

ORIGINAL ARTICLE

HEPATITIS C VIRUS (HCV) SEROPREVALENCE, ANTIGENAEMIA AND ASSOCIATED RISK FACTORS AMONG PREGNANT WOMEN IN NIGERIA

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ABSTRACT

Background & Aims: Hepatitis C viral infection is a significant public health challenge with potential risk of progressing to liver cirrhosis and hepatocellular carcinoma (HCC). Actively infected mothers can transmit the virus to their babies who may develop liver cirrhosis and HCC as young adults. We determined the seroprevalence of HCV, its antigenaemia and associated risk factors among pregnant women.

Methods: We recruited 400 pregnant women and tested their serum for HCV antibodies using immunochromatographic test and determined the HCV core antigenaemia among HCV sero-positives by enzyme-immunoassay (EIA). The bio-socio-demographic variables of the participants were statistically correlated to the test results.

Results: Seroprevalence of HCV was 5.8% (23/400) and the prevalence of HCV core antigenaemia was 73.9% (17/23). None of the bio-socio-demographic variables of the participants and other known risk factors evaluated had significant influence on either seroprevalence of HCV or its antigenaemia. Only the employment status of the participants' husbands ($p=0.01$) significantly affected seropositivity of HCV.

Conclusion: HCV core antigenaemia is high among pregnant women who have antibodies to HCV in our environment and this signifies an active hepatitis C virus infection.

Key Words: HCV, core antigen, antibodies, pregnancy, Nigeria

INTRODUCTION

HCV is a single stranded RNA virus. It is the only member of the genus hepacivirus and belongs to the family of flaviviridae (1, 2). HCV has emerged as the most important cause of acute hepatitis and jaundice in pregnancy (3). About 85.0% of infected individuals become chronic carriers unlike hepatitis B where 5.0-10.0% of the infected individuals become chronic carriers.(4) Chronic HCV infection often progress to liver cirrhosis and HCC making it the leading indication for liver transplant in United States of America (3). It is now recognized that HCV infection is associated with substantial morbidity and mortality hence, clearly represents a global public health challenge (2).

Each year, more than 350, 000 people die of HCV related conditions including liver cirrhosis and liver cancer because HCV is a leading cause of chronic

liver disease (5,6). An estimated 2.0-3.0% of the world's population is living with HCV infection (5).The world Health Organization (WHO) estimates that 170 million people are infected with HCV globally and 3.0-4.0 million infections occur per year (7). Africa has the highest WHO estimated HCV prevalence (5.3%) with Egypt being at the peak having prevalence ranging between 10.0-50.0% in different communities of that country (7-9).

HCV infection can be detected with antibody and antigen tests. Positive test result for HCV ribonucleic acid (RNA) using HCV reverse transcriptase polymerase chain reaction (HCV RT-PCR) is currently the acceptable indicator of active infections(10). HCV core antigen is an alternative to HCV RNA testing for detecting active HCV infection especially in a low resource setting (11). The measurement of HCV core antigen has been shown to be an indicator of active infection (12). It can be used for detection of pre seroconversion window samples especially in universal blood screening and screening of high risk

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patients. They are used for diagnostic test, reflex test after antibody testing to distinguish infected from non infected HCV seropositive individuals. It is also used for monitoring antiviral therapy in HCV positive patient (13). HCV core antigen has the advantage of being used for confirmation of infection(14).

