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**The Prevalence of Occult Hepatitis B Virus Infection among Hepatitis B Surface Antigen Sero-negative Blood Donors**

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**Abstract**

The signature of occult hepatitis B virus is in its ability to escape detection by hepatitis surface antigen (HBsAg) rapid kit which is the only kit certified across Nigerian health institutions. This phenomenon is a measure set back to haemotherapeutics with increased risk of transfusion with the hidden virus. This study aimed to determine the prevalence of occult HBV infection among blood donors. In this cross-sectional study, 250 well-screened HB-vaccinated and unvaccinated blood donors were enrolled at some selected secondary health facilities across the six Abuja District hospitals. A well-structured questionnaire was administered to obtain donor biodata and socio-demographic characteristics, CLIA, HB-5 panel check (Aria), HBV DNA analysis, and viral load was performed to ascertain the OBI. Serological confirmation using CLIA technique reveals 8 (3.2%), 11 (4.4%) HCV, 5 (2.0%) VDRL and 30 (12.0%) HBV detection among blood donors already certified free to donate with overall 54 (9.6%) positive blood unit out of 250 sample assayed. Virologic markers reveals 28 (11.2%) HBsAb, 1 (0.4%) HBeAg, 5 (2.0%) HBeAb and 5 (1.6%) HBcAb with 1 detectable viral of 37.0 (IU/ml) among HB-vaccinated blood donors. Similarly, a corresponding 51 (20.4%) HBsAb, 17 (6.8%) HBeAg, 27 (10.8%) HBeAb and 19 (7.6%) HBcAb with mean viral load of 129.8 ± 11.3 (IU/ml) among HB-unvaccinated blood donors. Our finding reports the prevalence of occult HBV DNA of 5.2% with the highest in male blood donors with post-secondary school educational status at mean age of 39.5 ± 15.9 years of age. This

result underscores the need to urgent intervention in the area of improving diagnostic sensitivity and to design new blood donor screening protocol to enhance transfusion safety.

**Keywords:** Blood donors, hepatitis B surface antigen, hepatitis B virus DNA

**Introduction**

Occult hepatitis B virus (OBI) is a hidden infection that standard HBsAg test kits fail to detect, posing significant risks to blood transfusion services and public health (Saita *et al.*, 2022). OBI can exacerbate chronic liver conditions (Chalid *et al.*, 2023), leading to cirrhosis, hepatocellular carcinoma (Harvala *et al.*, 2021; Tian *et al.*, 2022; Braccaccio *et al.*, 2023), and HBV reactivation in immunocompromised individuals, potentially causing acute hepatitis or fulminant hepatic failure. HBV genotypes (A–J) have distinct geographical distributions, with the E genotype being most prevalent in West Africa (Wang *et al.*, 2019; Kefeero *et al.*, 2023). Routine HBsAg screening does not eliminate the transmission of OBI, highlighting the need for sensitive screening methods (WHO, 2021; Sabeena *et al.*, 2022). Most patients with OBI are asymptomatic, and detection is often through viral DNA screening, as these patients usually have very low or undetectable serum HBV DNA levels (Tang *et al.*, 2018; Cappy *et al.*, 2021). Blood donor recruitment in Nigeria uses only the traditional HBsAg-dependent test kit despite varying prevalence of occult transmission of HIV, HCV, syphilis and HBV among blood

donors across health institutions (Ayorinde *et al.*, 2020; Steele *et al.*, 2020; WHO, 2021; Sobajo *et al.*, 2022;). This indicating the potential risk of HBV transmission through blood transfusions to blood recipients for medical procedures like gynecological-related complications, hemodialysis, and patients with thrombotic crisis (Steele *et al.*, 2020; Yasseen *et al.*, 2022). Real time-Polymerase Chain reactive techniques, which is the goal standard in occult hepatitis B virus detection are very expensive and not readily available for use in our primary and tertiary health institutions for effective case finding to reduce the risk of hepatitis B transmission through blood transfusion (Ayorinde *et al.*, 2021; Tian *et al.*, 2022). There were several attempts to assess the applicability of viral serological markers such as HBsAb, HBeAg, HBeAb, and HBcAb in predicting viral markers strongly associated detection of occult hepatitis B virus and to suggest it for deployment for use instead of the conventional single HBsAg test kit (Gnyawali *et al.*, 2022; Yasseen *et al.*, 2022; O'Brien *et al.*, 2023).

### **Hepatitis B serological viral markers**

Antibody to Hepatitis B surface antigen (Anti-HBs) is produced by the immune system in response to HBsAg (Lim *et al.*, 2020). Its presence indicates recovery from HBV infection or successful vaccination (Myran *et al.*, 2020). The detection of anti-HBs is a marker of immunity against HBV (Chalid *et al.*, 2023). Although hepatitis B core antigen (HBcAg) is part of the core protein of HBV is not usually measured directly due to its low levels in serum but detection of antibody to Hepatitis B core antigen (Anti-HBc) IgM and IgG are strong makers for viral replication (Sabeena *et al.*, 2022; Chalid *et al.*, 2023). IgM to anti-HBc appears early in the infection and indicates recent infection present of IgG to anti-HBc may persist for life and indicates past infection (Tsu *et al.*, 2019; Brancaccio *et al.*, 2023).

