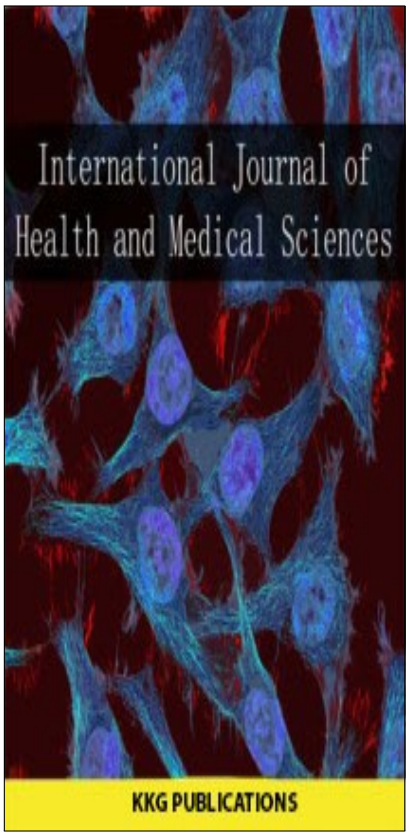

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A Survey on the Prevalence of Hepatitis B Virus and Predisposing Factors among Blood Donors in Two General Hospitals in Jigawa State Nigeria

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A SURVEY ON THE PREVALENCE OF HEPATITIS B VIRUS AND PREDISPOSING FACTORS AMONG BLOOD DONORS IN TWO GENERAL HOSPITALS IN JIGAWA STATE NIGERIA

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Abstract. This research was carried out to determine the prevalence state of Hepatitis B infection and predisposing factors among blood donors in Dutse and Gumel General Hospitals, Jigawa State, Nigeria. Five hundred and forty-six (546) blood samples were aseptically collected from consenting donors at two hospitals. Two hundred and seventy-three (273) samples were screened from each General Hospital for Hepatitis B Surface Antigen (HBsAg) using third generation enzyme immunoassay HBsAg kits. Out of 273 samples from Dutse General Hospital, 260 were males, and 13 were females while in Gumel General Hospital, 264 were males, and 9 were females. The prevalence rates in the two General Hospitals were 36 and 27 respectively. The peak age specific HBsAg sero-positivity occurred in the aged range of 31-40yrs and 21-30yrs for Dutse and Gumel General Hospitals respectively. Commercial donors constitute highest percentage of blood donors in the two Hospitals; 25% and 20% respectively. The finding indicates that there is no significant difference between gender and the occurrence of the infection among blood donors attending two General Hospitals $p > 0.05$. The result confirmed that there is no significant difference between the two hospitals based on the Hepatitis B infection at $p > 0.05$. Among the predisposing factors analysed statistically, the result shows that there is significant difference $p < 0.05$ between the level of education, occupational status, donors with history of Alcohol consumption, and the occurrence of Hepatitis B surface Antigen in both hospitals but there is no significant difference $p > 0.05$ between the Hepatitis B Virus (HBV) vaccination status and the occurrence of HBsAg among donors in both hospitals, so it is important to make thorough investigation among blood donors before transfusion of blood, because transfusion of blood saves life, but recipients are at high risk of contracting Hepatitis B virus infections through transfusion of infected blood and blood products. Mass screening, immunization against the virus, and public health education to enlighten the populace about infection and the routes of transmission are recommended.

INTRODUCTION

Hepatitis is an infection caused by toxic agents, viruses, drugs and an autoimmune response which is characterized by abdominal pain, liver inflammation, jaundice and fever, other causes of Hepatitis are viral and alcohol which lead to cirrhosis of the liver. Viral Hepatitis is a disease caused by different species of viruses which affect the whole body especially liver tissue. Acute viral Hepatitis is a common infection among adult and children which is caused by the following etiological agents; Hepatitis A, B, C, D, E, F, and G, cryptogenic (Caused by a virus as yet unidentified). More cases of Hepatitis viruses are being discovered and mostly less common. Other types of viruses, such as Cytomegalovirus (CMV) and Epstein Barr (EBV) are also involved [1]. Other categories of Hepatitis involve alcoholic Hepatitis, hemochromatosis, Wilson's disease, and autoimmune disease. Acute Hepatitis B refers to the first six months after someone is exposed to the Hepatitis B virus. Few people can fight the infection and clear viral Hepatitis while for the others the infection remains and leads to a chronic one [1].

