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Full Length Research Paper

Detection of Hepatitis C virus amongst pregnant women, in Kaduna state, Nigeria

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This study was undertaken to determine the prevalence of HCV among pregnant women in Kaduna State, Nigeria. Hepatitis C Virus (HCV) is an important cause of chronic liver disease and despite the introduction of an effective screening policy for all blood donations, transmission continues to occur by other means. In this study, we determined the prevalence of HCV to ascertain the risk of vertical transmission in kaduna state, Nigeria. Two hundred (200) blood samples (5ml) were tested for the presence of anti-HCV antibodies by HCV ELISA (Biotech Laboratories, UK). All the anti-HCV positive samples were tested for the present of HCV RNA by RT-PCR using HCV specific primers (Sacace Biotechnologies, Italy). Of the 200 pregnant women tested, 9 (4.5%) were positive for anti- HCV antibodies. All the anti-HCV positive samples were positive for HCV RNA. Genotype subtype 1b was found in the entire HCV RNA positive sample. HCV therefore affect pregnant women in Kaduna State, Nigeria with a prevalence rate of 4.5% posing the risk of vertical transmission.

Key words: Hepatitis C virus; pregnant women; vertical transmission HCV RNA; HCV genotype.

INTRODUCTION

Hepatitis C Virus (HCV) infection continues to be a major disease burden in the world (WHO, 2000). Hepatitis C Virus induced end stage chronic liver disease and it is the leading cause of liver failure (Hadzic, 2001; Beth et al., 2006). HCV is a member of the family flaviviridae, placed in a new monotypic genus-Hepacivirus (Nahum et al., 2004, Liang, 2007). Maternal screening for hepatitis C is a preliminary step in preventing vertical transmission of the virus. This helps in identifying asymptomatic women who might not present for medical attention until late in the course of the disease (Burn, 1999). The difficulty of obtaining accurate measurement of vertical transmission risk include persistence of maternal antibodies in the newborn, failure to identify all infected mothers at follow-up and loss of infants born to HCV positive mothers (Steven, 2006).

Vertical transmission has been correlated with higher maternal viral titre (Yeung, 2001, Airoldi and Berghella, 2006), an elevated alanine aminotransferase level in the year before pregnancy (Indolfi et al., 2006) and the presence of maternal cirrhosis (Kuroki, 1992). It is clear that children who are sero-positive at 18 months of age or older have been vertically infected with HCV, and that the presence of HCV RNA can be used in children less than 18 months of age to differentiate those with chronic HCV infection from those with clearance of HCV (Liang, 2007).

Robinson et al. (2008) reported that the mode of delivery (caesarian section/normal delivery) by HCV infected mother did not appear to alter the rate of HCV transmission. However, prolonged membrane rupture has been reported to be a major source of HCV mother-to-child transmission (Indolfi et al., 2006, Mast et al., 2005). Despite the fact that HCV RNA has been detected in breast milk, multiple observational studies suggest that breastfeeding does not appear to play an important role in the mother-to-infant transmission of HCV (Mast et al., 2005). One theory as to why transmission via breast milk never or rarely occurs is that gastric acid rapidly inactivates HCV (Indolfi et al., 2006). Women with HCV infection should be advised that unless there are other

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contraindications, they should consider breastfeeding their infants, safe though it is not possible to totally exclude the possibility that HCV transmission could occur via this route. The benefits of breastfeeding outweigh the theoretical, but unproven, risk of HCV transmission to the infant.

However, women with jaundice postpartum or women who develop cracked, bleeding nipples should stop breastfeeding (Robinson et al., 2008). Genome sequence comparison has revealed the existence of six major HCV genotypes and a large number of subtypes (Solange and Guilherme, 2003). Genotyping assignment helps in assessing disease prognosis and assists in establishing the appropriate duration of treatment (Michael et al., 2004). HCV genotypes 1, 2 and 3 are the most commonly detected types worldwide (Gismondi et al., 2004). Genotype 1 and 4 isolates were found to be less likely to respond to interferon (IFN) therapy than Genotype 2 and 3 (Jenny et al., 2004; Liang, 2007).

In Nigeria, the prevalence of HCV has been reported amongst pregnant women in Maiduguri (Baba et al. 2006). There is no data on the prevalence of HCV amongst pregnant women in the popular Kaduna State of Nigeria. This work was to determine the prevalence of HCV antibodies, genotypes and subtypes in pregnant women in Kaduna state.

