AJPMHS GLOBAL RESEARCH NETWORK

AMERICAN Journal of Pediatric Medicine and Health Sciences
Volume 3, Issue 4, 2025 ISSN (E): 2993-2149

Prevalence of Hepatitis B Virus Infection and Circulating Genotypes among HIV/AIDS Patients Attending Hospitals in Nasarawa State, Nigeria

Agok Sandra Ayong
Molecular Diagnostic Unit, Laboratory Department, Institute of Human Virology Nigeria

David Ishaleku, Ngene Istiphanus, Nicaise Ndembi
Microbiology Unit, Department of Biological Sciences, Nasarawa State University, Keffi, Nasarawa State, Nigeria

Abstract: Hepatitis B virus (HBV) prevalence remains high in sub-Saharan Africa, including Nigeria. We aimed to determine the seroprevalence of Hepatitis B Virus infection among HIV/AIDS patients attending some health care facilities in Nasarawa State, Nigeria. A total of 300 plasma of HIV/AIDS patients were randomly collected and screened for HBsAg using Rapid Test Kits. The overall prevalence of HBsAg in the selected hospitals among HIV/AIDS patients was 24 (8%) of which FMC Keffi, GH Nasarawa, GH Garaku had 7%, 6% and 11% respectively. The detection of HBsAg genotypes was carried out using Polymerase Chain Reaction technology (PCR). Of the 24 seropositive samples detected in the three hospitals, only 3 samples were detected molecularly to be positive which are all with genotype B. Of the 3 samples molecularly detected, 2 are from GHN and 1 from GHG.

Keywords: Hepatitis B Virus, Genotypes, HIV/AIDS.

INTRODUCTION

Hepatitis B virus (HBV) is the smallest known enveloped virus, characterized by a partially double-stranded DNA genome that belongs to the *Hepadnaviridae* family and is the causative agent of hepatitis B infection which primarily affects the liver (Seeger & Mason, 2015). HBV is a major global health concern, as it is one of the most prevalent infectious diseases worldwide, with millions of people chronically infected. The virus is responsible for significant morbidity and mortality, primarily due to complications such as cirrhosis and hepatocellular carcinoma (HCC) (Schweitzer et al., 2015).

Humans are the only known natural hosts of HBV, and transmission occurs through intravenous drug use, percutaneous, perinatal, and mucosal exposure to infected bodily fluids, including blood and sexual secretions (World Health Organization [WHO], 2023). People living with HIV (PLHIV) are at a higher risk of acquiring HBV due to these shared modes of transmission (WHO, 2021).

Hepatitis B virus (HBV) and human immunodeficiency virus (HIV) co-infection is a significant global public health concern, particularly in regions where both infections are endemic, such as sub-Saharan Africa and Southeast Asia (Kourtis et al., 2012).

There are about eight genotypes of HBV have been reported worldwide (Kramvis, 2014, Sunbul & Kao, 2015), but more recently, two additional genotypes (I and J) were tentatively proposed (Sunbul, 2014, Lin and Kao, 2015 & Rodgers, 2017). HBV genotypes A, B, C, D and F are known to cause hepatocellular carcinoma (Tong *et al.,* 2013). Infection by Genotype C or D is significantly more likely to lead to cirrhosis and hepatocellular carcinoma than genotype A or B (Kao, 2011).

In Africa, despite the high endemicity, there are limited data on the genotypes of HBV and their distribution (Musa *et al.,* 2015). Although genotype D is found throughout Africa, it is common more in the northern countries on the Mediterranean. The distribution of genotype E is restricted and confined to Western Africa including Nigeria, Angolo, Liberia, Senegal, Cote d'Ivoire, Cameroon, Ghana, Namibia and the Gambia (Velkov *et al.,* 2018). The predominant circulating genotypes in Nigeria are genotype E and, to a lesser extent, genotype A.

MATERIALS AND METHODS

Study Area and population

The study was carried out in three selected hospitals namely; Federal Medical Centre Keffi, General Hospital Garaku and General Hospital Nasarawa in the West Senatorial District of Nasarawa State. The Nasarawa West Senatorial District consists of five Local Government Areas but the study was carried out only in three LGAs (Keffi, Kokona and Nasarawa LGAs). Nasarawa State has a total land area of 27,117 km^2 and coordinates $8^0$32'N $8^0$18É (2006 population census and Wikipedia).

