TOPIC:

RAPID DIAGNOSTIC TEST BASED SCREENING FOR HBSAG: A COMMUNITY BASED STUDY.

PRESENTED BY:

J.A.NDAKO

AT THE 37TH ANNUAL CONFERENCE AND GENERAL MEETING OF THE NIGERIAN SOCIETY FOR MICROBIOLOGY(NSM).

2nd – 5th September, 2014.

ABSTRACT

Rapid Diagnostic Test Based Screening for HBsAg: A community based study.

NDAKO, J. A[1] ONWULIRI,F.C[2] DAHUNSI, S. O[.1] Fajobi,V.O[3]

[1] Department of Biological Sciences, Landmark University Omuaran, Kwara State Nigeria.

2 Department of PST, Faculty of Natural Sciences, University of Jos Nigeria.

3. Department of Health Services, Landmark University Omuaran, Kwara State Nigeria.

- **Background:** Hepatitis B Virus (HBV) infection is a major health problem and may lead to chronic hepatitis, cirrhosis and Hepatocellular Carcinoma (HCC). Detecting hepatitis B virus variants and antigenic variation of the HBsAg in relation to different geographic areas and process of treatment is fundamental for laboratory assay design, vaccine formulation, and prediction of progression of disease to chronic hepatitis and HCC.

- **Methods** HBV markers were assessed using serum from apparently healthy subjects. HBV markers included hepatitis B surface antigen (HBsAg), hepatitis B surface antibody, and hepatitis B core antibody (HBcAb).

- **Results** Samples from 200 volunteer subjects were tested.17 out of 200 (8.5%) showed evidence of HBV infection. Prevalence of various markers was also assessed among the population of study. Outcome of the Aminotransferase assays conducted showed high level of Transaminase.

- **Conclusion** HBV seroprevalence is high among our population of study. Routine screening for HBV is needed while an urgent public enlightenment is highly encouraged, alongside a regular vaccination schedule.

- **Key words**: HBV,Markers,Apparently Healthy Subjects.

Background

- Hepatitis B Virus (HBV) infection is a major health problem and may lead to chronic hepatitis, cirrhosis and Hepatocellular Carcinoma (HCC).**Detecting hepatitis B virus variants and antigenic variation of the HBsAg in relation to different geographic areas and process of treatment is fundamental for laboratory assay design, vaccine formulation, and prediction of progression of disease to chronic hepatitis and HCC.HBV markers were assessed using serum from apparently healthy subjects.**
- HBV markers included, HBsAg, Anti-HBs (HBsAb), Anti-HBc (HBcAb), HBeAg and Anti-HBe (HBeAb).
- Nigeria is a holoendemic area for HBV with carrier rate of 15-37% and an estimated 12% of the total population being chronic carriers of HBsAg (Alao et al., 2009; Ugwuja and Ugwu, 2010).

INTRODUCTION

- Hepatitis B Virus (HBV) infection is a well-recognized and major health problem, the global disease burden is substantial leading to significant morbidity and mortality worldwide especially in the developing countries.
- Approximately, 2 billion people in the world have been infected by HBV with 350 million chronic carriers worldwide. An estimated 500,000 to 1.2 million people die of HBV infection annually. (Zuckerman and Zuckerman, 2000).
- Hepatitis B infection is caused by hepatitis B virus (HBV), an enveloped, double – stranded circular DNA virus of complex structure. HBV is classified as an orthohepadna virus within the family Hepadnaviridae (Prescott et al., 2008).
- HBV infection is associated with different clinical features and leads to chronic carrier state in 5 to 10% of patients infected in adult life and 85 to 90% of those infected in infancy (Gust and Crowe, 1986).
- Infection with HBV can also lead to progressive liver disease, including liver cirrhosis and hepatocellular carcinoma (HCC) .(Ljunggren et al, 2004).

Introduction cont.