Mutations in the core antigen (HBcAg) can affect the production of anti-HBc and anti-HBs antibodies, potentially leading to false-negative results for these markers (Lim *et al.*, 2020). The detection of anti-HBc is done using ELISA or CLIA (Chalid *et al.*, 2023). Hepatitis B e antigen (HBeAg)

is a secreted protein produced by the virus and its presence indicates active viral replication and high infectivity (Chalid *et al.*, 2023; Brancaccio *et al.*, 2023). Antibody to Hepatitis B e antigen (Anti-HBe) is often produced in response to HBeAg and its presence indicates lower levels of viral replication and reduced infectivity (Sobajo *et al.*, 2022; Chalid *et al.*, 2023; Brancaccio *et al.*, 2023). Research literature proved that inability of HBsAg base rapid kit to detect occult hepatitis B virus is strongly correlated mutations, especially to hepatitis B virus surface antigenic proteins on Pre-S, Pre-core domain of 'a' determinant region of the hepatitis B surface antigen (Vermeulen *et al.*, 2021).

### **Materials and Methods**

Study design A cross-sectional study was done among consenting blood donors recruited by a convenient random sampling technique. 250 blood donors were recruited. The HBV DNA PCR was done at the molecular research laboratory in the University of Abuja, Teaching Hospital. Hepatitis B serological markers and hepatitis B virus viral load were determined using the Tianlong NAT PCR protocol (Shaanxi, China). Ethical clearance was obtained from the research and ethical committee of the University of Abuja and the federal capital territory research and ethics committee. Participants' informed consent and questionnaire were administered to obtain information like biodata, sociodemographic, and risk factors for occult hepatitis transmission.

### **Determination of hepatitis B serological markers using the Arial test kit.**

HBV 5 Panel test is a lateral flow chromatographic immunoassay for the qualitative detection of HBsAg, HBsAb, HBcAb, HBeAg, and HBeAb in human serum or plasma intended for use as a screening test to aid in the diagnosis of infection with HBV. The test is based on the principle of lateral flow chromatographic immunoassay consisting of 5 test panel strips assembled in one cassette. Each strip of the panel is composed of a sample pad, colloidal gold conjugate pad nitrocellulose membrane strip pre-coated with control and test absorbent pad. HBsAg is an antibody-based sandwich immunoassay, the conjugated pad impregnated with polyclonal anti-HBsAg

antibodies conjugated with colloidal and negative control membrane when adequate volume of test specimen migrates by capillarity action. HBsAg, if present binds to the anti-HBsAg gold conjugates for complexes which were captured on the membrane. Presence of burgundy color at both control and test line indicates a positive result while absence of test line indicates negative result. HBeAg strip is also antibody base sandwich immunoassay, the test utilizes a pair of anti-HBeAg antibodies to detect HBeAg in the specimen. a burgundy color indicates a positive result. HBsAb strip is an antigen-based sandwich immunoassay. The conjugate pad contains HBsAg conjugated with colloidal gold and nitrocellulose membrane is precoated with unconjugated HBsAg.

HBsAb, if present in the sample, binds to the HBsAg-gold conjugate to form complex which was captured by the nitrocellulose membrane forming burgundy color at control and test line indicating a positive reaction while absence of which is negative. HBcAb conjugate pad contained anti-HBc coated colloidal gold with nitrocellulose membrane precoated with HBcAg. A burgundy color at control and test line indicates a positive test while absence of which at test line indicates negative test. At HBeAb, the conjugate pad contained anti-HBeAb conjugated with colloidal gold and the nitrocellulose membrane precoated with HBeAg. If no HBeAb is present or its level in the specimen is below the test sensitivity, the HBeAb conjugate will have enough binding sites to bind the HBeAg coated on the nitrocellulose membrane before forming HBeAb - conjugate – HBeAg complex leading to burgundy color indicating positive test while absence of which indicate a negative test. If the level of HBeAb is higher in the specimen, it will bind HBeAg on the nitrocellulose membrane preventing the binding of HBeAb conjugated to the HBeAg. Therefore, absence of burgundy color at test line indicates positive test and presence of which indicate negative. Test Procedure: All specimens and test components were brought to room temperature. Frozen samples were allowed to thaw before gentle mixing. The test pouch was open at the indented notch and was placed on a clear flat bench surface. Both sample and the test cassette were labeled with each sample identification number. About 60µl of the sample was dropped at each of the

well labeled HBsAg, HBsAb, HBeAg, HBcAb and HBeAb respectively and allowed to incubate at room temperature for 15 minutes. Presence of burgundy color at control and test line indicates positive result except in the case of HBeAb and HBcAb the presence of indicators at the test line indicates a negative test result.

### **PCR determination of hepatitis B virus DNA viral load using Tialong PT-PRC.**

Tianlong RT-PCR is an automated reverse transcriptase real time (RT-PCR) based on dual-labeled hybridization probe targeted at precore and core region associated with HBV-DNA. It measures the affinity of DNA for silica-gel magnetic beads. HBV-DNA were examined in all samples that tested positive to HBsAb, HBeAg, HBeAb and HBcAb respectively using the primer sequence 3.8.3.2.1.