The pandemicity of the infections is common in Philippines, Africa and the middle-east, Asia, China [2]. The disease is common in North America and Europe with the 1in each 1000 known carriers of the infection. The prevalence of the disease is estimated to be 280 million carriers across the globe which represent more than 5% of the population worldwide [3]. In the recent estimate, World Health Organisation (WHO) reported that about 2 billion individuals are infected with Hepatitis B virus worldwide with 350 million peoples infected with chronic Hepatitis B infection [4]. However, twenty million peoples are infected with Hepatitis B virus in Nigeria out

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of which five million died due to consequences of the viral infection [5]. Transmission of infections results from exposure to infectious blood or body fluids [6].The acute illnesses cause inflammation of the liver, vomiting, jaundice and rarely death [7].

The route of transmission includes horizontal and vertical transmission [8]. The occurrence of chronic Hepatitis infection indicates the primary method of transmission in some endemic areas. However, areas with low prevalence such as the continental United States and Western Europe, the primary methods of transmission involve unprotected sex and intravenous drug use.

Similarly, the course of transmission has important clinical implications, because there is a very high probability of developing chronic Hepatitis B if the infection is acquired prenatally or around the time of child birth. More than 8-16 million Hepatitis B Virus infections occur each year in developing countries due to the use of unsafe hypodermic material, compared with 2.3-4 Million Hepatitis C virus infection and 80,000-160,000 Human immunodeficiency virus infection [9].

The Center for Disease Control studies confirmed that six hundred and twenty thousand individuals die annually (620,000) with 350 million individuals infected with Hepatitis viral infection globally. It is estimated that new cases of about 46,000 Hepatitis B occurred in the year 2006, United States reported cases of the infection with highest prevalence rate among the age group of 25-44 years with low prevalence among the age group of less than 15 years of age. However, unprotected sex, poor blood transfusion, and illegal use of drugs are the main routes of transmission in the United States [4]. Universal vaccination of children in the United States has led to 75%, decrease in newly diagnosed cases of HBV during the past decade, the infections account for estimated 500,000-700,000 annual deaths worldwide [5]. With a safe and effective vaccine available since 1982, much of this infection and death should be preventable [5].

Blood transfusion is described as a therapeutic method used to restore the volume of blood after extensive loss due to hemorrhage, burn or trauma; to increase the number and concentration of red-blood cells in persons with anemia in order to improve the oxygen-carrying capacity of their blood and to treat shock. Transfusion is a crucial adjunct in some types of surgeries in which patients lose large amounts of blood that need to be replaced [4].

However, before blood transfusion, blood screening needs to be done before transfusion so as to prevent the recipient from being infected with blood-related disease against such as HBsAg and HIV [10].

LITERATURE REVIEW

Hepatitis describes the inflammation of the liver, which is caused by a virus called HBV. The incubation period starts from the time of contact to the onset of symptoms that mostly starts between 6 weeks and 6 months. The virus may develop in highest and lowest concentrations of the blood and body products respectively. Hepatitis B Virus infection can be acute or chronic. Approximately half of the newly acquired infections are symptomatic, especially in adults and roughly 1% reported new cases result in acute liver failure and death [11].

