HEPATITIS C VIRUS AMONG PREGNANT WOMENPEOPLE LIVING WITH HIVAIDS ATTENDING CLINIC AT UNTH ITUKUOZALLA

BY

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MB/2008/368

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A RESEARCH PROJECT PRESENTED TO THE DEPARTMENT OF MICROBIOLOGY AND BIOTECHNOLOGY,

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SUPERVISOR: MR NWOBODO HUMPHREY

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CERTIFICATION

I certify that this research project was carried out by JideunoOnyinyechi W. (MB/2008/368) in the department of Microbiology and Biotechnology, faculty of Natural sciences Caritas University, Amorji-Nike Enugu. The department recognizes that JideunoOnyinyechi W. (MB/2008/368) bears full responsibility for this work.

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DEDICATION

Dedicated to almighty God for his love and mercy.

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ABSTRACT

A total of 50 blood (30 pregnant and 20 HIV) samples were obtained from pregnant women and PLWHA attending clinic at UNTH Ituku-ozalla. Samples were screened for hepatitis c viral infection using the rapid one step hepatitis C virus test strip. Two (6.66%) pregnant women were positive and four (20%) HIV patients were positive for Hepatitis C, giving an overall prevalence rate of (26.7%). The infection was the same in male (3) and in female (3). Those aged 23-34 years recorded a higher prevalence of (20%) than those in the age group 19-23. This higher prevalence of hepatitis C suggests that pregnant women and PLWHA may be at risk of hepatitis due to hepatitis C virus. Therefore routine screening of pregnant women and PLWHA should be instituted for early diagnosis and management of cases.

CHAPTER ONE

1.1INTRODUCTION

Hepatitis C infection is an infection of the liver caused by the hepatitis C virus (HCV). HCV is one of several viruses that can cause hepatitis. 'Hepatitis' means inflammation of the liver(Ryan and Ray,2004).It is unrelated to the other common hepatitis viruses (for example, hepatitis A or hepatitis B). HCV is a member of the*hepacivirus* genus in the family*Flaviviridae*. There are at least six distinctly different strains of the virus which have different genetic profiles (genotypes). In the U. S., genotype 1 is the most common form of HCV. Even within a single genotype there may be some variations (genotype 1a and 1b, for example). Genotyping is important to guide treatment because some viral genotype respond better to therapy than others (Wilkins *et al.* 2009).

According to Hepatitis C NSW (2010), here are some of the things that can happen once the body contract hepatitis C.

The body may deal with hepatitis C of its own accord and you may never get sick.About 25% of all people who contract hepatitis C will clear the infection (although this happen less commonly in people with HIV). For a range of reasons, hepatitis C infection is eradicated from the body in these people, usually within 12 months of having been infected.

Hepatitis C may remain present in the body. About three quarters of people who contract hepatitis C will be chronically infected. This means that they have detectable hepatitis C virus (measured by a PCR test) in their blood for a period of longer than twelve months. People in this group may be at risk of developing liver problems over time.

The genetic diversity of HCV is one reason that it has been difficult to develop an effective vaccine since the vaccine must protect against all genotypes. It is difficult for the human immune system to eliminate HCV from the body, and infection with HCV usually becomes chronic. Over decades, chronic infection with HCV damages the liver and can cause liver failure. Up to 85% of newly-infected people fail to eliminate the virus and become chronically infected. Infection is most commonly detected among people who are 40 to 60 years of age, reflecting the high rates of infection in the 1970s and 1980s. There are 8,000 to 10,000 deaths each year in the U.S. related to HCV infection. HCV infection is the leading cause of liver transplantation in the U.S and is a risk factor for liver cancer.

Most of the signs and symptoms of HCV infection relate to the liver. Less commonly, HCV infection causes conditions outside of the liver. Symptoms are generally mild and vague, including a decreased appetite, fatigue, nausea, fever. Headache, muscle or joint pains, and weigh loss.Hepatitis C after many years

becomes the primary cause of cirrhosis and liver cancer. About 10–30% of people develop cirrhosis over 30 years (Meisel, *et al.* 1995).

