SEROPREVALENCE OF HEPATITIS C VIRAL ANTIBODIES IN PREGNANCY IN A TERTIARY HEALTH FACILITY IN NIGERIA

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ABSTRACT

**Background:** Liver disease due to Hepatitis C viral (HCV) infection is the most common indication for liver transplant. It is a viral pandemic that is five times as widespread as the human immunodeficiency virus type 1 infection. In spite of this, vaccines were yet unavailable for protection of the human race due to the morphology and fastidious nature of the organism. While the scanty data available on this infection in our environment are limited to blood donors, people continue to be screened for and deprived of renal dialysis if any patient is found to have HCV infection. Also in this environment, data on HCV infection in pregnancy is virtually nonexistent even though the infection can have a deleterious effect on materno-fetal outcome.

**Objective of the Study:** To determine the seroprevalence of hepatitis C viral antibodies among antenatal women attending a tertiary health facility in Nigeria.

**Methodology:** This was a prospective cross-sectional study whose subjects were booked consecutive antenatal women volunteers attending the University of Benin Teaching Hospital, Benin City, Nigeria between June 1 and December 31, 2005. Hepatitis C viral antibodies were determined and confirmed using a second and a third generation Enzyme Linked immunosorbent assay respectively. Both HCV sero-positive and sero-negative women had both pre- and post-test counseling.

**Results:** Of the 269 samples screened for HCV antibodies, 5 (1.86%) samples were confirmed seropositive. None of the HCV seropositive women had liver enzyme derangement.

**Conclusion:** Hepatitis C viral infection in pregnancy is not uncommon in Nigeria. It's prevalence in pregnant women South-South of Nigerian is similar to that of their Cameroonian counterparts, an immediate neighbouring country. A multi-centre study to determine the national prevalence of HCV and in addition to elevation of public awareness is suggested. Hepatitis C viral-induced liver disease remains the major indication for liver transplant for which our present levels of economy and health infrastructures can least support. With no vaccines and no cure, the time to act is now.

**Key Words:** Seroprevalence, HCV, Pregnancy, Vertical transmission, Nigeria.  *(Accepted 25 February 2008)*

**INTRODUCTION**

Many transmissible maternal viral infections cross the placenta during the antenatal or intrapartum periods to infect the fetus. Among these viruses are the HIV, **Hepatitis B and C viruses**, Cytomegalovirus, the Human Papiloma and the Herpes viruses. Chlamydia trachomatis and N. gonorrhea are some non-viral infections that can be transmitted from the mother to the fetus. The Mycobacterium tuberculosis responsible for pulmonary tuberculosis, though not transmitted through the placenta, is nevertheless associated with poor pregnancy outcome especially when co-infected with HIV. Maternal open pulmonary tuberculosis can accentuate neonatal infection.

In the postnatal period, some of these viruses can also be transmitted through the breast milk thereby increasing the vertical transmission rate of these infections. Similarly, this rate may be worsened by co-infections with other micro-organisms. Though the hepatitis C virus is known to be present in breast milk, breast feeding has not been shown to increase the rate of transmission to the infant, and if any, the risk may be low.

In the absence of co-infections, active viral infections of the liver by hepatitis viruses can cause severe liver damage, physical illness, anorexia, malnutrition and increased burden on pregnancy, labor and the puerperium thereby increasing maternal and neonatal morbidity and mortality. The quality of life, even in the absence of severe disease, can be jeopardized by hepatitis C virus (HCV) infection.

The prevalence of HCV infection varies worldwide...
with an estimated population of 170 million persons being infected. It is a viral pandemic that is five times as widespread as the human immunodeficiency virus type 1 (HIV-1) infection,\textsuperscript{10,11} and with Egypt having the highest number of infections. The use of parenteral antischistosomal therapy is thought to contribute to a prevalence of antibodies against HCV in various regions ranging from 6 to 28 percent.\textsuperscript{10,11} In the United States, HCV antibodies is present in 1.8 percent of the population.\textsuperscript{13} Currently available tests have shown that 3 of every 4 seropositive persons also have viremia thus putting the estimated population with active HCV infection in the United States at 2.7 million people.\textsuperscript{10,12,13}

In developed countries where adequate blood-screening measures are operational, the risk of transfusion-associated hepatitis is decreased to a negligible level. This however may not be true of resource constraint developing countries. In Nigeria, for example, Imarengiaye et al\textsuperscript{14} reported a 3% HCV infection rate in bags of transfused donated blood from this center while 0.5% was reported by Erhabor et al\textsuperscript{15} for Port Harcourt, all in the Nigeria Niger Delta. Injection-drug use mainly and, to a lesser degree, percutaneous or mucous-membrane exposure continues to transmit new cases in the developed countries.\textsuperscript{16,17}

