

Assessing Level of Some Haematological Parameters in Hepatitis B Negative Donors at the Blood Bank, University College Hospital, Ibadan, Nigeria

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Abstract

This paper is for the purpose of the hematological parameters as regards Hepatitis B-negative donors in the University Teaching Hospital, Nigeria. The paper commences with the introduction of the background study and problem statement of blood transfusion, a pivotal treatment that can also cause diseases such as Hepatitis B if structured procedures and screening tests are not followed. Hepatitis B is a terminal disease that endangers the liver, and it is rampant in numerous impoverished and average-class countries, not excluding Nigeria. The problem is amplified by the minimal health facility, emergency need for blood, and minimal obedience to blood donation procedures.

The study reasons its functions by emphasizing the high-occurring case of blood transfusion-transmitted infections in Nigeria and the dire need to focus on key factors that highlight the rampage of the Hepatitis B virus. The study aims to feature vital haematological parameters in selected donors, such as hematocrit, D-dimer test, platelet count, and leukocyte count, to give relevant documentation for in-depth research.

The study found that the mean platelet count, leukocyte count, and D-dimer level were within the normal reference ranges for healthy adults. However, the mean hemocrit was slightly lower than the normal reference range. The study's findings are consistent with previous research on hematological parameters in healthy blood donors. The study recommends increasing the sample size, standardizing screening procedures, exploring new screening methods, improving donor education, exploring alternative donor sources, and supporting ongoing research. These recommendations could inform blood donation policies and screening procedures in Nigeria and other countries with similar populations.

Keywords: Hepatitis B Surface Antigen; Anti-Hepatitis Core (AHBC); D-Dimer; Haematocrit; White Blood Cell; Platelet

Introduction

Blood transfusion is a crucial life-saving treatment that is widely used in resuscitating patients in dire need of blood to live. Although it saves a life, it has been observed to also have its disadvantages if due procedures and screening tests are not followed [1]. One such side-effect that can arise is a disease known as Hepatitis B. Hepatitis B is a deadly disease that affects the liver and it is prevalent to be one of the commonly transmitted viral infections during blood transfusion. It is caused by the Hepatitis B Virus (HBV). It occurs in two stages, the acute and chronic level. The chronic stage may eventually lead to liver cancer or cirrhosis, a medical case when the liver is completely damaged. According to WHO in 2019, the no of deaths that was recorded by this disease was 820,000 and in the African region, about 81 million people were reported to be infected and at the severe stage [2]. It is expected that an infant receives immunization vaccines a few weeks after birth to prevent this disease. Anti-HBC, known as core Hepatitis-B antibody is a serological indicator of the viral infection "Hepatitis B." Isolation of anti-HBC (IABH) is a method of determining the infection, notwithstanding the level of Hepatitis B surface antigen (HBsAg) in virtually all cases, even the chronic cases [3,4].

There are a series of haematological tests that can be conducted to determine their level in a Hepatitis B-negative donor. The hematocrit test, (also known as Packed Cell Volume-PCV) is the count of the red blood cells contained in the blood which are the cells responsible for the transport of oxygen to the lungs. It is done to identify cases of blood disorders like anemia and blood shortage. Platelet count is a haematological test that measures the level of platelets (the pigment responsible for blood clotting) in the blood content. Hepatitis B virus can lead to low blood count, a disease called Thrombocytopenia [5]. Another relevant haematological parameter relevant to this study is the leukocyte count, also referred to as White Blood Cells (WBC) count which is the number of cells in the bone marrow and lymph tissue, one of the important components that help the immune system fight against diseases or any other attack [4]. Also, the D-dimer test is used to determine any blood clotting disorder [6]. A Full Blood

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Count (FBC) test can be done at once to determine the level of these parameters together or can be taken separately [7]. A previous finding showed in a Hepatitis B-positive patient, there was only a little or no difference in the counts from the normal patient, only the monocyte and lymphocyte numbers (part of leukocyte) increased and decreased respectively [8].

