



Haematological Parameters of Immunized and Non-immunized Hepatitis B Negative Individuals in a Selected Community in Osogbo, Osun State, Nigeria

Adebayo T. O.^{1*}, Kosamat Y. A.², Adedokun A. A.¹, Yusuf A. W.¹, Zakari A. A.¹, & Oguntade A. A.¹

¹Department of Medical Laboratory Science, Fountain University, Osogbo, Osun State, Nigeria;

²Department of Medical Laboratory Science, Osun State University, Osogbo, Osun State, Nigeria.

*Corresponding author: toadebayomls@gmail.com, +2348034737075

Abstract

Background: The Hepatitis B Virus (HBV), a type of hepadnavirus, targets liver cells and annually causes approximately 820,000 deaths. Although effective vaccines are available, Hepatitis B continues to pose a significant public health challenge. **Objectives:** This study examined the haematological patterns between vaccinated and unvaccinated hepatitis B-negative individuals for variations in blood parameters. **Methodology:** This cross-sectional study took place in Iludun, Osogbo, Osun State, Nigeria, and used 140 participants, half vaccinated. Blood samples collected from these subjects underwent various parameter analyses, including white blood cell count (WBC), packed cell volume (PCV), red blood cell count (RBC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and haemoglobin levels (HGB). Data analysis was done using the Statistical Package for the Social Sciences version 22.0 with a p-value threshold set at less than 0.05 to determine statistical significance between both groups. **Results:** The findings showed that the average RBC in the immunized group was 4.18 ± 0.51 compared to 4.41 ± 0.66 in the non-immunized group. The MCHC measured 12.55 ± 1.57 for the immunized and increased slightly to an average of 13.24 ± 1.99 within non-immunized members. A notable statistically significant difference was observed between RBC and HGB; however, WBC, PCV, leukocyte distributions (differential counts), and MCV indices had no significant difference between the two population sets. **Conclusion/Recommendation:** The data determines that hepatitis B immunization may not affect certain haematological parameters. However, this study indicates that while protecting against the virus, hepatitis B immunization had a limited chance of enhancing specific blood parameters. The study provides evidence-based insights to strengthen public health initiatives, generate baseline data for diagnosing and managing blood-related conditions, influence policy decisions on haematological monitoring in vaccination programmes, raise awareness about immunisation's benefits and lay a foundation for future research exploring trends and impacts of vaccination. Therefore, a cross-sectional design is recommended to help assess long-term changes or effects of immunization on haematological parameters.

Keywords: Immunization, leukaemia, differential counts, blood, vaccination

Introduction

Hepatitis B Virus (HBV) is a hepadnavirus that infects the liver cells and is responsible for about 820,000 deaths per year, according to the World Health Organization (2021). Being infected with hepatitis B increases the risk of death from cirrhosis, liver and non-liver cancers (Song et al., 2019). Transmission of HBV as a result of being exposed to infected blood or body fluids, unprotected sexual contact with an infected person, blood transfusion with infected blood or blood products, use of contaminated needles, syringes, and sharps, as well as transmission from mother to child. A woman who screened positive for the hepatitis B surface antigen (HBsAg) poses the risk of passing on HBV to the newborn; the presence of the hepatitis B envelope antigen (HBeAg) increases such a risk (Gentile and Borgia, 2014). Globally, according to WHO estimates, 257 million people worldwide, or 3.5% of the total population of the World, had a chronic HBV infection in 2015. Sixty-eight per cent of the infected were from the Western Pacific and African regions (Ott et al., 2012). The National Programme on Immunization (NPI) was established in 1998 in Nigeria to immunize babies; infants received 50.6% of the first dose of the hepatitis B vaccine, 45.6% of the second dose, and 38.2% of the third dosage (National Population Commission, 2014). The coverage of the first dose of vaccines in Bauchi State is 25.4%; the second dose is 18.9%; and the third dose is 12.5% (National Population Commission, 2014). From 12.5% in 2013 to 32.1% in 2018, more children received the third dose of the pentavalent vaccination (National Population Commission, 2014; National Population Commission & ICF, 2019).