### **Principle of the Test**

This is based on the detection of HBV nucleic acid by Real-time Polymerase Chain Reaction (RT-PCR) method. Conserved gene segment of HBV is used to design special primers and Taqman probe. This probe can bind specifically to a DNA template in the primers' amplification area. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes with the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye which is monitored by the real-time detection system during PCR. The PCR cycle at which an increase in the fluorescent signal is detected initially and is proportional to the amount of the specific PCR product in the reaction. The fluorescence intensities in real-time allow for the detection of the accumulating product without having to re-open the reaction tube after amplification. The internal quality control in the kit can effectively avoid false negative results. The plasmid inserted with an artificially synthesized competitive fragment is use as a template for internal control.

### **Procedure for HBV-DNA extraction using GeneflexExtraction Procedure**

The centrifuge has a refrigeration function, set the temperature to 25°C. 1.5 mL micro centrifuge tubes were labeled and 200µL Lysis Buffer L to

each tube. Add 200  $\mu\text{L}$  sample to the tube with Lysis Buffer L, vortex thoroughly. Centrifuging briefly, stay at room temperature ( $15^{\circ}\text{C}$ - $30^{\circ}\text{C}$ ) for 5 min. The column was placed on collection tube and label the column, transfer all liquid into the column, experiment room and ultra-clean workbench was treated with UV light regularly and at the end of each experiment. centrifuge at 12000 rpm for 30 seconds. The liquid was discarded in the collection tube and 500  $\mu\text{L}$  Washing Buffer A(WA) was added to the column and was centrifuged at 12000 rpm for 30 seconds. The liquid in collection tube was discarded add 450  $\mu\text{L}$  Washing Buffer B (WB) was added to the column, and centrifuge at 12000 rpm for 30 seconds. The above procedure was repeated. The liquid in the collection tube was centrifuge at the maximum speed for 2 minutes. The collection tube was discarded, and a column of 1.5 mL centrifuge tube was added. 100  $\mu\text{L}$  of elution Buffer was added at the center of the column membrane, and stayed for 1 minute before it was centrifuge at 12000 rpm for 2 min. The column was discarded, and the product (nucleic acid) was stored at  $2$ - $8^{\circ}\text{C}$  for inspection. The nucleic acid extracted was suggested for immediate detection or store at  $-80^{\circ}\text{C}$  for long time.

### PCR Amplification using CAP/CTM thermocycler

This is based on the detection of HBV nucleic acid by Real-time Polymerase Chain Reaction (RT-PCR) method. Conserved gene segment of HBV is used to design special primers and Taqman probe. This probe can bind specifically to a DNA template in the primers' amplification area. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes with the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye which is monitored by the real-time detection system during PCR. The PCR cycle at which an increase in the fluorescent signal is detected initially and is proportional to the amount of the specific PCR product in the reaction.

The fluorescence intensities in real-time allow the detection of the accumulating product without having to re-open the reaction tube after

amplification. The internal quality control in the kit can effectively avoid false negative results. The plasmid inserted with an artificially synthesized competitive fragment is used as a template for internal control.

### Result Analysis and Interpretation

Result is presented as follows, If the sample has no critical values and the ct value of internal control  $< 45$ , then the sample is negative

- I. If HBV DNA  $\geq 10\text{IU/ml}$ , the sample should be positive
  - a. If  $30\text{IU/ml} \leq \text{HBV DNA} < 1.0 \times 10^8\text{IU/ml}$ , report the quantitative result directly
  - b. If  $\text{HBV DNA} > 1.0 \times 10^8\text{IU/ml}$  make it into the effective range by appropriate dilution
  - c. If  $10\text{IU/ml} \leq \text{HBV DNA} < 30\text{IU/ml}$ , low viral load, only a qualitative indication, track carefully
- ii. HBV DNA  $< 10\text{IU/ml}$  and the ct value of internal control is less than 45, the result is less than the lower limit. Repeat the test and find the reason

### Hepatitis B virus DNA polymerase chain reaction

Plasma DNA isolation was done using a Tianlong extraction and amplification kit. RT-PCR was performed using specific primers derived from the regions coding for HBsAg using primer sequences and amplicon sizes. The first-round PCR was carried out in a final volume of 25  $\mu\text{l}$  containing 1 unit of Taq polymerase (Jena Bioscience, Germany), 200  $\mu\text{M}$  dNTP mix, 2.5  $\mu\text{l} \times 10$  Taq polymerase buffer, 15 mM  $\text{MgCl}_2$  and 10 pmol of each primer, molecular grade water (Jena Bioscience, Germany), and 2.5  $\mu\text{l}$  DNA template. The amplification was carried out for 35 cycles (20 s at  $94^{\circ}\text{C}$ , 30 s at  $55^{\circ}\text{C}$ , and 45 s at  $72^{\circ}\text{C}$ ) after initial denaturation for 2 min. A final extension step was performed for 10 min at  $72^{\circ}\text{C}$ . The second round PCR was carried out using 5  $\mu\text{l}$  of the First PCR product under the same condition as the first-round PCR except that 25 pmol of each internal primer was used. A 15- $\mu\text{l}$  aliquot of the PCR products was electrophoresed on 2% agarose gel at 170V for 30 min and then stained with ethidium bromide (Promega, USA) using  $\times 1$  tris boric acid EDTA buffer. The appropriate size band was visualized by gel documentation system (Alpha Imager and