Ethical Consideration and Approval

The ethical clearance for this study was obtained from the Health Research Ethics Committee of Federal Medical Center Keffi. Signed informed consent was also obtained from each of the subjects.

Exclusion and Inclusion Criteria

The study included HIV/AIDs patients attending Antiretroviral Therapy (ART) clinic in the selected hospitals. Children less than 11years of age and HIV negative patients were excluded for this study.

Sample Collection and Storage

Five (5) ml of whole blood samples of 300 HIV/AIDs patient were randomly collected into Ethylene Diamine Tetra acetic Acid (EDTA) tubes by venepuncture and centrifuged for 20 minutes at 30,000rpm. The plasma were separated using Pasteur/transfer pipettes and then transferred into a cryogenic vials (cryovials). The vials of plasma were stored at -20^o C until ready for analysis

Screening of Hepatitis B Surface Antigen

All the 300 plasma samples were screened for HBV using the HBsAg rapid test kit (LabACON, USA) following the manufacturer's instructions. The HBsAg one step test strip is a qualitative, lateral flow immunoassay for the detection of HBsAg in serum or plasma.

Hepatitis B Virus DNA Extractions

The DNA of HBV was extracted from the HBsAg seropositive samples using alkaline lysis method as described by Kaneko *et al*. (1989). Briefly, 10µl plasma was pipetted into 0.5 ml microcentrifuge tube and incubated with 1ul of 1M NaOH solution at 37^{o}C or 1 hour. The mixture was centrifuged for 15 sec in a tabletop microcentrifuge at 13,000 rpm. Subsequently, the solution was neutralized with 0.1M HCl and the resulting solution was centrifuge and the filtrate was used for amplification.

DNA purity and concentration was measured using a NanoDrop spectrophotometer. For DNA concentration, absorbance readings were performed at 260 nm (A_{260}) and the readings were observed to be within the instrument's linear range (0.1 – 1.0). DNA purity was estimated by calculating the A_{260}/A_{280} ratio and this was done by the spectrophotometer's computer software (where A_{260}/A_{280} ratio ranges from 1.7 – 1.9). A_{260}/A_{280} and A_{260}/A_{230} values greater than 1.7 were typically suitable for analysis.

DNA Amplification

The HBV genome was amplified by nested PCR using the universal primers (P1 and S1-2) for the outer primers, followed by two different mixtures containing type-specific inner primers as described above. The first PCR was carried out in a tube containing 40 ml of a reaction buffer made up of the following components: 50ng of each outer primer, a 200 mM concentration of each of the four deoxynucleotides, 1 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Norwalk, Conn.), and 13 PCR buffer containing 1.5 mM MgCl2. The AmpliTaq Gold DNA polymerase was used to obtain an automatic hot-start reaction. The thermocycler (GeneAmp PCR system 2400, 9600, and 9700; Perkin-Elmer) was programmed to first incubate the samples for 10 min at 95°C, followed by 40 cycles consisting of 94°C for 20 s, 55°C for 20 s, and 72°C for 1 min. The two second-round PCRs were performed for each sample, with the common universal sense primer (B2) and mix A for types A through C and the common universal antisense primer (B2R) and mix B for types D through F. A 1 µl aliquot of the first PCR product was added to two tubes containing the second sets of each of the inner primer pairs, each of the deoxynucleotides, AmpliTaq Gold DNA polymerase, and PCR buffer, as in the first reaction. These were amplified for 40 cycles with the following parameters: preheating at 95 °C for 10 min, 20 cycles of amplification at 94 °C for 20 s, 58°C for 20 s, and 72 °C for 30 s, and an additional 20 cycles of 94 °C for 20 s, 60 °C for 20 s, and 72 °C for 30 s.