MATERIALS AND METHODS

The study was conducted in Kaduna state, using hospitals in major towns of each of the three senatorial zones which include General hospital Kafanchan (Southern Kaduna), Yusuf Dantsoho hospital Kaduna (Central Kaduna) and Ahmadu Bello University Teaching Hospital Shika-Zaria (Northern Kaduna), between June and December 2008, after obtaining an ethical approval.

A structurally designed questionnaire was used for obtaining information from the patients. The inclusion criteria such as personal consent, pregnant woman, HIV and Hepatitis B Virus (HBV) negative were used. Exclusion criteria used are individual non consent, non pregnant woman, HIV and HBV positive patient.

Two hundred (200) blood samples (5ml) were collected into blood bottles containing anticoagulant after obtaining consent from the patients. The plasma were separated into sterile bottles and stored at -20°C immediately until required for analysis.

HCV antibody detection

The plasma samples were initially tested for the presence of Hepatitis C virus antibodies using a commercially available third generation HCV Enzyme-linked immunosorbent assay (Biotech Laboratories, UK). The procedure according to the manufacturer was used as follows: Specimen diluents (100µl) were pippeted into all test wells leaving 5 wells for control and blank. Negative control (100µl) was pippeted into duplicate wells that contain no diluents. Positive control (100µl) was pippeted into duplicate wells that contain no diluents. Specimen diluents (100µl) were pippeted into the first well and were left as blank. Test samples (10µl) were added in assigned wells and mixed.

The plates were sealed and incubated at 37^{o}C for 30 minutes. Each well was washed 5 times by filling each well with diluted wash buffer, then the plates were inverted vigorously to get water out and blocking the rim of wells on absorbent paper for 30 seconds. Enzyme conjugate (Horseraddish peroxides) (100µl) were added to each well except the blank and were mixed by swirling the microtitre plates. The plates were sealed and incubated at 37^{o}C for 30 minutes. The plates were washed 5 times as in number 7 above. Substrate solution (100µl) was pippeted to each well and was incubated at 37^{0}C for 10 minutes. Stopping solution (50µl) was added to each well to stop the colour reaction.

The intensity of the reaction was photometrically quantitated with a dual filter Enzyme Immuno Assay Reader (Sigma diagnostics EIA Multi-well Reader 11) immediately using O.D at 450nm, 630nm.

Reverse transcription polymerase chain reaction (RT-PCR)

The HCV RNA was extracted with ribo-sorb extraction kit (Sacace Biotechnologies, Italy) and manufacturer′s instructions were used. RT-G-mix-1 (5µl) was added into tube containing RT-Mix, vortexed for 10 sec and was briefly centrifuged. M-MLV (6µl) was added into tube with reaction mixed, vortexed and centrifuged for 7sec. This was immediately used for reverse transcription. Reaction mix (10µl) was added to each tube. The tubes with extracted RNA were re-centrifuged for 2 minutes at 16,000rpm. Supernatants containing extracted RNA (10µl) were taken to appropriate tubes. The tubes were placed into thermocycler (Techgene, model FTGENE 5D, Serial NO 121254-4) and were incubated at 37^{o}C for 30 minutes.

Each cDNA sample obtained was diluted 1:2 with T E-buffer [20µl of T E buffer was added to each tube] and was ready for used. Taq deoxyribonucleic acid (DNA) polymerase and moloney murine leukemia virus (M-MLV) reverse transcriptase were obtained from (Sacace Biotechonologies, Italy). Primers were specific for region of HCV genome with the 338bp for genotype 1a, 395bp for genotype 1b, 286bp for genotype 2, and 227bp for genotype antisense primer for all genotypes 3a:

5’-CAGTCACTGAGAGCGACATCCGTACG-3’- 1a
5’-AGGCCACTGCGGCCTGTCGAGCTGCGAA-3’- lb
5’-TATGTTCAACAGCAAGGGCCAGA-3’- 2

Table 1. Distribution of HCV among pregnant women per Senatorial zones.

Zone	Number Tested	Number (%) Positive	
		HCV Ab	HCV RNA
Southern	30	4 (13.3)	4 (13.3)
Central	100	3 (3.0)	3(3.0)
Northern	70	2 (2.8)	2(2.8)
Total	200	9(4.5)	9(4.5)

Chi square value = 5.975; P = 0.178 at 95% confidence interval P≤0.05

Table 2. Age distribution of HCV among pregnant women.