HCV infection can cause the body to produce unusual antibodies called 'cryoglobulins'. These cryoglobulins cause inflammation of the arteries (vasculitis) which may damage the skin, joints, and kidneys. In addition, these patients may develop Raynaud's phenomenon in which the fingers and toes turn color (white, then purple, then red) and become painful at cold temperatures(Iannuzzella, and Vaglio, 2010).

Two skin conditions, lichen planus and porphyria cutaneatarda, have been associated with chronic infection with HCV. HCV also is associated with B-cell lymphoma, a cancer of the lymph system.

Doctors use various tests to determine if a person has hepatitis C. One type of test measures antibodies in the blood, indicating that a person been exposed to HCV; the two most common antibody tests are called ELISA and RIBA. Viral load tests measure how much HCV genetic material is present in the blood; the two most common viral load tests are called PCR and bDNA.

Who to test for hepatitis c virus

According to MMWR (1998) Persons who ever injected illegal drugs,

Persons who were ever on chronic (long-term) hemodialysis;

Persons with persistently abnormal alanine aminotransferase level

Persons who were notified that they received blood from a donor who later tested positive for HCV infection;

Persons who received a transfusion of blood or blood components and organ transplant before July 1992; and

Healthcare, emergency medical and public safety workers after needle sticks, or mucosal exposures to HCV-positive blood

Children born to HCV-positive women.

1.2 **OBJECTIVES**

i. To determine the prevalence of hepatitis C virus in pregnant women attending ante-natal in UNTH Ituku-ozalla

ii. To determine the age distribution where the infection occurs most

iii. To determine the prevalence of hepatitis virus in people living with HIV and AIDS attending UNTH Ituku-ozalla

CHAPTER TWO

LITERATURE REVIEW

Hepatitis C virus is a small (55-65 nm in size)enveloped single stranded positive sense RNA virus The hepatitis C virus particle consists of a core of genetic material (RNA), surrounded by an icosahedral protective shell of protein, and further encased in a lipid (fatty) envelope of cellular origin. Two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope. It is a member of the *hepacivirus*genus in the family *flaviviridae* (Rosen, 2011).

2.1 **Mode of transmission and risk factors**

According to Maheswari, and Thuluvath (2010) the primary method of transmission in the developed world is intravenous drug use (IDU), while in the developing world the main methods are blood transfusions and unsafe medical procedures. Hepatitis C virus is spread parentally, sexually (Bryan Ogeneh, 2004).

IDU is a major risk factor for hepatitis C in many parts of the world. Of 77 countries reviewed 25 including the United States were found to have prevalence of hepatitis C in the intravenous drug user population of between 60% and 80%(Xia, *et al*. 2008).

Transfusion of blood products or organ transplantation without HCV screening may carry significant risks of infection. The United States instituted universal screening in 1992 and the risk subsequently has decreased from one in 10,000 to 10,000,000 per unit of blood down from a risk of one in 200 units of blood (Day, 2009) .

Those who have experienced a needle stick injury from someone who was HCV positive have about a 1.8% chance of subsequently contracting the disease themselves, the risk is greater if the needle in question is hollow and the puncture wound is hollow.

Sexual practices that involve higher levels of trauma to the anogenital mucosa, such as anal penetrative sex, or that occur when there is a concurrent sexually transmitted infection, including HIV or genital ulceration, do present a risk (Tohme, 2010).

The United States government only recommends condom use to prevent hepatitis C transmission in those with multiple partners.

Tattooing is associated with two to threefold increased risk of hepatitis C. This can be due to either improperly sterilized equipment or contamination of the dyes being used. Tattoos or piercings performed either before the mid-1980s, "underground," or nonprofessionally are of particular concern, since sterile techniques in such settings may be lacking (Jafari, 2010).

According to Lock, (2006), Personal-care items such as razors, toothbrushes, and manicuring or pedicuring equipment can be contaminated with blood. Sharing such items can potentially lead to exposure to HCV.

Vertical transmission of hepatitis C from an infected mother to her child occurs in less than 10% of pregnancies. It is not clear when during pregnancy transmission occurs, but it may occur both during gestation and at delivery (Lam, 2010).