Alpha Innotech). Oligonucleotide primers for the detection of occult HBV DNA through “nested”-PCR amplification

### Statistical analysis

Data obtained were analyzed using IBM SPSS (Statistical Package for Social Sciences, Inc.) statistics for Windows version 20.0 Armonk, New York, USA. Data were presented in percentages and analyzed using the Chi-square test and Pearson's correlation as appropriate, to get the P value. The differences were considered to be statistically significant, where  $P < 0.05$ . Results were also presented in tables and figures.

### Results

The results presented in the table demonstrate the positive frequency of HIV (S/CO), HCV (S/CO), VDRL (S/CO), and HBV (IU/ml) prescreened negative blood donors. The results reveal that 3.2% of the blood donors tested positive for HIV, while a vast majority (96.8%) tested negative. 4.4% of the blood donors tested positive for HCV, whereas 95.6% tested negative. 2.0% tested positive for VDRL, while 98.0% tested negative. HBV (IU/ml) screening shows that 12.0% of the blood donors tested positive for HBV, with 88.0% testing negative. This indicates that HBV shows the highest prevalence among the listed infections. Statistical analysis shows a Chi-square ( $X^2$ ) value of 22.670 and a P-value of less than 0.0001 indicating a statistically significant difference between the positive and negative cases for HCV. The total number of positive cases across all infections is 54 (9.6%), with 196 (78.4%) negative cases.

The result in table 2. show the distribution of serological viral markers among HBV vaccinated and un-vaccinated blood donors using HBV-5 panel rapid diagnostic kit among study population. The distribution of viral serological markers shows significant association ( $p < 0.0001$ ) in detection OBI the study population. The result showed that none of the 37 HBV and 213 HBV un-vaccinated and vaccinated blood donor show positive HBsAg using 5 panel HBV serological screening kit. Conversely, (11.2%) HBsAb, (0.4%) HBeAg, (2.0%) HBeAb and (1.6%) HBcAb were positive respectively. The results show that (3.6%)

HBsAb, (14.4%) HBeAg, (12.4%) HBeAb and (13.2%) HBcAb were negative. The distribution of OBI among un-vaccinated blood donors also shows significant association ( $p < 0.0001$ ). The result reveals, (20.4%) HBsAb, (6.8%) HBeAg, (10.8%) HBeAb and (7.6%) HBcAb were positive respectively. The result also shows that (64.8%) HBsAb, (78.4%) HBeAg, (74.4%) HBeAb and (77.6%) HBcAb were negative respectively. A viral load of 37IU/ml were recorded one sample among BHV-vaccinated blood donor sample while viral load mean  $\pm$  SD of  $129.8 \pm 11.3$  were reported among HBV-unvaccinated blood donors respectively.

The result in table 3 show the frequency occult hepatitis B virus detection based on age, gender, donor type, HB-vaccination status and viral serological markers among occult blood donors. The result reveals mean age of  $39.6 \pm 15.2$  in the blood donor population. distribution of OBI-positive cases is found only in males (4.8%), with no cases in females. The chi-square value (0.510) and p-value (0.475) suggest no significant difference in OBI distribution based on gender ( $p > 0.05$ ). evaluating educational status shows that most OBI-positive cases are found in individuals with post-secondary education (3.2%). OBI-positive cases are more common among commercial donors (3.6%) compared to family donors (1.6%) and voluntary non-remunerated donors (0.0%). The chi-square value (4.747) and p-value (0.093) suggest no significant difference in OBI distribution based on the type of donor ( $p > 0.05$ ). The analysis reveals that there are no statistically significant differences in the distribution of OBI among blood donors based on age, gender, educational status, or type of donor, as indicated by the chi-square and p-values. The findings underscore the importance of screening all donor groups for OBI, regardless of these demographic and socio-economic factors.

The result in table study analyzed the distribution of Hepatitis B viral markers among vaccinated and unvaccinated blood donors. The markers studied were Hepatitis B surface antibody (HBsAb), Hepatitis B e antigen (HBeAg), Hepatitis B e antibody (HBeAb), and Hepatitis B core antibody (HBcAb). The result reveals