- Although many cases of chronic HBV infection resolve spontaneously, some progress to cirrhosis, hepatocellular carcinoma, and eventual death.
- Prevalence varies greatly in different parts of the world, but is higher in tropical regions causing both acute and chronic liver disease. **In Nigeria, HBV infection rate is increasing by the day, the reason may be lack of proper health facilities or poor economic status and less public awareness about the transmission of this Virus.**
- Humans are the only known host for HBV. HBV is relatively resilient and, in some instances, **has been shown to remain infectious on environmental surfaces for more than 7 days at room temperature.**
- The clinical outcome of HBV infection depends upon the age at infection, the level of HBV replication and the immune status of the host (Kao, et al.,2000).

CASE STUDY

- Blood transfusion service (BTS) is an integral and indispensable part of the healthcare system. The priority objective of BTS is to ensure safety, adequacy, accessibility, and efficiency of blood supply at all levels (Islam, 2009).
- Transfusion of blood and blood components, as a specialized modality of patient management saves millions of lives worldwide each year and reduces morbidity. It is well known that blood transfusion is associated with a large number of complications, some are only trivial and others are potentially life threatening, demanding for meticulous pre-transfusion testing and screening.
- The use of unscreened blood transfusion keeps the patient at risk of acquiring many transfusion transmitted infections (TTI) like hepatitis viruses (HBV, HCV)

Case study cont.:

- Blood transfusion departments do not only screen TTI, but also give clue about the prevalence of these infections in healthy populations (Khan et al., 2007).
- Transfusion associated hepatitis B viral infection (TAHBV) continues to be a major problem even after adoption of mandatory screening of hepatitis B surface antigen (HBsAg) by enzyme-linked immuno-sorbent assay (ELISA).
- The high incidence of TAHBV is reported in patients receiving multiple blood transfusions. It has been demonstrated that some HBsAg negative donors who are anti-HBc positive (antibody to hepatitis core antigen) continue to replicate hepatitis B virus (Kumar et al., 2007). They may harbour and maintain HBV-DNA sequences in their liver and blood, thus, representing potential sources of HBV transmission (Kumar et al., 2007).

Markers of HBV infection

- HBV contains numerous antigenic components, including HBsAg, Hepatitis B core antigen (HBcAg), and Hepatitis B e antigen (HBeAg) CDC,2008.
- Surface antigen and HBV DNA are often the first detectable markers of acute infection, appearing before the onset of symptoms or elevation of alanine aminotransferase (ALT).
- By definition, HBV infection is chronic if surface antigen persists longer than 6 months. **HBV e antigen, is considered a marker of active replication and infectivity of the virus**.
- HBV core antigen cannot be detected in the sera samples but in liver cells to be used for biopsy, however antibodies to the core antigen such as first immunoglobulin M (IgM) and later immunoglobulin G (IgG) can be detected.CDC,2008

Markers of HBV infection.

- At present, HBsAg detection is the only diagnostic screening test for HBV infection identification in the blood transfusion centers of India.
- The prevalence of anti-HBc in the sera of healthy blood donors negative for HBsAg was not much considered.

Diagnosis:

1. HBsAg can be identified in serum 30 to 60 days after exposure to HBV and persists for variable periods.
2. Sera Samples are normally tested for HBsAg using immunochromatographic test strip, Enzyme immunoassay while clinical data, Liver function test, and HBV serum markers further assist in the diagnosis.
3. **Serological marker detection**: Serologic markers for HBV (Hepatitis B surface antigen [HBsAg], Hepatitis B e antigen [HBeAg] and antibodies to Hepatitis B core antigen [anti-HBc]) infection equally helps in precise diagnosis.

- **Development of rapid, simple, and standardized assays that can detect all known genotypes can accelerate progress in research on the clinical significance of HBV genotypes, and permits detection of the common as well as uncommon mutations. This is majorly for research purpose and drug formulation.**
- Samples from volunteer subjects were tested. Prevalence of various markers was also assessed among the population of study. Outcome of the assays conducted showed a high prevalence among the population studied.

