of the participants. **Conclusion:** Mean D-dimer is within the normal range and most of the potential donors were fit to donate suitable blood for transfusion, without the safety of both donors and prospective recipient being compromised.

KEYWORDS: Case control study, Hepatitis B, Hematological parameters, Immunodiagnostic, D-Dimer

INTRODUCTION:

Billions of people in the world are being infected by Hepatitis B virus (HBV)[1] and have consequently developed chronic and acute diseases, either of which may not show any symptom.[2]

Screening of donated blood for HBsAg was found to minimize transmission of hepatitis B virus through blood transfusion since the 20th century. Joint opinion had it that absence of HBsAg means no HBV infection. It is however pertinent to state that DNA strands of HBV have been found in the blood and liver

Corresponding Author:
Richard. P Akpan,
Department of Hematology, University College Hospital, Ibadan, Nigeria – 200109.

of anti-HBcAg and anti-HBsAg positive individuals according to several reports [3].

It is a fact that HBV infection leads to alteration of the hematological parameters, D-dimer inclusive as an aftermath of cells breaking down due to inflammation caused by the infection, and consequently results in physiological disorders. Liver damage due to the infection is another probable cause of abnormalities in the hematological system [4].

Liver which is a very important organ for metabolism has coagulation factors as part of its products. The liver produces chemicals which alter and render toxins less poisonous in order to prevent its harmful effects on body organs. Hence, damage to this organ by viral infections such as HBV may claim life [5].

D-dimer is a special metabolic product produced by fibrinolytic enzymes in plasma. This can be taken as one of the extra prognostic new markers for HBV-related cirrhosis mortality within thirty days. Abnormal D-dimer level has been related to poor prognosis in cirrhotic individuals which can also be used as another factor to pre-empt quick death for patients suffering from acute-on-chronic liver failure. D-dimer study is also essential to ensure safety of blood donors who may have coagulation disorder. Hence, this study seeks to determine D-dimer levels in selected Hepatitis B Negative blood donors [6].

MATERIALS AND METHOD:

Study Area

The study was conducted at the University College Hospital (UCH), Ibadan, Oyo State, Nigeria. Blood samples were collected at the Bio-repository Unit, while sample analyses were carried out at the Universal Laboratory of the hospital.

Subjects

The subjects comprised voluntary blood donors at the blood bank of UCH, whose consents were sought through forms which they filled and duly signed or thumb-printed.

Inclusion and Exclusion Criteria

Donors that tested negative for HBsAg were included, while those that tested positive were excluded. Also, donors above 18 years old were included while those below 18 years were excluded.

Ethics

The conduct of this study followed the guidelines of ethical committee of Oyo State Ministry of Health, Ibadan, Oyo State, Nigeria, and approval of the procedure was granted by same. Validated structured questionnaires were administered and blood samples were collected after the purpose of the research has been explained to and consent forms have been filled and signed by the subjects.

Sample collection

Validated structured questionnaire was used to obtain information on the demographic characteristics of the subjects and explanation was provided where necessary. 200 Hepatitis B negative donors were identified with the aid of ABON HBsAg rapid test kit using whole blood collected by fingerprick. 8 ml of blood sample was later collected from each subject by venipuncture. 4 ml was transferred into EDTA bottle for VITROS to determine Hepatitis B core antibody, and 4 ml into tri-sodium citrate container for D-dimer studies. Samples for anti-HBc and D-dimer studies were immediately spun with the aid of centrifuge and plasma was separated and collected and stored in a -80˚C Revco freezer till time for analysis.

Timeline for Sample Collection and Analysis

Blood samples were collected between August 2021 and February 2022. Sample analysis was done between March 2022 and May 2022, while statistical analysis was done between June 2022 and August 2022.

Sample Assay

Identification of HBsAg negative donors was done using ABON HBsAg rapid test kit. Fingerprick was used to collect approximately 75µl whole blood from the finger of the participant and added to the specimen well labeled (S) of the test device, then a drop of buffer (packaged with test device) (approximately 40 µl) was added to the specimen on the device. Stop watch was used to monitor time and the result was read at 15 minutes. Appearance of two distinct lines on the result region (marked as C-control and T-test) of the test device was interpreted as positive, one colored strip in the “C” area was interpreted negative, while one

colored strip in the "T" region or absence of colored lines in both regions was interpreted as invalid.

