

Original Research Articles

Title: Hepatitis C virus infection among pregnant women in Ibadan, Nigeria: Prevalence, correlates and co-infection with HIV and HBV.

Abstract

Aim

Vertical transmission of Hepatitis C virus, often enhanced in the presence of HIV and /or HBV co-infections, results in unidentified perinatally infected children who present in adulthood with long-term complications of chronic liver disease. This study was set out to determine the prevalence of chronic HCV infection, co-infection with HIV, HBV and associated risk factors among pregnant women in Ibadan, Nigeria.

Study design

A cross sectional study

Method

A total of 180 pregnant women attending the ante-natal clinic of the University College Hospital Ibadan, from March to August 2013, were screen for HCV, HBV using third generation Enzyme Linked Immuno-absorbent Assay (ELISA) and confirmatory assay using nucleic acid tests were done on positive samples. Anti-HIV-1 antibodies were identified using qualitative immunoassay determine test strips. Pretested validated questionnaire were used to obtain bio-data on sociodemographic characteristics and presence of possible risk factors for HCV infection. Data analyses was done using SPSS version 20.

Results

Overall seroprevalence of anti-HCV antibody was 1.7% (3/180), HBsAg was 8.3 % (15/180) and anti-HIV-1 antibody was 20.6 % (37/180). All HCV positive samples had HCV RNA identified in them but no detectable viraemia, while HBV DNA was identified in 86.7% (13/15) of HBsAg positive women. No co-infection between HCV/HBV and HCV/HIV was observed. Low level of education, marital status, and positive history of surgical procedures, blood transfusion and jaundice was significantly associated HCV infection.

Conclusion

Hepatitis C virus infection is less prevalent than HIV and HBV infection among pregnant women in Ibadan and the co-infection with HIV and HBV is uncommon.

Key words: Hepatitis C virus, HIV, HBV, prevalence, risk factors, pregnancy, vertical transmission.

1. Introduction

Hepatitis C virus, a blood borne pathogen found worldwide, is the leading cause of chronic liver disease and the commonest indication for liver transplant [1,2]. It was first characterized in 1989 as an enveloped single-stranded RNA virus of the Flaviviridae family and genus Hepacivirus, with about 6 major genotypes and over 50 subtypes identified [3, 4]. HCV is five times as widespread and ten times as infectious as HIV [5] and recent estimates of HCV disease burden show that about 185 million people worldwide are chronically infected [6], over 80 % of whom are asymptomatic and 55-85% progress to chronic liver disease in a slow and insidious manner [4-7]. This silent epidemic has become a major public health problem globally and deaths from liver disease secondary to HCV infection will continue to increase over the next 20 years [8]. In

resource-poor countries particularly in Sub-Saharan African region with a high HCV prevalence rate, HBV and HIV pandemic, and very limited access to accurate diagnosis and treatment; the morbidity and mortality will continue to be on the rise despite treatment outcomes that are comparable to those in well-resourced setting [4, 9,10].

Pregnant women and their infants fall among the groups less often discussed when considering the burden of HCV infection. The worldwide prevalence of HCV in the pregnant population ranges from 0.15% to 2.4% in the developed countries and estimated to be as high as 8.6% in the black populations [9-14]. Factors known to increase the risk of perinatal transmission include high maternal HCV viral loads, HIV and /or HBV co infection [4,14], transfusion of unscreened blood products, intravenous drug use [4,15], concurrent alcoholism and unsafe surgical procedures [4,16]. Sexual transmission is infrequent but can be facilitated by concurrent sexually transmitted diseases, and traumatic sexual practices [4,14]. Universal HCV screening among pregnant women is not in practice and considered unjustified given the absence of intervention to prevent vertical transmission of HCV, the absence of HCV vaccines or approved therapy to prevent HCV infection during pregnancy [1, 14]. Nevertheless, antenatal HCV testing will help identify asymptomatic women with chronic hepatitis at risk of transmitting the virus to their child and household, those who may benefit from antiviral therapy, monitoring of infected mother and child. There are no licensed treatments or guidelines for the treatment of infants or children infected with HCV infection [10,17].

In view of the HIV and HBV pandemic which has its peak in sub Saharan Africa, HCV co-infection with these viruses is very likely as they share the similar route of transmission, leading to increased risk of transmission of these viruses and hence, a vicious cycle. Multiple studies on rates of HCV co-infection among HBsAg carriers ranges from 1.3% to 18%, with an increased risk for cirrhosis, hepatocellular carcinoma (HCC) and even death [6,8,17]. In addition, maternal

68 HCV and HIV co-infection has shown that vertical transmission is 3- to 4-fold higher compared
69 to maternal HCV alone and transmission during breastfeeding occurs more often in patients co-
70 infected with HIV [14,18].