It utilizes standard ELISA format, it is easy to perform, cheap and less cumbersome(14). In a setting where samples need to be shipped to a laboratory, HCV core antigen could prove to be more stable than HCV RNA.(15) HCV RNA testing is technologically difficult, cumbersome, expensive and require more skill.(14) Hence, the need for HCV core antigen testing in low resource environment. Most of the HCV seropositive samples tested for HCV core antigen, the detection rate were between 50-76.7%.(10-14)

The transmission of HCV can follow exposure to infected blood and blood products including blood transfusion. Intravenous drug abuse, transplantation of infected organs as well as common cultural practices in some African countries such as scarification, circumcision and tattoos with unclean instruments constitute risks.(16-20)

In developed countries, the rapid improvement of healthcare conditions and the introduction of anti-HCV screening for blood donors have led to a sharp decrease in the prevalence of iatrogenic hepatitis C but the epidemic continues in developing countries where such control measures are inadequate or absent (15). Most previous studies, put the seroprevalence of HCV among pregnant women in our setting between 0.5 -9.2% (21-24), they involved assays for antibodies to HCV alone. The prevalence of HCV is important in pregnancy because of the risk of transmission to their neonate and it takes mothers with active HCV infection to transmit the infection to their neonates(25). Hence the need to determine the HCV seroprevalence, antigenaemia and associated risk factors among pregnant women at the University of Ilorin Teaching Hospital (UIH) Ilorin, Nigeria. Also there is paucity of information on HCV infection in pregnancy and most previous studies were antibody test especially in Nigeria.

MATERIALS AND METHODS

The study was carried out in the departments of Obstetrics and Gynaecology as well as Medical Microbiology and Parasitology of the University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria between 5th of December 2013 and 6th of March 2014.

The study was a cross sectional one comprised of consented pregnant women that attended the emergency, antenatal, and the labour wards as well as the antenatal clinic of the hospital. The participants were selected by simple random sampling technique. The calculated sample size using Leslie Fisher's formula (26) including 10.0% attrition was 392 but 400 participants were recruited. The power of the study was 0.85. About 650 pregnant women attended the clinic during the study period. The averages of 120 participants were recruited monthly.

All participants were counselled and informed consent was obtained before enrolment into the study. They had their peripheral venous blood specimens collected into sterile plain bottles. The prevalence of antibodies to HCV among pregnant women and the risk factors for its acquisition was determined. Five (5) ml of blood sample was aseptically collected into the specimen bottles from each of the participants. This was transported immediately into the laboratory where it was allowed to stand to clot on the bench at room temperature (23-25⁰C). The serum was aspirated after wards and divided into two equal halves. Serum in the first vial was used to test for antibodies to HCV (serology test) while the second sample was stored at -70⁰C for detection of HCV core antigen. Only samples that tested positive by serology were analysed for HCV core antigenaemia.

HCV antibodies were detected using immunochromatographic test Kits (Micropoint HCV kit) USA. HCV core antigen assay was done with EIA kit (Cell Biolabs Incorporation United States of America).(27) All tests were done and interpreted according to the manufacturer's instructions.

Consecutive seronegative pregnant women were recruited for the study as controls in the identification of the risk factors for acquisition of HCV. The socio-demographic variables as well as other relevant information were obtained in a study proforma specially designed for this purpose. Approval for the study was obtained from the Ethical Review Committee of the University of Ilorin Teaching Hospital and written informed consent was taken from all participants.

All data were entered into computer and analyzed using statistical package for social sciences (SPSS) software package version 20. The results were expressed as percentages and means with standard deviation. The continuous variables were analyzed using the student t-test while categorical variables were analyzed with chi square test. Correlation studies were done and statistical significance was set 0.05.

RESULTS

Bio-Socio-Demography: The mean age (\pm SD) of the 400 pregnant women studied was 29.2 (\pm 4.5) years and majority, 175 (43.8%) were in the age group 26 – 30years (Table 1A & 1B). Those married were 387 (96.8%) while only 1 (0.3%) was a widow. The mean estimated Gestational Age (\pm SD) of the participants was 38.0 (\pm 2.6) weeks. Primigravidas were 120 (30.0%), 225 (56.2%) were multigravidas while 55 (13.8%) were grandmultiparae (carrying their fifth or more pregnancies). Very few of the participants, 15 (3.8%) had no formal education and most were employed 225 (64.8%). More than half of the husbands' of the study participants 220 (55.0%) were unemployed, but had tertiary education 271(67.8%).

