

Seroprevalence of Hepatitis B and C among Pregnant Women attending Antenatal Care at Maternal and Children's Hospital Malumfashi, Katsina State, Nigeria

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Abstract

The research was conducted to determine the prevalence of hepatitis B and C virus infections among pregnant women attending antenatal care at Maternal and Children's Hospital (MCH) Malumfashi to assess the possible risk factors associated with hepatitis B and hepatitis C virus infections. Two hundred (200) pregnant women were screened for HBV and HCV infection. Blood samples collected were processed to get plasma samples. The plasma obtained was screened using the rapid immunochromatographic test kit to determine the presence of HBsAg antigen and HCV antibodies. Out of the 200 samples screened, 23 samples were HBsAg positive accounting for a prevalence of 11.5%. The highest prevalence of 18.1% was observed in the age group 16-20 and the lowest was 8.7% among the age group 41-45. Based on trimester, the highest prevalence was 21% among those in the 2nd trimester, while those in the 1st trimester had the lowest prevalence of 8.8%. Vaccination was observed to be the risk factor associated with HBV infection. Also blood transfusion and sharing of sharp object were associated with the infection. Of the 200 blood samples screened, 12(6.0%) were positive for HCV antibodies. The highest prevalence (20%) was observed in the age group 31-35 followed by 5.3%, 4.0% and 3.6% in the age groups 26-30,16-20 and 21-25 respectively. No HCV antibodies positive cases were detected in the age group 36-40 and 41-45. The risk factors associated with HCV infection, those with history of blood transfusion, sharing of sharp objects and pregnant women involved in polygamous marriage. Increased awareness on the risk factors and the need for vaccination and thorough immunization of all new born babies against hepatitis B and C will reduce the spread of HBV infection.

Keywords: Hepatitis, Seroprevalence, Pregnant women, Hospital

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 (Ugbebor *et al.*, 2011). In Nigeria, there is a strong

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relationship between HBV infection and various forms of chronic liver disease [CLD], including chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (Owolabi and Ojo, 2008). Hepatitis B virus infection, a major public health problem world-wide, is more prevalent in the developing countries. More than 2 billion people are infected world-wide while some 280 million are chronic carriers, harboring the virus in their liver (Emechebe *et al.*, 2009). About 2 million of these carriers die each year as a result of cirrhosis or primary liver cell cancer induced by the virus (Owolabi and Ojo, 2008).

HCV is 80nm diameter in size, enveloped, positive sense single stranded RNA virus in the family Flaviviridae and genus hepacivirus (Prescott *et al.*, 2008). It is an infectious disease causing liver inflammation. The infection is often asymptomatic but once established, chronic infection can progress to scarring of the liver (Fibrosis) and advanced scarring (Cirrhosis) which is generally apparent after many years. In some cases those with cirrhosis will go on to develop liver failure or other complications of cirrhosis including cancer (Ryan and Ray, 2004). Nigeria is one of the countries with the highest incidence of hepatitis, with a prevalence of 10-15% across the country (Owolabi and Ojo, 2008). Prevalence of HBV in normal population ranges from 2.7 % to 13.3 % (Kulkarni *et al.*, 1986; Muula, 2000). Medical personnel, especially surgeons and dentists are at the greatest risk of infection, while other health workers, commercial sex workers, and drivers have also been found to be at significant risk of getting infected (Owolabi and Ojo, 2008).

Transmission of HBV occurs when blood or body fluid of an infected person comes in contact with a susceptible individual, especially through a break in the skin or the mucous membrane (Emechebe *et al.*, 2009). Viral hepatitis during pregnancy is associated with high risk of maternal complications and has become a leading cause of foetal death (Ugbebor *et al.*, 2011). A Survey conducted among pregnant women attending antenatal clinic at Ahmadu Bello University Teaching Hospital (ABUTH), Shika-Zaria, Kaduna State, Nigeria reported a prevalence of 8.3 % (Ibrahim *et al.*, 2008). A similar study at the Ahmadu Bello University Health Care Service (Sick bay) Samaru campus, Zaria, Nigeria reported a prevalence of 13.3% (Lawal., 2009). Several studies elsewhere among blood donors reported the following prevalence; 13.3% in Lagos and Bauchi (Nasidi *et al.*, 1983), 7.3 % in Ile-Ife (Durosunmi *et al.*, 1993), 22 % in Maiduguri (Harry *et al.*, 1994). This study seeks to find out the seroprevalence of hepatitis B and hepatitis C (HBV & HCV) viral infections among pregnant women attending antenatal care at a maternal and children hospital in Malumfashi town of Katsina State. Being a town with quick access to cities like Kano, Zaria, Funtua etc coupled with seeming ignorance regarding the modes of transmission of the different hepatitis, a study of this type becomes imperative to know their prevalence as a starting point to control interventions.