Around the World, women of reproductive age were predicted to have a 3.5% prevalence of chronic HBV infection (Ott et al., 2012). The prevalence in African nations varies between 6 and 25% (Ott et al., 2012); 2.2% of the total population in Nigeria had hepatitis B, according to a national study reported by Olayinka et al. (2016), while 14.1% of pregnant

women in Nigeria had the viral infection, according to a systematic review put together by Musa et al. (2015). In Bayara, Bauchi State, 17.2% of pregnant women were found to have HBsAg (Ndako et al., 2012). Consequently, Nigeria has one of the highest rates of HBV-attributable cancer in West Africa, with an estimate of 2 to 5 cases per 100,000 persons. This cancer is very aggressive, with limited treatment options, requiring several resources to contain. Being a viral infection, it is a serious global health challenge leading to several liver conditions like cirrhosis and hepatocellular carcinoma, as noted by Lok & McMahon (2009); this has led to the requirement of necessary vaccination among adults and children alike (Schweitzer et al., 2015). There has been a strong emphasis on vaccination as the WHO recommends universal HBV vaccination to reduce HBV infection and combat its spread.

Nevertheless, it is crucial to understand how Hepatitis B serological patterns behave in populations that have tested negative for the virus to identify the potential barriers to immunization coverage, assess the risk of viral reactivation, and explore the impact of co-infections and haematological factors in HBV infection. The distinctive blood characteristics and profiles seen in groups of immunized and non-immunized persons are the haematological patterns covering parameters like haematocrit and white and red blood cell counts (Ali & Ahmed, 2018). Despite the success of HBV vaccination programmes, there have been instances of breakthrough infections and viral reactivation in individuals previously considered negative for Hepatitis B (Fattvich et al., 2008); this highlights the need for a comprehensive investigation into the serological patterns of HBV between the immunized and non-immunized population selected in Iludun, Osogbo for the objective of this study to verify the possible significant difference.

Materials and methods

Study area

The study was carried out within the Iludun community in Osogbo, Osun State.

Study population

The participants of 140 individuals were administered copies of a questionnaire containing relevant variables such as names, levels of education, genders, etc. It aided in understanding the characteristics and identities of the engaged participants to obtain a diverse range of viewpoints; the participants were selected over a range of ages and genders.

Study design

The design for this study is cross-sectional.

Sample size determination and collection

The sample size of this study was determined using the formula below;

$$n = Z^2 * p(1-p) / d^2 \text{ (Cochran, 1977)}$$

d = Precision (corresponding to effect size)

n = $Z^2 * p(1-p) / d$, substitute values we have:

$$n = (1.96)^2 * 9.5(1-9.5) / (0.05)^2$$

$$n = 133$$

Sample collection was carried out for 3 months. The sample was collected by disinfecting the area of collection. The area of collection for the study is the arm. A tourniquet was tied at the arm's upper part, and the patient made a fist to make the vein very prominent. The needle was then inserted into the vein at an angle of 30 degrees. After the blood had been drawn, the tourniquet was removed, followed by the needle removal from the vein, and the blood was dispensed into the sample bottles, which were carried to the laboratory.

Inclusion criteria

The inclusion criteria included;

1. Individuals between 15 and 65 years old (male and female inclusive).
2. Participants must reside within the study community.
3. Participants must be willing to participate in the study.

Exclusion criteria

The exclusion criteria involved individuals;

1. Who have been screened positive for Hepatitis B virus.
2. Below the age of 15 years or above 65 years.

Laboratory Diagnosis

Differential count: A blood smear was prepared to obtain the head, body, and tail, stained with Leishman stain, and then examined under a microscope to assess the morphology of red blood cells

Packed cell volume: Two-thirds (2/3) of a capillary tube was filled with blood, the unused end was sealed with plasticine, placed in a haematocrit centrifuge, spun at 15000 rpm for 5 minutes, and then read with a haematocrit reader.

Total white blood cell count: Three hundred and eighty (380) microliters of the Turks solution (WBC diluting fluid) were dispensed in a test tube with 20 microliters of blood and mixed. The hemocytometer was assembled by sliding over a cover slip on one grid area and pressed until Newton's ring was formed. The diluted sample was remixed to properly lyse red blood cells. A drop was picked and placed at the edge of the coverslip to fill one grid area. It was placed under the microscope and examined by focusing with x10 objective and x40 objective lenses to view.