S/N	Primers	Sequence	(position, specificity, and polarity)
1	First PCR		
2	P1	5'-TCA CCA TAT TCT TGG GAA CAA GA-3'	2823–2845, universal, sense
3	S1-2	.5'-CGA ACC ACT GAA CAA ATG GC-3'	685–704, universal, antisense
4	Second PCR		
5	Mix A		
6	B2	5'-GGC TCM AGT TCM GGA ACA GT-3'	67–86, types A to E specific, sense
7	BA1R	5'-CTC GCG GAG ATT GAC GAG ATG T-3'	113–134, type A specific, antisense
8	BB1R	.5'-CAG GTT GGT GAG TGA CTG GAG A-3'	324–345, type B specific, antisense
9	BC1R	5'-GGT CCT AGG AAT CCT GAT GTT G-3'	165–186, type C specific, antisense
10	Mix B		
11	BD1	5'-GCC AAC AAG GTA GGA GCT-3'	2979–2996, type D specific, sense
12	BE1	5'-CAC CAG AAA TCC AGA TTG GGA CCA-3'	2955–2978, type E specific, sense
13	BF1	5'-GYT ACG GTC CAG GGT TAC CA-3'	3032–3051, type F specific, sense
14	B2R	5'-GGA GGC GGA TYT GCT GGC AA-3'	3078–3097, types D to F specific, antisense

Table 3.1: Primer sequences used for Hepatitis B virus genotyping by nested Polymerase Chain Reaction

Hideo *et al*. (2001)

Agarose Gel Electrophoresis

Seven microliter of the amplified DNA was transferred into the wells of a 1.5% Agarose gel by stabbing the wells using a micropipette and this was done carefully to ensure that each well had only one sample. Each gel had one well which contained a DNA ladder (100 bp, Thermo Scientific) in order to estimate the size of the DNA amplicons. Electrophoresis was run at 125 volts for 20 min, after which the gels were viewed using ultra-violet trans-illuminator.

Data Analysis

Data were analyzed using Statistical Package for Social Sciences (Version 20). Chisquare test was used to compare differences among groups. Probability value of less than or equal to 0.05 was considered significant.

RESULTS

A total of 300 of HIV/AIDs patients' plasma were obtained from the selected hospitals (Federal Medical Center Keffi, General Hospital Nasarawa and General Hospital Garaku). Questionnaires were administered to each subject to obtain information used to assess the socio-demographic data and risk factors associated with the infection. The overall prevalence of HBsAg was 24 (8%). The prevalence of HBsAg with respect to age in the selected hospitals FMCK, GHN, GHG was high among 31-40yrs, 41-50yrs and 21-30yrs with 12.8%, 11.8% and 12.5% respectively. In relation to gender, the prevalence was high among females than males; FMCK (9%), GHN (8.8%) and GHG (12.5%) and was statistically significant ($p<0.05$). The prevalence of HBsAg in relation to occupation, marital status and multiple sex partners were highest among Civil servants in GHG (16.7%), widowed in GHN (20%) and those who have multiple sex partners in GHG (16.7%) respectively. The prevalence of HBsAg was statistically insignificant ($p>0.05$). In relation to the level of education, history of blood transfusion and scarification, the prevalence of HBsAg among HIV/AIDs patients was high in GHG with 41.2%, 21.4% and 17.2% respectively. The prevalence of HBsAg was statistically significant ($p<0.05$). The prevalence of HBsAg with respect to hepatitis awareness, history of hepatitis vaccination and history of sexually transmitted diseases among HIV/AIDs patients was high in GHG with 12%, 11.9% and 15.4% respectively. The prevalence of HBsAg was statistically insignificant ($p>0.05$). Of the 24 samples detected in the three hospitals, only 3 samples were detected molecularly which are all with genotype B. Of the 3 samples molecularly detected, 2 are from GHN and 1 from GHG.

