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Detection of Hepatitis B and C Virus by Strip Test and ELISA Method among Patients Attending Some Hospitals in Kaduna State

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ABSTRACT

Background: Hepatitis is an important cause of chronic liver disease despite the introduction of effective screening and treatment policy.

Objectives: This study was aimed at detecting Hepatitis B and C Virus toward comparing the sensitivity of Strip test and Enzyme Linked Immunosorbent Assay (ELISA) methods among patients attending some Hospitals in Kaduna State.

Methods: A descriptive cross sectional research design was adopted where blood samples from 240 respondents were collected within the three Senatorial Zone and screened for Hepatitis B (HBV) and Hepatitis C (HCV) positive by Strip test and ELISA methods. The obtained data were analyzed using the IBM-SPSS Version 24.00 Software. The Statistical Tests performed were Chi-square (x2) with significant difference at the threshold $p \leq 0.05$.

Results: Out of the total 240 samples collected, 48 (20.0%) and 20 (8.3%) were positive for HBV and HCV virus using Strip test, while 59 (24.6%) and 30 (12.5%) were positive for HBV and HCV using ELISA. The Hepatitis B and C virus co-infection was found to be 5 (2.08%) on both methods.

Conclusion: There was a high prevalence of HBV and HCV viral infection among the study population occurring either as single infections. Furthermore, ELISA test was more sensitive compared with rapid strip test; thus, rapid diagnosis is not recommended for testing HBV and HCV infection.

Keywords: Detection, HBV, HCV, Coinfection, Strip Test and ELISA.

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INTRODUCTION

Hepatitis B virus (HBV) is the causative agent of the infectious disease known as hepatitis B which affects the liver of an infected person (1). The virus has the ability to cause both acute and chronic infections (2) and is of serious global public health problem with approximately more than one billion people worldwide living with it (3). The HBV is a member of the hepadna virus family (4) having the virus particle (virion) consisting of an outer lipid envelop and an icosahedral nucleocapsid core composed of protein. These virions are of 30 -42nm in diameter with a viral DNA enclosed by the nucleopsid and DNA polymerase that has reverse transcripts activity. The outer envelope of the HBV contains embedded proteins that are involved in viral binding and entry into susceptible cells. The virus is one of the smallest enveloped animal viruses, and the 42nm virions which are capable of infecting liver cells known as hepatocytes are referred to as "Dane paticles". These particles are not infectious and are composed of lipid and protein that forms part of the surface of the virion, which is called the surface antigens (HBsAg) and is produced during the life cycle of the virus.

Hepatitis C virus (HCV) belongs to the Genus Hepacivirus, family Flaviviridae and is a positive stranded RNA virus (5). Consequently, HCV are RNA virus do replicate in the cytoplasm of the hepatocytes (6). The HBV and HCV have common rout of transmission such as blood transfusion, re-used needle for injection, injection drug users, sharing of barbing clipper, tattooing, unsterilized dental reuse of needle for mouth, ear and nose piercing, undertaking health procedures which requires invasive technique and having more than one sexual partner (7,8). Co-infection of HBV and HCV is more complex than mono infection with either HBV or HCV (6). HBV/HCV co-infection account for severe liver disease, the host's immunity plays a vital role in the coordination of each viral replication and viral interference which usually leads to predominance of one of the two virus (9). However, the actual prevalence of HBV/HCV co-infection is still unknown but some studies done have estimated the prevalence to be between 0.7% to 16% and these ranges over a wide interval in difference studies which depends on the geographical region and study population (9). Furthermore, the co-infection of HBV and HCV may occur superficially, that is one virus is acquired in an individual within pre-existence of chronic infection by the other virus (10). The most common medium of developing co-infection is by super infection which occur mostly in HCV super infection but rarely HBV super infection (10). Thus, treatment of these viruses may lead to complete disruption of their delicate coexistence; the treatment-induced eradication of each virus could result to reactivation of the other. The screening of HBV and HCV call for testing the serum or plasma specimens by ELISA tests which are generally costly as well as the chemicals and instruments are concerned. However, in the locality, rapid one step strip test devise (commercial diagnostic kits) has been used continuously due to cost (inexpensive). Now and then, the outcomes are deranged from those obtained using the rapid test kit. Such observations raised the need for quality control evaluation to rationalize uses of rapid test kits to satisfy the reliable benchmark of highly specific, sensitive and reproducible tests. In view of these, this research is sought to detect HBV and HCV toward comparing the sensitivity of both strip test and ELISA methods among patients attending some Hospitals in Kaduna State, Nigeria.