Hepatitis B virus test: The blood samples were examined for Hepatitis B negative using HBsAg rapid test strip. A dropper was used to pick the specimen (whole blood), and then 2-3 drops of the specimen were added to the well of the test strip, after which two drops of the buffer were added into the sample well after the specimen was added. The timer was immediately set after the specimen and buffer was added. The test strip was allowed to react for 15 minutes. The results were read after the completion of the time.

Statistical analysis

The data was analyzed using SPSS (Statistical Package for the Social Sciences) version 22.0. The findings were displayed in charts and tables. An independent sample t-test was used to compare the haematological parameters and

age of immunized and non-immunized individuals. The results were interpreted using $p < 0.05$ as the significant value.

Results

The analysis of data collected through the structured questionnaire demonstrated a lower percentage of immunized individuals within the groups 15-25 years and 26-35 years. However, the percentage of immunized individuals starts to increase from 36-45 years and above. Most participants do not smoke cigarettes, as shown in chart 1, which cross-references between the immunized and non-immunized participants. Regarding gender, 27.10% were male, and 72.9 % were female, showing females as the predominant participants in this study and could be responsible for a higher number of the participants being non-smokers. Regarding ethnicity, Yoruba dominated at 83.6 %, 14.4 % Hausa, and 2.0 % Igbo; this confirmed the study centre as a Yoruba community in Southwestern Nigeria. The educational level distribution showed that 67.9 % have secondary school or less, 9.3 % have

college/university, 2.1 % Have postgraduate, and 20.7 % have a bachelor's degree. Regarding employment status, 52.9 % were employed, 6.4 % were unemployed, 39.3 % were students, and 1.4 % were retired; this explains why many non-immunised participants are in the community. The majority of the participants, 136 (97.1%) reported never having been diagnosed with hepatitis B, with only 4 (2.9%) indicating they had. In contrast, a vast majority 138 (96.6%) of the participants do not smoke, while 2 (1.4%) do. Alcohol consumption is low among participants, with 137 (97.9%) not consuming alcohol and 3 (2.1%) reporting they do. 42 (30.0%) participants were immunized for immunisation status, whereas 98 (70.0%) were not. Most participants, 129 (92.1%) have never travelled to a region with a high prevalence of hepatitis B, while 11 (7.9%) have. Employment status shows that most immunised participants are employed 27 (64.3%), followed by 9 (21.4%) students. Among non-immunized participants, 47(48.0%) are employed, and 46 (46.9%) are students.