Methodology:

STUDY LOCATION

- Volunteer blood donors from local communities were recruited for the study.

STUDY POPULATION

- All the blood donors, donating blood in the blood bank were considered as the study population. About 200 samples were collected for the study during the one month period. The participant donors were from both urban and rural areas of the Community.
- **INCLUSION CRITERIA**
- All the donors who satisfied the qualifying criteria for the donation were included in the study. The study included both the voluntary blood donors and the replacement blood donors (blood donated to replace blood utilized.

EXCLUSION CRITERIA

- Persons belonging to high risk groups such as patients with history of sexually transmitted diseases, drug abusers, sex workers, pregnant women, etc. were excluded from the study..

Screening procedure :

QUESTIONNAIRE/ ETHICAL CONSENT

- Those individuals who volunteered to participate in the study were accepted alone after obtaining a written informed consent.
- A detailed pre-donation questionnaire was used to collect their socio-demographic characteristics including age, gender, occupation, marital status, pre-donation status, etc. They were also asked about family history of jaundice, previous surgical procedures, history of past or current use of intra venous drugs, tattooing, visiting community barber's shop in males, history of injections received in past one year, dental services under taken, etc.
- Some questions about high risk behaviours, such as premarital and extra martial sexual behaviour were also investigated.

Processing of samples:

- Ethical approval: The present study was approved by the ethical committee of National Blood Transfusion Services,Jos Plateau State.
- ***Processing of samples*** All the serum samples were screened for the HBsAg using ELISA (Omega diagnostics,) and for anti-HBc total, anti-HBc IgM, and anti-HBs (The 5-Panel Rapid test kit was used).
- The HBsAg negative samples were tested for the presence of antibodies to Hepatitis B core antigen (anti-HBc IgM). All the anti-HBc positive samples were retested with the same assay for confirmation of anti-HBc positivity.
- The presence of antibodies to Hepatitis B surface antigen (anti-HBs) was also investigated in all the positive sera samples.

RESULT:

- A total of 200 blood donors were screened during the study, and among them, a vast majority (184: 92%) were males, with a male to female ratio of 11.5:1. It was observed that there were 172 first time voluntary donors (males, 158 and Female, 14).
- Majority of the donors belong to the age group of 26 to 30 years.
- Out of the 200 samples studied, 17 of them were found to be positive for HBsAg. The prevalence rate of HBsAg was (17/200) 8.5%. All the positive cases were male and no female donors were found to be positive (Table 1). Age wise prevalence was found to be more in subjects aged 25 to 29 years with 6(3.0%) positivity(Table 2).

Table 1: Distributions of Blood Donors based on Sex of the subjects screened.

	Total Number Examined (%)	No. Positive (%)	No. Negative (%)	p-value
Male	147(74.0)	13 (6.5)	135(67.5)	
Female	52(26.0)	4(2.0)	48(24.0)	P= 0.0075

P>0.005

Table 2: Distributions of Blood Donors based on Age of the subjects screened.

	Total Number Examined (%)	No. Positive (%)	No. Negative (%)	p-value
Age (years)				
20-24	34(17.0)	4(2.0)	30 (15.0)	
25-29	52(26.0)	6(3.0)	46(23.0)	
30-34	49(24.5)	4(2.0)	45(22.5)	
35-39	30(15.0)	1(0.5)	29(14.5)	
40-44	35(17.5)	2(1.0)	33(16.5)	P= 0.1981

P <0.005

Table 3:Summary Distributions of Blood Donors Screened based on Vaccination, Socio Demographic Characteristics and other Risk Factors.