MATERIAL AND METHODS

Study Design: This study adopted a descriptive cross-sectional design to detect HBV and HCV using strip test and ELISA method among patients attending some Hospitals in Kaduna State, Nigeria.

Study Area

This research work covered some selected hospitals within the three geopolitical zones in Kaduna State: Sir Patrick Ibrahim

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Yakowa Memorial Hospital, Kafanchan, Yusuf Dantsoho Memorial Hospital, Tudun Wada and Hajiya Gambo Sawaba Hospital, Zaria. Kaduna state of Nigeria known as "the liberal state." Located in the north west of Nigeria and the capital city of the state is Kaduna. Kaduna state has a land mass of 44,567 square kilometers, and lies between latitude 100 20N and longitude 7045E. As at the last census of 2006, the estimated population is 6,113,503. The name Kaduna was derived from the word "Kada" a Hausa word meaning crocodile and was created in the year 1967 on the 27th may. The state has 23 local government areas (LGAS) till date, divided into three geo-political zones and senatorial districts which are; Southern Zone, Central Zone and Northern Zone (11). Kaduna state has one tertiary hospital, 3 secondary facilities and 255 primary health care facilities. In this study one secondary facility was used from each geopolitical zones namely: Sir Patrick Yakowa Memorial Hospital from Southern zone, Yusuf Dantsoho Memorial Hospital from Central zone and Hajiya Gambo Sawaba from the Northern zone.

Ethical Approval

This was obtained from. This study was carried out with the approval of the Health Research Ethics Committee of Kaduna State Ministry of Health Kaduna, Nigeria. The approval number was MOH/ADM/744/VOL.1/1043.

Inclusion and Exclusion Criteria

Consenting patients with Hepatitis test request were recruited in this study while the exclusion criterion were the non-consenting patients, those without hepatitis test request and children from 10 years and below. This is because most of these children have been immunized against Hepatitis B virus at birth.

Sample Size

The sample size was calculated based on previous prevalence of 5% co-infection reported by (12) among HIV patients in Keffi, Nasarawa State, using Cochran's formula.

$$n = \frac{z^2 pq}{d^2}$$

Where:

n = sample size, z = standard normal distribution at 95% confidence limit = 1.96, p = prevalence rate = 0.05%, q = 1 – p and d = allowable error = 0.05

$$n = \frac{(1.96)^2 x\ 0.05\ x\ (1-0.05)}{(0.05)^2} = 3\frac{8416\ x\ 0.05\ x\ 0.95}{0.0025} = 72.9904$$

Sum of sample size = 73

73 is the calculated sample size but 240 blood samples were collected to curtail dropout rate i.e. 80 blood samples from each hospital.

Sampling Techniques

The study employed multistage sampling technique. All the Hospitals in three (3) senatorial zones of Kaduna State were first clustered and three (3) Hospitals were selected through a balloting system. Structured questionnaire was administered to selected number of participants from the Hospitals to assess the status of Hepatitis in the area. The questionnaire provides information about the life history, behaviors and practice of the individual.

Sample Collection and Transportation

Five milliliters (5mL) of blood sample were collected aseptically via Vene puncture into a plain container from each consenting patient, immediately transported to Kaduna State University (KASU) Department of Microbiology Laboratory and processed.