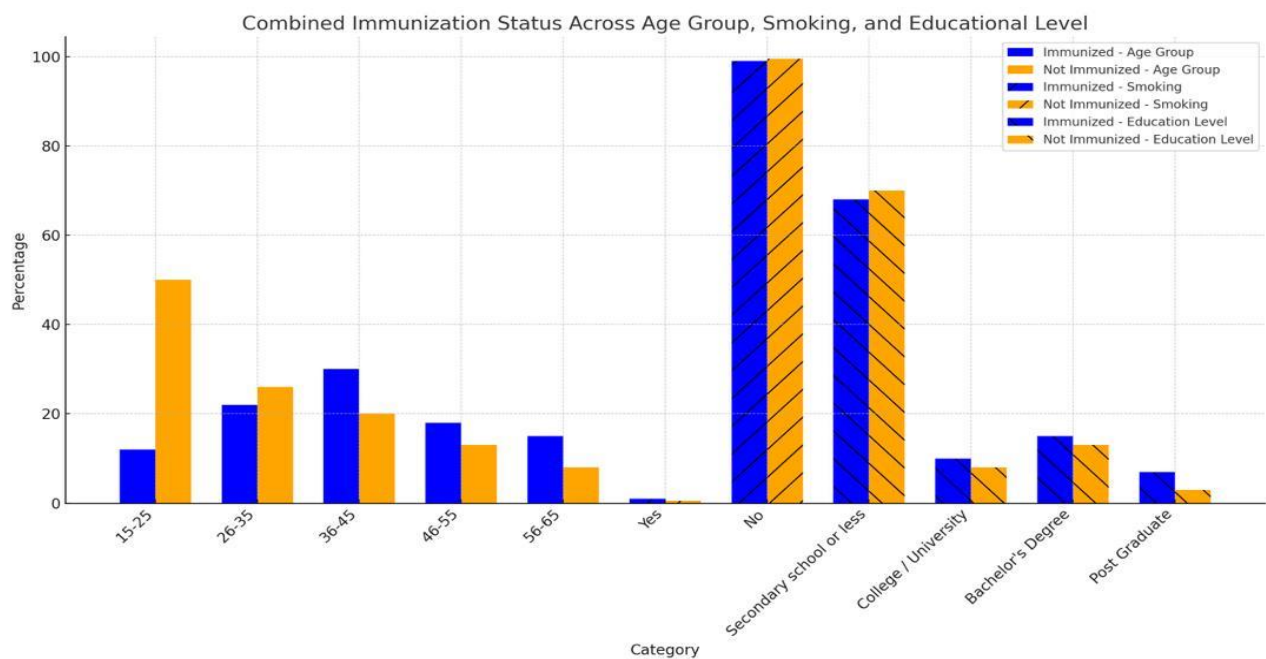


Chart 1: Combined immunization status across age groups, smoking, and educational level