VITROS was done following the procedure described in the operator manual of VITROS ECiQ immunodiagnostic system. The test was programmed on the system by selecting the test type "anti-HBc" and the tray to be used was also programmed by clicking on the tray icon and labeling the trays with alphabets. Specimen codes as written on the specimen container were entered for the trays specified for them. The specimens were then put in the trays assigned to them and the analysis was run. The principle involves the binding of anti-HBc in the sample with hepatitis B core antigen (HBcAg) coated wells. Unreactive sample is cleared by washing. Antibody conjugate (Horseradish peroxidase (HRP)) is then allowed to bound with the remaining exposed HBcAg on the surface of the well. Unbound HRP is also removed by washing. The bounded HRP conjugate is measured by a luminescent reaction. A reagent with luminogenic substrates (a luminal derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent increases the level and duration of the light produced. The VITROS ECi System read the light signal. The amount of HRP conjugate bound indicates the concentration of anti-HBc present in the sample. For interpretation, results were calculated as normalized signal over cut-off value (cut-off value already stored in the VITROS ECi system) (signal/cut-off, s/c). Based on the testing algorithm, > 1.10 values were interpreted as negative, < 0.90 as reactive/positive, while values ≥ 0.90 and ≤ 1.10 indicated need for retest.

D-Dimer studies followed the instructions in the user manual of LumiraDx D-dimer test instrument. *Patient Test* was selected from the device home screen and details of the participants were entered using the keyboard. The test strips were removed from their pouch and held upright by gripping the blue portion. When prompted, the instrument door was opened and the test strip was gently inserted with the thick black alignment rib of the strip on the left and lined up with the black line on the instrument. Sample type "plasma" and test type "D-Dimer" was selected on the instrument. The instrument was prompted and a 20 µl lithium-heparin anticoagulated transfer tube was used to transfer specimen to the specimen region of the strip. The door was closed when prompted by the instrument to continue the test. Results were displayed 6 minutes after sample application and were recorded. "Finish" was tapped to complete the testing and a prompt was followed to return to the Home Screen.

Statistical Analysis

Software SPSS (Statistical Package for the Social Sciences) version 23.0 was used for the analysis. The analyses included descriptive statistics of frequency count and percentages, mean and standard deviations of continuous variables (ages and values of D-dimers). Chart prepared with Microsoft excel was used to present categorical variables (negative and positive anti-HBc). Inferential statistics Chi-square was used to determine the relationships and association between categorical variables. P values > 0.01 were regarded as statistically significant.

RESULTS:

Most of the participants 194(97.0%) were males while only 6(3.0%) were female. Hence, male to female ratio was approximately 32:1. This implied that more males were actively involved in blood donation than their female counterparts.

Overall mean age and standard deviation was 30.7±7.1 with a range of 19 to 56 years old. Most 90 (45.0%) were in the age group 29-38, followed by age group 19-28 with 85 (42.5%), While the least 2 (1.0%) participants were in the age group 49-58. This implied that youths were more actively involved in voluntary blood donation than the aged ones. (**Table 1**)

Table 1: Sex and Age distribution of the participants.

Variables	Items	Frequency	Percent
Sex	Male	194	97
	Female	6	3
Age group	19-28	85	42.5
	29-38	90	45
	39-48	23	11.5
	49-58	2	1
Age(years)	mean±SD [range]	30.7±7.1 [19-56]	