HCV Prevalence: The seroprevalence of HCV in this study population was 5.8% (23/400). HCV core antigenaemia prevalence was 73.9% (17/23) among the HCV seropositive women (Figure 1).

Bio-Socio-Demographic characteristics as a risk for HCV: The age of the mothers was not significantly related to HCV sero-positivity (p-value = 0.869) neither was their marital status (p-value = 0.999) or any other maternal bio-socio-demographic data evaluated (Table 1A & 1B). Sero-positivity of HCV antibodies was however significantly (p=0.01) related to the employment status of the husbands of the participants

Table 1 A: Comparison of Bio-Socio-Demographic variables to HCV sero-positivity

Socio demographic variables	Screening of pregnant women for anti-bodies to Hepatitis C			χ^2	P value	OR (95% CI)
	Positive N (%)	Negative N (%)	Total N (%)			
Age-group						
15 – 20*	0 (0.0)	13 (3.4)	13 (3.2)			
21 – 25	3 (13.0)	72 (19.1)	75 (18.8)	0.54	0.925	0.00 (0.00 – 14.22)
26 – 30	9 (39.1)	166 (44.0)	175 (43.8)	0.03	0.869	0.00 (0.00 – 8.56)
31 – 35	7 (30.4)	99 (26.3)	106 (26.5)	0.11	0.740	0.00 (0.00 – 6.85)
36 – 40	4 (17.4)	24 (6.4)	28 (7.0)	0.76	0.288	0.00 (0.00 – 3.41)
> 40	0 (0.0)	3 (0.8)	3 (0.7)	-	-	-
Marital Status						
Single *	0 (0.0)	12 (3.2)	12 (3.0)			
Married	23 (100.0)	364 (96.6)	387 (96.8)	0.06	0.999	0.00 (0.00 – 7.31)
Widowed	0 (0.0)	1 (0.3)	1 (0.2)	-	-	-
Type of Family						
Monogamous	21 (91.3)	347 (92.0)	368 (92.0)			
Polygamous	2 (8.7)	30 (8.0)	32 (8.0)	0.02	0.705	0.91 (0.20 – 4.06)
Educational status						
None*	1 (4.3)	14 (3.7)	15 (3.8)			
Primary	0 (0.0)	32 (8.5)	32 (8.0)	0.15	0.319	-
Secondary	4 (17.4)	73 (19.4)	77 (19.2)	0.15	0.999	1.30 (0.15 – 10.70)
Tertiary	18 (78.3)	258 (64.8)	276 (69.0)	0.26	0.999	1.02 (0.15 – 7.15)
Occupational status						
Unemployed	6 (26.1)	80 (21.2)	86 (21.5)			
Employed	10 (43.5)	135 (35.8)	145 (36.2)	0.00	0.807	1.01 (0.31 – 3.17)
Self-employed	7 (30.4)	162 (43.0)	169 (42.2)	0.45	0.372	1.74 (0.50 – 5.99)
Total	23 (100.0)	377 (100.0)	400 (100.0)			

Table 1B: Comparison of Bio-Socio-Demographic variables to HCV sero-positivity (continued)

