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INCIDENCES OF HEPATITIS B SURFACE ANTIGEN AND HEPATITIS C ANTIBODIES IN DIABETICS PATIENT AT OOUTH

Owolabi, S. L.
Microbiology unit, Department of Science Laboratory Technology, Gateway Polytechnic (ICT), Saapade Ogun State, Nigeria.

ABSTRACT: *In recent time, controversy have been stirred up over the aetiology of liver failure in diabetic patients. Hence, this study was carried out to determine the incidence of Hapatitis B surface antigen and Hapatitis C antibodies in diabetic patients at OOUTH. 5ml venous blood samples was collected from the diabetic patients, centrifuged at 1500 rpm for 5minutes and screened for HBV and HCV using ELISA and Diaspot Kits respectively. Out of the 230 diabetic patients screened for Hepatitis Virus Infection, 49 (19.6%) of them were positive while 185 (80.4%) were negative. HBV infection was highest in frequency by 43 (95.6%), followed by HBV and HCV co-infection, with a frequency of 2 (4.4%). Gender and ages of diabetic patients were observed not to have significant relationship with the incidence of viral hepatitis infection (P>0.05). Incidence of viral hepatitis was significantly higher in uneducated and widowed diabetic patients (P<0.05). Higher incidence of viral hepatitis infection was found to be significantly associated with tattooing (P<0.05) while no significant association was observed between same infection and each of alcoholism, blood transfusion, previous surgery and number of sex partners (P>0.05). When the diagnostic test result of diaspot was compared with ELISA, sensitivity of 11.1% and 82.2% were respectively observed (P<0.05).In conclusion, this study has established that the incidence of viral hepatitis was higher among diabetic patients with no formal education, those who are widows, and tattoo. It was further discovered that diaspot is less sensitive compared to ELISA.*

KEYWORDS: HCV, HBV, ELISA, Aetiology,Diabetes ,Liver ,Hepatitis.

INTRODUCTION

Hepatitis is an inflammation of the liver characterized by the presence of inflammatory cells in the tissue of the organ. It may occur with limited or no symptoms, but often leads to jaundice, anorexia (poor appetite) and malaise. Hepatitis is acute when it lasts less than six months and chronic when it persist longer (Nkrumah, Owusu, Frempong and Averu, 2011). A group of viruses known as the hepatitis viruses cause most cases of hepatitis worldwide, but it can also be due to toxins (notably alcohol, certain medications and plants), other infections and autoimmune diseases (Wild, Roglic, Green, Sicree and King, 2004). The hepatitis virus is found in the blood and other body fluids and is transmitted from person to person .The most mode of infection occurs via transfusion of blood and blood product where there is no screening for blood-borne viruses, medical or dental interventions in countries where equipment is not adequately sterilized, mother to infant during childbirth, sexual transmission (in the case of hepatitis B), sharing equipment for injecting drugs, sharing straws, for snorting cocaine, sharing razors, toothbrushes or other sharp household articles, tattooing and body piercing if done using unsterile equipment. (Allison, Wreghitt, Palmer and Alexander, 1994).

The Hepatitis B virus is spread between people through contact with the blood or other body fluids (i.e. semen, vaginal fluid and saliva) of an infected person, while the Hepatitis C virus is spread through direct contact with infected blood. Very rarely, it can also be passed on through other body fluids. Many people

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infected with Hepatitis B or C rarely displays any symptom, although they can still transmit the virus to others (Naing, Mak, Ahmed and Maung, 2012).

Hepatitis B is a major disease of serious global public health proportion. Of the 2 billion people who have been infected with the hepatitis B virus (HBV) globally, more than 350 million have chronic infections. (Jadoon, Shahzad, Yaqoob, Hussain and Ali, 2010). Over 20 million people are infected annually with this virus (Balogun, Adeleye, Akinlade, Kuti and Otegbayo, 2006).