Age (Years)	No Tested	Number (%) Positive	
		HCV Ab	HCV RNA
≤ 20	25	1(4.0)	1(4.0)
21 – 30	132	6(4.6)	6(4.6)
31 – 40	42	2(4.8)	2(4.8)
41 – 50	1	0(0)	0(0)
51 -60	0	0(0)	0(0)
Total	200	9(4.5)	9(4.5)

Chi – Square Value = 2.006; P = 0.735 at 95% Confidence Interval P≤0.05

5'-CTCGGACCCTGACTTTCT-3'- 3a
5'-CCTGGTCATAGCCTCCGTGAA-3'-

Tubes (12) of PCR Mix-1 genotypes 1a/1b tubes and 12 of PCR Mix-1 genotypes 2/3a including 1 tube for negative control and 1tube for positive control were prepared. PCR Mix-2 (10µl) was added to each tube. cDNA samples obtained after RT step (5µl) were added to appropriate tubes. DNA buffer (5µl) was added to negative control tube of amplification. cDNA 1a (5µl) was added to the PCR-Mix -1 genotype 1a/1b tube. cDNA 1b (5µl)were added to the PCR-Mix -1 genotype 1a/1b tube. cDNA 2 (5µl) were added to the PCR Mix-1 genotypes 2/3a tube. cDNA 3a (5µl) were added to the PCR Mix-1 genotypes 2/3a tube. The tubes were closed and transferred to the thermocycler only when temperature reached 95°C. The reactions were started after the thermocycler was programmed. Step (Initial denaturation): 95°C, 5 minute. Step 2 (42 cycles): Denaturation at 95°C, 1 minute; annealing at 68°C, 1 minute; extension at 72°C, 1 minute. Step 3 final extension at 72°C, 1 minute and finally 10°C for storage temperature.

The amplified cDNAs were detected by agarose gel electrophoresis and bands were identified by markers and control cDNA. Genotypes were determined by HCV genotype specific primers using HCV genotype kit (Sacace Biotechnologies, Italy). The data were analyzed using SPSS soft ware and chi square test was used to test for association of pregnant women and HCV infection.

RESULTS

A total of 200 pregnant women were included in this study. Out of the 200 pregnant women tested, 9(4.5%) were positive for anti- HCV antibodies by ELISA and all the 9(4.5%) anti- HCV positive specimens were HCV RNA positive (Table 1). Table 1 shows HCV distribution among senatorial zones, HCV prevalence in the southern zone was 13.3% followed by 3.0% in the central zone and the northern zone had the least (2.8%) HCV prevalence.

Table 2 shows the age distribution of HCV among pregnant women in Kaduna State, Nigeria. Majority of the HCV RNA positive pregnant women were between age 31 and 40 years with HCV RNA prevalence rate of 4.8% followed by age 21 to 30 who had prevalence rate of 4.6%. The less than or equal to 20 age group had the least prevalence rate of 4.0%.

DISCCUSSION

The prevalence of 4.5% HCV infection among pregnant women in this study is higher than the 2.5% HCV prevalence reported among pregnant women in Maiduguri (Baba, 2006). However it is within the worldwide prevalence range of 0.1% to 4.5% (Steven, 2006). Robinson et al. (2008) reported that approximately 5% of pregnant women with chronic HCV infection will transmit the infection to their infants. This shows that 5%

of the 4.5% HCV infected mothers will vertically transmit the HCV to their infants. The prevalence of 4(13.3%) HCV infection from the Southern zone is the highest follow by 3 (3.0%) HCV infection in the central zone and the least 2(2.8%) HCV prevalence was found in the Southern zone.

This shows a consistent pattern as the prevalence of HCV increases progressively from Northern zone to Southern zone. The factor responsible for this trend is not defined in this study. The low prevalence of 4.5% among women could be as a result of high clearance rate of HCV infection in women. Bakr (2006) reported that a significant number of persons clear HCV from their blood after infection with a high clearance rate in females than in males. The highest HCV cases was found among the age group of 31 to 40 having 2(4.8%) HCV prevalence followed by 21 to 30 with 6(4.6%) HCV prevalence. The least prevalence was found among age ≤ 20 age group with 1(1.4%) and no HCV prevalence was found among age 41 to 50 (Table 2).

By this finding the middle age group (31 to 40) has the highest prevalence of HCV infection. It has also shown that the prevalence rate of the disease is lower with the lower age and decline with age from 51 and above. The highest prevalence rate of 4.8% HCV infection found among 31 to 40 age group, is in agreement with earlier report that HCV infection occurs among all ages, but the highest incidence of acute Hepatitis C infection is found among persons of ages 20 to 39 years (CDC, 1998). The bases for the modal age group of 20 to 39 years may be youthfulness which is associated with adventure, quest for recreation, which manifest in substance abuse and multiple sexual partners.