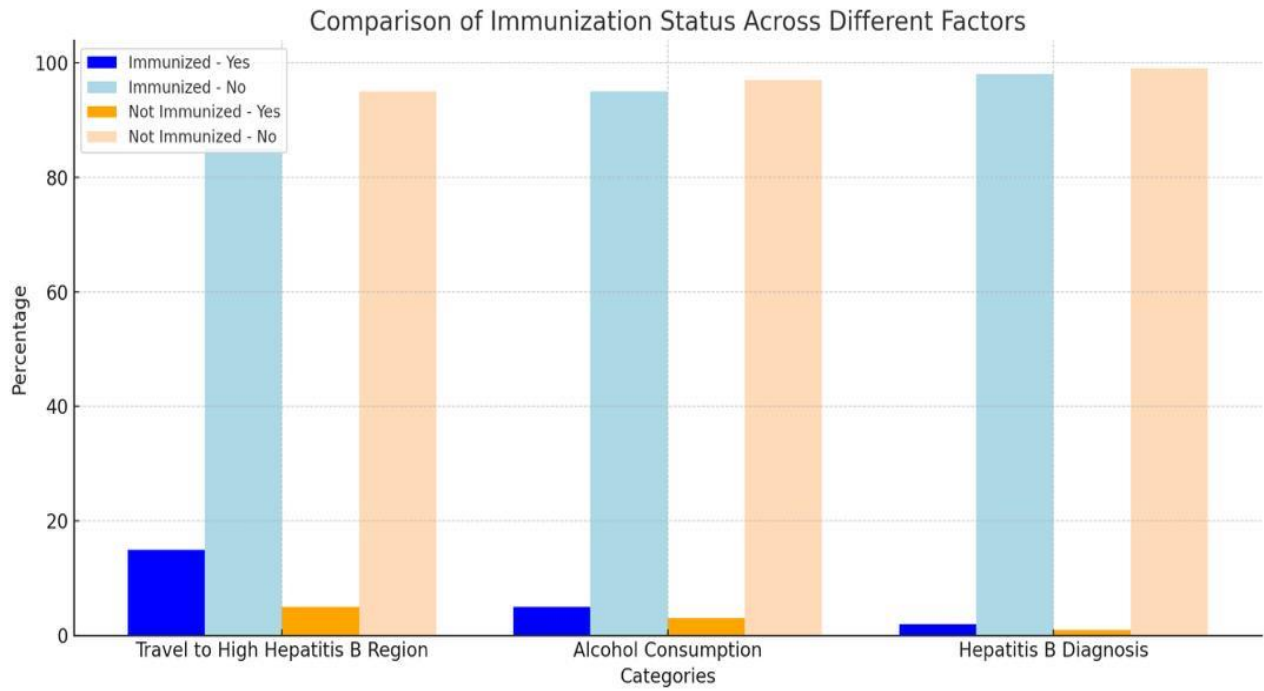


Chart 2: Comparison of immunization status across different factors

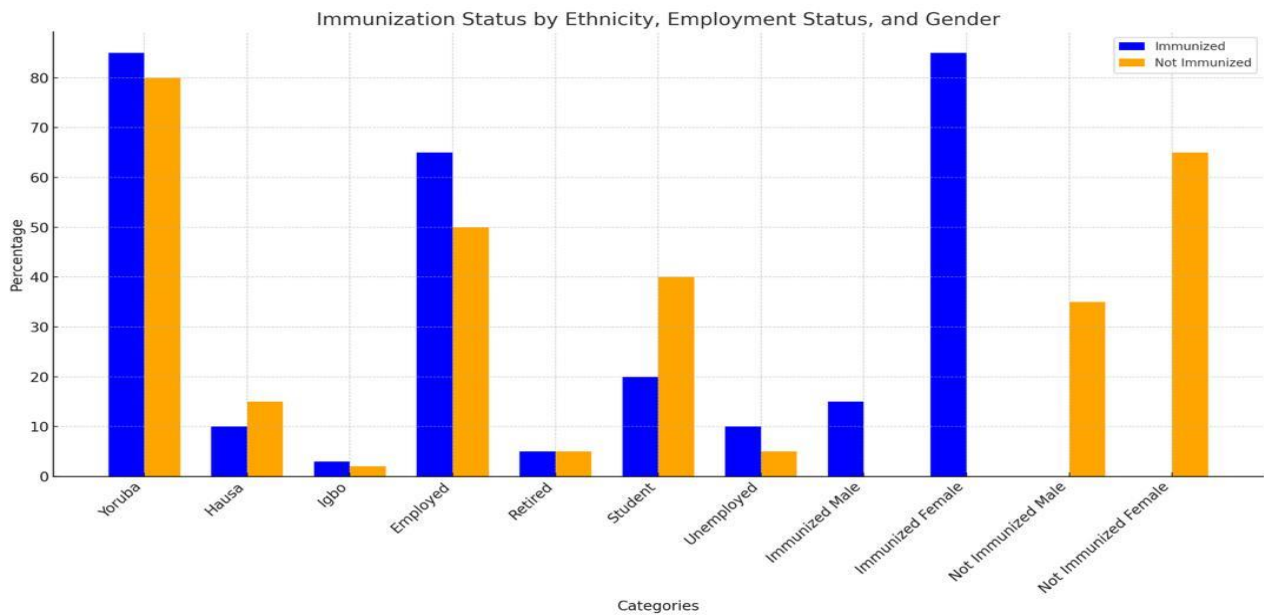


Chart 3: Immunization status by ethnicity, employment status, and gender

For the ethnicity, it shows that among immunized participants, Yoruba makes up 37 (88.1%), Hausa 4 (9.6%) and Igbo 1 (2.3%). Among non-immunized, Yoruba make up 80 (81.6%), Hausa 16 (16.3%) and Igbo 2 (2.1%). For the immunized, 42 (100%) reported No, and none reported Yes to smoking. Among the non-immunized participants, 2 reported Yes (2.0%) to smoking, and 96 (98.0%) reported No to smoking. For the immunized, 3 (7.1%) reported Yes to consuming alcohol, and 39 (92.9%) reported No. Among the non-immunized participants, 98 (100%) reported No to consuming alcohol, and none reported Yes. For the immunized, 2 (4.8%) reported Yes to having been diagnosed with hepatitis B, and 40 (95.2%) reported No. Among the non-immunized participants, 2 (2.0%) reported Yes to having been diagnosed with hepatitis B, and 96 (98.0%) reported No. Among the non-

immunized participants, 93 (94.9%) reported No to travelling to a region with a high prevalence of hepatitis B, while 5 (5.1%) reported Yes. For the immunized, 36 (85.7%) reported No to travelling to a region with a high prevalence of hepatitis B, while 6 (14.3%) reported Yes. Table 1 above shows that the non-immunized group has an average age of 29.04 years; the immunized group is noticeably higher, with an average age of 37.36; this implies that older people might be more likely to get immunized, maybe as a result of better access to healthcare or more excellent health knowledge. The immunized group has an average white blood cell count of 5.41 cells/L, a little more significant than the non-immunized group (4.90 cells/L). This slight white blood cell count variation could suggest a stronger immune response.

Table 1: Comparison of age and haematological parameters between the immunized and non-immunized participants using independent samples t-test.