Materials and Methods

Study Area:

The study was carried out at the Department of Haematology, Maternal and Children's Hospital Malumfashi, Katsina State, which is located on *latitude* 100.27N and 100.30N and *longitude* 70.24E and 70.24E.

Ethical Approval:

Ethical approval was obtained from the Katsina State hospital services management board located at Shaikawa quarters near emir palace mosque Katsina, and informed consent was sought from the pregnant women.

Study Population:

The study population comprises two hundred (200) women who are pregnant and fall between the ages of 18-47, attending antenatal care at the maternal and child hospital (MCH) Malumfashi during a period of three month as indicated above. These subjects were also selected randomly.

Sample Collection:

Sample collection was carried out on working days (Monday to Friday) for a period of three months (July to September 2017). Blood sample were collected aseptically by venipuncture using 2ml sterile disposable syringe and needles. The samples were quickly transferred in to anticoagulant container and plasma was separated by centrifugation at 3000 rpm for 5 minutes. The plasma were extracted in to the screw cap tubes, and refrigerated at -2°C until used.

Screening for HBsAg and HCV Antibodies:

Each serum was screened for the presence of HBsAg by one step HBsAg test strips, which is a rapid chromatographic immunoassay for qualitative determination of HBsAg in the serum. All plasma samples and test strips were allowed to stand at ambient temperature (25°C) prior to use. The test strips were removed from the sealed pouch and used for the test, each plasma sample was screened by immersing the strips vertically in the plasma for 15 seconds. The maximum line on the strip was not exceeded when the strip was immersed in the plasma. The strips were then placed on a clean, flat, non-absorbent surface and allowed to stand for 15 minutes after which the result was read (Shahin *et al.*, 2009)

Result

A total of 200 pregnant women were examined and the prevalence of Hepatitis B antigen (HB Ag) in relation to age showed that 23 tested positive. The age groups 16 - 20, 21 - 25, 26 -30, 31 - 35, 36 - 40, 41 - 45 and 46 - 50 recorded prevalence of 18.1, 12.8, 14.3, 9.6, 9.7, 8.7, and 12.5 respectively (Table 1). Prevalence of HB Ag by trimester revealed that the first trimester had 8.8%, the second trimester had 21.4% and the third trimester 14% (Table 2).

On the other hand, 12 women were positive for Hepatitis C Virus (HCV) antibodies across the age groups. The result obtained from prevalence studies of Hepatitis C Virus antibodies in pregnant women in relation to age, showed that age groups 16-20, 21-25, 26-30, 31-35, 36-40, 41-45 and 46-50 recorded prevalence of 4.0, 7.1, 5.3, 20.0, 0.0, 0.0 and 0.0 respectively (Table 3). Based on trimesters of the subjects the prevalence of HCV antigen was 4.2%, 9.8% and 2.9% for the first, second and the third trimesters respectively (Table 4).

A total of 800 pregnant women were screened for risk factors, which included vaccination, blood transfusion, sharing of sharp objects and polygamous marriage. The pregnant women with vaccination history summed up to 84, whereas those without vaccination sum up to 116. Pregnant women with and without blood transfusion each were up to 68 and 132 respectively. The pregnant women with the history of sharing sharp objects summed up to 41, while those

without summed up to 159. Finally, pregnant women with polygamous marriage summed up to 92, whereas those without summed up to 108. The number of infected (positive) and prevalence of pregnant women with and without vaccination were 1(1.2) and 22(18.9) respectively. The number of infected (positive) and prevalence of pregnant women with and without blood transfusion were 5(7.4) and 18(13.6) respectively. The number of infected (positive) and prevalence of pregnant women with and without sharing sharp objects were 9(22.0) and 14(8.8) respectively. Finally, the number of infected (positive) and prevalence of pregnant women with and without polygamous marriage was 11(12.0) and 12(11.1) respectively (Table 5).