More so, the age group of 20 to 39 coincides with the age of child bearing and this is a signal that HCV should be taken seriously. Genotype 1b was found in all the HCV antibody positive specimens. HCV Genotype 1 is the most difficult to treat and thus another reason why HCV must be taken seriously. The prevalence of 4.5% HCV infection among pregnant women indicated that HCV infect pregnant women in Kaduna state with risk of vertical transmission.

RECOMMENDATION

The health system should be strength to support all HCV infected pregnant women medically and socially, as well as supporting vaccine development research. HCV screening should also be included among blood borne pathogen for pregnant women.

REFERENCE

Airoldi J, Berghella V (2006). Hepatitis C and Pregnancy. Obst Gynecol Surv., 61: 666-672.

Baba MM, Onwuka IS, Baba SS (2006). Hepatitis B and C Virus Infections among Pregnant Women in Maiduguri, Nigeria. J. Public Health, 7(2): 60-62.

Bakr I, Rekacewicz C, El-Hosseiny M (2006). Higher Clearance of Hepatitis C Virus Infection in Females Compared with Males. Gut., 55(8): 1183-1187.

Beth B, Marc G, Jay H, David K, Jake L, John M, and Leonard S (2006). Chronic Hepatitis C: Current Disease Management. Nation Digest Disease Information, 7: 1-20.

Burn DN, Minkoff H (1999). Hepatitis C Virus Screening in Pregnancy. Obstetric and Gynecol., 94: 1044-1048.

Centre for Disease Control and Prevention (1998). Recommendations for Prevention and Control of HCV Infection and HCV Related Disease. Mortality and Morbidity Weakly Report, 47(19): 1-39.

Gismondi MI, Estela IT, Saul G, Maria CG, Maria VP (2004). HCV Infection in Infants and Children from Argentina. J. Clinical Microbiol., 42(3): 1199-1202.

Hadzic N (2001). Hepatitis C in Pregnancy. Disease Child, Neonatal J., 84: F201-F204.

Indolfi G, Azzari C, Moriondo M, Lippi F, de Martino M, Resti M (2006) Alanine Amino Transaminase Levels in the Year Before Pregnancy Predict the Risk of Hepatitis C Virus Vertical Transmission. J. Med. Virol., 78: 911-4.

Jenny M, Yoann M, Sylvie A, Andre C, Chistelle R, Frederic L, Andre E, Serge P, Philippe G, Odile B, Roger C, Emmanuel G, Paul D, Raymond C (2004). HCV Genotypes in Caribbean Land of Martinique. J. Clinical Microbiol., 42(2): 784-791.

Kuroki T, Nishiguchi S, Fukuda K (1992). Transmission of Hepatitis C Virus from Mothers with Chronic Hepatitis C Without Human Immunodeficiency Virus. J. Infectious Disease, 166: 1192-3.

Liang TJ (2007). Shortened Therapy for Hepatitis C Virus Genotype 2 or 3 – is Less More? The New England J. Med., 357(92): 176-278.

Mast EE, Hwang LY, Seto DS (2005). Risk Factors for Perinatal Transmission of Hepatitis C Virus (HCV) and the Natural History of HCV Infection Acquired in Infancy. J. Infectious Disease. 192: 1880-9.

Nahum M, Daniel M, Norbeto CC, Jesus B, Richardo C, Misael U (2004). Prevalence of HCV Infection Among Hemodialysis Patients at a Tertiary Care Hospital in Mexico City, Mexico. J. Clinical Microbiol., 42(9): 4321-4322.

Robinson JL, Robert B, Richmond JF, Moore DL, Samson LM (2008). Vertical Transmission of Hepatitis C Virus. Paediatric Child Health. 13(6): 529-534.

Solange B, Guilherme O (2003). Molecular Epidemiology of the HCV in Brazil. Genetics and Molecular Res., 2(1): 117-123.

Steven KH (2006). Mother to Child Transmission of HCV. Medical Writer Circle. 18: 1-5.

World Health Organization (WHO) (2000). Hepatitis C.; http://www.who.int/mediacentre/factsheets/fs164/en/1/3/2008.

Yeung LT, King SM, Roberts EA (2001). Mother-to-Infant Transmission of Hepatitis C Virus. Hepatol., 34: 223-9.