Socio demographic variables	Screening of pregnant women for antibodies to Hepatitis C			χ^2	P value	OR (95% CI)
	Positive N (%)	Negative N (%)	Total N (%)			
Ethnicity						
Yoruba*	20 (87.0)	341 (90.5)	361 (90.2)			
Ibo	1 (4.3)	18 (4.8)	19 (4.8)	0.21	0.999	1.06 (0.14 – 22.26)
Hausa	1 (4.3)	1 (0.3)	2 (0.5)	1.36	0.113	0.06 (0.00 – 2.24)
Others	1 (4.3)	17 (4.5)	18 (4.5)	0.28	0.999	1.00 (0.13 – 21.08)
Gravidity						
Primigravid*	5 (21.7)	115 (30.5)	120 (30.0)			
Multigravid	14 (60.9)	211 (56.0)	225 (56.2)	0.64	0.425	0.66 (0.20 – 2.01)
> 4	4 (17.4)	51 (13.5)	55 (13.8)	0.25	0.464	0.55 (0.12 – 2.58)
Husband's Occupation						
Unemployed*	2 (8.7)	35 (9.3)	37 (9.2)			
Employed	13 (56.5)	207 (54.9)	220 (55.0)	0.07	0.999	0.91 (0.00 – 4.53)
Self-employed	8 (34.8)	135 (35.8)	143 (35.8)	4.72	0.01*	0.96 (0.13 – 5.25)
Husband's educational status						
None *	2 (8.7)	39 (10.3)	41 (10.2)			
Primary	1 (4.3)	23 (6.1)	24 (6.0)	0.23	0.999	1.18 (0.08 – 34.90)
Secondary	3 (13.0)	61 (16.2)	64 (16.0)	0.18	0.999	1.04 (0.12 – 8.17)
Tertiary	17 (73.9)	254 (67.4)	271 (67.8)	0.12	0.999	0.77 (0.12 – 3.66)
Total	23 (100.0)	377 (100.0)	400 (100.0)			

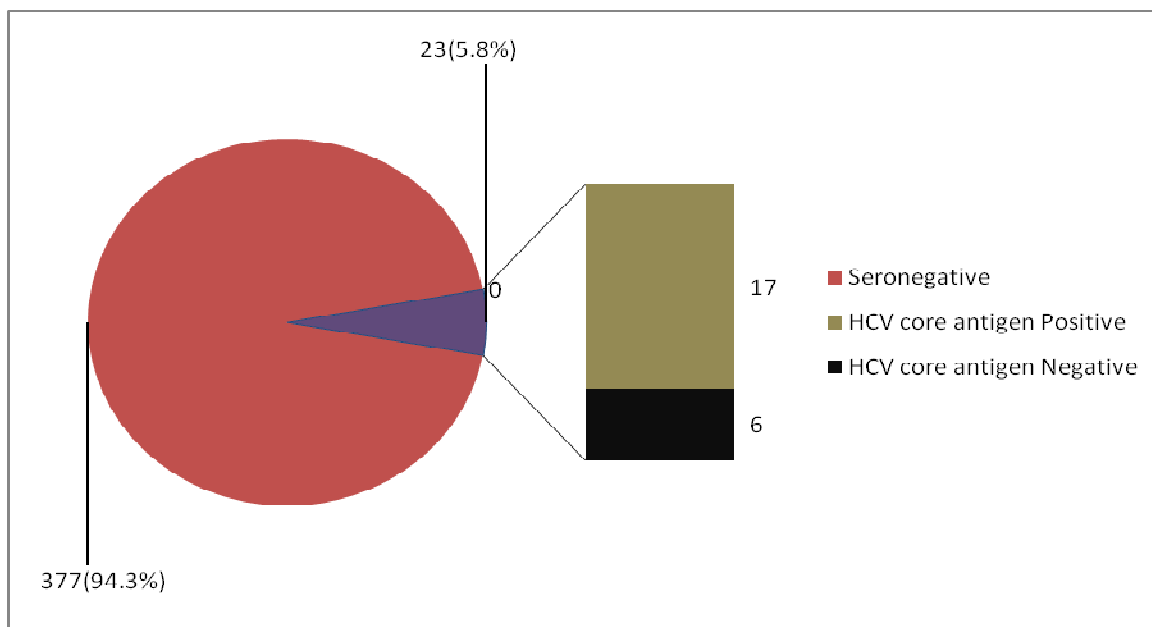


Figure 1: Prevalence of antibodies to Hepatitis C virus among pregnant women and prevalence of HCV core antigenaemia

Clinical Risk behaviours and HCV Sero-positivity:

The clinical risk behaviours of HCV infection such as blood transfusion (p value= 0.88), scarification Marks/ tattoo (p value =1.00), multiple Sexual Partners (p value=0.401), sharing of Blades (p value= 0.731), sharing of Manicuring and Pedicuring equipment (p value = 0.726). Previous history of surgical operation (p value = 0.546) and history of jaundice/ hepatitis in sexual Partner (p value = 1.00) did not significantly affect HCV sero-positivity (Tables III). None of the women in this study had history of intravenous drug abuse.