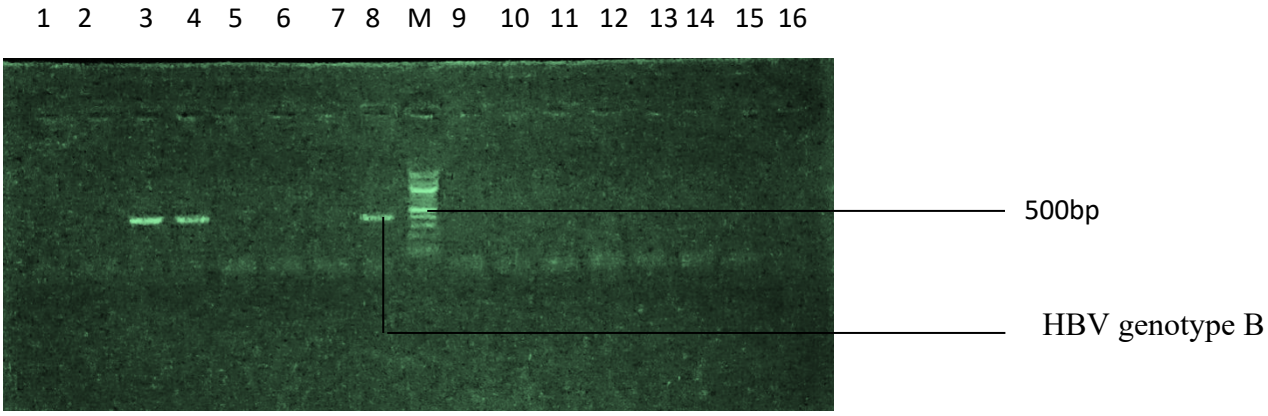

Plate 1: Agarose gel electrophoresis showing the HBV subtype. Lane 3, 4 and 8 showed the genotype B bands at 345bp while other lanes showed no band for any HBV genotypes. Lane M represents the 1500bp molecular ladder.

Table 1: The occurrence of Hepatitis B virus genotypes among HIV/AIDs patients in the selected hospitals.

Hospitals	No. of positive Samples examined	No. (%) HBV genotypes				
		A	B	C	D	E
FMCK (100)	7	-	-	-	-	-
GHN (100)	6	-	2 (25.0)	-	-	-
GHG (100)	11	-	1 (10.0)	-	-	-
Total = 300	24	-	3 (12.5)	-	-	-

DISCUSSION

From this study we observed that not all HBsAg seropositive samples were confirmed HBV positive using PCR techniques and this finding suggest that it could be due to cross-reactivity of the antigen/antibodies. In addition, these findings also agree with the fact that PCR techniques for detection of HBV are more specific than the rapid kits methods.

The detection of only HBV genotype B in HIV/AIDS patients in this study is unexpected, given that HBV genotype B is predominantly found in Asia, particularly in regions such as Japan, Taiwan, China, Indonesia, Vietnam, Northern Canada, and Greenland (Lin & Kao, 2015; Sagnelli et al., 2016; Kramvis, 2016). This finding contrasts with established epidemiological data, which typically associate HBV genotypes E and A with sub-Saharan Africa, including Nigeria. HBV genotypes E, B, A, C and D are the prevalent genotypes in Zaria, Nigeria, as they occur in single genotype and in mixed-genotypes pattern (Ahmad *et al.,* 2019)

Moreover, this result contradicts previous studies conducted in West Africa, such as those by Abdou et al. (2010), which reported a higher prevalence of HBV genotypes E and A among HIV-positive individuals in the region. The absence of these expected genotypes in the present study raises questions about possible factors influencing genotype distribution, including migration patterns, sample selection, or viral evolution. Although genotype D is found throughout Africa, it is common more in the northern countries on the Mediterranean (Lin & Kao, 2015 and Velkov, 2018). This also shows that these genotypes may likely have been circulating in the study location.

Further investigation is necessary to determine whether this unexpected finding is due to regional variations in HBV genotype distribution, the impact of HIV co-infection on genotype selection, or potential limitations in the study's methodology. Genetic sequencing of additional samples and epidemiological studies focusing on migration history and transmission dynamics could provide more clarity.

CONCLUSION

The prevalence of HBsAg among HIV/AIDs patients was high and prevalence in relation to socio-demographic and other risk factors were statistically insignificant. In addition, not all seropositive HBsAg were genotypically confirmed HBV and HBV subtype B was the only HBV subtype detected in seropositive HBsAg.

REFERENCES

1. 2006 Population Census, Federal Republic of Nigeria, National Bureau of Statistics. Archived from the original on 2009-03-25.
2. Abioye, J.O.K., Akpan, I., & Adogo, L.Y. (2017). Prevalence of HBsAg among HIV patients attending Limi Hospital Abuja. *International Journal of Scientific & Research Publications.* Vol. 7: 689 – 693
3. Ahmad, A. E., Bakari, A. G., Musa, B. O. P., Mustapha, S. K., Jamoh, B. Y., Abdullahi, I. N., Tahir, M. I., Olatunji, A. O., Maishanu, S. H., Suleiman, A. B., Tolulope, A., Hawkins, C., Sagay, A. S., Zoakah, A., & Olayinka, A. T. (2019). Pattern of Prevalent Hepatitis B Virus Genotypes in Zaria, Nigeria. *Nigerian Postgraduate Medical Journal*, *26*(2), 80-86. https://doi.org/10.4103/npmj.npmj_59_19
4. Baumert, T. F., Thimme, R., & von Weizsäcker, F. (2007). Pathogenesis of hepatitis B virus infection. *World journal of gastroenterology*, *13*(1), 82-90.
5. Cheng, S., & Chang, M.H., (2010). Epidemiology and Natural History of Hepatitis B in Children. *Springer.* ISBN: 978-1-60761-372-5. pp. 13
6. Chiekulie, K.D., Emmanuel, C.O., Oguamanam, O.E., Jerome, E.A., & Nathan C.N., (2013). Sero-prevalence of hepatitis B virus and hepatitis C virus among HIV patients in a suburban