Viral infections account for more than 50% cases of acute Hepatitis in the United States [12], and the risk for chronic infection is inversely related to the age at infection. Approximately 90% of the infected infants and 30% of the infected children aged < 5 years become chronically infected. Among persons with chronic HBV infection, the risk for premature death from liver cirrhosis is 15-25%. Based on the epidemiological survey, it has been confirmed that 15-40% infected individuals may develop liver cirrhosis or Hepato cellular carcinoma. It is estimated that chronic Hepatitis B Virus carriers bear a potential 100-fold increased risk for HCC development compared with non-carriers [13]. Since 1997, Hepatitis B Virus infection has become the 10th leading cause of death in the world [1], and HCC has been ranked as the 5th most frequent cancer [14]. In China, more than one thirty million (130m) people are suffering from Chronic Hepatitis B [15], and HCC has been ranked as the second major cause of cancer-related death since 1990 [16]. The infections account for estimated 500,000-700,000 annual deaths worldwide [17]. With a safe and effective vaccine available since 1982, much of this infection and death should be preventable [5].

Studies show that blood transfusion in Nigeria is facing serious challenges. In the first instance, the Federal Government of Nigeria is limited to the creation and operation of National Blood Transfusion Services (NBTs) and funding of the blood bank units of our various federal medical centers and teaching hospitals, [18]. Secondly, most of the transfusions of blood in most of our various clinics are gotten from paid donors and families of patients in need of the blood after cross-matching and screening. At the grass root level, ordinary citizens still don't see voluntary blood donation as a lifestyle that should be imbibed and encouraged in the best interest of all especially the sick. Most of the state ministries of health are only interested in HIV/AIDS [19]. However, the current situation is so pathetic that the ordinary, Nigerian citizens don't even think of blood donation. One of the reasons responsible for this is the low level of awareness of why it is necessary and advisable to donate blood [19].

Blood transfusion is one of the common sources of transmission of Hepatitis B infection, but the improvement of laboratory diagnostic procedure and progressively extensive screening for Hepatitis B Virus infection in the recent years has dramatically reduced the risk of acquiring HBV infection through transfusion of blood. Other sources of infection include contaminated hypodermic needles, organ transplant, and other surgical equipment [9].

Public health workers, dentists, and others who frequently have contact with infected blood or blood products are at the highest risk of the infection [9]. This present study is established to survey the prevalence state of Hepatitis B virus and predisposing factors whose status needs to be defined and ruled out among blood donors attending Dutse and Gumel General Hospitals. This information is necessary for any attempt to donate blood safely to recipients. However, there is scanty information on the epidemiological characteristics of Hepatitis B virus infection from some countries belonging to these endemics regions. For example, in Nigeria at present, it is difficult to assess the true rate of infection with HBV among the Nigerian population; this is associated with the limited number of studies that have been reported [20].

MATERIAL AND METHODS

Study Area

The study area involved two districts namely Dutse and Gumel. The Dutse and Gumel General Hospitals handle most of the referral cases from the neigbouring hospitals and clinics in Jigawa state, located at latitude 12^0N to 13^0S and longitude 9^0E to 10^0N. The areas fall within Sahel savanna vegetation zone of the sub-Saharan tropical climate with scanty rainfall during the year. The two hospitals lie approximately 150 kilometers from each other [21].

Collection and Handling of Specimens

Five hundred and forty-six (546) samples were aseptically and randomly collected from 2 General Hospitals (Dutse and Gumel) by taking five milliliters (5ml) of venous blood samples. Sera were separated by centrifugation machine for 5 minutes. Sera samples were removed and introduced into 1.8ml cryovial container labeled and then stored at 2-8^{0}C.

Reagents Preparation

All reagents were prepared in accordance with the manufacturer's specifications of the ELISA kits used.

91 wells were selected out of 96 wells to introduce the blood sample. The remaining 5 wells were positive control into 1st well, low positive control into 2nd and 3rd wells, and negative control in 4th and 5th wells [23].

The content of the working water solution bottle was mixed thoroughly with diluted volume of concentrated washing solution (25-folds) of purified water prior to use [22].

Preparation of working solution of conjugate-1: The required volume of concentrated conjugate-1 (11 folds) was diluted thoroughly with the corresponding volume of conjugate-1 diluent and was shaken thoroughly without foaming [23].

Preparation of working solution of conjugate-2: 11-folds of the concentrated conjugate-2 was mixed thoroughly with the (11 folds) of conjugate-2 diluent [22].