A long labor is associated with a greater risk of transmission. There is no evidence that breast-feeding spreads HCV; however, to be cautious, an infected mother is advised to avoid breastfeeding if her nipples are cracked and bleeding, or her viral loads are high (Alter, 2006).

2.2 **pathogenesis of hepatitis C virus**

Hepatitis C virus enters a susceptible host either directly through needle inoculation or transfusion of contaminated blood or inadvertently through breakage of a percutaneous barrier (as exemplified by sexual or perinatal transfusion) (Alter, 1997).

The virus then enters hepatocytes or other susceptible cells probably through a unique surface molecule as the viral receptor (Pileri*et al*. 1998).

After uptake, the virus uncoats and releases the genome to begin replication. The viral genome first serves as a template for the translation of polyprotein. The processed non-structural protein then form a complex with the genome and initiate synthesis of the negative strand which in turn function as the template for positive strand synthesis. The replication complex probably resides in a membraneous compartment in the cytoplasm. The RNA replicative intermediate matures and interact with the core and envelope proteins to assemble into a virion.

Incubation period

A viraemia can be detected within 1 to3 weeks of transfusion of HCV contaminated blood. The viraemia lasts for 4 to 6 months in people with acute infection and longer than 10 years in persistent infection. The average time to seroconversion after exposure to HCV is 8 to 9 weeks(Bryan Ogeneh, 2004).

2.3 **Epidemiology**

WHO (2011) Estimated that 130–170 million people, or 3% of the world's population are living with chronic hepatitis C. About 3–4 million people are infected per year, and more than 350,000 people die yearly from hepatitis C-related diseases. Rates have increased substantially in the 20th century due to a combination of IDU and intravenous medication or poorly sterilized medical equipment.

Hepatitis C virus is found among people of all ages but the incidence is high among the ages of 20-39 and males predominate (Alter, 1990).

2.4**Signs and symptoms**

Hepatitis C infection causes acute symptoms in 15% of cases and chronic symptoms in 80% cases. Symptoms are generally mild and vague, including a decreased appetite, fatigue, nausea, muscle or joint pains, and weight loss. Most cases of acute infection are not associated with jaundice (Maheswari, 2008 and Degenhardt, 2011).

2.5**Diagnosis of hepatitis C**

There are a number of diagnostic tests for hepatitis C including: HCV antibodyenzyme immunoassay or ELISA, recombinant immunoblot assay, and quantitative HCV RNApolymerase chain reaction (PCR).HCV RNA can be detected by PCR typically one to two weeks after infection, while antibodies can take substantially longer to form and thus be detected (Ozaras, 2009).

Serology

According to Wilkins, *etal*. (2010), Hepatitis C testing typically begins with blood testing to detect the presence of antibodies to the HCV using an enzyme immunoassay. If this test is positive, a confirmatory test is then performed to verify the immunoassay and to determine the viral load. A recombinant

immunoblot assay is used to verify the immunoassay and the viral load is determined by a HCV RNA polymerase chain reaction. If there is no RNA and the immunoblot is positive it means that the person had a previous infection but cleared it either with treatment or spontaneously; if the immunoblot is negative, it means that the immunoassay was wrong. It takes about 6–8 weeks following infection before the immunoassay will test positive.

Liver enzymes are variable during the initial part of the infection and on average begin to rise at seven weeks after infection. Liver enzymes are poorly related with disease severity.

Biopsy

Liver biopsies are used to determine the degree of liver damage present; however, there are risks from the procedure. The typical changes seen are lymphocytes within the parenchyma, lymphoid follicles in portal triad, and changes to the bile ducts. There are a number of blood tests available that try to determine the degree of hepatic fibrosis and alleviate the need for biopsy. (Rosen, 2011)

Screening

It is believed only 5–50% of those infected in the United States and Canada become aware of their status. Testing is recommended in those at high risk, which includes those with tattoos. Screening is also recommended in those with

elevated liver enzymes as this is frequently the only sign of chronic hepatitis. Routine screening, however, is not recommended in the United States. There are however considerations by the United States Centers for Disease Control and Prevention (CDC) as of 2012 regarding a recommendation for a single screening test for those born between 1945 and 1965.