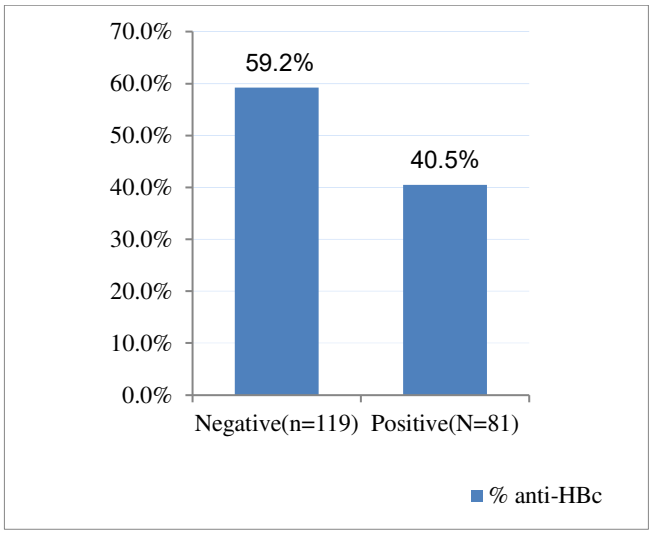

Fig. 1: Distribution of Anti-HBc

59.2% of the participants were anti-HBc negative, while 40.5% were positive (**Fig.1**). 29 (58.0%) of those who are negative for anti-HBc have normal D-dimer level while 90 (60.0%) of them have abnormal level of D-dimer. 21 (42.0%) of those who test positive for anti-HBc have normal D-dimer level, while 60 (40.0%) have abnormal D-dimer level. Chi-square showed that there was no significant relationship between the D-dimer levels (Ug/ml) and anti-HBc status of the participants at 1% significant level. (**Table 2**)

Table 2: Relationship of the results of D-dimer (Ug/ml) with outcome of Anti-HBc test

Variable	Items	Anti HBc		Total	χ^2	P-Value
		Negative	Positive			
		N (%)	N (%)			
D-dimer (Ug/ml)	normal	29(58.0)	21(42.0)	50	.062	.803
	Abnormal	90(60.0)	60(40.0)	150		

Mean with a standard deviation of D-dimer (Ug/ml) was [1.477±1.0] with range from 0.2-5.9. Mean D-dimer level (Ug/ml) was not significantly affected by either negative or positive anti-HBc ($p > 0.01$). (**Table 3**)

Tables 3: Comparison of mean D-dimer level (Ug/ml) with negative and positive anti-HBc results

Variable	Statistics	Anti-HBc		Total	t-test	p-value
		Negative	Positive			
D-dimer (Ug/ml)	Mean ±SD	1.451±1.0	1.516±1.1	1.478±1.0	.441	.660
	Range	.2-4.2	.2-5.9	.2-5.9		

Chi-square revealed that age did not significantly determine negative or positive Anti-HBc status of the participants and did not also have significant influence on the D-dimer level, all at 1% significant level. (**Table 4**)

Table 4: Relationship of age with results of D-dimer (Ug/ml) and Anti-HBc

Anti HBC	Age group (years)	D-dimer (Ug/ml)		Total	χ^2	P-Value
		Normal	Elevated			
		N (%)	N (%)			
Negative	19-28	14(28.0)	36(72.0)	50	4.095	.251
	29-38	14(27.5)	37(72.5)	51		
	39-48	1(5.9)	16(94.1)	17		
	49-58	0	1(100.0)	1		
Total		**29**	**90**	**119**		
Positive	19-28	10(28.6)	25(71.4)	35	2.683	.443
	29-38	11(28.2)	28(71.8)	39		
	39-48	0	6(100.0)	6		
	49-58	0	1(100.0)	1		
Total		**21**	**60**	81		

DISCUSSIONS:

This study was aimed to determine D-dimer level in hepatitis B negative blood donors. 200 hepatitis B negative blood donors were identified and 90% of them were males while only 3% were females. This indicates that males are more fit and acceptable for blood donations than females. This is based on wide belief that donated bloods from females have certain negative implications on both the donors and recipients. According to Ugwu et.al, 2018, women generally lack awareness about their fitness, having been misled by even scientists that they will have less adequate Hb level to donate blood, which have made many families to shield their females from blood donation [7]. Doro et.al, (2015) and Mremi et.al, (2021) also recorded in similar research that male donors were more than female donors [8, 10].

The mean age was 30.7±7.1 with a range of 19 – 56 years. This age bracket follows the pattern in many research findings [7]. Specifically, it is in line with the finding of Ugwu et.al, 2018 which observed the average age of donors to be 28.8±8.5 and a range of 19 – 56 years. Onyekwelu et.al, (2022) observed the same pattern in similar research, while Mremi et.al, (2021) also found out that blood donation was common among males who were not too young and too old than females in the same age bracket. This also corroborated the finding of Doro et.al, (2015) [5, 8, 10].