	Total Number Examined (%)	No. Positive (%)	No. Negative (%)	p-value
History of Vaccination				
Yes	32(16.0)	2 (1.0)	30 (15.0)	
No	168(84.0)	15(7.5)	153(76.5)	P= 0.3116
Risk factors based on family history of HBV				
Yes	15(8.5)	5 (2.5)	12(6.0)	
No	185(92.5)	12(6.0)	173(86.5)	P= 0.2033
History of Blood transfusion				
Yes	8(4)	4(2.0)	4(2.0)	
No	192(96.0)	14(7.0)	178(68.5)	P= 0.2230
Previous History of surgery				
Yes	4(2.0)	2(1.0)	2(1.0)	
No	196(98.0)	157(7.5)	181(90.5)	P= 0.4643
History of Alcoholic consumption.				
Yes	22(11.0)	7(3.5)	15(7.5)	
No	178(89.0)	168 (84.0)	10(5.0)	P= 0.1957

Table 4:Prevalence of HBV markers among Positive subjects screened

		HBsAg	HBeAg	Anti-HBs	Anti-HBe	Anti-HBc	P-Value
CATEGORY	Results	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
Blood donors	Positive	17 (85)	1 (0.5)	10(5.0)	9 (4.5)	4 (2.0)	
	Negative	183 (91.5)	199 (95.5)	190 (95.0)	191 (95.5)	191 (98.0)	
	Total	200 (100.0)	200 (100.0)	200 (100.0)	200 (100.0)	200 (100.0)	P<0.0001

Table 5:Overall Determination Of Serum Alanine Aminotransferase (ALT) Levels On Positive Subjects Screened:

Table 5:Overall Determination Of Serum Alanine Aminotransferase (ALT) Levels On Positive Subjects Screened:

CATEGORY	SEX		Total Number of Positive Subjects Screened (%)	Total Number of abnormal ALT Level (%)	Total Number of Normal ALT Level (%)	P- Value
	MALE (%)	FEMALE (%)				
BLOOD DONORS	13(76.4)	4(23.6)	17(100.0)	8(47.0)	9(53.1)	

Table 6:Summary Distributions of Blood Donors Screened based on Sex, Age, Vaccination, Socio Demographic Characteristics and other Risk Factors.

	Total Number Examined (%)	No. Positive (%)	No. Negative (%)	p-value
Sex				
Male	147(74.0)	13 (6.5)	135(67.5)	
Female	52(26.0)	4(2.0)	48(24.0)	P= 0.0075
Age (years)				
20-24	34(17.0)	4(2.0)	30 (15.0)	
25-29	52(26.0)	6(3.0)	46(23.0)	
30-34	49(24.5)	4(2.0)	45(22.5)	
35-39	30(15.0)	1(0.5)	29(14.5)	
40-44	35(17.5)	2(1.0)	33(16.5)	P= 0.1981
History of Vaccination				
Yes	32(16.0)	2 (1.0)	30 (15.0)	
No	168(84.0)	15(7.5)	153(76.5)	P= 0.3116
Risk factors based on family history of HBV				
Yes	15(8.5)	5 (2.5)	12(6.0)	
No	185(92.5)	12(6.0)	173(86.5)	P= 0.2033
History of Blood transfusion				
Yes	8(4)	4(2.0)	4(2.0)	
No	192(96.0)	14(7.0)	178(68.5)	P= 0.2230
Previous History of surgery				
Yes	4(2.0)	2(1.0)	2(1.0)	
No	196(98.0)	157(7.5)	181(90.5)	P= 0.4643
History of Alcoholic consumption.				
Yes	22(11.0)	7(3.5)	15(7.5)	
No	178(89.0)	168 (84.0)	10(5.0)	P= 0.1957

Risk factors:

- Risk factors among blood donors screened showed that 7(3.5%) of subjects that tested positive had history of alcoholic consumption, this was followed by those who had Family history of HBV 5(2.5%) P>0.005.Those with History of Blood Transfusion recorded a prevalence of 4(2.0%) while 2(1.0%) that tested positive had history of surgery.