University Teaching Hospital in South-East Nigeria. *Pan African Medical Journal* – ISSN: 1937- 8688

7. Emechebe, G.O., Emodi, I.J., Ikefuna. A.N., Ilechukwu, G.C., Igwe, W.C., Ejiofor, O.S., *et al*. (2009). Hepatitis B virus infection in Nigeria. *Niger Med J*. 50:18-22.
8. Frietas, S.Z., Soares, C.C., Tanaka, T.S., Lindenberg, A.S, Teles, S.A *et al,* (2014). Prevalence, risk factors and genotypes of hepatitis B infection among HIV infected patients in the state of MS, Central Brazil. *Braz J. Infect Dis.* 18:473-480
9. Gyar, S.D., Agbo, P., & Reuben, C.R. (2014). Assessment of Hepatitis B Co-infection among HIV/AIDS Patients Attending Antiretroviral Therapy (ART) Clinic in Garaku, Central Nigeria. *Research Journal of Microbiology, 9: 232-238.*
10. Hamza, M., Samaila, A.A., Yakasai, A.M., Musa, M.B, & Abdulrazaq, G.H. (2013). Prevalence of Hetatitis B and C virus infections among HIV-infections in a tertiary hospital in North-Western Nigeria. *Niger J Basic Clin Sci* Vol. 10:76-81.
11. Ishaku, A.A., Godwin, O., & Ishaleku, D. (2013). Seroprevalence of Hepatitis B and C co-infection among cohort seropositive HIV patients accessing Health care in Nasarawa State, North Central Nigeria. British journal of psychological Research. Vol1:pp15-24
12. Kramvis, A. (2016). The clinical implications of hepatitis B virus genotypes and HBeAg in pediatrics. *Reviews in Medical Virology*, *26*(4), 285–303.
13. Lin, C.L., & Kao, J.H. (2015). Hepatitis B virus genotypes and variants. *Cold Spring Harb Perspect* Med 5: a021436.
14. Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S.Y., *et al.*, (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010
15. Musa BM, Bussell S, Borodo MM, Samaila AA, Femi OL. Prevalence of hepatitis B virus infection in Nigeria, 2000-2013: a systematic review and meta-analysis. Niger J Clin Pract. 2015 Mar-Apr;18(2):163-72.
16. Nkrumah B, Owusu M, Frempong H.O, & Averu P (2011). Hepatitis B and C viral infections among blood donors from rural Ghana. *Ghana Med J.* 9;45(3):97-100
17. Ojide, C.K., Kalu, E.I., Ogbaini-Emevon, E., & Nwadike, V.U. (2015). Co-infections of hepatitis B and C with human immunodeficiency virus among adult patients attending human immunodeficiency virus outpatients clinic in Benin City, Nigeria. *Niger J Clin Pract.* 18:516-21.
18. Okocha, E. C., Oguejiofor, O. C., Odenigbo, C. U., Okonkwo, U. C., & Asomugha, L. (2012). Prevalence of hepatitis B surface antigen seropositivity among HIV-infected and non-infected individuals in Nnewi, Nigeria. *Nigerian medical journal : journal of the Nigeria Medical Association*, *53*(4), 249-53.
19. Olayinka, A. T., Oyemakinde, A., Balogun, M. S., Ajudua, A., Nguku, P., Aderinola, M., … Nasidi, A. (2016). Seroprevalence of Hepatitis B Infection in Nigeria: A National Survey. *The American journal of tropical medicine and hygiene*, *95*(4), 902–907. doi:10.4269/ajtmh.15-0874
20. Oluremi, A.S., Opaleye, O.O., Ogbolu, D.O., Babalola, B.A., Shittu, T., & Adesiyan, A.A. (2014). Prevalence of hepatitis B virus infection among HIV patients in Ikole Ekiti, South-Western Nigeria. *Asian Pac J Health Sci.* 1(4):507-511
21. Pennap, G.R., Osanga, E.T., & Ubam, A. (2011). Seroprevalence of hepatitis B surface Antigen among pregnant women attending antenatal clinic in Federal Medical Centre Keffi, Nigeria. *Research Journal of Medical Sciences*; 5(2):80-82.