A total of 600 pregnant women were examined for prevalence of Hepatitis C Virus (HCV) in relation to risk factors, which included, blood transfusion, sharing of sharp objects and polygamous marriage. The number of pregnant women examined for HCV in relation to blood transfusion was 24 and number that tested positive was 4 with a prevalence of 16.7, and number examined without transfusion was 176 out of which 8 tested positive with prevalence of 4.6. The numbers of pregnant women examined for HCV in relation to sharing of sharp objects were 66 out of which 6 tested positive with prevalence of 9.1, whereas those without sharing sharp objects were 134 out of which 6 tested positive with a prevalence of 4.5. The number of pregnant women examined for HCV in relation to polygamous marriage were 108 out of which 6 tested positive with prevalence of 5.6, whereas those without polygamous marriage were 92 out of which 6 tested positive with a prevalence of 6.5 (Table 6).

Table 1: Prevalence of HBsAg in Pregnant Women in Relation to Age

Age	Range (years)	Number Examined	Number Positive (%)
16 - 20		11	2 (18.1)
21 - 25		47	6 (12.8)
26 - 30		28	4 (14.3)
31 - 35		52	5 (9.6)
36 - 40		31	3 (9.7)
41 - 45		23	2 (8.7)
46 - 50		8	1 (12.5)
Total		200	23 (11.5)

Table 2: Prevalence of HBsAg Among Pregnant Women by Trimester

Trimester (months)	Number Screened	Number Positive (%)
First (1 - 3)	34	3 (8.8)
Second (4 - 6)	28	6 (21.4)
Third (7 - 9)	138	14 (10.1)
Total	200	23 (11.5)

Table 3: Prevalence of HCV-Antibodies in Pregnant Women in Relation to Age

Age Range (years)	Number Examined	Number Positive (%)
16 - 20	50	2 (4.0)
21 - 25	56	4 (7.1)
26 - 30	38	2 (5.3)
31 - 35	20	4 (20.0)
36 - 40	26	0 (0.0)
41 - 45	7	0 (0.0)
46 - 50	3	0 (0.0)
Total	200	12 (6.0)

Table 4: Prevalence of HCV among Pregnant Women by Trimester

Trimester (months)	Number Screened	Number Positive (%)
First (1 - 3)	48	2 (4.2)
Second (4 - 6)	82	8 (9.8)
Third (7 - 9)	70	2 (2.9)
Total	200	12 (6.0)

Table 5: Prevalence of HBsAg Among Pregnant Women in Relation to Risk Factors

Factors	Response	Number Examined	Number Infected (%)
Vaccination	Yes	84	1(1.2)
	No	116	2(18.9)
Blood Transfusion	Yes	68	5(7.4)
	No	132	18(13.6)
Sharing of Sharp objects	Yes	41	9(22)
	No	159	14(8.8)
Polygamous marriage	Yes	92	11(12)
	No	108	12(11.1)
Total		800	72(9.0)

Table 6: Prevalence of HCV Among Pregnant Women in Relation to Risk Factors

Factors	Response	Number Examined	Number Positive (%)
Blood Transfusion	Yes	24	4(16.7)
	No	176	8(4.6)
Sharing of Sharp objects	Yes	66	6(9.1)
	No	134	6(4.5)
Polygamous marriage	Yes	108	6(5.6)
	No	92	6(6.5)
Total		600	36(6.0)

Discussion

The result of this study showed a prevalence rate of 11.5%, this is in contrast with the findings by Nasidi *et al.* (1983), who reported a prevalence rate of 13.8% in Lagos. Similarly, 11.6% prevalence was reported in Maiduguri (Harry *et al.*,1994), 8.3% in Zaria (Luka *et al.*,2008), 4.8% in Porthacourt (Akani *et al.*, 2005) and 5.7% in Ilorin (Agbede *et al.*,2007). While a similar study among pregnant women attending antenatal clinic at Ahmadu Bello University, Zaria Main Campus Clinic, Samaru showed a prevalence rate of 13.3% (Lawal, 2009). From surveys in other parts of Nigeria, the prevalence of HBsAg range from 2.7-13.3% (Kulkarmi *et al.*, 1986; Muula., 2000).