Similarly, univariate analysis did not identify any significant association with any of the established clinical risk behaviours and HCV sero-positivity or antigenaemia (Tables IV & V).

Table II: Sero-Prevalence of HCV according to age groups and age related Prevalence of HCV core antigenaemia

Age-group	Antibody (N = 23)	Core Anti-gen (N = 17)
	n (%)	n (%)
15 – 20	0 (0.0)	0 (0.0)
21 – 25	3 (13.0)	3 (17.6)
26 – 30	9 (39.1)	6 (35.3)
31 – 35	7 (30.4)	6 (35.3)
36 – 40	4 (17.5)	2 (11.8)
> 40	0 (0.0)	0 (0.0)

Table III: Sero- Prevalence of HCV and the clinical risk behaviours

Risk factors	Screening of pregnant women for antibodies to Hepatitis C			χ^2	p value	OR (95% CI)
	Positive	Negative	Total			
Blood transfusion						
Yes	5 (21.7)	45 (11.9)	50 (12.5)	1.905	0.188	2.049 (0.725 – 5.790)
No	18 (78.3)	332 (88.1)	350 (87.5)			
Scarification marks or tattoos						
Yes	11 (47.8)	176 (46.7)	187 (46.8)	0.011	1.000	1.047 (0.451 – 2.432)
No	12 (52.2)	201 (53.3)	213 (53.2)			
Multiple sexual partners						
Yes	7 (30.4)	86 (22.8)	93 (23.2)	0.706	0.401	1.480 (0.590 – 3.715)
No	16 (69.6)	291 (77.2)	307 (76.8)			
Intravenous drug abuse						
Yes	0 (0.0)	0 (0.0)	0 (0.0)	0.626	1.000	-
No	23 (100.0)	377 (100.0)	400 (100.0)			
Sharing of blades						
Yes	6 (26.1)	111 (29.4)	117 (29.2)	0.118	0.731	0.846 (0.325 – 2.202)
No	17 (73.9)	266 (70.6)	283 (70.8)			
Sharing of manicuring and pedicuring equipment						
Yes	3 (13.0)	40 (10.6)	43 (10.8)	0.134	0.726	1.264 (0.360 – 4.442)
No	20 (87.0)	337 (89.4)	357 (89.2)			
Previous history of surgical operation						
Yes	4 (17.4)	86 (22.8)	90 (22.5)	0.365	0.546	0.712 (0.236 – 2.150)
No	19 (82.6)	291 (77.2)	310 (77.5)			
History of Jaundice/ Hepatitis in partner						
Yes	1 (4.3)	17 (4.5)	18 (4.5)	0.001	1.000	0.963 (0.122 – 7.569)
No	22 (95.7)	360 (95.5)	382 (95.5)			
Total	23 (100.0)	377 (100.0)	400 (100.0)			

Table IV: Univariate Logistic regression to determine risk factors for HCV sero-positivity

Mothers; N (%)				
Risk Factors (exposure)	Total N=400	HCV Antibodies	OR (95% CI)	p value
Blood transfusion	50 (12.5)	5 (10.0)	0.488 (0.173 – 1.379)	0.176
Scarification marks or tattoos	187 (46.8)	11 (5.9)	0.955 (0.411 – 2.219)	0.915
Multiple sexual partners	93 (23.2)	7 (7.5)	0.676 (0.269 – 1.695)	0.403
Intravenous drug abuse	0 (0.0)	0 (0.0)	-	-
Sharing of blades	117 (29.2)	6 (5.1)	1.182 (0.454 – 3.078)	0.732
Sharing of manicuring and pedicuring equipment	43 (10.8)	3 (7.0)	0.791 (0.225 – 2.781)	0.715
Previous history of surgical operation	90 (22.5)	4 (4.4)	1.404 (0.465 – 4.237)	0.547
History of Jaundice/ Hepatitis in partner	18 (4.5)	1 (5.6)	1.039 (0.132 – 8.169)	0.971