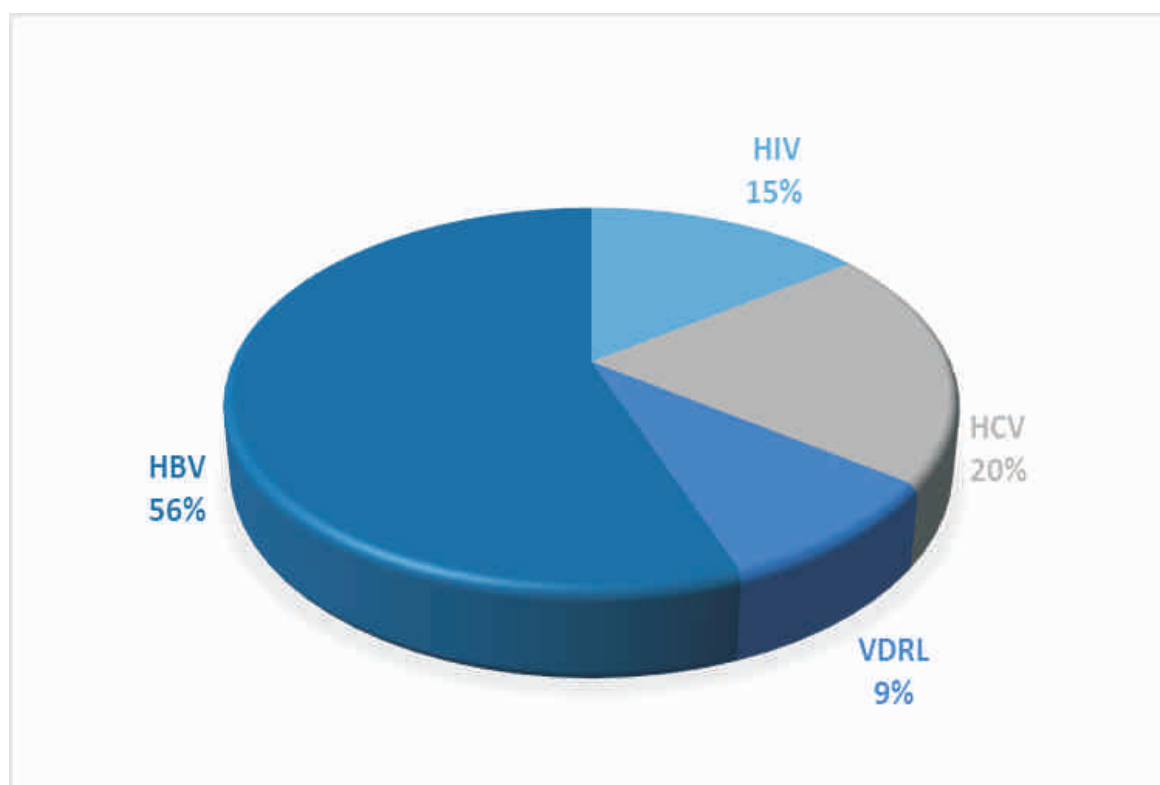
HBsAb Positive: 75.7% of vaccinated donors and 23.9% of unvaccinated donors. HBeAg Positive: 2.7% of vaccinated donors and 6.8% of unvaccinated donors have HBeAg, HBeAb Positive: 13.5% of vaccinated donors and 10.8% of unvaccinated donors have HBeAb. Similarly,

HBcAb Positive: 10.8% of vaccinated donors and 7.6% of unvaccinated donors have HBcAb. Statistical Analysis shows Chi-square (X<sup>2</sup>): HBV-vaccinated (65.317), HBV-Unvaccinated (34.462) P value: <0.0001 for both groups.

**Table 1. Frequency distribution of other transfusion transmissible occult viral infection HIV, HCV VDRL and HBV in the general population.**

| CLIA Diagnostic kit | Positive<br>n (%) | Negative<br>n (%) | X <sup>2</sup> | P-value  |
|---------------------|-------------------|-------------------|----------------|----------|
| HIV (S/CO)          | 8 (3.2)           | 242 (96.8)        | 29.833         | P<0.0001 |
| HCV (S/CO)          | 11 (4.4)          | 239 (95.6)        |                |          |
| VDRL (S/CO)         | 5 (2.0)           | 245 (98.0)        |                |          |
| HBV (IU/ml)         | 30 (12.0)         | 220 (88.0)        |                |          |
| Total               | 54 (9.6)          | 196 (78.4)        | -              | -        |

**Key:** CLIA = Chemiluminescent microparticle immunoassay, OBI= occult hepatitis B, n= Number of subjects, %=Percentage, X<sup>2</sup>= Chi square, P-value = prevalence value, HIV= human immunodeficiency virus, HBV= hepatitis B virus, HCV= hepatitis C virus, VDRL= general disease research laboratory.

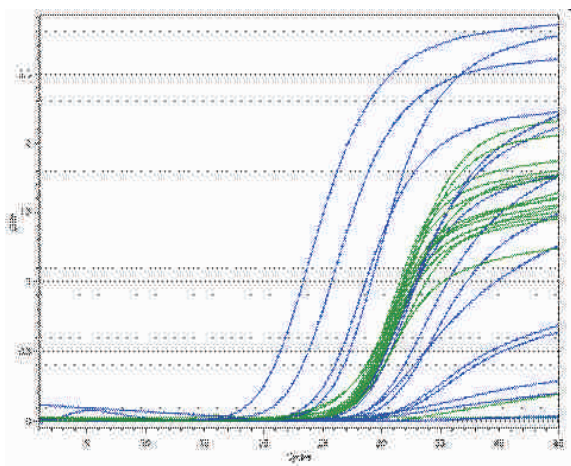


**Figure 1: Distribution of other transfusion transmissible viral infection (HIV, HCV And VDRL) among HBV vaccinated and un-vaccinated blood donors.**