Sample Processing

With a centrifuge (Centurion Scientific; PRO-Hospital.GP6; 3218721-322; U), the samples were spun at 1000 rpm for 5min. The serum was separated and divided into two equal volumes. One aliquot was for RDT (LabACON; HaugzhouBiotest-Biotech CO., Ltd. Lot:RP5110211) for HBsAg and (LabACON; HaugzhouBiotest-Biotech CO., Ltd. Lot No: HCV2003002) for HCsAg and the other aliquot for ELISA test (Highton HBs-Ag ELISA; Qingdao Hightop Biotech CO., Ltd; Lot:E0620622) and (RecombiLISA HcvIgGELISA; CTKBiotech. Inc.; CA92064, LISA; Lot:E1502162) with a sensitivity of 97.5% and a specificity of 95% for HBsAg and 95% and 95% for HCs Ag. The sera were stored in the Department refrigerator at -20oC and later used for analysis. The samples were packaged in an ice packed cooler at a temperature of 4oC and were immediately transported for further analysis.

Sample Analysis

Rapid Diagnostic Test (RDT)

Principles of HBV Test: One step HBS Ag test strip is a qualitative lateral flow immunoassay for detection of HBSAg in plasma or serum. The membrane is pre-coated with anti-HBS Ag antibodies on the test line region of the test strip. During testing, the Serum or plasma specimen reacts with the partied coated with anti-HBS Ag antibody. The mixture then migrates upwards on the membrane chromatographically by capillary action to react with anti-HBSAg antibodies on the membrane and generate a coloured line. The presence of this coloured line in the test region indicates a positive result. To serve as a procedural control, a coloured line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred (13).

Principle of HCV Test

HCV one step test device is also a qualitative membrane-based immunoassay for the detection of antibody to HCV in serum or plasma. The membrane is coated with protein A on the test line region of the device. During testing, the serum or plasma specimen react with the recombinant HCV antigen coated particles. The mixture migrates upward on the membrane by capillary action to react with protein A on the membrane and generate a coloured line. Presence of this coloured line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred (14).

HBS Ag and Anti-HCV Test Procedure

After collecting the blood sample serum was separated immediately to avoid haemolysis. The test device was removed from the foil pouch and used immediately within an hour. The test device was placed on a clean and level surface. With a pipette, 5µL of the serum was transferred into the test device wells. Two (2) full drops of buffer (approximately 80µL) were added. The result was read within 15 minutes using an accurate timer. Precautionary measures were observed throughout the procedure and standard procedure for proper waste disposal was followed. Trapping of air bubbles in the specimen well(s) was avoided. No result was interrupted after 30minutes. The test was carried out at room temperature of 37℃ to avoid adverse effect of humidity and temperature on the results.

Data Analysis

The collected data were analyzed using Statistical Package for

social sciences (SPSS) version 24.00. The responses of the respondents were presented with frequency counts and percentages. The Chi-square (χ^2) statistics were used to examine significant association on the prevalence and coinfection of HBV and HCV and P value ≤ 0.05 was considered significant.

RESULTS

The prevalence of HBV, HCV and HBV-HCV co-infection revealing out of 240 samples tested for hepatitis B via the test strip method, only 48 were positive; that corresponded to a 20.0% prevalence of HBV. The ELISA test returned a higher prevalence of HBV with a value of 24.6% as 59 of the 240 samples returned positive of ELISA. The strip test was 81.4% sensitive and 26.5% specific for HBV antigen when compared to the ELISA detection method. Twenty (20) of the 240 samples tested positive for HCV when the test strip was used giving a prevalence of 8.33%. Using ELISA, HCV prevalence of 12.5% was recorded as 30 out of the 240 sampled tested using the ELISA technique were positive for the antigen. There was also no significant difference ($p > 0.05$) between the prevalence of HCV recorded using the test strip or the ELISA technique. Relative to the ELISA method, the test strip was 66.7% sensitive, and 9.52% specific for, HCV antigen. The co-infection of HBV and HCV was detected in 5 out of the 240 samples giving a prevalence of 2.08% co-infection with the two viruses; prevalence recorded in test strip and ELISA was 2.08%; the sensitivity recorded was 100% for HBC-HCV co-infection when the test strip was compared with ELISA technique (Table 1).