Substrate mixture: 16 folds of tetramethylbenzidine [TMB3'5, 5'] (Concentrated 16-fold) were diluted with 16-fold volume of substrate buffer [22].

Low positive control: Low positive control was prepared by diluting the content of the vial with the volume of purified water as indicated on the label of the vial. The solution was kept at room temperature for five minutes [22].

Storage of unused reagents: The unused reagents were kept at 2-8^{0}C.

Assay Procedure

(i) The coated strips were washed three times, by adding 380-400l of working washing solution and allowed to dry for 40 seconds and then aspirated. An automatic microplate washer was used to wash the plate [22].

(ii) Out of 96 wells, 100μl of positive control were introduced into A1 well, 100μl of low positive control into A2 and A3 wells, 100μl of negative control into A4 and A5 wells, and 100μl of serum were introduced into the remaining 91 wells of the plate (Plate-1). Then covered by plate lid and incubated at (42.0$\pm$0.5^{0}C) for 30 minutes [22].

(iii) After 30-minute incubation at (42.0$\pm$0.5^{0}C), 50μl of working solution of conjugate-1 was added into each well in the plate. The colour changed in negative and low positive controls to bright yellow except in positive control. Then the plates were shaken. The plates were re-incubated in microplate incubator at (42.0$\pm$0.5)^{0}C for 45 minutes.

(iv) 50μl of working solution of conjugate-2 was added to each well after re-incubation mixed thoroughly. Then incubated in a micro plate at (42.0$\pm$0.5)^{0}C for 45 minutes.