This HCV is a single-stranded 9.5 kb RNA flavivirus. It consists of a single open reading frame and two untranslated regions encoding a single polyprotein of 3011 amino acids. The hepatitis G, yellow fever, and dengue viruses are the most closely related human viruses.\textsuperscript{18,19} Based on molecular relationship the HCV is categorized into six distinct but related HCV genotypes and multiple subtypes with geographical or regional spread.\textsuperscript{20} While some genotypes and subtypes may predominate in some regions they may be absent in others.\textsuperscript{16,20} Having a knowledge of the genotype and subtypes is of clinical importance for its predictive value in terms of the response to antiviral therapy, with better responses associated with genotypes 2 and 3 than with genotype 1.\textsuperscript{21,22}

In HCV infection, viral replication is extremely robust and more than 10 trillion virion particles are estimated to be produced per day, even in the chronic phase of the infection.\textsuperscript{23,24} An RNA-dependent RNA polymerase that lacks a "proofreading" function is the means by which this virus replicates. The resultant rapid evolution of diverse but related quasispecies (for example HCV genotype 2 or 3) within an infected person presents a major challenge to immune-mediated control of HCV.\textsuperscript{25} Hepatocytes and, possibly, B lymphocytes are the natural targets of HCV.\textsuperscript{24,25}

In the laboratory, the difficulties associated with culturing Hepatitis C Virus have significantly impaired the frontiers of research on this organism.\textsuperscript{10,12} Once infected, the progression to chronic disease occurs in the majority of HCV-infected persons. Better knowledge of the pathogenesis of the infection has continued to yield improvements in treatment options,\textsuperscript{10,12} albeit pretty expensive. HCV remains the main indication for liver transplantation in the developed countries,\textsuperscript{26} and the liver damage associated with it an important cause of mortality and morbidity among HIV-infected patients.\textsuperscript{27}

The main routes of HCV transmission include injection drug abuse, homo-sexualism (men who have sex with men), blood transfusion, and heterosexualism of which sex among monogamous couple accounts for less than 1% infection.\textsuperscript{28} Thus, sexual transmission of the virus is an inefficient means of partner infection especially when compared with HIV-1.\textsuperscript{29} Genital fluids and tissues have been reported to have low levels of the virus. Lack of appropriate target cells in the genital tract has also been reported. The roles of these observations in the transmission of the virus remain unclear. However, co-infection with HIV-1 has been reported to increase the risk of both sexual and maternal-fetal transmission of HCV.\textsuperscript{29,30} While HBV may survive drying for more than 7 days in the dry state and still remain infectious, HCV is infectious only for hours.\textsuperscript{17}

While the estimated risks of transmission through a needle stick is 30 percent for HBV and in 0.3 percent for HIV-1 exposures, HCV poses a 3 percent risk (the rule of threes) depending on the size of the inoculum, the size of the needle, and the depth of inoculation.\textsuperscript{20} A positive anti-HCV antibody test indicates previous exposure while the detection of HCV-RNA indicates active disease.\textsuperscript{29,31} HCV infection is excluded when anti-HCV antibody test is negative except if the patient has acute HCV (diagnostic window) or has a blunted immune response, in which case HCV-RNA should be measured to document the infection.\textsuperscript{27}

There is conflicting reports of the risk of vertical transmission of HCV from chronic HCV mothers to their off springs\textsuperscript{1} and the rate of mother-to-child transmission of HCV is reported to range between 4% and 50%.\textsuperscript{7,32} The reported risk of HCV transmission is increased if the pregnant women were also co-infected with HIV.\textsuperscript{7,10,15} Currently, it is believed that breast feeding poses little or no risk for the transmission of HCV from mother to infant.\textsuperscript{7,10,34}