71 Vertical transmission in HCV RNA-negative pregnant women is about 0% to 3% [11], about 4%
72 to 15% in HCV RNA-positive women [1,2], but can be as high as 19% in HIV-coinfected
73 mothers [11,18]. The role of confirmatory diagnosis of chronic HCV infection by HCV RNA
74 detection is pivotal in predicting risk of transmission, progression of disease. While some
75 patients positive for HCV antibodies, who also have persistently normal liver enzymes have
76 spontaneously cleared their acute HCV infection, they continue to have detectable HCV
77 antibodies in their blood stream [19], giving a false positive result.

78 Pregnant women at high risk for HCV infection ought to be screened for anti-HCV antibodies
79 and if positive, must have HCV RNA testing done, however these are not practiced in Nigeria.
80 There is a dearth of published data on confirmed chronic HCV infection among pregnant women
81 in Nigeria and limited knowledge on actual risk of transmission from mother to child. Given that
82 most perinatal HCV infections are silent with long-term complications presenting later in
83 adulthood, with increased morbidity, mortality, and significant financial burden on the society,
84 there is need for accurate and early detection, prevention and control practices to reduce the risk
85 of vertical transmission from infected mothers.

86 This study was set out to determine prevalence of Hepatitis C virus infection among pregnant
87 women, the rate of co-infection with HIV and/or HBV infection as well as risk factors which
88 serves as identifiers of women at high risk of HCV infection. The findings are expected to
89 preempt the review of obstetrics policies in Nigeria with respect to testing, counseling and
90 follow-up of pregnant women positive for HCV infection as well as their infants.

2. Materials and Method

2.1 Study Design

This was a cross-sectional study in which consenting pregnant women, attending Ante natal clinic at the University College Hospital in southwestern Nigeria were recruited between March 2013 and August 2013 and their blood samples taken for analysis. Semi structured questionnaires which had been pretested and validated, was used to obtain socio-demographic characteristics and risk factors for HCV infections.

2.2 Study Area and Population

The University College Hospital, the pioneer tertiary health institution in Nigeria was established in 1957 with the aim of research, training and provision of health services. The hospital is located in Ibadan, south-western Nigeria, about 437 km from Abuja the Federal Capital Territory. The participants were pregnant women attending antenatal clinic in the University College Hospital and hailed from different parts of the country but largely comprised of the indigenes of Southern Nigeria. The practices of the people such as polygamy, early age of first sexual intercourse, female circumcision, are seen among these women who also visit local beauty parlor where tattooing, manicure, pedicure and fixing of artificial hair were done using shared needles and possibly contaminated sharp instruments. These women are also possible recipients of unscreened blood and blood products.

Sample size was calculated to give a 95% confidence level, a margin of error of +/- 5%, using a previous survey of HCV seroprevalence found among pregnant women attending antenatal clinic in a Medical Centre in Benin City, in southern Nigeria which was taken as 5% [20]. A total of a hundred and eighty consenting pregnant women with ages ranging between 22 and 44 years were

recruited. A written informed consent was obtained after careful explanation, in a clear language, of the concept of the study to each pregnant woman before their inclusion in the study. Ethical clearance was sought and obtained from the Joint Ethical committee of the University of Ibadan and University College Hospital Ibadan before the commencement of the study.

2.3 Specimen collection and handling

About 5mls of venous blood was collected aseptically by venipuncture into plain bottles and allowed to clot. The sera was separated by centrifugation at room temperature at 3000 rpm and stored in aliquots in the freezer at -20⁰C. This was done on every visit to the Ante natal clinic.

2.4 Laboratory Investigations

All samples were screened, using a sandwich third generation enzyme linked immunosorbent assay ELISA for anti HCV antibody (DIAPRO Diagnostic Bioprobes Milano Italy) and HBsAg, (Monolisa Ultra HBsAg by BIORAD FRANCE), while anti-HIV antibody was identified using Uni-Gold Recombigen and ALERE determine. All samples found to be positive for anti HCV antibody were re-tested with a second third generation ELISA and further analyzed for HCV nucleic acid identification and quantification using a real time quantitative polymerase chain reaction (Roche). While HBV DNA was identified using a thermocycler (Robocycler gradient 40) to run the polymerase chain reaction and gel electrophoresis to identify the amplified gene of interest. All tests were carried out according to the manufacturer's instructions as outlined in the package inserts.

2.4.1 HCV, HBV and HIV-1 detection by Enzyme Linked Immunoassay

A third generation enzyme linked immunoassay was used to identify presence of anti- HCV antibody (DIAPRO ITALY), HBsAg (Monolisa Ultra HBsAg by BIORAD FRANCE) as well as

anti-HIV-1 antibodies (Uni-Gold Recombigen and ALERE determine). It is a solid-phase simultaneous sandwich immunoassay, which employs specific monoclonal antibodies and polyclonal antibodies. Protocol for the measurement was done according to the manufacturer's instruction and reading was done at O.D. of 450 nm with an EIA plate reader. The tests ran were validated and results were interpreted according to the manufacturer's instruction.