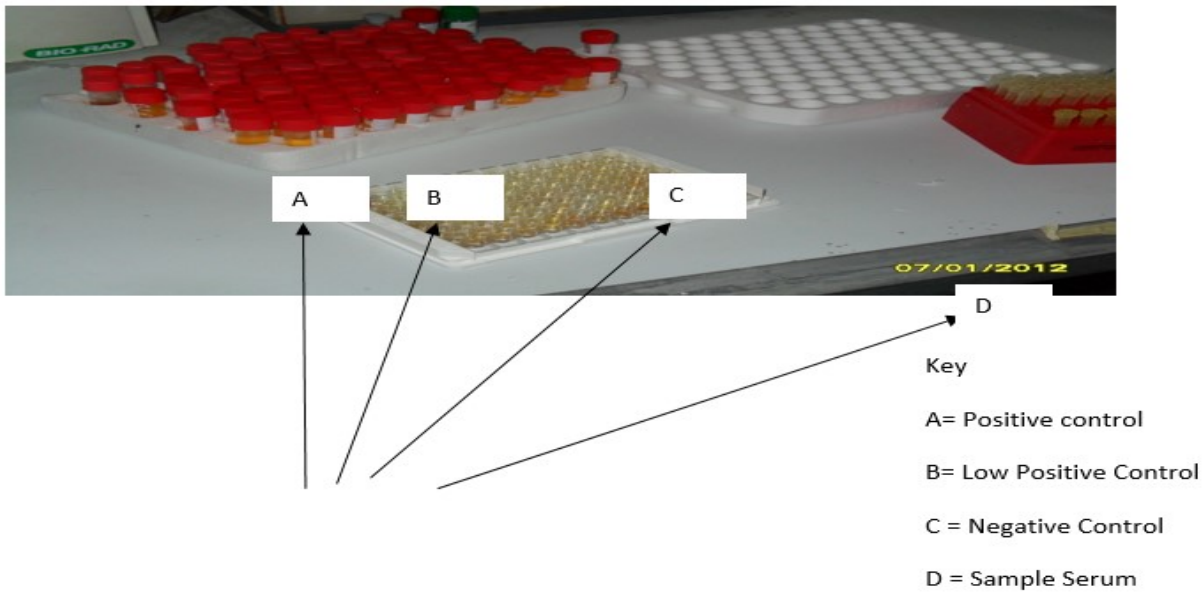

Fig. 1 . Shows the Elisa plate after dispensing the serum and controls

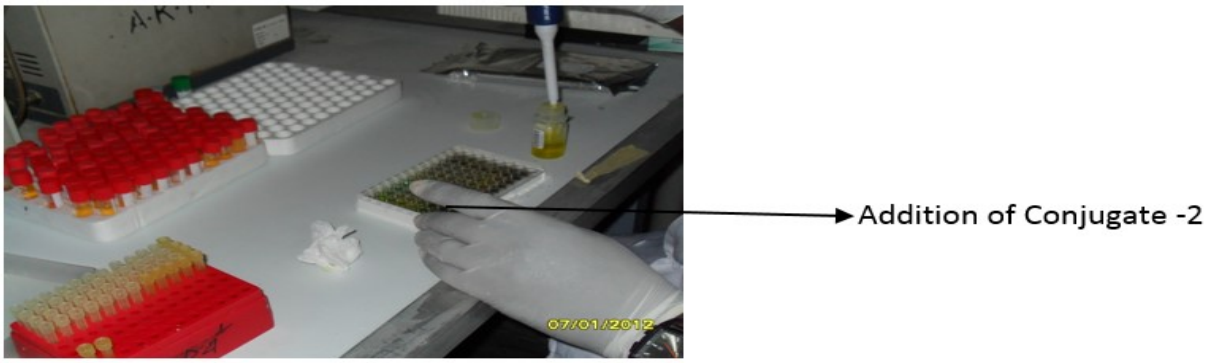

Fig. 2 . Shows the addition of 50μl conjugate 2 into well

(vii) Then the content of the well in the plate was poured off and washed 7 times by the use of automatic washer with working solution. The plates were dried by turning them upside down on absorbent paper.

(viii) 150μl of substrate mixture was added into each well and kept in a dark place at 18-24^{0}C for 25 minutes.

(ix) 50μl of stopping solution was added into the wells and after 2-3 minutes, the optical density was recorded at 450/620nm [24].

Calculation

The Cut-off Value (COV) was obtained by adding Absorbance density (OD) of two negative controls of each plate and divided by two. The final value was added to a constant (0.06) as recommended in accordance with the manufacturer's instruction [23].

Interpretation of the Result

For the interpretation of ELISA results, the COV = the result of a well would be positive if OD450 of sample $\geq$ COV and the results would be negative if OD450 sample $<$ COV.

Statistical Analysis

Statistical analysis was performed to find the association between the socio-demographic, behavioral, biochemical indicators, and prevalence of HBV infection using simple percentage and SPSS software. The prevalence rates of Hepatitis B Virus in two districts were calculated at 95% Confidence Interval (CI). The demographic variables assessed include age, marital status, residence (urban or rural), and gender.

RESULTS

The results show that there is significant difference in all the three (3) socio-demographic variables, statistically analysed; p =78.141 for Educational level, p = 90.737 for Occupational status, p = 48.19 for history of Alcohol consumption, and no significant difference in Vaccination of HBV infection with p = 0.45 at 95% confidence interval.

TABLE 1
PREVALENCE OF HBSAG IN RELATION TO SOCIO-DEMOGRAPHIC VARIABLES IN JIGAWA STATE

	Variables	Positive (Reactive HBsAG) Percentage (%)	Negative (Non-Reactive) Percentage (%)	χ^2	p Value
Educational Level	Non Formal Edu.	27	8	78.141	0.05
	Primary	3	15		
	Secondary	2	27		
	Tertiary	0.7	39		
Occupational Status	Farmers	7.7	14	90.737	0.05
	Student	1	22		
	Business	2	14.3		
	Civil Servant	0.7	38		
Alcohol Consumption	Consume Alcohol	4	70	48.19	0.05
	Consume Non-alcohol	7	17		
Vaccination of HBV	Vaccinated	2	18	0.45	0.05
	Not Vaccinated	9.5	69		