**Table 2: Frequency distribution of hepatitis B viral markers pattern and OBI viral load among HBV vaccinated and un-vaccinated blood donors**

| HBV Vaccinated blood donors (N=37) |              |              |                |          | HBV Unvaccinated blood donors (N=213) |              |                |
|------------------------------------|--------------|--------------|----------------|----------|---------------------------------------|--------------|----------------|
| Viral Marker                       | Positive (%) | Negative (%) | X <sup>2</sup> | P- value | Positive (%)                          | Negative (%) | X <sup>2</sup> |
| HBsAg                              | 0 (0.0)      | 0 (0.0)      | 65.856         | <0.0001  | 0 (0.0)                               | 0 (0.0)      | 29.61          |
| HBsAb                              | 28 (11.2)    | 9 (3.6)      |                |          | 51 (20.4)                             | 162 (64.8)   |                |
| HBeAg                              | 1 (0.4)      | 36 (14.4)    |                |          | 17 (6.8)                              | 196 (78.4)   |                |
| HBeAb                              | 5 (2.0)      | 31 (12.4)    |                |          | 27 (10.8)                             | 186 (74.4)   |                |
| HBcAb                              | 4 (1.6)      | 33 (13.2)    |                |          | 19 (7.6)                              | 194 (77.6)   |                |
| HBeAg                              | 1 (0.4)      | 36 (14.4)    |                |          | 17 (6.8)                              | 196 (78.4)   |                |
| Viral load (copies/ml) (Mean ±SD)  |              |              |                |          | 129.8± 11.3                           |              |                |

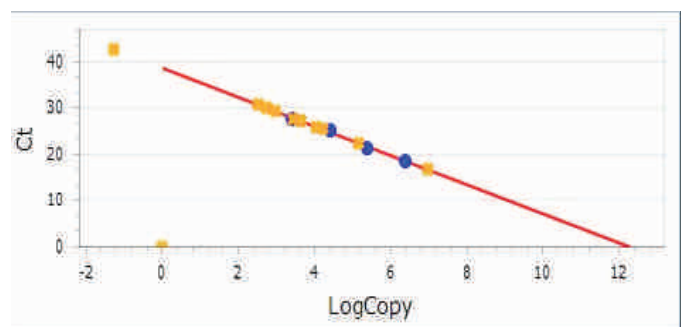
**Key:** n= Number of subjects, % =Percentage, X<sup>2</sup>= Chi square, P-value = prevalence value, df= degree of freedom, HBsAg= hepatitis B surface antigen, HBsAb= hepatitis B surface antibody, HBeAg= hepatitis B envelop antigen, HBeAb=hepatitis B envelop antibody, HBcAb=hepatitis B core antibody.



Std Curve: Project ID: hbvTarget: ic

Formula:  $y = 0.01 * x + 21.97$ ; R: 0.0006E: -100.00%

|        |            |           |          |         |
|--------|------------|-----------|----------|---------|
| Copies | 2500000.00 | 250000.00 | 25000.00 | 2500.00 |
| Ct     | 21.88      | 22.75     | 21.16    | 22.36   |



**Plate 1: Standard curve of  $y = 0.01 * x + 21.97$ : R 0.0006E: -100.00% different viral load copies at different Ct values**

**Table 3: Frequency Occult Hepatitis B virus detection base on age, gender, donor type, HB-vaccination status and viral serological markers among occult blood donors**

| Characteristic             | Analysis    | OBI Positive | OBI Negative | P-value |
|----------------------------|-------------|--------------|--------------|---------|
| Age (years)                | 39.6 ± 15.2 | 13 (5.2)     | 237 (94.8)   |         |
| <b>Gender</b>              |             |              |              |         |
| Male                       | 241(96.4)   | 13 (5.2)     | 228 (91.2)   | 0.4836  |
| Female                     | 9 (3.6)     | 0 (0.0)      | 9 (3.6)      |         |
| <b>Type of donors</b>      |             |              |              |         |
| Family donors              | 100 (40.0)  | 4 (1.6)      | 96 (38.4)    | 0.0932  |
| Commercial donors          | 108 (43.2)  | 9 (3.6)      | 99 (39.6)    |         |
| Voluntary donors           | 42 (16.8)   | 0 (0.0)      | 42 (16.8)    |         |
| <b>Vaccination status</b>  |             |              |              |         |
| HBV-Vaccinated donors      | 37 (14.8)   | 2 (0.8)      | 35((14.0))   | 0.9615  |
| HBV-unvaccinated donor     | 213 (85.2)  | 11 (4.4)     | 202 (80.8)   |         |
| <b>Serological markers</b> |             |              |              |         |
| HBsAg                      | 0 (0.0)     | 0 (0.0)      | 0 (0.0)      | 0.0001  |
| HBsAb                      | 79 (31.6)   | 0 (0.0)      | 79 (31.6)    |         |
| HBeAg                      | 18 (7.2)    | 2 (0.8)      | 16 (6.4)     |         |
| HBeAb                      | 32 (12.8)   | 3 (1.2)      | 19 (7.2)     |         |
| HBcAb                      | 23 (9.2)    | 8 (3.2)      | 15 (6.0)     |         |

**Key:** n= Number of subjects, % =Percentage, X<sup>2</sup>= Chi square, P-value = Prevalence value, df= degree of freedom, HBsAg= hepatitis B surface antigen, HBsAb= hepatitis B surface antibody, HBeAg= hepatitis B envelop antigen, HBeAb=hepatitis B envelop antibody, HBcAb=hepatitis B core antibody.