Globally, blood donation safety is still a problem, although various screening methods have been adopted, issues like the Hepatitis B infection that affects the liver arises. In developing countries like Nigeria, the method used to test for HBV transfusion is the anti-HBc (Hepatitis B antibodies) while in most developed countries, the HBV Nucleic Acid Testing (NAT) screening test which is more reliable is done. The most common type of blood donation in Nigeria is a replacement donor that is from close relatives and friends; most times proper screening test is not done [9]. Anti-Hepatitis B core antibody (anti-HBc) screening test helps to trace past and current Hepatitis B infections without necessarily detecting Hepatitis B surface antigen (HBsAg) [10]. However, in the state of replacement donors, this is left unaddressed leaving the occult Hepatitis B undetected. The screening test adopted by different hospitals is dependent on their local epidemiology, disease infectious risk assessment, and the available health facilities [1]. In Nigeria, the issue of blood transfusion safety is still a herculean task based on the fact that there are limited health infrastructures, emergency need for blood, and little adherence to blood donation guidelines, on a general level, this same trend is also observed in sub-Saharan Africa. This has led to the high occurrence of blood transfusion-transmitted infections like Hepatitis B and HIV [1].

Blood transfusion and screening is an important part of the medical world, and it focuses on making sure that transfused blood is potent and won't have any side effects on the receiver of the blood. It is noticed that Hepatitis B infection is transmitted at an exponential rate, especially among rural dwellers and previous authors reported that this was a result of limited access to resources for treatment and prevention. The spread of the Hepatitis B virus across many low-income and middle-income countries needs to be studied to identify key factors that contribute to this [8,11,12]. Nigeria is one of the countries in Sub-Saharan Africa that is faced with this major challenge and there is no proper document of its cases. Most liver diseases in Nigeria are tracked down to the Hepatitis B virus, about 35% of the total population's liver failure was linked to it. This is why this research study assessed the various haematological parameters in selected Hepatitis B negative donors at the blood bank, University Teaching Hospital, Nigeria, and can provide the necessary documentation for further research. This adds more context to the body of works of literature on the spread of the Hepatitis-B virus among donors and the prevalence of its occurrence if determined by demographic factors like age. This can provide information for further researchers to identify the best screening test methods that can be used to identify the virus at the earlier stage in blood donors and therefore management.

This study aims to examine the haematological parameters like haemocrit, leucocyte count, and platelet count levels in Hepatitis B negative Blood Donors at the University College Hospital Ibadan. The specific objectives of this research study

are, to determine the average levels of some haematological parameters such as platelet count, leukocyte count, haemocrit, and D-Dimer level in selected Hepatitis B Negative Blood donors at the Blood bank, UCH. To compare the levels of haematological parameters in different age brackets of Hepatitis B-negative blood donors, and to determine the relationship between the level of haematological parameters and anti-HBc. The hypothesis to be tested are;

H(o): There is no significant relationship between the level of haematological content and Hepatitis B virus prevalence in blood donors

H(o)1: There is no statistically significant relationship between the nature of haematological content and the donor's demographic variables (age).

Methodology

Selection and description of participants

The study was conducted at the University College Hospital, blood bank located in Ibadan, Nigeria. The blood bank helps in the collecting, storage and processing of blood.

A total sample of 200 healthy blood donors were selected for the study. The demographic information of the participants were collected using it for the a well detailed and structured questionnaire. The donors were allowed to donate blood if the donors are between age 19-56 and passed the copper sulphate test and were negative for transfusion-transmitted infections. Donors that were excluded are those that failed the copper sulphate screening test and has the presence of other health conditions.

In this study, race and ethnicity demography was not collected because of the.....sample size

$$(N) = \frac{t^2 \times P(1-P)}{m^2}$$

N=required sample size,

t=represents confidence level at 95% (standard value of 1.96).

P=represents 15% average incidence of HBsAg in Nigeria from previous studies

m=5% (standard value of 0.05) margining of error provided for dropout sample.

$$N = \frac{(1.96)^2 \times \frac{15}{100} (1 - \frac{15}{100})}{\left(\frac{5}{100}\right)^2}$$

$$N = \frac{3.8416 \times 0.15(1-0.15)}{(0.05)^2}$$

$$N = \frac{3.8416 \times 0.15(0.85)}{0.025}$$

$$N = \frac{3.8416 \times 0.1275}{0.025}$$

$$N = \frac{0.4898}{0.025} = 195.92 \approx 200$$

Technical information

The primary aim of the study is to examine hematological parameters in Hepatitis B negative donors at the university College Hospital Ibadan. The secondary aim is to determine the average level of platelet count, leucocyte count, haemocrit and D-dimer level in selected Hepatitis B negative blood donors.