2.4.2 Protocol for HCV RNA extraction, detection and quantification

RNA extraction from serum was done using the miTotal RNA extraction miniprep system (Green bioresearch) in accordance with manufacturer's instructions. The Bioneer's AccuPower® HCV quantitative RT-PCR Kit was used, which was composed of target specific primers, fluorogenic probe, DNA polymerase, dNTPs, reaction buffers and stabilizers. The reaction mixture for RT-PCR was prepared in a single tube and contained: HCV pre mix (50 mM KCl, 10 mM Tris-HCl, 0.01 mM EDTA, 60 nM Passive Reference [pH 8.3]), 5 mM MgCl₂, 20 pmoles/ µl of primer, each deoxynucleoside triphosphate at a concentration of 0.3 mmol, 0.4 U of RNase inhibitor/ µl, 0.025 U of Taq Gold Polymerase/ µl) was added in 45 µl of Dep C water and vortexed, thereafter 2.5 µl of extracted RNA was added to 22.5 µl of premix to make 25 µl. About 2.5 µl each of the 5 standards (positive) controls were loaded and the mixture was incubated at 45°C for 15 minutes to convert the RNA into cDNA. About 20 µl of the cDNA was added to the RT-PCR capillary tubes, labeled and loaded into the machine. Amplification was done with the following protocol; pre denaturation at 95°C for 5minutes, then 60 cycles of denaturation at 95°C for 7secs, annealing at 52°C for 7secs, extension at 72°C for 7secs and cooling at 42°C for 2secs. 5 standards were used to calculate the viral load from 200IU/ml to 2×10⁶IU /ml of sample

2.4.3 Protocol for HBV DNA extraction amplification and detection

The E.Z.N.A blood DNA Mini Kit was used for DNA extraction in accordance with manufacturer's instructions. Primer sequences used for HBV were Hep B gen 1 5' TGC GGG GTT TTA TCA TCT TCC T-3', Hep B gen 2 5'-GTT TAA ATG TAT ACC CAA AGA C-5' and Hep B gen 3 5'-CAG CGG CAT AAA GGG ACT CAA G-5'. PCR round one set up comprised; 3µl DNA extract, 1.5 µl of primers Hep gen 1 and 2, 15.5 µl of PCR water were added to the Bioneer Accu power Taq PCR premix (MgCl₂, dNTPs, Taq polymerase, Taq buffer, reaction buffer) all in a tube. While the PCR round 2 was 2µl DNA extract, 36 µl of PCR water, 2µl of primers Hep gen 2 and 3. All tubes were sealed and briefly centrifuged before amplification in PCR machine (Robocycler gradient 40). Amplification was done with the following protocol; pre denaturation at 95°C for 5minutes, then 30 cycles of denaturation at 95°C for 30secs, annealing at 50°C for 30secs, extension at 72°C for 40secs and final extension at 72°C for 5 minutes. After amplification, electrophoretic separation of PCR products was performed on 1.5% agarose gel stained with ethidium bromide, and visualized by UV illumination. Known HBV positive samples with DNA product read at 450 bp were used as positive control. To confirm the absence of contamination, a negative control reaction was included every time the gel was run. For this purpose, the RNase/DNase free water was used and no bands were expected.

2.5 Data Analysis

Data collected were subjected to descriptive and inferential statistical analysis using SPSS version 20. (SPSS Inc. Illinios, USA). The Mean, standard deviation and test of comparison using student's t-test was derived for continuous variables, while categorical variables were summarized as proportions, and further analyzed using Chi square and Fisher's exact test to assess association between the variables. Test of association using logistic regression was done

to describe the relationship between the predictor variables (risk factors for maternal infection found to be statistically significant) and the outcome variable (anti-HCV antibody). P values $\leq$ 0.05 was considered significant.

3. Results

3.1 Sociodemographic characteristics of the participants

The mean age of the one hundred and eighty participants finally enrolled was 32 (SD 4.8) years. The youngest was 22 years and the oldest was 44 years and more than half (55.6%) aged between 29 and 35 years. Almost all the women 172 (95.6%) were married and 172 (95.6%) were in a monogamous relationship. All participants had some level of education, with more than half of them 119 (66.1%) having tertiary-level education and about 77 (42.8%) of them were employed in the formal sector while 33 (18.3%) were unemployed. Multiparous respondents constitute the majority with 127 (70.6%) of the participants, with majority 149 (82.8%) in their beyond their first trimester. The socio-demographic characteristics are as illustrated in table 1.