Table 1: Prevalence of HBV, HCV and HBV-HCV Co-Infection

	No. Examined	No. Positive (%)		Sensitivity (%)	Specificity (%)
		Test strip	ELISA		
HBV only	240	48 (20.0)	59 (24.6)	81.4	26.5
HCV only	240	20 (8.33)	30 (12.5)	66.7	9.52
HBV - HCV co -infection	240	5 (2.08)	5 (2.08)	100.0	2.13

The prevalence of HBV, HCV and HBV and HCV co-infection in the three senatorial zones of Kaduna State using the Test strip method was significantly higher ($p < 0.05$) for HBV than those of either HCV or HBV-HCV co-infection with prevalence of 21.3, 5.0 and 2.5%, respectively (Table 2). Similarly, the incidences of HBV recorded at Kaduna North was significantly higher ($p < 0.05$) than those recorded for HCV with prevalence values of 11.3 and 1.3%, respectively; not case of HBV-HCV co-infection was recorded with Kaduna North senatorial zone during the study. Cases of HBV infection recorded with Kaduna South senatorial zone were also higher ($p < 0.05$) than those of HCV or HBV-HCV co-infections. The respective prevalence of HBV, HCV, and HBV-HCV co-infection recorded within Kaduna South senatorial zone was 27.5, 18.8, and 3.8%. The highest prevalence of HBV was recorded at Kaduna South with a value of 27.5%, followed by that recorded at Kaduna Central senatorial zone with a prevalence of 21.3%; while the least prevalence of HBV was recorded at Kaduna North senatorial zone with a prevalence of 11.3%. The difference in HBV prevalence between the three senatorial zones was statistically significant ($p < 0.05$). There was significant difference ($p < 0.05$) in the prevalence of HCV between the three senatorial zones with the highest prevalence recorded at Kaduna South senatorial zone with a value of 8.33%, followed by Kaduna Central with a prevalence of 5.0%; the least occurrence of HCV was detected at Kaduna North with a prevalence of 1.3%. Cases of HBV and HCV co-infection were detected at Kaduna central and Kaduna South senatorial zone, while none was detected at Kaduna North. The prevalence of co-infection with HBV and HCV was higher at the Southern senatorial zone than at the Northern zone with prevalence values of 3.8 and 2.5%, respectively (Table 2).

Table 2: Prevalence of HBV, HCV and HBV and HCV Co-Infection in the Three Senatorial Zones of Kaduna State using the Test Strip Method

Locations	No. Examined	No. of Test Strip Positive (%)		
		HBV	HCV	HBV -HCV
Kaduna Central	80	17 (21.3)	4 (5.0)	2 (2.5)
Kaduna North	80	9 (11.3)	1 (1.3)	0 (0.0)
Kaduna South	80	22 (27.5)	15 (18.8)	3 (3.8)
Total	240	48 (20.0)	20 (8.33)	5 (2.08)

HBV: $\chi^2_{(2)} = 5.863$; $p = 0.053$; **HCV:** $\chi^2_{(2)} = 19.726$; $p = 0.000$; **HBV-HCV:** $\chi^2_{(2)} = 4.103$; $p = 0.129$

The prevalence of HBV, HCV and HBV/HCV co-infection in the three senatorial zones of Kaduna State using the ELISA method show the significantly difference of either HBV, HCV or HBV/HCV co-infection with prevalence values of 12.5, 1.3 and 0.00% was recorded at Kaduna North respectively, no case of HBV-HCV co-infection was recorded with Kaduna North senatorial zone during the study. Cases of HBV infection recorded with Kaduna South senatorial zone were also higher ($p < 0.05$) than those of HCV or HBV-HCV co-infections. The respective prevalence of HBV, HCV, and HBV-HCV co-infection recorded within Kaduna South senatorial zone was 38.8, 27.5, and 3.75%. The highest prevalence of HBV was recorded at Kaduna South with a value of 38.8%, followed by that recorded at Kaduna Central senatorial zone with a prevalence of 22.5%; while the least prevalence of HBV was recorded at Kaduna North senatorial zone with a prevalence of 12.5%. The difference in HBV prevalence between the three senatorial zones was statistically significant ($p < 0.05$). There was significant difference ($p < 0.05$) in the prevalence of HCV between the three senatorial zones with the highest prevalence recorded at Kaduna South senatorial zone with a value of 27.5%, followed by Kaduna Central with a prevalence of 8.80%; the least occurrence of HCV was detected at Kaduna North with a prevalence of 1.3%. Cases of HBV and HCV co-infection were detected at Kaduna central and Kaduna South senatorial zone, while none was detected at Kaduna North. The prevalence of co-infection with HBV and HCV was higher at the Southern senatorial zone than at the central zone with prevalence values of 3.75 and 2.5%, respectively (Table 3).