In epidemiological studies, the age of acquiring infection is a major determinant of the incidence and prevalence rate of HBV (Zali *et al.*, 1996). The prevalence of HBSAg in this study was not limited to a particular age group it was rather distributed among all age groups that are reproductively active. The low prevalence observed in the age group 31-35, 36-40 and 41-45, could probably be due to natural immunity (Fong and Schooenfield, 2006), meanwhile the highest prevalence among the age group 16-20 may be attributable to the fact that this age group could be less informed on prevention or rather could be more adventurous in behaviour which could lead to higher exposure to infection. HBV being sexually transmissible infection could have been transmitted during sexual intercourse; similarly, the observed prevalence of

HBV in age groups 21-25, 26-30 and 46-50 years may be associated to their active reproductive age (Makroo *et al.*, 1989).

The prevalence of HBV on the basis of trimester in this study showed that those in the second trimester had the highest prevalence rate of 21%, while those in the first and third trimester had prevalence rate of 8.8% and 10.1% respectively which is in consonant with the findings by Livavati *et al.* (2004), who reported higher prevalence in third trimester and low prevalence in first trimester. Our findings are in contrast with the study by Lawal (2009), who reported the higher prevalence in the second trimester compared to first and third trimester. The observed variation may be attributable to degree of exposure to the risk factors.

Study has also shown that, the likely hood of contacting the infection is strongly reduced by receiving vaccine against hepatitis B, and avoidance of sharing sharp object particularly non sterilized this is in contrast with the findings by Halima and Ajayi (2000) who reported the use of unsterilized sharp object as a risk factor in the transmission of HBV.

Even though, polygamous couple are previously consider to have a risk of spreading the HBV due to heterosexual partners (Lawal, 2009). Also in the case of previous blood transfusion is not though as a significant mode of transmission, except where the mechanisms of ensuring blood safety are lacking, HBV may be prevalence in that community. There is also a problem of 'window period' when the antigens or antibodies are not yet demonstrable, the blood can still transmit the infection (Liaw *et al.*, 1984). Community and hospital based researches in Nigeria have reported high prevalence of HBsAg ranging from 7.4% to 26% (Ekpo *et al.*, 1995) and from 2.7% to 13.8% (Muula, 2000) this is consistent with the findings in our study.

An overall seroprevalence of hepatitis C virus in pregnant women in this study was 6.0% which is similar to that of other African countries, such as Gabon with prevalence of 6.5% (Delaporte *et al.*, 1993) and Democratic republic of Congo with prevalence rate of 6.6% (Laurent *et al.*, 2001). The seroprevalence obtained also concurs with similar community based study in San Juan, Puerto Rico which showed estimated prevalence of 6.3% (Perez *et al.*, 2005).

However, the overall seroprevalence of HCV in this study is in contrast with that of Egypt which recorded a very high prevalence of 28% (Saeed *et al.*, 1991), the seroprevalence in this study is also in contrast with that in Singapore with low prevalence of 0.54% (Durosunmi *et al.*, 1993) and that in Belgium with prevalence of 0.87% (Van *et al.*, 2002).

In a similar study among pregnant women in different parts of Nigeria, different prevalence rate of HCV has been reported, 2.5% in Maiduguri (Baba *et al.*, 1999), 1.6% in Abuja and South West of Nigeria with prevalence of 9.2% (Ogunro *et al.*, 2007).

The Seroprevalence of HCV in this study was highest in the age group 31-35 with 20.0% followed by 7.1%, 5.3% and 4.0% in the age groups 21-25, 26-30 and 16-20 respectively. However no HCV-antibodies positive cases were detected in the age group 36-40 and 41-45. The absence of HCV-antibodies in the age group 36-40, 41-45 and 46-50 is in contrast with the report of Atome *et al.* (2008) who reported highest prevalence in the age group 35 and above. The absence of HCV of HCV-antibodies in the age group 41-45 concurs with the report of (Njouom *et al.*, 2003).