**Discussion**

The data presents the prevalence of transfusion-transmissible infections (HIV, HCV, VDRL, and HBV) among blood donors using the CLIA diagnostic kit. The **prevalence** of HBV (IU/ml) shows a notably higher prevalence of 12.0%. The higher prevalence of HBV points to a significant public health challenge. Given that HBV can lead to chronic liver disease, cirrhosis, and hepatocellular carcinoma, this higher infection rate necessitates enhanced surveillance, vaccination programs, and public enlightenment based on the positivity and negativity of OBI (Occult Hepatitis B Infection). The prevalence of HIV (S/CO) among the population tested is 3.2%, indicating a relatively low prevalence and significance. However, HIV remains a critical public health concern due to its lifelong implications and requirement for continuous medical management. The data presented reveals the prevalence of HCV is 4.4%. The prevalence of VDRL (indicative of syphilis infection) is 2.0%. **Although** the prevalence is

lower than HIV and HCV, the presence of VDRL indicates ongoing transmission of syphilis. This underscores the need for continued public health efforts to identify, treat, and prevent syphilis infections, especially among high-risk group. The data highlights the need for targeted interventions for HCV and HBV due to their relatively higher prevalence and statistical significance. Public health strategies should include comprehensive screening programs, targeted education and awareness campaigns, vaccination initiatives, especially for HBV and ensuring safe blood transfusion practices. While the prevalence rates for HIV and VDRL are lower, continued efforts are essential to improve access to testing and treatment services and address the underlying risk factors for transmission. These findings can inform policy makers and healthcare providers in allocating resources effectively to combat these infections. Priority should be given to addressing HBV and HCV due to their higher prevalence and potential long-term health impacts. The data underscores the importance of

comprehensive public health strategies to address transfusion-transmissible infections.

The overall impact with 9.6% of blood donors testing positive for at least one infection, the screening process helps in maintaining a safer blood supply. The high percentage of negative cases (78.4%) also reflects the effectiveness of the screening protocols. The data presented underscores the importance of continuous monitoring and improvement of blood screening processes to protect public health. It also highlights the need for awareness and education campaigns to encourage safe blood donation practices. The screening of blood donors for TTIs is a critical component of blood transfusion safety. However, detection of occult HIV, HCV, VDRL, and HBV among blood donors prescreen negative is a major setback to transfusion safety, thereby increasing the risk of transfusion-transmitted infections among blood supply for recipients. Confirmation of blood donor screening for TTIs is crucial especially among blood donors to ensure safe blood for transfusions.

The analysis of Hepatitis B viral markers among vaccinated and unvaccinated donors reveals critical insights. HBsAb (Hepatitis B Surface Antibody) reveals a significantly higher percentage of vaccinated donors are HBsAb positive (75.7%) compared to unvaccinated donors (23.9%). This demonstrates the effectiveness of the Hepatitis B vaccination in inducing protective antibodies. HBeAg (Hepatitis B e Antigen) present a lower percentage of vaccinated donors are HBeAg positive (2.7%) compared to unvaccinated donors (6.8%). HBeAg is associated with active viral replication and higher infectivity, indicating that vaccination reduces the risk of active infection. Similarly, our finding on HBeAb (Hepatitis B e Antibody) among the blood donors reveals a slightly higher percentage of vaccinated donors are HBeAb positive (13.5%) compared to unvaccinated donors (10.8%). More so, HBeAb presence indicates a response to HBeAg and often a lower level of viral replication. Our result shows a higher percentage of vaccinated donors are HBcAb positive (10.8%) compared to unvaccinated donors (7.6%). This finding of HBcAb presence

indicates previous or ongoing infection, and its presence in vaccinated donors may reflect past exposure. Although statistical analysis shows that both groups (vaccinated and unvaccinated) show significant differences in the distribution of Hepatitis B markers, with chi-square values of 65.317 and 34.462 and p-values of  $< 0.0001$ . These results highlight the protective effect of the Hepatitis B vaccination and the importance of vaccination programs in reducing the prevalence of active and chronic infections.

The findings from this study underscore the importance of comprehensive screening for OBI and other Hepatitis B markers among blood donors. Vaccination plays a crucial role in reducing the prevalence of active Hepatitis B infections and increasing the presence of protective antibodies. Continuous efforts in public health education, awareness, and vaccination programs are essential to ensure the safety and efficacy of blood transfusion practices.

The data shows that HBV vaccination has a significant impact on the presence of viral markers among blood donors. Vaccinated individuals generally show lower rates of active infection and viral replication markers (HBeAg, HBcAb) and higher rates of immunity markers (HBsAb) compared to unvaccinated individuals. The statistical values ( $X^2$  and P value) indicate a significant difference between vaccinated and unvaccinated groups, with P values less than 0.0001 for HBsAb, reflecting the effectiveness of vaccination in preventing hepatitis B infection and its markers.