Table 3: Prevalence of HBV, HCV and HBV and HCV Co-Infection in the Three Senatorial Zones of Kaduna State using the ELISA Method

Locations	No. Examined	No. of ELISA Positive (%)		
		HBV	HCV	HBV -HCV
Kaduna North	80	10 (12.5)	1 (1.3)	0 (0.0)
Kaduna Central	80	18 (22.5)	7 (8.80)	2 (2.5)
Kaduna South	80	31 (38.8)	22 (27.5)	3 (3.75)
Total	240	59 (24.6)	30 (12.5)	5 (2.08)

HBV: $\chi^2_{(2)} = 15.147$; $p = 0.001$, **HCV:** $\chi^2_{(2)} = 26.517$; $p < 0.001$, **HBV-HCV:** $\chi^2_{(2)} = 2.860$; $p = 0.239$

DISCUSSION

The prevalence of HBV (20%) recorded among the study population was well within the reported national prevalence range for HBV of 2 to 25%. However, it is higher than the global average prevalence of 3.9% (15). The high prevalence of HBV among the study population, relative to the global average, indicated that HBV is still endemic in the survey area. The present result for HBV was higher than those of (16), who in retrospective case-control study reported a prevalence of 8.83% among women attending a tertiary hospital in Ibadan. This result was also higher

than that of Mustapha et al. (2020) who recorded an overall HBV prevalence of 6.7% in their study among antenatal care attendees in Gamawa Local Government Area of Bauchi State, Nigeria (17). Magaji et al. (2020) had also reported a lower prevalence (7.4%) of HBV among attendee of a tertiary hospital in Jos, Plateau State, Nigeria (18). In a study conducted in the Niger-Delta region of Nigeria, Umego et al. (2020) observed HBV prevalence of 4.6% (19). Sobajo et al. (2023) in a survey of hepatitis virus among hospital attendees in two South-western states in Nigeria reported a prevalence of HBV infection 12.4% (20). Among a sample of pregnant women in rural community in Southern Nigeria, Orluwene et al. (2021) reported a prevalence of 8.1% for HBV (15). A much higher prevalence (41.6%) of HBV infection was reported by (21). The variations in prevalence of HBV recorded in this study compared to other authors may be connected with the type of diagnostic kits or tests adopted as variations exist in the sensitivity and specificity of the kits or test methods. Other factors that influence the occurrence of HBV include cultural habits which facilitate horizontal transmission of the virus such as unprotected sex, night gatherings, sharing of sharp objects (needles and razor blades), alcohol intake, drinking from same cup, tribal marks, or the practice of circumcision (22). Prevalence of HBV is also affected by geographical region (23). Orluwene et al. (2021) added that low immunization against the virus, poor health awareness and education, and government's low investment on prevention of the disease contribute to the high prevalence of HBV recorded (15).