While 59.2% of the participants were anti-HBc negative, 40.5% were positive. This is evident that less than half of the participants in this study have been previously infected with HBV or have received vaccine against it. In similar research by Olayinka et.al, (2016), more than half of the studied population in a national survey had evidence of previous infection or vaccination [9]. Doro et.al, (2015) claimed that fewer prospective blood donors were positive for HBV among those screened for seroprevalence and this was corroborated by the finding of this research.[10] That substantial part of the prospective donors have traces of previous infection suggested that additional test be carried out in order to totally ensure absence of the virus' DNA in the donated blood from such persons before rendering them suitable for transfusion without any risk of infecting the recipient.

The result showed no significant relationship between D-dimer levels (Ug/ml) and Anti HBc status of the participants. This disagreed with the result of Al-Dahmoshi et.al, (2021) that D-dimer had significantly high increase in HBV patients than healthy persons [6]. This result also disagreed with that of Onyekwelu et.al, (2022) that liver disease induced by hepatitis B significantly increase prothrombin time, INR, APTT, D-dimer [5].

Age did not significantly determine D-dimer levels of Anti-HBc positive or negative participants at 1% level of significance, with D-dimer level been normal for lower age group than older age group and also elevated in the same direction irrespective of positive or negative Anti-HBc status. This disagreed with the claim of Doro et.al, (2015) that age groups and infections were statistically associated with each other and anti-HBc positive is higher in older age group. Mremi et.al, (2021) also claimed that the possibility of infection is higher in aged donors than younger age group. This result is partially in line and partially deviated from the results of Gomes et.al, (2021) who recorded that anti-HBc decreased by 43% per year increase in age, and there is significant association between age and anti-HBc [10,11]. It was also found from other studies that the D-dimer level with prevalence of elevated D-dimer increased with age [12-15]. Tita-Nwa et.al, (2011) recorded that D-dimer statistically increased with age [16]. These disagreed with the result of this study.

Cao and Mei (2022) recorded that there was significantly positive association between D-dimer levels of patients with HBV-related acute-on-chronic liver failure [17]. This was also not in line with the results of this study.

CONCLUSION:

The study was aimed to investigate D-dimer levels of Hepatitis B negative blood donors. Less than half of the participants have either been previously infected by Hepatitis B or received vaccine against it. This suggests that additional screening be conducted to ascertain total disappearance of the virus. Mean D-dimer level of 1.477±1.0 is within the normal range. Based on the results, most of the prospective donors were fit to donate blood. The study also emphasized the importance of thorough blood screening to ascertain the suitability of donated blood for transfusion, while the safety of the donor is not compromised.

REFERENCES:

[1]. Heymann DL. Hepatitis B In: Control of Communicable Diseases Manual. 20th ed. Washington, DC: American Public Health Association; 2015. p. 257-64.

[2]. Committee on Infectious Diseases, American Academy of Pediatrics. Hepatitis B. Red Book: 2021–2024 Report of the Committee on Infectious Diseases. 32 ed. Elk Grove Village, IL: American Academy of Pediatrics; 2021. 381 -399 pages.

[3]. Hennig H., Puchta I., Luhm J., Schlenke P., Goerg S., and Kirchner H. Frequency and Load of Hepatitis B Virus DNA in First-time Blood Donors. Blood, 2002, 100, 7: 2637-2641.

[4]. Obu, S I, Amilo G I, Ibeh N C, Ehiaghe A F, Chkwuanukwu R C, Ehiaghe J I, Erhunmwun U R, Nosahkare O O, Ogenyi S and Unimnebeshi A O. Evaluation of Some Haemostatic Parameters as Co-Markers to Viral Load in the Management of HBV Infection Treatment Outcome in Delta State, Nigeria. Journal of Bioanalysis & Biomedicine 2022; 14, 1: 304 – 307.

[5]. Onyekwelu K A. Case Report of Ds DNA Negative with Ana Positive SLE Induced Neuropsychosis Diagnosis Dilemma. Annals of Medical and Health Sciences Research 2022; 12: 336-343.