Hepatitis C is a viral infection of the liver and is the most common blood-borne infection acquired by direct contact with human blood. The major sources of HCV infection worldwide are (use of unscreened blood for transfusions and re-use of needles and syringes that have not been adequately sterilized. The world health organization (WHO) estimates that about 3% of the world populations (200 million people) have so far been infected with the Hepatitis C virus (Chen, Li, Chen, See, Lee, 2006).

Almost 50 Variable frequencies of hepatitis B and C infections, have been reported throughout the world but higher in developing countries such as Nigeria where it is hyper endemic. The prevalence has been reported in Jos, in Port Harcourt, Maiduguri and Lagos in Nigeria. Hepatitis B and C co-infection and diabetes mellitus are two major public health problems that causes devastating health and financial burdens worldwide. Infections due to hepatitis B and C viruses are of public health significance around the globe. Worldwide viral hepatitis is the commonest cause of hepatic dysfunction, morbidity and mortality. Detection of antibodies to various hepatitis viral antigens indicates infection. And in most cases, it portrays a chronic infection.

MATERIALS AND METHODS

Study Area
This study was conducted at Olabisi Onabanjo University Teaching Hospital, (OOUTH) Sagamu, Ogun State, Nigeria. Frequency of hepatitis positivity in diabetic patients was compared among the various risk factors using Chi (χ^2). The level of significance was determined at 95% (i.e. $\alpha = 0.05$).

Study Population
The study population was all diabetic patients attending DAME at Olabisi Onabanjo University Teaching Hospital, Sagamu, and Ogun State.

Sample size
Total population technique was used, the total number of patients attending Lady caroline Adebutu Diabetes clinic, between October 2014 and may 2015 was 230 patients.

Sampling Technique.
All patients were recruited by purposive sampling technique, in which only Diabetic patients attending OOUTH were consecutively enrolled. Demographic data of the patients were obtained by questionnaires.

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Specimen Collection and Analysis.
Serological testing for HBV and HCV was performed at the medical microbiology Laboratory, Olabisi Onabanjo University,Ago Iwoye

Detection of HBsAg in Patient's Serum
Hepatitis B surface antigen (HBsAg) was detected in patients' serum using diaspot (blumbery 1971). The methodology was strictly performed according to the manufacturers manual instruction, the HBsAg was performed using serum, the pouch was brought to room temperature before opening, with the arrows on the strip pointing towards the serum, the strip was immersed vertically in the serum for at least 10-15secs, the test strip was later placed on a non absorbent flat surface, the timer started while waiting for the red line(s) to appear. The result was read at 15 minutes.

3.5.3 Procedure for HBsAg ELISA
Reagents were allowed to reach room temperature (18-30^{o}c), the wash buffer was diluted (20x) as indicated in the manual:
Step 1: Three wells marked as negative control, two wells as positive control, and one blank.
Step 2: 50micro litre of positive control, negative control, and specimen in to their respective wells except the blank. Separate disposal pipette tip was used for each specimen negative control, positive control, to avoid cross-contamination. It was mixed by tapping the plate gently.
Step 3: 50 micro litre of HRP-conjugate was added in to each well except the blank, and mixed by tapping the plate gently.
Step 4: The plate was covered with the plate cover and incubated for 60 minutes at 37c
Step 5: The plate was removed from the incubator and the cover was discarded, each well was washed 5 times with diluted washing buffer, the micro wells were soaked for 30-60seconds at each time. After the final washing cycle, the plate was turned down on to blotting paper or clean towel and tapped to remove any remainders.
Step 6: 50microlitre of chromogen A and B solutions in to each well including the blank. The plate was incubated in a dark room for 15minutes at 37c
Step 7: 50 micro litre of stop solution was added in to each well via multichannel pipette and mixed gently

Step 8: The plate reader was calibrated with the blank well and the absorbance was read within 10minutes at 450nm.The cut –off value was calculated, and the result was evaluated.