Table 1. Socio-demographic characteristic of the pregnant women

N=180

VARIABLE	FREQUENCY	(%)
Age group (years)		
22-28	40	22.2
29-35	100	55.6
$\geq$ 35	40	22.2
Type of Family		
Monogamous	172	95.6
Polygamous	8	4.4
Level of Education		
Primary	5	2.8
Secondary	56	31.1
Tertiary	119	66.1

Marital Status		
Married	172	95.6
Single	8	4.4
Employment Status		
Employed(government/private)	77	42.8
Self Employed	70	38.9
Unemployed	33	18.3
Religion		
Christian	137	76.1
Islam	43	23.9
Gestational age		
1 st trimester	31	17.2
2 nd trimester	111	61.7
3 rd trimester	38	21.1
Parity		
Primiparous	53	29.4
Multiparous	127	70.6

199

200 3.2 Prevalence of HCV and co-infection with HIV, HBV

201 Anti- HCV antibody was detected in three (1.7%) of the pregnant women, all of whom had HCV
 202 RNA identified in their serum but had undetectable viral load levels (LLD of 200 IU/ml). HBsAg
 203 was detected in fifteen (8.3%) of the participants, thirteen of whom (86.7%) had positive serum
 204 HBV DNA identified. The overall HIV seroprevalence was 20.6% (37/180). No co-infection
 205 between HCV/HBV and HCV/HIV was observed among the participants. The OD450nm values
 206 of the HCV and HBV positive samples are shown respectively in tables 2 and 3 below, as well as
 207 the cut off values for the controls.

208

209 **Table 2.** The OD values of HCV ELISA positive samples

Sample	OD450nm value	Sample OD/ Cut off	Interpretation
Sample 1	1.252	3.202	Positive
Sample 2	0.686	1.754	Positive

Sample 3	0.573	1.465	Positive
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210

211 Positive control = 2.068 OD450nm (Greater than 1.000- Accepted)

212 Negative control = 0.039-0.042 OD450nm

213 Mean value= 0.041 OD450nm (less than 0.05- Accepted)

214 Cut off = Negative control + 0.350 = **0.391**

215 Sample OD/ cut off < 0.9 = Negative

216 Sample OD/cut off > 1.1 = Positive

217

218 **Table 3.** The OD values of HBV ELISA positive samples

Sample	OD450nm value	Sample OD/ Cut off	Sample	OD450nm value	Sample OD/ Cut off
Sample 1	1.718	17.010	Sample 9	1.322	13.089
Sample 2	0.910	9.010	Sample 10	0.950	9.406
Sample 3	0.875	8.663	Sample 11	2.412	23.881
Sample 4	0.946	9.366	Sample 12	1.655	16.386
Sample 5	1.788	17.702	Sample 13	0.900	8.911
Sample 6	1.124	11.129	Sample 14	1.614	15.980
Sample 7	1.020	10.010	Sample 15	1.318	13.050
Sample 8	1.321	13.079			

219

220 Positive controls = 1.714-1.723-1.811 OD450nm

221 Mean value = 1.749 (Greater than 1.000- Accepted)

222 Negative controls = 0.040-0.055-0.069 OD450nm

223 Mean value= 0.051 OD450nm (less than 0.08- Accepted)

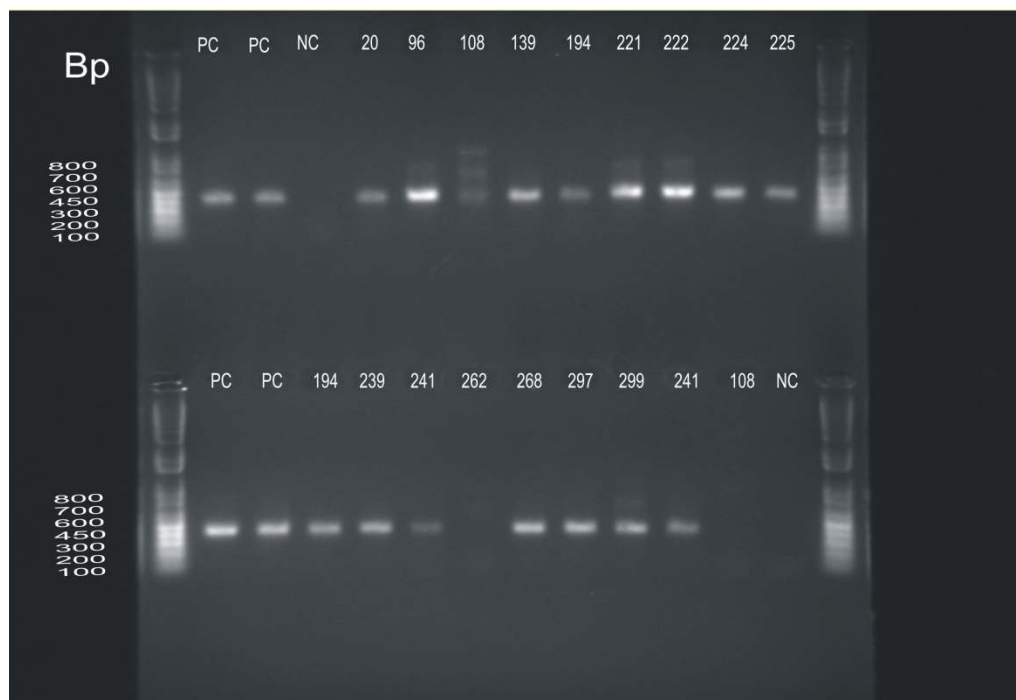
224 Cut off = Negative control + 0.05 = **0.101**