[6]. Bayram Ahmed Abdul-Abbas, Al-Dahmoshi Hussein O M, Al-Khafaji Noor S K, Al Mammori Raheem Tuama Obayes, Al-Shimmery Ali Husain Shilib and Saki Morteza. "Study of the D-dimer, C-reactive protein, and autoantibodies markers among HBV infected patients in Babylon province, Iraq," BioMedicine 2021; 11, 4, Article 8.

[7]. Ugwu A.O., Madu A.J., Efobi C.C. and Ibegbulam O G. Pattern of Blood Donation and Characteristics of Blood Donors in Enugu, Southeast Nigeria. Nigeria Journal of Clinical Practice 2018; 21, 11: 1438-1443.

[8]. Mremi A, Yahaya JJ, Nyindo M, Mollel E. Transfusion-Transmitted Infections and associated risk factors at the Northern Zone Blood Transfusion Center in Tanzania: A study of blood donors between 2017 and 2019. PLoS ONE 2021; 16, 3: e0249061.

[9]. Olayinka A.T., Oyemakinde A., Balogun M.S., Ajudua A., Nguku P., Aderinola M, Egwuenu-Oladejo A, Ajisegiri S.W., Sha'aibu S, Musa B O P, Gidado S and Nasidi A. Seroprevalence of Hepatitis B Infection in Nigeria: A National Survey. "The American Journal of Tropical Medicine and Hygiene 2016 Oct. 5; 95; 4: 902 – 907.

[10]. Basma Doro, Wajdi M. Zawia, Walid M. Ramadan Husien, Nagi Meftah Gerbil Abdalla, Adam M. Rifai, Enase Dourou, Fatma J. Amar and Abdulwahab N. Aboughress. International Blood Research & Reviews 2015; 4; 3: 1-8, 2015, Article no. IBRR.20272.

[11]. Gomes L., Cordeiro Gomes Sanson M., Brainin P, de Melo M D C V, de Souza R M, Mazaro J, Lima K O, Resende J S, Vieira I V M, Mesquita E D S, Matos L O, Dutra I C S, Palmisano G, Wrenger C, Marinho C R F and da Silva R D S U (2021). Levels of Hepatitis B antibody Titers are affected by Age and Doses Gap Time in Children from High Endemic Area of Western Amazon. Public Library of Science – PLoS ONE 2021. Volume 16, issue 7: e0253752. Published online 2021 July 1.

[12]. Dacie JV, Lewis SM. Miscellaneous tests. In: Dacie JV, Lewis SM, editors. Practical Haematology. London Churchill Livingstone; 1995; 559–574.

[13]. [13] Saadeh C. The erythrocytes sedimentation rate: old and new clinical applications. Southern Medical Journal; 1998; 91: 220–225.

[14]. Righini M, Goehring C, Bounameaux H, Perrier A. Effects of age on the performance of common diagnostic tests for pulmonary embolism. The American Journal of Medicine, 2000; 109: 357–361.

[15]. Righini M, Le Gal G, Perrier A, Bounameaux H. The challenge of diagnosing pulmonary embolism in elderly patients: influence of age on commonly used diagnostic tests strategies. Journal of the American Geriatrics Society. 2005; 53: 1039–1045.

[16]. Tita-Nwa F., Bos A., Adjei A., Ershler W., Longo D.L. and Ferucci L. (2011). Correlates of D-dimer in Older Persons. Aging Clinical and Experimental Research. 22, 1: 20 – 23.

[17]. Cao and Mei (2022): Predictive Value of Elevated Serum D-Dimer for Short-Term Prognosis In HBV-Aute-On-Chronic Liver Failure. Experimental and Therapeutic Medicine; 2022. 24: 472.

Cite of article: Akpan Richard P, Ugbomoiko Daniel O, Ajugwo Anslem O, Akpan Victoria O. D-dimer levels in Hepatitis B negative blood donors at the blood bank of university college hospital, Ibadan. Int. J. Med. Lab. Res. 2023;8,2:1-7.
http://doi.org/10.35503/IJMLR.2023.8201

CONFLICT OF INTEREST: Authors declared no conflict of interest
SOURCE OF FINANCIAL SUPPORT: Nil