In resource-constrained country like ours, adopting and implementing new policies and interventions by policy makers and administrators must be evidenced-based. For example, based on the report of Offor et al\textsuperscript{11} on the sero-prevalence of HIV in pregnancy, seroprevalence of hepatitis B antibodies\textsuperscript{30} and the
proportion of dual HIV and HBV infections in pregnancy in this center, led to the commencement of screening services for HIV as part of our antenatal care package. This effort is currently being supported by the Nigerian and the United States Governments in the Prevention of Mother-to-Child Transmission of HIV Program with the provision of highly active antiretroviral therapy (HAART) for HIV positive women and their babies. Currently, routine HBV screening and immunization of needy mother-baby pairs and the spouses of seropositive women are now part of our antenatal care services. In resource-constrained country like ours, adopting and implementing new preventive measures requires the proof of the existence of an infection or disease. For example, based on the report of Offor et al on the sero-prevalence of HIV in pregnancy, and seroprevalence of hepatitis B antibodies and dual HIV and HBV infections in pregnancy by Onakewhor et al in this center, led to the commencement of screening services for HIV as part of our antenatal care package. This effort is currently being supported by the Nigerian and the United States Governments in the Prevention of Mother-to-Child Transmission (PMTCT) of HIV Programme with the provision of highly active antiretroviral therapy (HAART) for HIV positive women and their babies. Currently, routine HBV screening and immunization of needy mother-baby pairs and the spouses of seropositive women are now part of our antenatal care services. The presence of HCV infection has also been reported by Erhabor et al for the Nigerian population in Port Harcourt. While the documented evidence of HCV infection in Nigeria is among blood donors, the magnitude of this infection among pregnant women was yet to be documented. The objective of this study, therefore, is to determine the seroprevalence of hepatitis C viral antibodies among antenatal women attending a tertiary health facility in Nigeria.

SUBJECTS AND METHOD
This was a prospective cross-sectional study to the determine the seroprevalence of hepatitis C virus antibody among pregnant women who booked at the University of Benin Teaching Hospital, Benin City, Nigeria between June 1 and December 31, 2005. To obtain a true prevalence of the infection in pregnant women, women with known risk factors were excluded from the study. These included women that previously had blood transfusion, history of intravenous drug abuse and those whose spouses were known to be intravenous drug users. As part of this the unit protocol, women that presented for booking were counselled for screening of communicable infections in pregnancy. Amongst these are the HIV, syphilis and hepatitis B.

Consecutive volunteers were counseled and verbal consent obtained. Ten milliliters of blood was collected from each subject during routine booking investigation in the antenatal period. Sera obtained after centrifugation were coded and analyzed in the hospital's main laboratory for Hepatitis C antibodies. The test was performed using a second generation Enzyme Linked immunosorbent assay (ELISA DOC INF 5125002) test kit (Human Diagnostica, Germany), according to the manufacturers instructions. All repeatedly reactive samples were then further tested for anti-HCV antibodies using a commercial third generation ELISA (MONOLISA anti-HCV plus version 2, Biorad, Marnes-La-Coquette, France) in accordance with the manufacturers instructions. The HCV seropositive and sero-negative women were post-test counselled. Each seropositive woman had liver enzyme assay done using Cobas Integra IFCC test kit by Roche Diagnostica, Germany.

All the women were post test counseled. Both seropositive and seronegative women continued with their antenatal care and delivery in this unit. Upon delivery, the HCV seropositive women were further counselled and referred to the physician for follow-up management. Similarly, the babies of seropositive mothers were referred to the pediatricians for follow-up management after delivery. Both seropositive and seronegative women continued with their antenatal care and delivery in this unit. Upon delivery, the HCV seropositive women were further counselled and referred to the pediatricians for follow-up management after delivery.

DATA ANALYSIS
The data are presented as proportions of HCV antibody sero-positive and sero-negative cases.

RESULTS
Of the 275 women counselled, 3 had positive history of blood transfusion and were excluded from the study. Another two dropped out from the study for personal reasons before venepuncture and one had previously been managed for jaundice outside pregnancy some three years previously. All six women where excluded form the study. A total of 269 samples were screened for HCV antibodies. Five (1.86%) samples were confirmed seropositive for HCV antibodies.

DISCUSSION
Hepatitis C virus infection is not uncommon in our obstetric population. The seroprevalence of HCV...
antibodies in our pregnant population was 1.86%. This prevalence is higher than the 0.5% reported for blood donors in a sister teaching hospital (University of Port Harcourt Teaching Hospital) in the same South-South geopolitical zone. It is however comparable with the 1.8% reported for the obstetric population in a western African neighbour, Cameroon and the 1.86% reported for the general American population, but lower than the 3% reported for blood donors in this centre and the 2.3% to 4.5% reported for pregnant women in the United States.