Prevalence of Markers among positive subjects screened:

- The highest rate of positivity recorded with the HBsAg showed 8.5% among Blood donors. 0.5% among Blood donors. Anti-HBs which indicates antibody to the HBsAg showed 5.0% while Anti-HBe positivity among the subjects screened recorded 4.5%.Anti-HBc resukt among subjects screened recorded 2.0%.

Discussion

- The 8.5% prevalence recorded in this study is higher compared to the result obtained by Okonko et al, (2010) in a study conducted at Abeokuta-Nigeria, where a prevalence rate of 6.6% for HBsAg in young adults aged 15-29 years was reported.
- However, The positive in this study is lower than the 20.0% found by Alao *et al*. (2009) in Otukpo, an urban area of Benue State; the 18.6% reported by Buseri et al. (2009) in Osogbo, Nigeria; the 14.5% overall HBsAg seroprevalence reported by Lawal *et al.* (2009) in Ibadan) among truck drivers in Sagamu, Ogun State, Nigeria; the 13.5% reported by Opaleye et al. (2010) in Osogbo, Osun State, Nigeria; the 13.2% found by Fasola et al (2009) in Ibadan, South-western, Nigeria; the 13.2% reported for HBsAg by Pennap et al. (2010) in Keffi, Nassarawa State, Nigeria; the 11.0% reported by Sule *et al*. (2010) in Ayingba.

DISCUSSION

- **The discovery of the HBsAg was a major breakthrough in decreasing the incidence of post transfusion hepatitis**.
- Following infection by the hepatitis B virus (HBV), the first serological marker to appear in the blood is the HBV DNA, followed by HBsAg, the DNA polymerase and the hepatitis B ‘e’ antigen (HBeAg).
- Thereafter, the antibodies to the hepatitis B core antigen (anti-HBc), hepatitis B ‘e’ antigen and the HBsAg can be detected.
- Screening of donated blood by ELISA for HBsAg is the common method for detecting hepatitis B infection.
- **Screening of blood for the detection of this viral marker, however, does not rule out the risk of transmission of hepatitis B totally, because during the host serological response to infection, there is a phase during which the HBsAg cannot be detected in the blood, although, hepatitis B infection is present.**

Discussion cont.

- This phase is called the ‘window period’. It represents a carrier state of the disease. **Therefore, a definite hazard of transmission of hepatitis B to recipients of such units of donated blood exists.**
- During this ‘window period’, detection of the anti-HBc serves as a useful serological marker for hepatitis B infection. The IgM class of the anti-HBc is the first to appear, and indicates a recent infection.
- From this study, It is therefore strongly suggested that a marker must be utilized for the screening of blood in the population to detect the presence of hepatitis B during the window period (Hoofnagle et al., 1978; Doglas et al., 1993).

CONCLUSION:

- This study showed that the prevalence of HBsAg among blood donors is generally comparable to results obtained from similar studies carried out earlier.
- It is very important, especially for healthcare providers and policy makers, to recognize the risk factors of HBV infection and design effective preventive programs to drastically minimize the spread of HBV infection.
- Similarly, low rate of screening by the populace could be attributed to lack of perceived utility, lack of funds, or both (Allain *et al*,2003).
- Furthermore, systematic study of donors and recipient populations should be undertaken so as to provide the basic data for accurate estimation of transfusion-related risks of HBV infection in high-prevalence areas of Africa.
- Routine screening for HBV is needed while an urgent public enlightenment is highly encouraged, alongside a regular vaccination schedule.

Sample documentation in progress.

Sample documentation cont.

Sample Assay progress.

HBV Elimination Strategies:

A comprehensive strategy to eliminate hepatitis B virus transmission as recommended by CDC includes:

- Prompt and proper screening of infected individuals.
- Prenatal testing of pregnant women for HBsAg to identify newborns who require immunoprophylaxis for prevention of perinatal infection.
- Identification of household contacts who should be vaccinated and proper screening of blood donors.
- Routine vaccination of infants, vaccination of adolescents, and vaccination of adults at high risk to infection, CDC 2008.
- Rigorous public enlightenment/awareness programs to communities, especially at endemic zones.