2.6**Prevention of hepatitis C**

As of 2011, no vaccine protects against contracting hepatitis C. However, a number are under development and some have shown encouraging results (Halliday, 2011).

A combination of harm reduction strategies, such as the provision of new needles and syringes and treatment of substance use, decrease the risk of hepatitis C in intravenous drug users by about 75%. The screening of blood donors is important at a national level, as is adhering to universal precautions within healthcare facilities. Not sharing personal care items that may contain blood, such as razors and toothbrushes and always using latex condoms during sexual intercourse, particularly if you or your partner have Hepatitis C

Not donating blood, organs or tissues if you are HCV positive.

In countries where there is an insufficient supply of sterile syringes, medications should be given orally rather than via injection.

2.7**Treatment of hepatitis C**

Current treatment is a combination pegylated interferon alpha and the antiviral drug ribavirin for a period of 24 or 48 weeks, depending on HCV genotype. When combined with ribavirin, pegylated interferon-alpha-2a may be superior to pegylated interferon-alpha-2b, though the evidence is not strong. Improved outcomes are seen in 50–60% of people. Combining either boceprevir or telaprevir with ribavirin and peginterferonalfa improves antiviral response for hepatitis C genotype 1(Alter, 2007).

Adverse effects with treatment are common, with half of people getting flu like symptoms and a third experiencing emotional problems. In people with thalassemia, ribavirin appears to be useful but increases the need for transfusions.

Several alternative therapies are claimed by their proponents to be helpful for hepatitis C including milk thistle, ginseng, and colloidal silver. However, no alternative therapy has been shown to improve outcomes in hepatitis C, and no evidence exists that alternative therapies have any effect on the virus at all.

2.8**Hepatitis C virus and pregnancy**

Women with HCV are concerned about the risk of transmitting the virus to their babies during pregnancy or birth. Studies consistently show that the rate of

perinatal or vertical transmission is low, about 5% or 1 in 20. Vertical transmission is most likely to happen when the mother has a high HCV viral load; several studies have shown that no transmission occurred when women had undetectable viral loads. Studies also show that women who are co-infected with both HCV and HIV have a higher risk (15-35%) of transmitting HCV to their infants. One British study has suggested that the risk of vertical HCV transmission may be reduced through Cesarean delivery; however, according to the Society of Obstetricians and Gynecologists of Canada, "routine Cesarean section is not recommended as a specific measure to reduce the risk of vertical transmission of HCV" (Alter, 1999).

According to (Tajiri*et al.* 2001),Although HCV has been detected in breast milk in some studies, there is no indication that breastfeeding transmits the virus. Most experts do not discourage HCV-positive women from breastfeeding. But women may wish to exercise caution if their nipples are cracked or bleeding. HCV is not transmitted from mothers to children through normal household contact.

According to Terrault, who treats many women with HCV, being pregnant does not adversely affect the progression of hepatitis C. Likewise, women with HCV do not have a higher rate of pregnancy or birth complications compared to uninfected women. However, women with severe, advanced liver disease may experience difficulties during pregnancy.

Universal prenatal screening of women for HCV is not currently recommended. Babies of HCV-positive women should be tested for HCV after 12-18 months. According to the Centers for Disease Control and Prevention, most infants infected with HCV at birth have no symptoms and do well during childhood. Studies suggest that infants are more likely than adults to completely clear the virus from their bodies. HCV treatment has not been well studied in infants and children.

Ribavirin is known to cause miscarriages and birth defects, so pregnant women should not take this drug. Most doctors also recommend that interferon should not be taken during pregnancy, because its effect on the human fetus is not well known. Mothers taking Rebetron (interferon and Ribavirin therapy) should not breast feed because of the potential for an adverse reaction from the drug in their infant.

Pregnant patients with hepatitis C should be advised to;

Obtain vaccination against hepatitis viruses A and B as indicated.

Abstain from alcohol use.

Avoid hepatotoxic drugs such as acetaminophen (Tylenol) that may worsen liver damage.

Inform the infant's pediatrician of the mother's hepatitis C status.

Not donate blood, body organs, or tissue.