225 Sample OD/ cut off < 1 = Negative

226 Sample OD/cut off > 1 = Positive

227

228



230

231 **Figure 1:** Gel electrophoresis on PCR product for HBV DNA

232 Bp - DNA ladder at 100 bp gradient

233 PC- positive control

234 NC- negative control

235 The numbered wells represent different participant samples

236 Positive samples: 20, 96, 139, 194, 221, 222, 224, 225, 239, 241, 268, 297, 299,

237 Negative samples: 108, 262

238

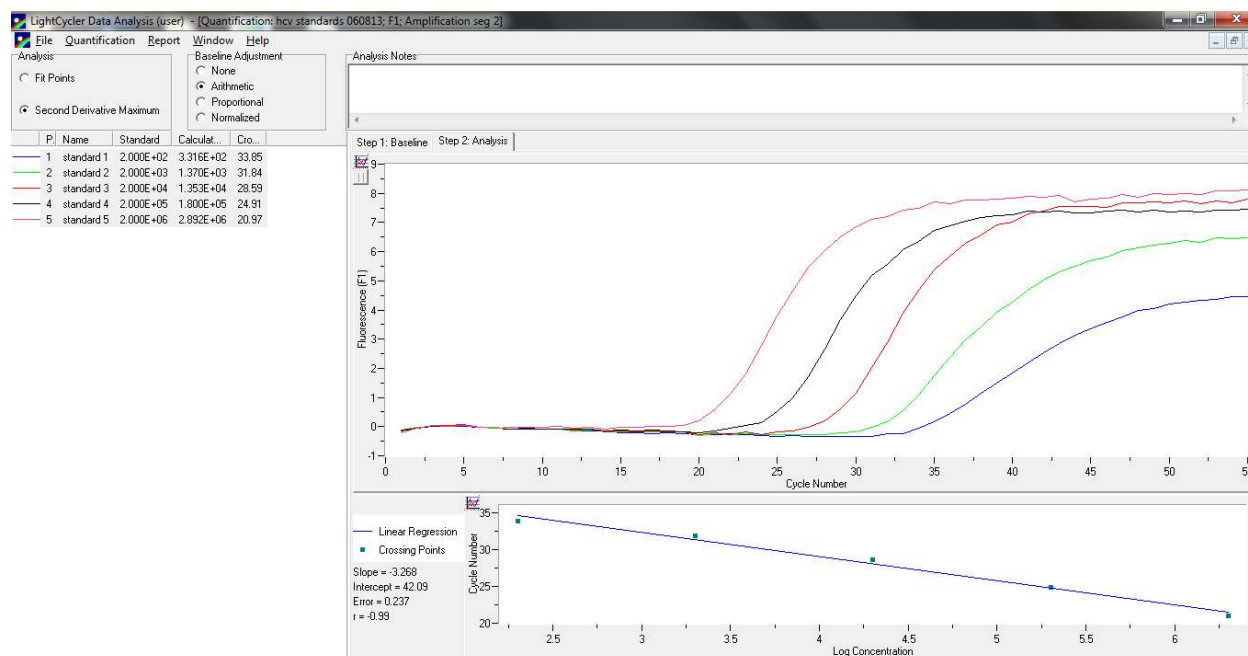


Figure 2: Graph showing the 5 standards used with their viral loads in quantification of HCV

3.3 Risk factors for HCV infection among the participants

The mean age of anti HCV sero-positive pregnant women was 29.3(±5.51) years as compared with 32.1.05(±4.78) years among the sero-negative respondents in the study, student *t* test did not show a statistically significant difference between the mean age (P=0.321). Marital status of the respondents and their level of education was found to be significantly associated with the patients' HCV infection status ($X^2=5.995$, P-value=0.014) and ($X^2=10.733$, P-value=0.005) respectively. However, no significant positive association was found between factors such as religion, gestational age and parity and the likelihood of having Hepatitis C virus infection. This is further illustrated in table 4 below

Blood transfusion was found to be significantly associated with HCV infection ($X^2=7.106$, P-value = 0.05), likewise positive surgical history ($X^2=5.232$, P-value= 0.022) and history of jaundice ($X^2=7.978$, P-value= 0.003). However, this study shows that the number of current and lifetime sexual partners, age at first sexual experience, previous contact with HBV, HCV

infected persons and other sexually transmitted infection were not associated with Hepatitis C virus infection. Similarly, no significant relationship was observed between HCV infection and HIV status or consistent use of condom. This is further illustrated in table 5 below.