TABLE 2
PERCENTAGE OF POSITIVE AND NEGATIVE HBsAG AMONG DONORS IN DUTSE AND GUMEL GENERAL HOSPITALS JIGAWA STATE

Sex	No. of Screened Blood Donors in Dutse	No. of HBsAg. Positive in Dutse	No. of HBsAg. Negative in Dutse	No. of Screened. Blood Donors in Gumel	No. of HBsAg Positive in Gumel	No. of HBsAg Negative in Gumel
Male	260	36(13%)	237(86%)	264	26(9.5%)	246(90%)
Female	13	-	13(4.8%)	9	1(3.6%)	8(2.9%)
TOTAL	(n=273)	36(13%)	250(98%)	(n=273)	27(9.89%)	254(92.9%)

Percentage prevalence of HBsAg reactive is 36 in Dutse and 27 in Gumel General Hospitals respectively. However, the result analysed using t-test indicates that there is no significant difference between infected individuals in two General Hospitals respectively with p = 0.491 at 95% confidence interval.

TABLE 3
NUMBER OF COMMERCIAL AND FAMILY DONORS IN DUTSE AND GUMEL GENERAL HOSPITALS

Hospitals	Number Screened	Number of Commercial Blood Donors	Number of Family Donors	Number of Unspecified Donors
Dutse	273	69(25%)	37(13%)	167(61%)
Gumel	273	58(23%)	23(8.3%)	192(70%)
Total	(n=546)	127(48)	60(21.3%)	359(66%)

Commercial donors constitute the highest donors in Dutse 69 and Gumel 58 General Hospitals compared to family replacement donors 37and 23 respectively. While the number of unspecified donors was 167 and 192 respectively.

TABLE 4
PREVALENCE OF HBSAG IN RELATION TO ABO BLOOD GROUPS AND RHESUS FACTORS

Hospitals	Blood Group A	Blood Group B	Blood Group AB	Blood Group O	Rhesus Factors
Dutse	43	84	20	126	D+
Gumel	39	63	31	140	D+
Total	82	147	51	266	D+

Blood group O showed highest donors in both Hospitals and Rhesus factors for the entire subject tested showed D.

DISCUSSION

Currently, blood transfusion in Nigeria is facing interesting challenges; with the emergence of transfusing transmissible Hepatitis B virus infection. In this present study, we observed HBsAg percentage prevalence of 13% and 9.8% in Dutse and Gumel General Hospitals respectively among blood donors as indicated in Table 2. This research confirmed that HBV infection through transfusion could be a major route of acquiring Hepatitis B infection in Dutse and Gumel General Hospitals. The results have lower prevalence when compared with that reported by [7] in Karamojong, Uganda. 26.7% but appear higher when compared with work done by [18], [12], and [1] [1.1%; 4.83% and 10%] respectively.

Probable explanation of the variations observed was attributed due to differences in socio-demographic characteristics (educational status, residence, occupational status, poverty, cultural reasons) and other associated risk factors. The finding showed a higher infection rate of 6% among blood donors within the age range of 31-40 years in Dutse and 5% within 21-30 years in Gumel. People at these age groups are at high-risk behaviors and intravenous drug used which make them more prone to Hepatitis B infection [18].

This was uncorroborated with an earlier report by [18] who reported that the highest age specific to HBsAg positivity rate occurs mostly among 18-27 years age group (4.2%) in Niger Delta, Nigeria, and the work of [7] whose report indicates that 40% of youth in Uganda, mostly by the age of 15-19 years, have indicated the sign and symptom of the infection and adults are more vulnerable to the infection.

This shows that factors influencing Hepatitis B virus infection differ from one locality to another. Cultural reasons and socio-economic factors may be the predisposing factors as one locality may be more exposed at earlier age than other [7] [25]; [15-19 years (40%)] and [16-25years (6.0%)] respectively. The study showed prevalence of 10% HBsAg among commercial donors in Dutse and 7% in Gumel General Hospitals respectively. These figures are 5 and 10 folds higher when compared to family replacement donors 2% and 0.7% respectively see (Table 3).