Another important factor considered in this study was the stage of pregnancy of the pregnant women, the results from the study showed that those in the second trimester had the highest prevalence rate of 9.8% than those in their first trimester 4.2% and third trimester 2.9%.

The prevalence of HCV-antibodies among those with history of sharing sharp objects and those involved in polygamous marriage is relatively low 9.1% and 5.6% respectively compared to that of blood transfusion.

Conclusion

The age group of 16-20 years was the highest carrier group for Hepatitis B virus infection. This consists of pregnant women in the second trimester of pregnancy. However the age group of 31 to 35 carries the highest burden of Hepatitis C infection in this study. Avoiding practices that appear to increase the risk of infection including the sharing of sharp objects and avoiding sexual promiscuity can help curb the spread of infection. Appropriate screening of blood donors and pregnant women using sensitive method should therefore be ensured to prevent further transmission of hepatitis B and Hepatitis C.

References

- Agbede O.O, Iseniyi J.O, Kolawale M.N and Ojuowa A. (2007): Risk factor and Seroprevalence of hepatitis B antigenemia in mothers and their preschool age children in Ilorin, Nigeria. *Therapy*.4 (1):67-72.
- Akani C.I, Ojule A.C, Oporun H.C, and Ejilemele A.A. (2005): Seroprevalence of HBsAg in pregnant women in Porthacourt, Nigeria. *Nigeria postgraduate medical journal*. 12(4) 266-270.
- Atome G., Makuwa M., Njouom R. and Kazanji M. (2008). Hepatitis C virus prevalence and genetic diversity among pregnant women in Gabon Central Africa. *BioMed* 8:82 – 86
- Baba M., Onwuka S.and Baba S. (1999). Hepatitis C virus infection among pregnant women in Maiduguri, Nigeria. *European journal of public health* 7:60-62
- Cheesbrough M. (2006): *District laboratory practice in tropical countries. 2nd edition*. Cambridge University Press, U K. 250-252.
- Delaporte E., Thiers V., Dazz M., Romeo R., Cabanne N., Aptel I., Schrijvers D., Brechot C. and Lourouze B. (1993). High level of hepatitis C endemicity in Gabon equatorial Africa. *Journal of medical hygiene* 87:636-637.
- Durosunmi, G., Zuridah, H. and Ariffin M. Y. (1993). Prevalence of hepatitis C virus antibodies in blood in Malaysia. *Malaysian Medical Journal*, 48:313 – 316
- Emechebe, G.O., Emodi, I.J., Ikefuna, A.N., Ilechukwu, G.C., Igwe, W.C., Ejiofor, O.S. and Ilechukwu, C.A. (2009). Hepatitis B virus infection in Nigeria - A review. *Nigeria Medical Journal*, 50:18-22
- Fong T., and Schoenfield L.J. (2006): Hepatitis B. www.medinet.com
- Halima N.K. and Ajayi O.I. (2000): Risk factors and seroprevalence of hepatitis B and C antibodies in blood donors in Nigeria. *East Africa medical journal*. 77:410-412.
- Harry T.O., Bajani M.D. and Moses A.E. (1994): Hepatitis B virus infection among blood donors and pregnant women in Maiduguri, Nigeria. *East Africa medical journal*. 70:596-597.
- Ibrahim, M.B., Luka, S.A. and Iliya, S.N. (2008): Seroprevalence of HBsAg among pregnant women attending antenatal care at ABUTH, Zaria, Nigeria. *Nigerian journal of parasitology*. 29(1):38-41.
- Kulkarmi S., Alowala F.O. and Wayo G.G. (1986): Prevalence of HBsAg in Northern Nigerian blood donors. *Voxsanger*. 50:151-153.
- Laurent C., Henzel d, Mulanga-kabeya C, Maertens G, Larouze B and Delaporte, E. (2001). Seroepidemiological survey of hepatitis C virus among commercial sex workers and pregnant women in Kinshasa, Democratic Republic of Congo. *International Journal of Epidemiology*, 30(4): 872-877.
- Lawal N.D. (2009): *Seroprevalence of HBsAg among pregnant women attending antenatal care at ABU main campus clinic*. An Undergraduate Project, Department of Biological Sciences Ahmadu Bello University, Zaria, Nigeria, 11-15.
- Liaw, Y.F., Huang, M.J., Chu, C.M., Sheen, I.S. and Lin, D.Y. (1984). The Window Period Between Hepatitis B e Antigen and Antibody in Chronic Type Hepatitis. *Hepatology*. 4(4): 619-621.
- Lilavati G., Chandra M.P., Snehajja L.I. and Umakanta N. (2004): Incidence of HBsAg carrier state in pregnancy in Eastern Orissa. *Journal of obstetrics and gynecology*. India. 54(2):136-138.
- Luka S.A., Ibrahim, M.B., and Iliya S.N. (2008) Seroprevalence of hepatitis B surface antigen among pregnant women attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. *Journal of Parasitology*, 29:38-41.