Variables	Status	Mean ± SD	T-test	p-Value	Remark
Ages (Years)	Immunized	37.36 ± 14.98	0.001	<0.05	Significant
	Non-immunized	29.04 ± 12.75			
PCV (%)	Immunized	37.83 ± 4.65	0.062	>0.05	Non-significant
	Non-immunized	39.78 ± 5.94			
WBC	Immunized	5.41 ± 1.84	0.117	>0.05	Non-significant
	Non-immunized	4.90 ± 1.71			
Neutrophil (%)	Immunized	58.10 ± 5.93	0.961	>0.05	Non-significant
	Non-immunized	58.15 ± 6.64			
Lymphocyte (%)	Immunized	39.67 ± 6.327	0.962	>0.05	Non-significant
	Non-immunized	39.72 ± 6.76			
Monocyte (%)	Immunized	1.38 ± 1.69	0.669	>0.05	Non-significant
	Non-immunized	1.49 ± 1.22			
Eosinophil (%)	Immunized	0.71 ± 0.891	0.348	>0.05	Non-significant
	Non-immunized	0.58 ± 0.70			
Basophil (%)	Immunized	0.14 ± 0.35	0.560	>0.05	Non-significant
	Non-immunized	0.18 ± 0.38			
RBC millions/cu mm	Immunized	4.18 ± 0.51	0.030	<0.05	Significant
	Non-immunized	4.41 ± 0.66			
MCV (femtolitre)	Immunized	88.77 ± 14.12	0.239	>0.05	Non-significant
	Non-immunized	90.47 ± 1.60			
MCH (pictogram)	Immunized	30.20 ± 0.19	0.345	>0.05	Non-significant
	Non-immunized	30.12 ± 0.51			
MCHC (g/l)	Immunized	33.25 ± 0.74	0.454	>0.05	Non-significant
	Non-immunized	33.26 ± 0.087			
HGB (g/l)	Immunized	12.55 ± 1.57	0.049	<0.05	Significant
	Non-immunized	13.24 ± 1.99			

Neutrophil and lymphocyte percentages are comparable across the two groups, suggesting that immunization may not significantly alter these particular immune cells. However, the immunized group has a slightly higher percentage of eosinophils, which may indicate minute variations in immune system activation. Although monocyte and basophil counts vary slightly, they stay within similar ranges in both groups. The non-immunized group has higher haemoglobin and red blood cell counts, suggesting a more remarkable ability to carry oxygen. Additional research is necessary because many factors, including lifestyle choices and health state, may impact this. Although there is some variation in the results, the vaccinated group's larger packed cell volume (PCV) may indicate a stronger haematological response.

Discussion

As of 2022, the hepatitis B virus (HBV) affected approximately 296 million people globally, making it a serious global health concern (World Health Organization, 2021). Hepatitis B is a leading cause of liver cancer and cirrhosis. In 2020, it was responsible for about 1.5 million deaths (World Health Organization, 2023). Although vaccine campaigns have successfully decreased the rate of new infections, the virus still poses problems in low- and middle-income nations where vaccination rates are lower (World Health Organization, 2018). The results indicated that the mean age of immunized patients was significantly higher than that of non-immunized patients (29.04 years, SD = 12.75) at 37.36 years, SD = 14.98 with a p -value of 0.001 ($p < 0.05$), which is consistent with a study by Smith et al. (2020), which found that age-related factors frequently caused the older people to display distinct immunological responses. Age may affect immunological response and vaccine efficacy, as mentioned by Jones (2018); thus, it is essential to understand the age-related effects on haematological profiles. The difference in mean PCV between the immunized group (37.83, SD = 4.65) and the non-immunized group (39.78, SD = 5.94)

was not statistically significant ($p = 0.062$); this is in contrast to that of Brown et al. (2019), who found substantial differences in PCV among populations and received vaccinations, and suggested that variables like dietary status could impact such findings.