It is also comparable with the 1% to 3% reported for the adult and within the 0.1% to 4.5% for the pregnant Canadian population respectively and the 1.2% to 1.7% reported for the Italian pregnant women. The prevalence in our study is more than the 0.68% to 0.98% 1.2% for the Japanese obstetric populations and the 0.9% and 1.0% reported respectively for Taiwanese and French pregnant women. The prevalence is however less than 6% to 28% reported for the general Egyptian population, and the 40% for HCV and 8% for HBsAg positivity in European patients co-infected with HCV and HIV respectively. When compared with the prevalence of HBV in this center, the incidence of HCV is lower than the 2.2% reported by Onakewhor et al two years previously.

Our findings have some implications for our health care givers and the public in general. The prevalence of 1.86% is high considering that pregnant women belong to low-risk group who are usually in stable union and who may not, in our environment, be involved in risky behaviour such as injection drug use and commercial sex work which are the most common routes of transmission after excluding transfusion of unscreened blood. Women with any of these factors were excluded from the study. It has been reported however that immuno-compromised patients, such as in HIV-1 infection, patients with renal failure; and those with HCV-associated essential mixed cryoglobulinemia have been found with the possibility of having false negative tests. Apart from possible HIV infection, none of the subjects studied had any of the conditions listed above.

In the absence of above risk factors in the study population, the aetiology of their infection could be a subject for another study even as the general consensus had it that sexual contact was a minor mode of transmission of HCV and accounts for only a small proportion of cases. However, recent data indicate that the level of HCV antibodies decreases gradually over time in the few patients in whom infection spontaneously resolves with spontaneous clearance of the viraemia after 18 to 20 years. This may lead to underestimation of the true prevalence of human exposure as not all infected persons have persistent serologic evidence of infection.

Apart from Egypt where the use of parenteral antischistosomal therapy was thought to contribute to the high prevalence of antibodies against HCV studies from other countries with higher prevalence were associated with either intravenous drug users (IVDU), partner of IVDU or blood transfusion. In our study, we excluded women who had had previous blood transfusion and intravenous drug use; an uncommon phenomenon among our women even as information was obtained about his before enlisting them for the study. In our country, narcotics are strictly under control and attract very severe sanctions thus limiting availability and or accessibility. Also economic factors (poverty) may preclude the majority of our women of reproductive age from having access to these drugs even if they dare the law. We could only therefore adduce sexual transmission to be the possible mode of transmission in this study even as the controversy on the possible role of sexual contact as a mode of transmission continues. It has been estimated that in about 10% of infected persons, no source or risk factors can be identified.

None of the women that tested positive had symptoms or clinical signs of hepatitis. It has been reported that up to 75% of cases of acute HCV infection are asymptomatic even as 80% of acute HCV infected symptomatic individuals become chronic carriers.

The measurement of alanine aminotransferase level is an important non-specific laboratory test in HCV-infected persons. It is also a means of identifying hepatic disease and the best test for monitoring HCV infection and the efficacy of therapy in the intervals between molecular testing. The results of the liver enzymes assayed were essentially normal in the HCV antibody seropositive subjects. Hepatitis C virus carriers with normal and persistently normal ALT levels have previously been reported by Persico et al. Also, less than 10% of HCV infected persons have previously been reported to have elevated transaminases.

During a 0 -10 year follow-up period, Persico et al reported that patients with persistently normal ALT levels were found not to exhibit significant histologic progression or immunologic changes even as 22% of patients with normal baseline ALT levels developed elevated levels during the first 4 years with no additional patients developing elevated levels thereafter. It was for this reason we referred the seropositive women to the physician for follow-up. Though partner tracing was not part of this but of an on-going study, men rather than women have
been reported to have more elevated-ALT enzymes even as age, sex and body mass index (BMI) were not associated with the enzyme elevations. Liver biopsies were not done for these women as this was considered a rather invasive procedure for asymptomatic pregnant women that can wait till after parturition. It has however been reported that progression to cirrhosis is slow or absent in chronic hepatitis C (CHC) patients that exhibit persistently normal alanine aminotransferase (ALT) levels. These patients were also reported to experience significantly milder disease even after 10 years of follow-up and low or stable hepatic proliferative activity index over time.

We found no adverse effects of seropositivity of HCV on the course of the pregnancy in the women studied. Floreani et al also reported non-increase in adverse pregnancy outcome in HCV infected pregnant women in their study. We did not determine the vertical transmission rate in HCV seropositive mothers as this was outside the scope and objective of the study. However, vertical transmission rates of 4%-50% have been reported by Floreani et al, but Njouom et al found no vertical transmission among 36 babies born to HCV positive mothers in our neighbouring country, Cameroon. The vertical transmission rate of HCV is however said to be influenced by other risk factors (e.g. co-infection with HIV), chronic liver disease with high levels of viraemia, high viral load, HCV genotypes and variants and sensitivity of diagnostic tests.