- Makroo R.N., Hussain G. and Kaul A. (1989): Seroprevalence of HBsAg in Kashmir blood donors, *Indian journal of medical research*, 89:310-313.
- Muula A.S. (2000): Tackling HIV/AIDS in Africa-another perspective. *African health*. 23(1)5-6.
- Nasidi A., Harry T.O., Vyazor S.O., Numumbe G.M.R., Azzan B.B. and Ancinlevva. (1983): Seroprevalence of HBsAg in two different geographical areas of Nigeria. *Proceeding of the first international conference*, (12-15 December, 1983): Lagos Nigeria.
- Njouom R., Pasquier C., Ayouba A., Saune K., Lobe M., Tene G., Thonnon J. and Nerrienet E.(2003). Hepatitis C virus infection among pregnant women in Yaunde, Cameroon: prevalence, viremia and genotypes. *Journal of Medical Virology* 69:384-390.
- Ogunro P., Adekanle D., Fadero F., Olabisi T., and Olorunyomi S.(2007). Prevalence of hepatitis C virus antibodies in pregnant women and their offspring in a tertiary hospital in South Western Nigeria. *Journal of infectious disease* 1(3):333 - 336.
- Owolabi, H.A, Ojo, A.S. (2008). Hepatitis B virus and Chronic Liver disease in Nigeria: a brief review of literature. *IFEMED Journal*, 14 (1): 6-10.
- Perez, C. M., Saurez E., Torres E. A., Roman, K. and Colon, V. (2005). Seroprevalence of hepatitis C virus and associated risk behaviours : a population based study in San Juan, puerto Rico. *Journal of viral hepatology* 34 (3): 593 - 599.
- Prescott L. M., Harley J. P. and Klein D. A. (2008) *Microbiology, 7th edition*. McGraw Hill pp 936- 940.
- Ryan, K. J. and Ray C. G. (2004). *Medical microbiology, 4th edition*. McGraw Hill pp 551-552.
- Saeed, A. A., Al-Admawi A. M., Al-Rasheed A., Fairclough D., Bacchus R., Ring C. and Garson J. (1991). Hepatitis C in Egyptian blood donors in Riyadh. *Lancet* 33: 359 - 360.
- Shahin, M., Hourri, R., Mehdi, N., Elham, J., Hassan, A., Amir Reza, R., Hanieh, Zi., Sedigheh, A., Mahtab, M., Akram, P., Reza, M. and Saeed, E. (2010). Seroprevalence of hepatitis C virus: the first population-based study from Iran, *International Journal of Infectious Diseases, Vol. 14(3)*: e113-e116
- Ugbebor, O., Aigbirior, M., Osazuwa, F., Enabudoso, E. and Zabayo.O. (2011).The prevalence of hepatitis B and C viral infections among pregnant women. *North American Journal of Medical Science*, 3(5):238-241.
- Van D. P., Thyssen A. and Van F. (2002). Epidemiology of hepatitis C in Belgium present and future. *Actagastroenterol journal Belgium* 65 : 78 - 79.
- Zali, M.R. (1996). Epidemiology of Hepatitis B in the Islamic Republic of Iran, *Eastern Mediterranean Health Journal*, 2(2), 290-298.