Quality Control and Calculation of the Results.
The result was calculated by relating each specimen absorbance value to the cut-off value of the plate.

Assay of HCV in Patients Serum
Hepatitis c virus was detected in patient serum using diagnostics kit from Blumbery (1971)

Procedure for HCV
The manufacturers' instruction was strictly followed.
Test strip was dipped in to fresh serum specimen for 2-3 seconds with the arrow end pointing downward. Strip was later laid flat on a clean, dry, non absorbent surface. Result was read within 10-20 minutes.

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Procedure for HCV ELISA

All the kit reagents and samples were brought to room temperature ((18-30^{o}c), for 30 minutes and mixed carefully before the assay.

Three wells were marked as negative control, two wells were marked as positive control, and one was reserved for blank. 50micro litre of positive control, negative control, and specimen were pipetted in to their respective wells except the blank. Separate disposal pipette tip was used to introduce specimen in to their respective wells, it was mixed by tapping the plate gently. 50 micro litre of HRP-conjugate was added in to each well except the blank, and mixed by tapping the plate gently. The plate was covered with the plate cover and incubated for 60 minutes at 37c

After incubation, each well was washed 5 times with diluted washing buffer, the micro wells were soaked for 30-60seconds at each time. After the final washing cycle, the plate was turned down on to blotting paper and tapped to remove any remainders. 50microlitre of chromogen A and B solutions were added in to each well including the blank. The plate was incubated in a dark room for 15minutes at 37c . 50 micro litre of stop solution was added in to each well via multichannel pipette and mixed gentle The plate reader was calibrated with the blank well and the absorbance was read within 10minutes at 450nm.The cut –off value was calculated, and the result was evaluated The result was calculated by relating each specimen absorbance value to the cut off value of the plate.

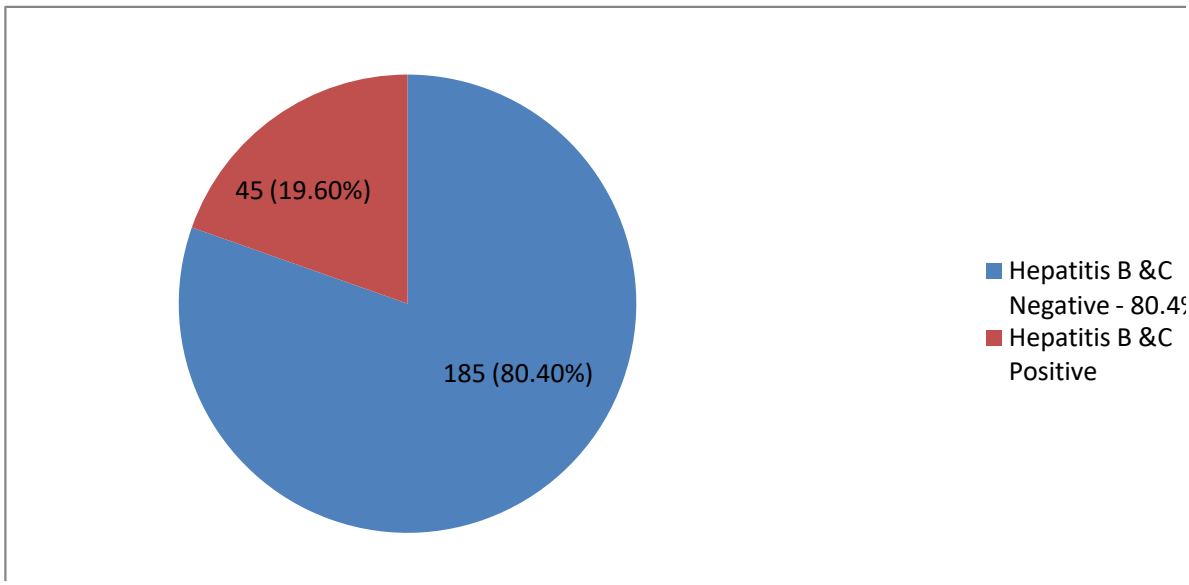

Figure 4.1:Frequency distribution of hepatitis B and C infections among diabetic patients attending OOUTH