The prevalence of HCV (8.33%) recorded in this study was higher than those of Adesina et al. (2016) who recorded HCV prevalence of 1.65% in a study conducted at the University College Hospital, Ibadan, Nigeria (16). Nnakenyi et al. (2020) also reported a lower prevalence of HCV (4.7%) in a study conducted among HIV patients at a tertiary health institution in South East Nigeria (24). The present result was also higher than those who reported HCV prevalence of 0.5% among HIV-infected children among students of University of Maiduguri in Northern Nigeria (25). The present result for HCV prevalence was, however, within the national average (7 to 15%) derived in a comprehensive systematic analysis (22). The prevalence of mono-infection with HBV or HCV varied significantly between the senatorial zones. HBV infections were detected in all three senatorial zones of the state with the highest prevalence recorded in the Southern zone (27.5%) and the least in the Northern zone (11.3%). On the other hand, no case of HCV was detected in the northern zone of the state. Similarly, the highest prevalence of HCV mono-infection was recorded in the Southern zone (8.33%) while the least was recorded in the North (1.3%). These differences could be explained, partly, by some of the risk factors associated with each of the zones. The responses from the questionnaires indicated that while the southern and also the central zones scored higher on awareness and knowledge of hepatitis and its mode of transmission (compared to the northern zone), the northern zone recorded least percentage of individuals who consume alcohol consumption or had any sexual experience; these two factors have been strongly linked with the prevalence of viral hepatitis. Alcoholism predisposes individuals to hepatitis infection, while sexual intercourse provides a route of horizontal transmission of the virus.

The investigation of coinfection prevalence of HBV and HCV according to Khan et al. (2014) is crucial because it may accelerate the course of the liver damage, increase the risk of hepatocellular carcinoma, and affect therapeutic response to antiviral molecules (26). The present results indicated a prevalence of 2.08% for HBV-HCV coinfection, a finding which was higher than the reports by Adesina et al. (2016) (0.19%) and Omosigho et al. (2022) (11.0%) (16, 27). In a study carried out in Bauchi State, Nigeria, Alkali et al. (2018) recorded zero prevalence of HBV and HCV coinfection despite recording cases of single infection with either HBV or HCV (28). Adesina et al. (2016), working at Ibadan, South-West Nigeria identified 0.19% cases of coinfection with HBV and HCV (16). Nnakenyi et al. (2020) examined the prevalence of hepatitis viral antigen among HIV patients at a tertiary health institution in South East Nigeria, and recorded a 0.58% prevalence of HBV and HCV coinfection (24). According to Desikan et al. (2017), prevalence of HBV and HCV coinfection is high in patients with chronic liver disease, HIV positive individuals, persons with kidney disease, and among person who inject drugs (10). It was noted that socio-demographic characteristics such as the age, level of education, and marital status, may account for the different prevalence of HBV and HCV coinfection (27). The relative prevalence of HBV and HCV coinfection across the three senatorial zone of the state indicated that co-infection with the viruses were detected only in the South and Central zone, as no case of coinfection was detected in samples collected from the Northern zone. The HBV and HCV coinfections are usually found in regions where the viruses are endemic, or in areas where there are increased risks of parenteral transmission (blood transfusion, intravenous drug use, indiscriminate and unprotected sexual activities (29). The present result alluded to the endemicity of the viral infections in both the southern and central zones. These zones of the state recorded higher prevalence of HBV and HCV mono-infections; the northern zone recorded the least cases of mono-infection with HBV, without any detected case of HCV infection. Endemicity of hepatitis viral infection could facilitate superinfection or co-infection. In superinfection, an individual harboring a chronic infection with either HBV or HCV virus becomes infected with the second virus, while in a strictly co-infection case, the individual is simultaneously infected by both viruses (30). It is also reported that hepatitis viral coinfections are more complex than mono-infections, and usually has implications for disease severity, clinical manifestations and management; higher rates of liver cirrhosis and increased severity of liver disease have been reported with coinfection of HCV and HBV compared to mono-infections with either virus. Furthermore, coinfection with HBV and HCV has been associated with increased risk hepatocellular carcinoma (31).

CONCLUSION

It is concluded that there was a high prevalence of HBV and HCV infection in Kaduna State, Nigeria, occurring either as single infections or co-infections. When cases of single infections were considered separately, the prevalence of HBV was higher than that of HCV. Upon comparing the two methods, ELISA test was more sensitive than one step rapid test strip device for assessing the presence of HBsAg and anti-HCV among patients attending some Hospitals in Kaduna State. Consequently, it is strongly recommended that, ELISA method be used to confirm test results obtained from the one step rapid test strip, when screened for chronic liver disease (CLD).

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