OR: Odds ratio; 95% CI: 95% Confidence Interval

Table V: Univariate Logistic Regression to determine Risk Factors for HCV core Antigenaemia

Mothers; N (%)				
Risk Factors (exposure)	Total N=23	HCV core an- tigen positive	OR (95% CI)	p value
Blood transfusion	5 (21.7)	4 (80.0)	0.650 (0.058 – 7.324)	0.727
Scarification marks or tattoos	11 (47.8)	8 (72.7)	1.125 (0.175 – 7.243)	0.901
Multiple sexual partners	7 (30.4)	4 (57.1)	3.250 (0.461 – 22.927)	0.237
Intravenous drug use	0 (0.0)	0 (0.0)	-	-
Sharing of blades	6 (26.1)	4 (66.7)	1.625 (0.213 – 12.422)	0.640
Sharing of manicuring and pedicuring equipment	3 (13.0)	2 (66.7)	1.500 (0.111 – 20.299)	0.760
Previous history of surgical operation	4 (17.4)	3 (75.0)	0.933 (0.078 – 11.177)	0.957
History of Jaundice/ Hepatitis in partner	1 (4.3)	0 (0.0)	-	-

OR: Odds ratio; 95% CI: 95% Confidence Interval

DISCUSSION

The seroprevalence of HCV was 5.8% in this study. This is within the worldwide seroprevalence of HCV in pregnancy of 1.0–8.0%(28). The prevalence obtained in this study is however lower than the prevalence of 10.0–50.0% in Egypt. (7,8) The high prevalence in Egypt has however been attributed to contaminated needles and syringes used during the mass schistosomiasis treatment campaigns of 1960s – 1980s in that country. (8)

The seroprevalence of HCV in the present study is lower than 9.2% derived from a study in South Western Nigeria despite the fact that the study was conducted in a similar teaching hospital setting. (29) It is on the other hand close to 6.0% found in Maiduguri²⁴ but higher than the 3.6% in Benin(21), 0.5% in Niger Delta(22) and 4.5% of Kaduna(23). Even though, studies above were also carried out in Nigeria, the variations noticed in the prevalence of HCV in different regions may be due to the differences in the socio-cultural practices, environmental factors and the mode of transmission. The low prevalence in our study may be related to the fact that most of the participants were educated and well informed. They might have been taking precautionary measures against acquiring the virus.

Of the 23 HCV seropositive pregnant women tested for hepatitis C core antigen, 17(73.9%) were HCV core antigen positive suggesting active infection in this group of participants. This finding is in contrast to the finding in a study done in Kaduna, Nigeria where all the HCV seropositive pregnant mothers were positive for HCV RNA (18). This may be an affirmation of the superiority of HCV RT-PCR over HCV core antigen assay by EIA. Our finding however is similar to the finding by Cicioglu et al in Demirel where 72.17% of HCV seropositives participant were HCV core antigen positive¹⁰. Our finding is also similar to that of Dale et al in Vellore, where 76.7% of HCV seropositives participants were HCV core antigen positive(14). Furthermore, our finding is higher than 50.3% found by Daniel H et al (11). It is also higher than 31.0% found by Kumar in Egypt (30). The finding in this study is within the affirmation that more than 50% of anti HCV positive persons will be HCV core antigen positive (15). Variation in findings may be due to the method of testing, sensitivity and specificity of the test kits. The advantages of HCV Core antigen assay are that being an

immunoassay, it does not require sample processing as in molecular assays and a positive results confirms an infection(31).