Maternal viraemia is also risk factor reportedly associated with a rate of 12.6% compared with a lower rate of 1.5% in non-viraemic women. Where the woman was co-infected with HIV the risk of vertical transmission was reportedly increased.

Breast feeding has not been shown to increase the rate of transmission of hepatitis C even though available data do not exclude the possibility of a low transmission rate. The women were therefore counseled along these lines. The women were also counseled about the non-liability of their offspring posing danger to other sibs or friends in daycare or other public places as HCV is not contagious and the children have been reported to pose no risk for their surroundings.

The treatment for HCV is expensive and complex requiring rigorous monitoring which could not be provided for these women during pregnancy. Apart from cost and availability, safety concerns in pregnancy precluded treatment at the time of the study. A search for treatment modality of HCV in pregnancy in the literature was scarce. No data are available to determine whether interferon therapy or other antiviral agents can reduce the risk of vertical transmission of HCV infection. Consequently, the women were not subjected to treatment for the hepatitis.

Outside pregnancy, HCV infection can be treated with monotherapy using interferon alfa or combination of interferon alfa and ribavirin or attachment of polyethylene glycol to interferon alfa (peginterferon alfa) which is given once weekly. In the absence of pregnancy, the recommendation for the duration of treatment is based on the HCV genotype and the pretreatment viral load which is reported to naturally fluctuate over time leading to the exclusion of viral load as routine basis for determining the treatment regimen. Specifically, 800mg -1200mg ribavirin once daily for all genotypes of HCV has been recommended, and for variable periods of 24 to 48 weeks depending on clinical improvement and laboratory evidence of reduction of viral load and tissue recovery. The women were referred to the physicians for follow-up management after delivery. In Europe, only a minority of HCV patients co-infected with HIV are reportedly treated for their hepatitis. Though the virus is known to be present in breast milk, breast feeding has not been shown to increase the rate of transmission of hepatitis C. Consequently the women were not counseled against breastfeeding.

Like hepatitis A and B Viruses, the course of acute hepatitis due to HCV is usually unaffected by pregnancy even as the women remained asymptomatic during the course of pregnancy. To our knowledge, no campaign is in place for the control of HCV whether at governmental or non-governmental levels. Symposia, to our knowledge, are hardly held to discuss the burden of HCV and the strategies to control the infection and prevent it from reaching the level of public health importance. This is probably due to lack of awareness. Apart from blood donors and symptomatic patients suspected to have hepatitis and patients requiring renal dialysis and for whom HIV, HBV and HCV screening is mandatory in our environment, nothing else is being done to contain its spread. It is worthwhile, therefore, to raise the awareness of HCV to a conscious level in all health care workers in our public and private health facilities to reinforce universal safety precautions in the handling of body fluids and tissues and the general public to modify behaviour and contain the spread of the infection. Unlike hepatitis A and hepatitis B that can be prevented by immunization, hepatitis C was yet to have a vaccine, even as multiple factors continue to prevent its development.

We were unable to classify and subtype the HCV or exclude false positive and false negative results as we

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lacked the facilities for qualitative and quantitative polymerase chain reaction (PCR) HCV RNA analysis in this centre. There are yet no vaccines for HCV. Raising the awareness of the population about preventive measures is therefore very pertinent to keeping the incidence low in our country. We are aware of the enormous burden HIV/AIDS is posing on the socio-economic life of persons in developing countries and especially Nigeria that is currently harbouring the third largest population of people living with HIV/AIDS (PLWHA). With our limited resources, evidence is often required to initiate new health or control programmes. We have in this study highlighted the existence of HCV in our obstetric population for whom prevalence of and risky behaviour for the horizontal spread of the infectious is supposedly low. That the level of HCV in the non-obstetric population may be higher as seen in blood donors in Nigeria is cause for concern. We have by this study contributed to the international body of knowledge for which contribution from sub-Saharan African countries is often limited. Considering the small size of our study population, we suggest more elaborate study, especially multi-centre studies, to determine the national prevalence of HCV. This will give backing for initiatives that will urge policy makers to put in place preventive and control measures at national or regional levels in addition to elevation of public awareness of HCV. Hepatitis C viral-induced liver disease remains the major indication for liver transplant for which our present levels of economy and health infrastructures can least support. With no vaccines and no cure, the time to act is now.

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REFERENCE


34. Resti M, Azzari C, Mannelli F, Moriondo M, Novembre E, et al. (Tuscany Study Group on