References:

- Arauz-Ruiz , Norder PH, Robertson BH, Magnius LO, Genotype H: (2002) **A new Amerindian genotype of hepatitis B virus revealed in Central America.** *J Gen Virol* **83:**2059-2073.
- Chu CJ, Lok ASF: (2002)**Clinical significance of hepatitis B virus genotypes.** *Hepatology* **35:**1274-1276
- Ljunggren K, Myhre E, Bläckberg J(2004) **Clinical and Serological Variation between**
- **Patients Infected with Different Hepatitis B Virus Genotypes.** *.J. Clin Microbiol* **42:**5837-5841.
- Neurath AR, Thanavala Y: (1990) **Immunochemistry of viruses. II. The basis for serodiagnosis and vaccines.** In *Hepadnaviruses*. Edited by: Van Regenmortel MHV, Neurath AR. Elsevier, Amsterdam, the Netherlands:403-458.
- Norder H, Courouce AM, Coursaget P, Echevarria JM, Leef SD, Mushahwar IK, Robertson BH, Locarnini S, Magnius LO: (2004). **Genetic Diversity of Hepatitis B Virus Strains Derived Worldwide: Genotypes, Subgenotypes, and HBsAg Subtypes.** *Intervirology* 47:289-309.
- Norder H, Courouce AM, Magnius LO: (1994) **complete genomes, phylogenic relatedness and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology* 198:**489-503.
- Stuyver L, De Gendt S, Van Geyt C, Zoulim F, Fried M, Schinazi RF, Rossau R: (2000)**A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness.** *J Gen Virol* **81:**67-74.

References Cont.

- Norder H, Courouce AM, Magnius LO: (1994) complete genomes, phylogenic relatedness and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. Virology 198:489-503.
- Norder H, Courouce AM, Coursaget P, Echevarria JM, Leef SD, Mushahwar IK, Robertson BH, Locarnini S,
- Magnius LO: (2004). Genetic Diversity of Hepatitis B Virus Strains Derived Worldwide: Genotypes, Subgenotypes, and HBsAg Subtypes. Intervirology 47:289-309.
- Odemuyiwa SO, Mulders MN, Oyedele OI, Ola SO, Odaibo GN, Olaleye DO, and Muller CP: (2001) Phylogenetic analysis of new hepatitis B virus isolates from Nigeria supports endemicity of genotype E in West Africa. J Med Virol 2001 65:463-469.
- Prescott, L.M. Harley, J.P. and Klein, D.A. (2008). Viral Hepatitides. In: Willey, J.M., Sherwood, L.M. and Woolverton,C.J. Review of Microbiology (7th edition). McGraw-Hill Companies Inc. New York, pp 936-939.
- Sibnarayan, D., Arup, B., Chandra, P.K., Biswas, A., Panigrani, R., Mahapatra, P.K., Panda, C.K., Chakrabarti, S., Bhattacharya, S.K. and Chakravarty, R. (2008). Analysis of hepatitis B virus X gene phylogeny, genetic variability and its impact on pathogenesis: Implications in Eastern India HBV Carriers. Virology, 382:190-198.
- Stuyver L, De Gendt S, Van Geyt C, Zoulim F, Fried M, Schinazi RF, Rossau R: (2000)A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J Gen Virol 81:67-74
- Usuda SH, Okamoto H, Iwanari K, Baba F, Tsuda Y, Miyakawa Mayumi M (1999): 1Serological detection of hepatitis B virus genotypes by ELISA with monoclonal antibodies to type-specific epitopes in the preS2-region product.J Virol Methods, 80:97-112.
- Zuckerman JN, Zuckerman AJ: (2000,) Current topics in hepatitis B. Journal of infections 41(2):130-136.

THANK YOU
FOR LISTENING
AND

PLEASE HELP SREAD THE PREVENTIVE MEASURES.