Table 4. Socio-demographic data and outcome of anti –HCV antibody tested among pregnant women at the University College Hospital

Variable	Anti-HCV antibody positive(N%) N=3	Anti-HCV antibody negative(N%) N=177	X^2	P value
Age group(years)				
22-28	1(33.3%)	39(22%)	0.015	0.633
29-35	2(66.7%)	98(55.4%)		
≥ 35	0(0%)	40(22.6%)		
Type of Family				
Monogamous	3(100%)	169(95.5%)	0.142	0.872
Polygamous	0(0%)	8 (4.5%)		
Level of Education				
Primary	1(33.30%)	4 (2.3%)	10.755	0.005*
Secondary	1(33.3%)	55(31%)		
Tertiary	1(33.3%)	118(66.7%)		
Marital Status				
Married	2(66.7%)	170(96%)	5.995	0.014*
Single	1(33.3%)	7 (4%)		
Religion				
Christian	2(66.7%)	135(76.3%)	0.150	0.561
Islam	1(33.3.%)	42(23.7%)		
Gestational age				
1st trimester	0(0%)	31(17.5%)	1.896	0.387
2nd trimester	3(100%)	108(61%)		
3rd trimester	0(0%)	38(21.5%)		
Parity				
Primiparous	0(0%)	53(29.9%)	1.273	0.349

Multiparous	3(100%)	124(70.1%)
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***Significant at 5% level of significance**

Table 5. Risk factors and outcome of anti-HCV antibody tested among pregnant women in University College Hospital Ibadan.

Variable	anti-HCV antibody positive(%) N=3	anti-HCV antibody negative(%) N=177	X ²	P value
Sexual contact with known HCV positive persons				
Yes	0(0%)	6 (3.4%)	0.105	0.903
No	3(100%)	171(96.6%)		
Current sex partners				
One	2(66,7%)	155(87.6%)	1.157	0.338
More than one	1(33.3%)	22(12.4%)		
Lifetime sex partners				
One	2(66.7%)	107(60.5%)	0.048	0.657
More than one	1(33.3%)	70(39.5%)		
Blood Transfusion				
Yes	2(66.7%)	23 (13%)	7.106	0.05*
No	1(33.3%)	154 (87%)		
Age at First sexual exposure				
<15years	0(0%)	4 (2.3%)	0.069	0.934
>15years	3(100%)	173(97.7%)		
Condom use				
Yes	0(0%)	62(35%)	1.603	0.279
No	3(100%)	115(65%)		
Past STI ¹				
Yes	1(33.3%)	31(17.5%)	0.505	0.446
No	2(66.7%)	146(82.5%)		
Surgical procedure				
Yes	2(66.7%)	29(16.4%)	5.232	0.022*
No	1(33.3%)	148(83.6%)		
HIV Status				
Positive	0(0%)	37(20.9%)	0.789	0.499
Negative	3(100%)	140(79.1%)		
History of Jaundice				

Yes	2(66.7%)	4(2.3%)	7.978	0.003*
No	1(33.3%)	173(97.7%)		

*Significant at 5% level of significance.

¹ Sexually Transmitted infection

In table 6 below, logistic regression analysis was done to assess predictors of Hepatitis C virus infection. A stepwise multivariate analysis showed that the presence of anti-HCV among pregnant women was independently associated with a previous history of jaundice (P = 0.011), while blood transfusion, level of education, marital status and previous surgical history lost their statistical significance.

Table 6: Logistic regression analysis of risk factors for Hepatitis C virus infection

	Bivariate analysis				Multivariate analysis			
	B	P-value	Odd's ratio	95% CI	B	P-value	Odd's ratio	95% CI
Level of education								
Primary (REF)			1.000				1.000	
Secondary	-2.621	0.082	0.073	0.004-1.392	-0.192	0.915	0.826	0.024-27.827
Tertiary	-3.384	0.024	0.034	0.002-0.645	-0.683	0.750	0.531	0.011-26.267
Marital Status								
Single (REF)			1.000				1.000	
Married	-2.497	0.052	0.082	0.007-1.020	-2.759	0.144	0.063	0.002-2.568
Blood Transfusion								
Yes	2.595	0.037	13.391	1.167-153.656	2.618	0.102	13.707	0.597-31.811
No(REF)			1.000				1.000	
Past surgical history								
Yes	2.323	0.061	10.207	0.896-116.314	1.308	0.383	0.270	0.014-5.098
No(REF)			1.000		-2.522		1.000	
History of Jaundice								
Yes	4.460	0.001	86.500	6.44-1161.20	3.810	0.011*	4.460	1.325-11.330

274

275 REF-REFERENCE VARIABLE

276 *-STATISTICALLY SIGNIFICANT

277

278 **4. Discussion**

279 A hospital-based cross sectional study was carried out at the ante-natal clinic of the University
280 College Hospital Ibadan in southwestern Nigeria. One hundred and eighty pregnant women were
281 recruited, their serum analyzed for anti-HCV antibody, HCV RNA, HBsAg, HBV DNA, as well
282 as anti-HIV-1 antibody, while socio demographic characteristics and risk factors for HCV
283 infection was assessed with pretested questionnaires.