This also corresponds with reported work by [18] in Niger Delta, Nigeria, who stated that there was higher HBsAg of 1.7% prevalence among commercial donors compared to family replacement donors with prevalence rate of (0.7%). This finding is in line with the work done by [4] who confirmed that commercial donors are more vulnerable to transmit transfusion-transmissible infection because most of the paid donors are more prone to the high risk behavior of the infection.

The result demonstrates that there is no significant difference between those who donate blood and the prevalence of the infection in relation to gender in two general hospitals ($p > 0.05$). The result shows similarity with the previous work of [26] who reported that there was no significant difference ($p > 0.05$) between gender and the occurrence of HBsAg among blood donors in Ekiti State, Nigeria.

It has been observed that no significant difference is observed between the hospitals understudy based on the prevalence of the infection at $p > 0.05$. This can be attributed to the fact that the cohorts in both Hospitals enjoy similar socio-demographic characteristics, hence displaying similar pattern of presentation.

This supports [1] who reported that there was no significant difference between the two study areas having common risk factor for HBsAg infection like history of injection, drug use, early sexual debut, and cultural practices. Based on the chi-square analysis, it has been found that there is positive association between the level of education and exposure to surface antigen in both district Hospitals. The result corroborated with [4] which states that socio-economic status among the

poor and less educated, especially those who reside in the rural community, may contribute to the HBV exposure. The result agrees with the work of [27] who reported that those with little or no education had higher percentage prevalence than the more educated ones. The result shows that there is no significant difference ($p > 0.05$) between the HBsAg sero-positive blood donors attending Dutse and Gumel General Hospitals because the socio-economic, independent, and demographic variables among the two districts are almost identical.

Among the subjects, the result showed that there was significant difference between HBsAg and occupational status among subjects in both districts ($p < 0.05$). This is because most of the infected persons are peasant farmers who live with HBV risk factors compared to other civil servants who were more educated about the possible route of HBV transmission.

This was consistent with who concluded that the occupational status of the people who are predominantly farmers has contributed to the high rate of HBV infection and could lead to epidemic in the society through association with person having sex with infected person, needles, syringes, and other hypodermics instruments. This observation corresponds with previous report of [28] who reported that blood transfusion and consumption of alcohol were significantly associated with the infection. The finding concurs with recommendations made by [29] who reported that those with infection are recommended to avoid taking alcohol so as to reduce the resurgence of the infection. The findings also discovered that there is no significant difference between vaccination of the infection and occurrence of Hepatitis B Virus infection ($p > 0.05$) among blood donors in both district hospitals, may be because the incorporation of Hepatitis B Virus vaccine in National programme is not more than 34 years hence, those interviewed had already escaped the immunization.

CONCLUSION

Hepatitis B virus infection is endemic in Dutse and Gumel Districts of Jigawa State with infection rates of (13%) & (9.8%) respectively. Similarly, the work showed no variation existing between the Southern and Northern regions of the state due to common demographic and socio-economic variables. The finding also explored that the infection rate of Hepatitis B infection was high in farmers, non-vaccinated individuals, and those with history of Alcohol consumption. Additionally, commercial donors constitute high rate when compared to family replacement donors in both districts which the present study focused on.

Apart from the above, the study also unfolded that there is prevalence rate of Hepatitis B infection among individuals not formally educated people compared with the uneducated ones across the two districts under study. Against the background above, one can conclude that the level of education has a serious overbearing on the transmission of Hepatitis B infection in Jigawa state, Nigeria.

Recommendations

Based on the current findings, the following recommendations are offered:

- The research recommends that a routine immunization programme be sustained as the best method for controlling infection in the study areas.
- Public information programmes should be strengthened to help in creating awareness of the routes of Hepatitis B infection in the state.
- There is also the need for sensitization campaigns on the prevention of sexual transmission. This if properly mounted would help to reduce the incidence of infection in Jigawa state.
- Effort made by the Federal Government of establishing the National Blood Transfusion services fueled by voluntary non-remunerated, low risk blood donors to address the acute shortage and safety of blood should be encouraged and should be sustained with all the logistics required by the centers.

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— This article does not have any appendix. —