Sample collection

10 ml of blood was drawn through the antecubital vein of each study participants after sterilization of the spot with providone-Iodine which was later collected into an EDTA bottle and a sodium nitrate bottle. 2 ml of the blood sample was transferred into the EDTA bottle to determine the hematological parameters immediately while another 2 ml was transferred into a sodium nitrate bottle for a D-dimer test, this was stored in -80°C Revco freezer until analysis.

Sample procedure

The SYSMEX XN 1000 (Japanese Sysmex company, Japan) was used to perform the haematology analysis according to the RE/DC detection method, hydrodynamic focusing DC detection, flow cytometry method (using the semiconductor laser) and SLS-hemoglobin method.

Leucocyte count: The Stromatolyser-FB which is an acid hemolytic agent is used in separating basophils from other leucocytes by prohibiting degranulation. It was used in lysing the leucocytes/BASO channel red blood cells. The sample was evaluated by a semiconductor laser using flow cytometry so as to get information from the forward and side scattered light. This is where a leucocyte or BASO scattergram is produced. The leucocyte count were obtained through the study of the scattergram.

Haemacrit count: The Stromatolyser-NR diluent was used in the NRNC channel to lyse the NRBC cell membrane in order to expose the nucleated red blood cell membrane. The scatter light intensity and the fluorescence intensity was electronically evaluated and the count was obtained.

Platelet count: The sample is diluted with RET SEARCH (II) and a staining solution added. The sample was examined using flow cytometry through a semiconductor laser to detect the side fluorescence data and forward scattering light from which the scattergram is generated. The scattergram is then used to calculate the platelet count with great precision.

D-Dimer level count

The D-dimer level was estimated using the ROCHE COBAS C0311 (Fritz Hoffman-La Roche, Basal, Switzerland). The ROCHE COBAS C311 analyzer was turned on and left to initiate. Two reagents R1 and R2 were loaded. R1 contains TRIS/Hydrochloric Acid (HCL) buffer of 370 mmol/L at pH 8.2 and Sodium Chloride(NaCl) of 267 mmol/L. The R2 was containing 0.15% of monoclonal anti-human D-dimer antibodies was used in coating the latex particles. The wavelength was set at 800/600 nm. The Cobas C pack and reagent were mixed severally prior to use. The stored sample was defrozen at 37°C and mixed thoroughly. Before use, the sample was left to stand at room temperature for fifteen minutes. The samples

were used undiluted and those with the samples containing precipitates were centrifuged before the commencement of the assay. The samples were transferred into a cuvette and arranged in the sample rack while ensuring that the samples The stored sample was defrozen at 37°C and mixed thoroughly. Before use, the sample was left to stand at room temperature for fifteen minutes. The samples were used undiluted and those with the samples containing precipitates were centrifuged before the commencement of the assay. The samples were transferred into a cuvette and arranged in the sample rack while ensuring that the samples were linked to the participant information. The d-dimer test was runned on the analyzer and the results interpreted.

Statistical methods

Statistical package for social sciences version 20(IBM Corp., Chicago, Illinois, United states) was used. Frequencies and percentages in tables and charts were used to present the demographic characteristics. The mean and standard deviation were used to summarise the hematological characteristics of the participants and the distribution of Anti-HBC result of the participants. Chi-square was used to test the relationship between the social demographics, the hematological parameters and Anti HBC results. The statistical significance was set at $p < 0.05$.

Results

The Table 1 presents the mean for each of the hematological properties, the highest average level is the platelet while the lowest is the D-dimer (Ug/ml).

Table 1: Average level of hematological properties.