284 The prevalence of HCV infection was found to be 1.7% which is consistent with the 2.1% quoted
285 for Nigeria in the Hepatitis C global prevalence data, published in 2010 [2]and similar to the
286 1.86% found among pregnant women in University of Benin Teaching Hospital by Onakewhor
287 *et al* [12]. It is however lower than the 5% reported in Benin City by Duru *et al* [20], while lower
288 rates of 0.5% reported in Gwagwalada by Agarry *et al* [21] and 0.4% in Calabar by Mboto *et al*
289 [22] were observed in Nigeria. When compared with findings from other African countries, it is
290 similar to the prevalence rate of 1.8% found among pregnant women in Cameroon [5], the 2%,
291 2.1% and 2.6% reported in Burkina Faso, Gabon and Côte d'Ivoire respectively [23-25].
292 However it is lower than 17-26% in Egypt and 5% in Pakistan [9, 21]. In other parts of the
293 world, it is higher than the 0.3% reported by Todd *et al* in Afghanistan [26].

294 About 8.3% of the pregnant women tested positive for HBsAg, this is similar to findings in other
295 hospital based studies in Nigeria [27,28]. Lower prevalence rates ranging from 2.2%- 6.7%
296 [29,30] and higher prevalence ranging from 9.3-15.8% [21,31], were observed in different parts

of the country. This depicts a trend that follows a low prevalence from the southern parts of the country increasing to its highest of 15.8% in the northern parts.

Hepatitis C virus is transmitted vertically in 5-15% of all pregnancies in HCV seropositive women [1,2]. In this study, all participants positive for anti-HCV antibody had HCV-RNA identified in their serum suggesting high rate of active infection and increased risk of vertical transmission among these women. All anti-HCV positive participants in this study had undetectable viral load, a finding not uncommon in sub-Saharan Africa [18]. Fluctuation in HCV RNA levels, low sensitivity of the PCR machine, technicalities in sample processing/storage, and the heterogeneity and complex nature of the virus may account for the low rate of detectable viraemia; these are few of the challenges encountered in sub-Saharan Africa. Determination of HCV viraemia in pregnancy permit more accurate prediction of infant outcome as it is likely that women found consistently PCR negative during pregnancy have a near zero risk of vertical transmission [14]. About 86.7% of the HBsAg sero-positive pregnant women had HBV DNA identified in their serum, majority (73.3%) of whom presented in their second and third trimester. In the absence of early detection and treatment in these pregnant women, the risk of transmitting HBV to their newborns at birth is nearly 100% [32].

This brings to mind the need for high quality diagnostic methods such as nucleic acid testing for accurate identification of women with asymptomatic viral infections who are likely to transmit the virus to their newborn and household. It is also vital in the management and prognostication of infected individuals and will help reduce incidence of false positive results, thereby giving a more robust prevalence rate reports. It should be established in every tertiary institution across the country, however very few health care institutions have facilities/equipment for molecular diagnosis of viral infections in Nigeria, this may be due to limited resources and lack of trained personnel.

The mean age of 29.3($\pm$ 5.51) was found among the anti-HCV antibody sero-positive women, all of whom were below 35 years of age. Age is a known risk factor for hepatitis C infection; seropositivity has been reported to increase until the age 40 and then declines over time [24]. The finding in the study is in keeping with similar studies observed in Gabon, Burkina Faso [24], as well as Japan [1] and can be explained by the greater probability of exposure of these women to risk factors such as multiple sexual partners, multiparty, surgical procedure and blood transfusion.

Majority (66.1%) of the pregnant women tested had tertiary education. This may be because this study was tertiary hospital-based and in an urban center. Increasing level of education was noted to be inversely related with HCV and HBV infection, this finding was in agreement with that found in Nnewi by Eke *et al* [27], Kano by Yakassai *et al* [28], Port-Harcourt by Obi *et al* [29], and in Minna by Ndams *et al* [31]. This may be explained by the fact that a higher educational status is associated with greater awareness of the infection, its route of transmission and prevention, indicating the positive influence of education and public enlightenment/awareness on the carrier rate of HCV/HBV infection.

The presence of cosmetic alterations in the form of body piercing or tattooing, cultural practices that allow female circumcision and scarification marks as well as unsafe injection practices should be taken into consideration whenever assessing the risk of an individual having HCV infection. The presence of these risk factors were increased among the sero-positive women in the study, however they were not statistically significant. This might be due to a decline in these practices as a result of the nationwide public enlightenment and higher educational level. Nevertheless, with the strength of the sample size, and the urban setting of the study, caution must be applied, as the findings might not be transferable to larger studies done in rural

communities where these cultural practices persists and patronage of chemist manned by unqualified medical personnel is common.

Blood transfusion was a significant risk factor for the acquisition of Hepatitis C infection in this study. This was also observed in Congo as reported by Laurent *et al* [33] but varied from findings reported by Mboto *et al* in Calabar [22]. The use of paid blood donors is a major source of HCV infection in the developing countries; unfortunately, there are several countries that do not consistently screen blood donors for HCV. This calls for the strengthening of the national regulatory policy on universal screening of blood and blood product with the view of curtailing transmission through this route.