Not share any personal items that may have blood on them (e.g., toothbrushes and razors).

Discuss the low but present risk for transmission with their partner and discuss the need for counseling and testing. However, HCV-positive persons with one long-term, steady sex partner do not need to change their sexual practices (CDC 1998).

(Davies *et al*. 2003) "Amniocentesis in women infected with hepatitis C does not appear to significantly increase the risk of vertical transmission, but women should be counseled that very few studies have properly addressed this possibility.

Risk of vertical transmission of HCV appears to be related to the level of viraemia in the pregnant mother and not to the route of delivery. The virus does not appear to be transmitted when a woman's titer is < 10^6/mL or is negative. Although (Tejari et al and Conte et al 2000) did not find cesarean section to be protective against transmission of HCV to the neonate(Gibb et al 2000) have found the HCV maternal to child (MTC) transmission rate to be reduced in patient delivered by elective cesarean. The latter study has yet to be confirmed. Most women become pregnant during the years between 20 and 40, which is also the age group in which the incidence of hepatitis C infection is rising most quickly. Any woman with risk factors for hepatitis C (such as exposure to transfusions, contaminated needles, or injected drug use) should be

screened for hepatitis C before and during pregnancy (Hunt and Christine, 1999).

2.9 **Hepatitis C virus and HIV**

There is still some debate about whether hepatitis Caffects HIV. Some studies have suggested that hepatitis Cinfection can lead to more rapid progression of HIVbut others have not.

Hepatitis C is a more serious illness in people with HIVdisease as it progresses more rapidly in these patients.The reasons for this are not known completely butprobably relate to the possibility that your body'sability to control hepatitis C may be reduced if you haveHIV infection.

HIV can still be treated even if you also have hepatitis C.In fact, it's really important to be aware of your HIVviral load and your CD4 count, and to treat HIV tokeep it under control.Hepatitis C can affect HIV treatment choices, becauseof the potentially toxic effects some HIV drugs haveon the liver. There's no accepted list of "best HIVtreatments" for people with both HIV and hepatitis Cvirus. Ritonavir (in full doses)and nevirapine are two HIV drugs which are morelikely to affect the liver.Your doctor is likely to regularly check up on howyour liver is coping, to make sure the HIV drugs aren'tcausing any problems.

Taking care of the liver withcoinfection

According to Hepatitis C NSW (2010), one of the really important things is to have the liver function carefully and regularly tested, inaddition, avoidingother risky things which can damage or stress yourliver. This can include:

Binge drinking or heavy drinking;

Use of some prescription and other drugs: as your doctor for more information about drugswhich can affect your liver.

Many people with hepatitis C use non prescribedalternative treatments like herbs. One herb which should not be usedif you have hepatitis C related liver problems is kava.

Maintaining a healthy, balanced diet is a good idea,although there is no evidence that a special diet isneeded for people with hepatitis C.

Vaccination against hepatitis A and B is essential forpeople with both hepatitis C and HIV. These preventableforms of hepatitis can cause serious complicationsfor people who already have chronic hepatitis C.

CHAPTER THREE

3.1**Materials**

HCV test strip, centrifuge, timer, tourniquet, rack, syringe, cotton wool, test tube, EDTA bottle, gloves

32**Study Population**

This study was a cross-sectional seroprevalence study involving pregnant women attending ante-natal and HIV patients attending the General out-patient clinic of the University of Nigeria Teaching Hospital, Ituku-Ozalla.

3.3**Sample collection**

Venous blood (3 mls) was obtained from the participants and tested for antibody to HCV (anti-HCV) using hepatitis C virus one step rapid diagnostic test using the serum or plasma. The serum or plasma was separated from blood as soon as possible to avoid haemolysis**.**

3.4Method

Serology

The test strip, serum or plasma specimen wasallowed to equilibrate to room temperature (15-30^{o}c) prior to testing.

The pouch was brought to room temperature before opening it. The strip was removed from the sealed pouch and used as soon as possible

With arrows pointing towards the serum or plasma specimen, the strip was immerse vertically into the serum or plasma for at least 10-15 seconds.do not pass the maximum line on the strip when immersing it.