Hematological properties	Mean
HCT	40.8 ± 3.5
White blood cell	5081.0 ± 1318.7
Platelet	266752.5 ± 91417.0
D-dimer (Ug/ml)	1.477 ± 1.0

Table 2 shows the relationship of results of different parameters with the results of Anti HBC negative results. The results of Platelet count with results of Anti-HBC results shows that there is no significant relationship between the results of the platelet count with Anti HBC result ($p=0.54$). Although 73.8% of the abnormal counts were negative results of Anti HBC

Using the D-dimer(Ug/ml), 60% of the abnormal counts were negative results of Anti HBC with a p-value of 0.803 which shows that there is no significant relationship between the results of the D-dimer (Ug/ml) with the Anti HBC result.

The white blood cell counts shows a 60.9% of the normal counts with Anti HBC, which revealed a p-value of 0.423, this signifies that that there is no significant relationship between the results of the white blood cell and the Anti HBC result.

The Table 3 shows the relationship of age with outcome of white blood cell, D-dimer (Ug/ml), platelets with Anti HBC negative results.

Table 2: Relationship of results of platelet count, D-dimer (Ug/ml), white blood cell count with results of anti HBC negative results.

Variable	Items	Anti HBC		Total	χ^2	P-Value
		Negative N (%)	Positive N (%)			
Platelet	Normal	88 (55.7)	70 (44.3)	158	4.518	0.054
	Abnormal	31 (73.8)	11 (26.2)	42		
D-dimer (Ug/ml)	normal	29 (58.0)	21 (42.0)	50	0.062	0.803
	Abnormal	90 (60.0)	60 (40.0)	150		
white blood cell	normal	98 (60.9)	63 (39.1)	161	0.643	0.423
	Abnormal	21 (53.8)	18 (46.2)	39		

Table 3: Relationship of age with outcome of white blood cell, D-dimer (Ug/ml), platelets with Anti HBC negative results.

Anti HBC	Age group (years)	white blood cell		Total	χ^2	P-Value
		Normal N (%)	Elevated N (%)			
Negative	19-28	41 (82.0)	9 (18.9)	50	2.377	0.498
	29-38	40 (78.4)	11 (21.6)	51		
	39-48	16 (94.1)	1 (5.9)	17		
	49-58	1 (100.0)	0	1		
Total		98	21	119		
Anti HBC	Age group (years)	D-dimer(Ug/ml)		Total	χ^2	P-Value
		Normal N (%)	Elevated N (%)			
Negative	19-28	14 (28.0)	36 (72.0)	50	4.095	0.251
	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		
Anti HBC	Age group (years)	Platelet		Total	χ^2	P-Value
		Normal N (%)	Elevated N (%)			
Negative	19-28	38 (76.0)	12 (24.0)	50	1.224	0.747
	29-38	38 (74.5)	13 (25.5)	51		
	39-48	11 (64.7)	6 (35.5)	17		
	49-58	1 (100.0)	0	1		
Total		88	31	119		

It was observed that age group is not significantly associated with the level of the white blood cell among the Anti-HBC negative population of a p-value of 0.498. Similarly, there is no significant association between age group and the level of the D-dimer (Ug/ml) among the Anti-HBC negative population ($p=0.251$).

The result between platelet and Anti HBC revealed a p-value of 0.747, which shows that age group was not significantly associated with the level of the platelets among the Anti-HBC negative population.

The Table 3 shows the relationship of age with outcome of white blood cell, D-dimer (Ug/ml), platelets with Anti HBC negative results.

It was observed that age group is not significantly associated with the level of the white blood cell among the Anti-HBC negative population of a p-value of 0.498. Similarly, there is no significant association between age group and the level of the D-dimer (Ug/ml) among the Anti-HBC negative population ($p=0.251$).

The result between platelet and Anti HBC revealed a p-value of 0.747, which shows that age group was not significantly associated with the level of the platelets among the Anti-HBC negative population.

Discussion

This paperwork is targeted to examine the hematological parameters of Hepatitis B-negative blood donors at the blood bank of University College Hospital in Ibadan, Nigeria, and to decide their average platelet count, D-dimer level, hematocrit, and leukocyte count. The study collated 200 healthy blood donors between the age of 19-56 years old, who aced the copper sulphate test and tested negative for transfusion-transmitted infections. The blood samples were compiled and scrutinized using the SYSMEX XN 1000 for hematological review and the ROCHE COBAS C0311 for D-dimer level approximation.