Similar to findings reported by Laurent *et al* [33], sexual behavioural risk factors such as early age at sexual debut, multiple sexual partners, inconsistent condom use, were not significant predictors of HCV infection in this study, however, these women are at risk of other STIs. The efficacy of transmission of HCV infection via sexual intercourse is lower than it is for other sexually transmitted viral infections [1, 2,11,21], and some experts are of the opinion that HCV and HIV are not transmitted via the same routes in sub-Saharan Africa [18]. Some studies have reported very low intra-spousal transmission of HCV among monogamous couples and the CDC does not recommend the use of barrier precautions among heterosexual monogamous couples to prevent HCV transmission [1,2,11]. Nevertheless the role of sexual transmission in the spread of HCV should no longer be overlooked and there is increasing need for education of the general population on the evils of risky sexual behaviour.

A previous history of jaundice has been identified as independent risk factor for HCV infection in this study. This is in agreement with studies that have identified contact with persons with Jaundice as independent risk factor to HCV infection [11, 15, 21]. It is therefore important to adequately screen all pregnant women with history of jaundice. Past surgical history was found

significant on univariate analysis and this is in order in view of the various route of transmission viz a viz blood transfusion, use of poorly sterilized instruments, that comes with surgical procedures.

This study indicates that HCV/HBV and HCV/HIV co-infection are uncommon among pregnant women in Ibadan, this is similar to findings reported in Gwagwalada and Benin City both in Nigeria [21,34]. However, studies done in Yaoundé Cameroon by Njouom *et al* [5] revealed HCV/HBV and HCV/HIV co-infection rates of 7.9% and 6.7% respectively, while HCV/HIV co-infection rate of 1.3% was reported in Burkina Faso [23]. In a recent meta-analysis of HCV infection in sub-Saharan Africa by Rao *et al*, HCV/HIV co-infection rate of 5.7% was reported [18], with lower co-infection rates among pregnant women and low risk cohorts. In a different population like among blood donors, commercial sex workers, intravenous drug users, and haemodialysis patients, high co-infection rates has been reported [35].

Co-infection of HBV and HIV is a more common finding, and the prevalence of both viruses in the general population is higher than HCV infection, this may account for the finding in this study. The low sexual transmission rate of HCV infection when compared with HBV and HIV may explain the low co-infection rate observed. Approximately 21% (37/180) of the participant in this study were positive for HIV-1 and positive patients with low CD4 cell count are often unable to produce antibodies against other pathogens such as HCV, thus HCV RNA or viral load test will be required to identify those with HCV infection. This is important because once the phase of immunocompetence is initiated via the use of antiretrovirals, the course of Hepatitis C is aggravated and more often than not will go undetected leading to significant morbidity and mortality. Hence, HCV-HIV and HCV-HBV co-infection is of grave concern in sub-Saharan Africa where these viral infections are endemic. If unchecked, the increased vertical

transmission, accelerated liver disease progression and increased rate of chronicity and hepatocellular cancer would enforce the HCV pandemic with devastating consequences.

While some countries practice selective screening of “high risk” women in pregnancy, experts are advocating universal HCV screening in pregnancy as some studies have demonstrated the failure of selective antenatal screening policy to identify some previously undetected HCV infections [23]. In developing countries with limited resources, non-identification of asymptomatic HCV infection may be due to failure to identify and screen all women at high risk of HCV infection, and refusal to HCV test. In Nigeria, there is need for a nationwide survey, with high quality diagnostic method, to accurately determine the prevalence and risk factors for HCV/HBV and HIV infections among pregnant women, as well as a review of obstetrics policies as regards antenatal screening.

There are a few limitations in our study. The study was not a community based study, but rather a hospital based study carried out among pregnant women attending the antenatal clinic (ANC) of the hospital, and thus may not be used as a true reflection of Ibadan as a whole. However, University hospital provides ANC services to majority of Ibadan pregnant women. The selection of participants for the study was limited to the first 180 consenting pregnant women who met the eligibility criteria and this may have introduced a bias. It is unlikely that bias was introduced, however, owing the use of a randomized sampling technique in selection of participants.

5. Conclusion

There is a low prevalence of chronic HCV infection among these women; hence HCV-HBV and HCV-HIV co-infection is uncommon despite a high prevalence of HBV and HIV infection.

While parenteral transmission including blood transfusion and unsafe surgical procedures, possibly plays a major role, sexual transmission of HCV among pregnant women seems to be limited. It is important to keep educating the masses on mode of spread of these viruses, implementation of universal precautions and avoidance of high-risk sexual behavior. Preventing exposure to infected blood or blood products, safe sexual practices, the use of condom during sexual contact, adequate sterilization of hospital equipment and materials, needle-exchange programs for injecting drug users may help to limit the spread of HCV infection. There is need for larger surveys with nucleic acid testing for more robust prevalence estimates to inform development of health care policies on prevention, control and treatment programmes. Pregnant women at high risk for HCV infection should be screened for anti-HCV, and if positive, HCV RNA, HBV and HIV testing should be performed. Infants of women with hepatitis C infection should be screened and followed up [11].

Consent: Consent was obtained from all the study participants.

Ethical Approval: The study protocol was approved by the Institutional Review Board of the University of Ibadan, and the University College Hospital both in Oyo State, Nigeria.

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