22. Peter, A.R., & Stephen, A.L. (2016). New Perspectives on the hepatitis B virus life cycle in the human liver. The journal of clinical investigation 126(3):833-836.\

23. Rodgers MA, Vallari AS, Harris B, et al. Identification of rare HIV-1 Group N, HBV AE, and HTLV-3 strains in rural South Cameroon. Virology. 2017 Apr;504:141-151.

24. Sagnelli, E., Alessio, L., Sagnelli, C., Gualdieri, L., Pisaturo, M., Minichini, C., Di Caprio, G., Starace, M., Onorato, L., Macera, M., Scotto, G., & Coppola, N. (2017). Hepatitis B virus genotypes, epidemiological characteristics, and clinical presentation of HBV chronic infection in immigrant populations living in Southern Italy. *Hepatitis Monthly, 17*(8), e13260.

25. Sahajian F, Venhems P, Bailly F, Fabry J, Trepo C, Sepatjam M (2007). Screening campaighn of Hepatitis C among underpriviledged bpeolpeconsulting in health centres of Lyon area, France. Eur. J. Pub. Health 17: 263-271.

26. Schweitzer, A., Horn, J., Mikolajczyk, R. T., Krause, G., & Ott, J. J. (2015). Estimations of worldwide prevalence of chronic hepatitis B virus infection: A systematic review of data published between 1965 and 2013. *The Lancet, 386*(10003), 1546–1555. https://doi.org/10.1016/S0140-6736(15)61412-X

27. Seeger, C., & Mason, W. S. (2015). Molecular biology of hepatitis B virus infection. *Virology, 479*(1), 672–686. https://doi.org/10.1016/j.virol.2015.02.031

28. Shepard, C.W, Simard, E.P, Finelli, L, Fiore, A.E, Bell, B.P. (2006). Hepatitis B virus infection : Epidemiology and Vaccination. *Epidemiologic Reviews*, Vol. 28, Issue 1, 1 August 2006, pp. 112–125

29. Tremeau-Bravard, A., Ogbukagu, I. C., Ticao, C. J., & Abubakar, J. J. (2012). Seroprevalence of hepatitis B and C infection among the HIV-positive population in Abuja, Nigeria. *African health sciences*, *12*(3), 312-7.

30. Uleanya, N.D., & Obidike, E.O. (2015). Prevalence and risk factors of hepatitis B virus transmission among children in Enugu, Nigeria. *Niger J Paed.* 42(3):199-203

31. Umego, C.F., Mboto, C.I., Mbim, E.N., Edet, U.O., George, U.E., & Tarh, J.E. (2018). Epidemiology of Hepatitis B virus infection in South-South, Nigeria. *International STD Research & Reviews.* 7(1):1-17

32. Uneke, C.J., Ogbu, O., and Inyama, P.U. (2005). Prevalence of hepatitis B surface antigen among blood donors and human immunodeficiency virus-infected patients in Jos, *Nigeria.Mem. Inst. Oswaldo Cruz*, 13:6.

33. Velkov S, Ott JJ, Protzer U, Michler T. The Global Hepatitis B Virus Genotype Distribution Approximated from Available Genotyping Data. Genes (Basel). 2018 Oct 15;9(10). pii: E495.

34. Weinbaum, C.M., William, I., Mast, E.E., Wang, S.A., Finelli, L., Wesley, A., Neitzel, S.M., Ward, J.W. (2008). Recommendations for identification and public health management of persons with chronic hepatitis B virus Infection. Morbidity and Mortality Weekly Report 57:1-20.

35. World Health Organization. (2018). Hepatitis B Fact sheet No. 416. Accessed 17 August 2018.

36. World Health Organization. (2023). *Hepatitis B fact sheet*. Retrieved from https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

37. Wu, C.C., Chen, Y.S., Cao, L., Chen, X.W., Lu, M.J. (2018). Hepatitis B virus infection: Defective surface antigen expression and pathogenesis. *World J Gastroenterol* 24(31): 3488-3499