The strip was placed on a non-absorbent flat surface, start the timer and wait for the red line to appear. The resultshould be read at 10 minutes. Do not interpret the result after 20 minutes.

Interpretation of result;

Positive: two distinct red lines appear. One should be in the control region and the other line should be in the test region.

Negative: one red line appears in the control region. N apparent or red line appears in the test region.

Invalid: the control line fails to appear. This is due to insufficient specimen volume or incorrect procedural technique. Review the procedure and repeat the test with a new test strip.

CHAPTER FOUR

RESULT

It was observed that out of 30 pregnant women (6.67%) tested positive for hepatitis C. as shown in table 4.1. 12 pregnant women in the age group 19-23 tested negative . In the age group 23-28 (9.09%) tested positive to HCV. In the age group 29-33(14.3%) tested positive to HCV.

Table 4.2 shows the sex distribution of HCV in PLWHA. A total of 20 patients were examined of which 7 were males and 13 females. (20%) of them were positive for HCV, (42.9%) of the male and (7.69%) of the female tested positive.

In table 4.3 a total of 7 males were examined for HCV and 42.9% tested positive. No sample was collected from the age group 20-24 and35-39. 2 samples were collected from the age group25-29 and (50%) of them tested positive.Five (5) samples were collected from the age group 30-34 and examined (40%) of them were positive.

The table 4.4 shows the age distribution of HCV in females living with HIV and AIDS. Thirteen (13) patients were examined for HCV and (7.7%) tested positive. In the age group20-24 and 35-39 no samples were collected. In the age group 25-29, 7 samples were examined for HCV and 14.3% tested positive. In

the age group 30-34,6 samples were examined for HCV and none tested positive.

Table 4.1Age distribution of HCV in pregnant women

Age group	number examined	number infected (%)
19-23	12	0
24-28	11	9.09
29-33	7	14.3
34-38	0	0

Table 4.2Sex distribution of HCV in HIV patients

Sex	number examined	number infected (%)
Male	7	42.9
Female	13	7.69
total	20	20

Table 4.3Age distribution of HCV in male HIV patients

Age group	number examined	number infected (%)
20-24	0	0
25-29	2	50
30-34	5	40
35-39	0	0

Table 4.4Age distribution of HCV in female HIV patients

Age group	number examined	number infected (%)
20-24	0	0
25-29	7	14.28
30-34	6	0
35-39	0	0

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 **DISCUSSION**

The result showed that a total of 50 samples were collected; 30 from pregnant women and 20 from PLWHA. It was observed that the prevalence of hepatitis C virus was higher in those within the age range of 24-34 than in those within the age range of 19-23 and 38-40due to exposure to exposure to the risk factor of hepatitis C virus (Alter 1990).

It was also observed that (42%) of the men living with HIV and AIDS tested positive for HCV than the women (7.69%) because men are mostly infected than women due to men are likely to have risk factor for exposure to hepatitis C virus (alter 1990).

The prevalence of HCV is higher in PLWHA (20%) than in pregnant women (6.67%) and this can be due to reduced immunity and similar routes of transmission for HIV and HCV.

5.2 CONCLUSION

Though the prevalence of hepatitis C virus is high from this work, the infection has an adverse effect in all susceptible individual; men, women and

children.Though there is no vaccination against this virus, medical management and ant-viral therapy should be employed to reduce or control this infection.

5.3 **RECOMMENDATION**

Since the prevalence of hepatitis C virus is high, it is recommended that Primary prevention activities such as screening and testing of blood, organ, tissue and semen donors can reduce the potential risk of HCV transmission from blood or blood component, intravenous drug use, multiple sex partners, tattooing.

Secondary prevention activities such as identifying HCV infected persons through diagnostic test, medical management and anti-viral therapy and providing appropriate medical follow-up and promoting healthy life styles and behavior can reduce risk for chronic infection.

Professional and public education; Health care emergency medical and public safety workers should be educated regarding the risk for contacting HCV.

Immunization against HCV is not available; therefore identifying persons at risk but not infected with HCV provides opportunity for counseling on how to reduce the risk of becoming infected.

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