The data presented gives a better understanding of the serological HB-viral markers and OBI viral load among HBV vaccinated and unvaccinated blood donors. HBsAg (Hepatitis B surface antigen) both vaccinated and unvaccinated donors show 0% positivity for HBsAg. This suggests that none of the donors are currently infected with active HBV as HBsAg is typically a marker for acute or chronic hepatitis B infection. HBsAb (Hepatitis B surface antibody) vaccinated Donors: 28 (11.2%) are positive, while 9 (3.6%) are negative. This is a strong indicator of immunity post-vaccination, as HBsAb is the antibody that typically indicates recovery or immunity from HBV. Unvaccinated Donors: 51 (20.4%) are positive,

and 162 (64.8%) are negative. A notable number of unvaccinated individuals also show positivity, which might be due to previous natural infection leading to the development of immunity. Regarding HBeAg (Hepatitis B e antigen), 1 (0.4%) of vaccinated donors was positive, indicating a very low presence of active viral replication among vaccinated donors. However, among unvaccinated donors: 17 (6.8%) were positive, suggesting that there are more unvaccinated individuals with ongoing viral replication. HBeAb (Hepatitis B e antibody) testing indicated that 5 (2.0%) of vaccinated Donors were positive, while 31 (12.4%) are negative. This shows that a small fraction of vaccinated donors have developed antibodies against HBeAg, indicating the control of infection. Unvaccinated Donors: 27 (10.8%) are positive, and 186 (74.4%) are negative, suggesting a higher level of past exposure in the unvaccinated group compared to the vaccinated group. HBcAb (Hepatitis B core antibody) Vaccinated Donors: 4 (1.6%) are positive, indicating past or ongoing infection in a small number of vaccinated individuals. Unvaccinated Donors: 19 (7.6%) are positive, suggesting a higher rate of past or chronic infection in unvaccinated donors. Viral Load (copies/ml), Vaccinated Donors: Mean  $\pm$  SD not specified but marked as 37. Unvaccinated Donors: Mean viral load is  $129.8 \pm 11.3$  copies/ml, suggesting that unvaccinated individuals have a measurable level of HBVDNA, indicating ongoing viral replication.

The data in this research reveals several important characteristics and trends regarding the distribution of OBI among blood donors based on age, gender, educational status, type of donors and vaccination status among blood donors. This work reveal mean age of blood donors is  $39.6 \pm 15.2$  years and all the OBI-positive cases were only found in males (4.8%), with no cases in females. However, the chi-square value (0.510) and p-value (0.475) suggest no significant difference based on gender. This highlights the need for continuous screening in both genders despite observed trends. Evaluation of OBI based on educational Status reveals OBI-positive cases are more common in individuals with post-secondary education (3.2%). These were blood donors with educational attainment of N.C. E, ND, HND, B.Sc, M.Sc and PhD respectively. This could suggest that educational awareness programs need to be reinforced across all educational levels. Our database on type of Donor shows that OBI-

positive cases are more frequent among commercial donors (3.6%) compared to family donors (1.6%) and voluntary non-remunerated donors (0.0%). Although not statistically significant, these findings emphasize the importance of rigorous screening processes across all donor categories. Analysis of vaccination status of blood donor's reveals that, out of 37 (14.8%) HBV-vaccinated blood donor enrolled, on 2 (0.8%) were positive for occult hepatitis B virus while of 213 (85.2) HBV-unvaccinated blood donors 11(4.4%) were tested negative for OBI using CLIA methods. No significant differences in OBI distribution based on age, gender, educational status, or type of donor (chi-square and p-values  $> 0.05$ ). This underscores the importance of universal screening protocols irrespective of demographic or socio-economic factors.

### Conclusion

The study highlights the prevalence of transfusion-transmissible infections (TTIs) among blood donors, with a focus on occult HIV, HCV, VDRL, and HBV. The data reveals that HBV has the highest prevalence, followed by HCV, HIV, and VDRL. This underscores the need for targeted interventions, including enhanced surveillance, vaccination programs, and public health initiatives to reduce the prevalence of these infections. The findings also emphasize the importance of comprehensive screening protocols and public health education to ensure the safety of blood transfusions.

### Limitations

- 1. Sample Size and Selection:** The study is limited by the sample size and the selection process of blood donors, which may not be representative of the general population.
- 2. Diagnostic Accuracy:** The use of the CLIA diagnostic kit, while effective, may have limitations in sensitivity and specificity, potentially leading to false-positive or false-negative results.
- 3. Data Scope:** The study focuses on specific transfusion-transmissible infections and may not account for other potentially infectious agents in the blood supply.
- 4. Cross-Sectional Design:** A cross-sectional study only provides a snapshot in time and does not account for temporal changes in

infection rates or the long-term impact of interventions.

- 5. Self-Reported Data:** Information on vaccination status, type of donors, and other demographic factors may be subject to bias as it is self-reported by the donors.

These limitations suggest that while the study provides valuable insights, further research with larger and more diverse populations, longitudinal data, and advanced diagnostic methods are necessary to fully understand and address the prevalence and impact of TTIs among blood donors.

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