The seroprevalence of HCV in the present study is highest among the age group 26–30years (39.1%) and 31–35years (30.4%). These age groups correspond to the age of child bearing where there is risk of exposure to HCV from sexual intercourse and blood transfusions from Obstetric complications like postpartum haemorrhage. This finding is similar to the observation among women of childbearing age in Vom, Plateau State Nigeria where women in these age groups had the highest seroprevalence of HCV. (32) However, the relationship between the age group and HCV seropositivity was not statistically significant ($p=0.869$, $OR=95\%$, $CI=0.00-8.56$). Few previous studies have similarly demonstrated no significant relationship between the age of the patients and the HCV prevalence (28,32). These however are in contrast to the study done in Egypt where HCV infection was significantly related to the old age⁸. None of the participants in this study whose age is above 40 years were HCV seropositive. Probably a national survey on the relationship between the age and HCV seroprevalence may provide the needed answer on this issue. Thus, high prevalence of HCV infection in the younger population of pregnant women in this study, constitute a future risk of paediatric HCV infection in our environment.

All the HCV seropositive pregnant women (100%) were married in this study as found elsewhere. Most (78.3%) had tertiary education probably because of teaching hospital setting where the study was carried out since most educated women would prefer to consult specialists/Obstetricians. This is however in contrast to another report also from Nigeria where women with low level of education had the highest seroprevalence.(32)

For our study, the relationship between the HCV seropositivity and the education level ($p= 0.99$), occupation ($p=0.807$), and other bio-socio-demographic variables of the participants were not significant as previously documented. The participants' husbands employment status significantly affected HCV seroprevalence of their wives (p value = 0.001, $OR= 0.96$, $CI 0.13 -5.25$).

Of the HCV seropositive pregnant women, 21.7% had history of blood transfusion. Blood transfusion has been recognized as a risk factor for acquisition of HCV as seen in a study among women in Pakistan. (33) However, no significant association was found

between blood transfusion and HCV sero-positivity (p value=0.188, OR= 2.049, 95% CI= 0.725– 5.790). This finding has been similarly documented elsewhere(32-33). Since 78.3% of HCV seropositives in our study did not have history of blood transfusion and yet they were HCV seropositive, it is a pointer to other possible route of acquisition of HCV.

History of multiple sexual partners was found in 30.4% of HCV seropositive women in this study as was also identified in some other studies³²⁻³³ although it did not significantly (p= 0.4) affect HCV sero-positivity. Furthermore, none of the HCV seropositive women had history of intravenous drug abuse probably because it is not a common practice in our environment. Intravenous drug abuse has been found as one of the major risk factors for HCV.(29)

Scarification marks making and tattooing was recorded among 47.8% of the HCV seropositive women in our study as previously established³² but this finding was similarly not significant (p value = 1.00). History of surgical operation has been identified as a risk for HCV. (34) In our study, 17.4% of HCV positive women had positive history of surgery which was not significantly related to the infection. Furthermore, sharing of blades as well as manicure and pedicure equipment found in 26.1% and 13.0% of HCV positive pregnant women were not significantly associated with HCV sero-positivity.

Generally, the known risk factors for HCV infection were not significantly related to either HCV sero-positivity or antigenemia in this study. The low prevalence of these factors in the study population may be due to the fact that many of the participants were highly educated. This might have influenced their socio-cultural practices such as scarification, body piercing and tattooing that increase the risk of infection. Only one of the women reported past history of jaundice /hepatitis in her partner and she was HCV core antigen negative. It may be that, others were not aware of their partners' status or the infection. This further suggests the need for health education on hepatitis, its complications and prevention in our environment.

CONCLUSION: HCV core antigenaemia is high among pregnant women who have antibodies to HCV in our environment. This finding poses a potential danger of neonatal HCV infection and its chronic complication in the affected mothers' babies. The significant relationship between participants' husbands' employment status and HCV seropositivity of their wives also calls for an educational intervention as well as empowerment of the husbands.

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