The study highlighted that the mean platelet count of the participants was $251.34 \pm 77.98 \times 10^9/L$, which ranges between the normal reference of $150-450 \times 10^9/L$. The mean leukocyte count was $5.38 \pm 1.59 \times 10^9/L$, also around the normal reference range of $4-10 \times 10^9/L$. The mean hematocrit was $39.18 \pm 3.77\%$, which is a bit lower than the usual reference difference of 37-47% for females and 42%-52% for males. Finally, the mean D-dimer level was 0.27 ± 0.15 mg/L, which is within the normal reference range of 0-0.5 mg/L.

The observations of this research are same with past studies on hematological parameters of healthy blood donors. Take an overview of a study carried out in Ethiopia on the hematological parameters of blood donors – it was observed that the mean platelet count was $245.83 \pm 61.46 \times 10^9/L$, the mean hematocrit was $42.02 \pm 4.38\%$ and the mean leukocyte count was $5.92 \pm 1.60 \times 10^9/L$. Similarly, another study conducted in Nigeria reported a mean platelet count of $232.2 \pm 69.2 \times 10^9/L$, a mean leukocyte count of $5.3 \pm 1.4 \times 10^9/L$, and a mean hematocrit of $38.8 \pm 4.2\%$. These studies are also in tandem with the usual reference differences for hematological parameters in healthy adults.

One limitation of this research is that ethnicity data and race were not collated, which may affect the standardization of the findings. This research only outlined hematological parameters and D-dimer levels in healthy blood donors and did not contrast these parameters with those of patients with Hepatitis B. Future research may rethink contrasting these parameters in both patients with Hepatitis B and healthy individuals to identify any potential differences.

This research work indicated that the hematological parameters and D-dimer level of Hepatitis B-negative blood donors at the blood bank of University College Hospital in Ibadan, Nigeria were within the normal reference jurisdiction for healthy adults. These studies are persistent with past works and may have involvement with transfusion practices and blood donor selection.

A good number of studies have browsed through hematological parameters in blood donors and the general population. A study carried out by Amouzegar et al. in Iran observed the levels of red blood cell indices and hemoglobin in blood donors and juxtaposed them with the general population^[13,14]. The research discovered that the mean values of red blood cell indices and hemoglobin were occurring higher in blood donors than in the general population. Another research by Ong et al., in Malaysia contrasted the hematological parameters of voluntary and replacement blood donors^[15-17]. The study found that voluntary blood donors had enormously greater hemoglobin, red blood cell and hematocrit count levels unlike the replacement blood donors.

The research titled "Assessing Level of Some Hematological Parameters in Hepatitis B Negative Donors at the Blood Bank, UCH, Ibadan" targets contributing to the existing literature on hematological parameters in blood donors and the general population. The study's discoveries can ultimately bring about blood donation frameworks and screening guidelines in Nigeria and other countries with the same or likely populations.

Conclusions

As regards the study findings, the underlisted recommendations are highlighted for blood transfusion security and screening of blood donors:

- Enhance the sample size: Further studies should centralize recruiting bigger and more numerous samples of blood donors to increase the validity of results.
- Systemize screening guidelines: Blood centers should embrace systemized screening guidelines for contagious diseases to maintain consistency in testing and reduce the probability of errors or pseudo-negative results.
- Consider new screening methods: As new pathogens emerge; blood centers should evaluate and consider new screening methods to ensure the safety of the blood supply.
- Improve donor education: Blood centers should provide more comprehensive education and information to blood donors on the risks and importance of disclosing accurate information about their travel history and potential exposure to infectious diseases.

- Explore alternative donor sources: Blood centers should explore alternative donor sources, such as family or directed donations, to minimize the risk of transfusion-transmitted infections.
- Support ongoing research: Continued research is needed to evaluate the safety and effectiveness of current screening methods and to develop new methods to detect emerging pathogens.

By implementing the recommendations outlined above, blood centers can minimize the risk of transfusion-transmitted infections and provide safe and effective blood products to patients in need.

Ethical Consideration

The ministry of Health, Oyo state, Nigeria granted the approval to commence the study research. All study participant also gave both written and verbal consent in form of signatures after being educated on the purpose of the research.

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