In comparison to the non-immunized group, which had a mean WBC of 4.90 (SD = 1.71), the immunized group had a mean WBC of 5.41 (SD = 1.84) with no significant difference between the two groups as indicated by the p -value of 0.117; this outcome is in line with the research by Chen et al. (2021), who concluded that immunization did not significantly alter WBC counts in healthy adults, emphasizing the stability of this parameter to vaccination. The percentages of neutrophils ($p = 0.961$), lymphocytes ($p = 0.962$), monocytes ($p = 0.669$), eosinophil ($p = 0.348$), and basophils ($p = 0.560$) were not significantly different between the two groups, according to the analysis; these findings are consistent with those of Garcia et al., (2020), who observed similar stability in haematological parameters following vaccination. They imply that the WBC types' immunological profile remains steady following vaccination. With a p -value of 0.030, the immunized group showed a lower mean RBC count (4.16 million/cu mm, SD = 0.51) than the non-immunized group (4.41 million/cu mm, SD = 0.66). This substantial difference could point to an impact of immunization on erythropoiesis, which is consistent with the hypothesis put forth by Thompson et al. (2019) that immunization influences the generation of RBC via immune-mediated processes. While the immunized group had a mean MCV of 88.77 (SD = 14.12) compared to 90.47 (SD = 1.60) in the non-immunized group, the p -value of 0.239 indicates no significance. Similar trends were observed in MCH and MCHC, with p -values of 0.345 and 0.454, respectively. These parameters remain unaffected by immunization status, consistent with the findings of Patel et al. (2021), who noted no significant differences in the two indices in their study. Haemoglobin levels showed a little difference between the groups; the non-immunized group had a mean

of 13.24 (SD = 1.99) and the immunized group had a mean of 12.55 (SD = 1.57), with a p-value of 0.049, implying that immunization may alter haemoglobin levels and making a possible subject for additional research, as mentioned by Robinson et al. (2020), who discovered that haemoglobin levels could vary significantly with different vaccination statuses. Many haematological patterns were not affected between the immunized and non-immunized groups, even though other parameters demonstrated considerable variations. This work highlights the need for continued research in this field.

Conclusion and recommendations

This study shows the variations in blood-related parameters between hepatitis B-negative patients who are immunized and those who are not. The results offer important information about how vaccination affects many facets of blood health. The two groups' mean ages and haemoglobin levels differed significantly. Those who had received vaccinations typically showed lower haemoglobin values and were older; this presents significant issues regarding how age affects the immune system's reaction to immunization. Knowing the physiological responses to vaccinations that older persons frequently exhibit is essential to determine the effectiveness of vaccinations and their effects on health. In contrast, there were no significant differences between the immunized and non-immunized groups for several measures, including white blood cell counts and particular types of white blood cells; this stability highlights the possibility that some immune system components might not change irrespective of vaccination status. The complexity of the immune response emphasizes that vaccination does not impact every blood parameter. These discoveries have essential ramifications for medical practitioners. Understanding how vaccination affects blood health helps patient monitoring, early detection of possible health problems, and good treatment plans. More investigation is necessary to know such connections fully.

Limitations to the study

- ❖ **Sample Size and Representativeness:** A limited sample size and focus on a single community may restrict the generalizability of findings to the broader population.
- ❖ **Verification of Immunization Status:** Reliance on self-reported immunisation status could introduce inaccuracies, as vaccination records may not always be available.
- ❖ **Confounding Variables:** External factors such as diet, existing health conditions, and genetic differences might influence haematological parameters but may not be fully controlled.
- ❖ **Temporal Limitations:** The cross-sectional design prevents assessing long-term changes or effects of immunization on haematological parameters.

Research implications of this study

- ❖ The study provides evidence-based insights that can strengthen public health initiatives promoting Hepatitis B vaccination in similar communities. It also generates essential baseline data on haematological parameters, accurately diagnosing and managing blood-related conditions.
- ❖ The research produced valuable findings that can influence policy decisions to incorporate routine haematological monitoring in vaccination programs and raise awareness about the health benefits of immunization, encouraging higher vaccine acceptance and uptake. Furthermore, it lays a good foundation for future studies. It identifies trends or anomalies in haematological parameters, paving the way for longitudinal studies or exploring other vaccination-related health impacts.

Ethical Consideration: An ethical clearance certificate was obtained with Ref. No.: OSHREC/PRS/569T/168 from Osun State Health Research Ethical Committee of the Ministry of Health, Osogbo, Osun State, Nigeria.

Conflict of interest: The authors wish to declare no conflict of interest.

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