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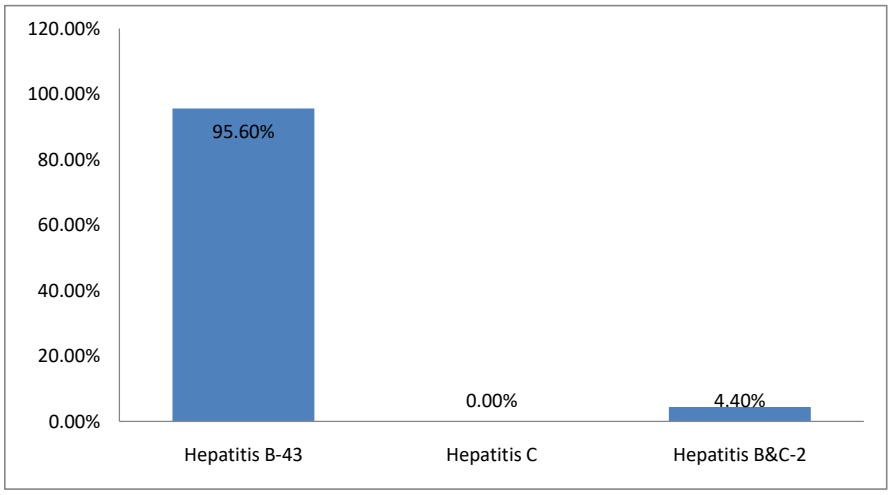

Figure 4.2: Frequency distribution of hepatitis B and C infections among the 45 hepatitis positive patients.

Table 4.1: Relationship between hepatitis infection and gender of diabetic patients

Subject	Gender				Total
	Males		Females		
	n	(%)	n	(%)	
Hepatitis Positive	9	(14.3)	36	(21.6)	45
Hepatitis Negative	54	(85.7)	131	(78.4)	185
Total	63	(100.0)	167	(100.0)	230

$\chi^2 = 1.54$, $P > 0.05$

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Table 4.2: Relationship between hepatitis infection and ages of diabetic patients

Subjects	Ages ≤ 40		41-50		51-60		≥ 61		Total
	n	(%)	n	(%)	n	(%)	n	(%)	
Hepatitis Positive	3	(10.3)	11	(17.2)	15	(24.6)	16	(21.1)	45
Hepatitis Negative	26	(89.7)	53	(82.8)	46	(75.4)	60	(78.9)	185
Total	29	(100.0)	64	(100.0)	61	(100.0)	76	(100.0)	230

$\chi^2 = 2.88$, $P > 0.05$Table 4.3: Relationship between hepatitis infection in diabetic patients and their levels of education.

Subjects	Levels of education. No formal		Primary		Secondary		Tertiary		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Hepatitis Positive	15	(46.9)	17	(20.2)	11	(12.8)	2	(7.1)	45	
Hepatitis Negative	17	(53.1)	67	(79.8)	75	(87.2)	26	(92.9)	185	
Total	32	(100.0)	84	(100.0)	86	(100.0)	28	(100.0)	230	

$\chi^2 = 20.44$, $P < 0.05$

Table 4.4: Relationship between hepatitis infection in diabetic patients and their marital status.

Subjects	Marital status Married		Single		Widowed		Total
	n	(%)	n	(%)	n	(%)	
Hepatitis Positive	43	(20.5)	0	(0)	2	(33.3)	45
Hepatitis Negative	167	(79.5)	14	(100.0)	4	(66.7)	185
Total	210	(100.0)	14	(100.0)	6	(100.0)	230

$\chi^2 = 4.24$, $P > 0.05$

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Table 4.5: Relationship between hepatitis infection in diabetic patients and their body tattooing.

Subject	Tatoo				Total
	Yes		No		
	n	(%)	n	(%)	
Hepatitis positive	21	(29.6)	24	(15.1)	45
Hepatitis negative	50	(70.4)	135	(84.9)	185
Total	71	(100.0)	159	(100.0)	230

$\chi^2 = 6.54$, $P < 0.05$

Table 4.6: Relationship between hepatitis infection in diabetic patients and alcoholism

Subject	Alcoholism				Total
	Yes		No		
	n	(%)	n	(%)	
Hepatitis positive	32	(23.2)	13	(14.1)	45
Hepatitis negative	106	(76.8)	79	(85.9)	185
Total	128	(100.0)	92	(100.0)	230

$\chi^2 = 2.88$, $P > 0.05$

Table 4.7: Relationship between hepatitis infection in diabetic patients and Blood transfusion.

Subject	Blood transfusion				Total
	Yes		No		
	n	(%)	n	(%)	
Hepatitis positive	42	(20.9)	3	(10.3)	45
Hepatitis negative	159	(79.1)	26	(89.7)	185
Total	201	(100.0)	29	(100.0)	230

$\chi^2 = 1.79$, $P > 0.05$

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Table 4.8: Relationship between hepatitis infection in diabetic patients and previous record of surgery.

Subject	Previous surgery				Total
	Yes		No		
	n	(%)	n	(%)	
Hepatitis positive	43	(19.7)	2	(16.7)	45
Hepatitis negative	175	(80.3)	10	(83.3)	185
Total	218	(100.0)	12	(100.0)	230

$\chi^2 = 0.07$, $P > 0.05$

Table 4.9: Relationship between hepatitis infection in diabetic patients and number of sexual partnerS

Subject	Number of sexual partner				Total
	Single		Multiple		
	n	(%)	n	(%)	
Hepatitis positive	40	(19.0)	5	(26.3)	45
Hepatitis negative	171	(81.0)	14	(73.7)	185
Total	211	(100.0)	19	(100.0)	230

$\chi^2 = 0.60$, $P > 0.05$

Table 4.10: Comparative assessment of sensitivity results between ELISA and Diaspot

Test kit	Sensitivity	Specificity
ELISA	5/5x100(100%)	185/225x100(82.2%)
DIASPOT(test)	5/45x100(11.1%)	185/185x100(100%)

$\chi^2_{MN} = 42.03$, $P < 0.05$

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DISCUSSION

Result from this study, showed significant relationship between hepatitis B and C infection and the level of education in diabetic patient s. This result strongly emphasized on the influence of education in the control of the infection because diabetic patients with no formal education(illiterate) have the highest rate of hepatitis infection (46.9%) while diabetic patients with tertiary education have the list case (7.1%) of hepatitis infection

A significant relationship between hepatitis Band C infection and tattooing in diabetic patients strongly suggests the implicative role of skin scarification with unsterilized devices in the incidences of hepatitis Band C infections in diabetic patients (29.6%)Higher incidence (20.9%patitis) of hepatitis infection was observed in diabetic patients with previous history of blood transfusion, than in diabetic patients without previous history of blood transfusion (10.3%) but the association was insignificant($P>0.05$).This observation demonstrated that blood transfusion may although be a co factor in promoting the incidences of hepatitis infection, but it is not a major factor because blood transfusion can only be a major source of hepatitis infections in subjects, if the administered blood was not screened.

Comparative assessment of sensitivity results between ELISA(Gold standard) and Diaspot (Test) in this study showed a significant difference, that Diaspot test kit, is less effective in detecting positive cases of hepatitis Band C than ELISA. Since the duration of hepatis B and C infections varies from one subject to another, couple with varying degree of antibody production, it will be difficult to conclude that the use of Diaspot should be condemned in hepatitis diagnosis, its relevance of use may how ever be restricted to a particular stage of hepatitis infection which could not be determined In this study

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