SEROPREVALENCE AND FACTORS ASSOCIATED WITH HEPATITIS B VIRUS INFECTION IN PREGNANT WOMEN ATTENDING ANTENATAL CLINIC IN KARAGWE DISTRICT COUNCIL, KAGERA REGION

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SEROPREVALENCE AND FACTORS ASSOCIATED WITH HEPATITIS B VIRUS INFECTION IN PREGNANT WOMEN ATTENDING ANTENATAL CLINIC IN KARAGWE DISTRICT COUNCIL, KAGERA REGION, 2017

By

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A Dissertation Submitted in (partial) Fulfillment of the Requirement for the Degree of Master of Sciences (Epidemiology and Laboratory Management) of

Muhimbili University of Health and Allied Sciences

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled "***Seroprevalence and factors associated with Hepatitis B virus infection in pregnant women attending antenatal care clinic in Karagwe District Council, Kagera Region, 2017***" in (partial) fulfillment of the requirement for the degree of Master of Science (Epidemiology and Laboratory Sciences) of the Muhimbili University of Health and Allied Sciences.

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Prof. Said Aboud (Supervisor)

Date_____

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Ms. Loveness Urio (Supervisor)

Date_____

DECLARATION AND COPYRIGHT

I, **Denis Rabanus Kapinga**, declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature_____ Date_____

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DEDICATION

I dedicate this dissertation to my lovely wife Beatrice, my children Frank, Calorine and Exavery, my father Rabanus and my mother Evelina, and all the members of my family for their love, support and encouragement during this process.

ABSTRACT

Background: Hepatitis B virus (HBV) infection is a common serious liver disease affecting millions of people throughout the world. HBV infection in pregnant women can result in maternal complications and poor neonatal or infant outcomes. HBV infection in infancy increases the risk of developing liver cirrhosis and hepatocellular carcinoma.

Broad Objective: To determine the seroprevalence of and factors associated with HBV infection in pregnant women attending antenatal clinic in Karagwe District Council in Kagera region.

Materials and Methods: Clinic-based cross-sectional study was conducted on 600 pregnant women attending first visits in four ANC clinics in Karagwe District Council, Kagera Region, Tanzania during March–April, 2017.Blood samples were tested for hepatitis B surface antigen (HBsAg) using rapid test.

Results: The overall seroprevalence of HBV infection was 5.2% (95% CI: 3.6 – 7.3). In Bivariate analysis, history of multiple sexual partners ($p<0.01$), blood transfusion ($p<0.01$), alcohol consumption ($p<0.01$) and scarification ($p<0.02$) were associated with HBsAg positivity. In multivariate logistic regression analysis, history of multiple sexual partners ($p<0.01$) and drinking of alcohol ($p=0.03$) were significant predictors of HBV infection in pregnant women.

Conclusion and Recommendations: Seroprevalence of HBV infection in Karagwe District Council was intermediate. History of multiple sexual partners and drinking of alcohol predicted significantly HBV infection in pregnant women. Screening of pregnant women for HBsAg should be part of ANC visits followed by at birth-

hepatitis B vaccination of new born babies of pregnant mothers who tested positive for HBsAg so as to reduce and prevent the spread of HBV infection.

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LIST OF ABBREVIATIONS/ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
ALAT	Alanine Aminotransferase
ANC	Antenatal Clinic
ART	Antiretroviral Therapy
DMO	District Medical Officer
DPT	Diphtheria, Pertussis and Tetanus
ELISA	Enzyme-Linked Immuno Sorbent Assay
HAV	Hepatitis A Virus
HBeAb	Hepatitis B Envelope Antibodies
HBeAg	Hepatitis B Envelope Antigen
HBcAb	Hepatitis B Core Antibody
HBcAg	Hepatitis B core Antigen
HBcIgM	Hepatitis B Core Immunoglobulin M
HBV	Hepatitis B Virus
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MNH	Muhimbili National Hospital
MUHAS	Muhimbili University of Health and Allied Sciences
N	Number of sample size

NACP	National AIDS Control Program
PCR	Polymerase Chain Reaction
RMO	Regional Medical Officer.
WHO	World Health Organization

DEFINITION OF TERMS

Acute hepatitis B virus: New symptomatic HBV infection.

Antibody to HBsAg (anti-HBs): The protective antibody that develops following recovery from HBV infection and after vaccination

Antibody to hepatitis B core antigen (anti-HBc): Antibody produced in all HBV Infections (indicating infection sometime in the past)

Antenatal care: The systemic medical supervision of women during pregnancy with aim to preserve the physiological aspect of pregnancy and labor and to prevent or detect, as early as possible, all that is pathological (2006).

Blood-borne pathogens: Infectious microorganisms present in blood that can cause diseases in humans. These pathogens include hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (OSHA, 1999),

Combination vaccine: A vaccine made by combining antigens that prevent different Diseases (e.g. DTP)

Chronic HBV infection: Persistent (long-term) infection of Hepatitis B virus.

Cirrhosis: Permanent liver damage (scarring).

Hepatitis B core antibody (HBcAb): A serological maker that shows either acute or chronic Hepatitis B virus by presence or absence of Immunoglobin G to HBcAg.

Hepatitis B e antigen (HBeAg): A serological marker of increased infectivity in persons who are infected with hepatitis B virus

Hepatitis B surface antigen (HBsAg): A serological marker present in persons who are currently infected with HBV i.e. persons with both recent and chronic infection.

IgM class antibody to hepatitis B core antigen (IgM anti-HBc): Antibody detectable for four to six months following recent infection with HBV

Monovalent vaccine: A vaccine-containing antigen for protection against a single Disease.

Sexually transmitted infections (STIs): Infectious disease which is passed from one person to another through intimate sexual contact with infected person.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Hepatitis B virus (HBV) infection remains a global public health problem [1]. Approximately 350-400 million people are chronically infected with HBV [1-4]. The prevalence of HBV infection is categorized into three levels based on their endemicity, ranging from high (≥8%) to intermediate (2-7%) and low (<2%) [5,6]. It is most commonly transmitted through blood transfusions, sexual contact, from mother to child, and through open wound and other blood contact related activities [7,8].

HBV infection in pregnant women can result in maternal complications, including anemia, urinary tract infection, mental health conditions, hypertension, gestational diabetes mellitus, and obesity and weight gain, but may also lead to poor neonatal and infant outcomes, including very low birth weight and increased oxygen requirements [9,10]. HBV infection in infancy increases the risk of developing both liver cirrhosis and hepatocellular carcinoma [4, 11]. Health service provision may help improve outcomes in women and children with HBV infection through screening, management, and follow-up [12].

HBV infection is determined by screening for the presence of hepatitis B surface antigen (HBsAg). Prevalence of HBsAg varies geographically from<1% of the pregnant women in Germany [13] and India [14], to 2% in northern Turkey [15], to 4% in Logos Nigeria [16], to 11% in Yemen [17], and to 32% in South Korea [18].

In Africa, the prevalence of HBV infection among pregnant women ranges from 3-17% [16-24] and in Tanzania ranges from 2-8% [25-27].

Karagwe District Council is considering plans to introduce immunization against HBV infection in women and infants at birth, though there is little information concerning seroprevalence of HBV infection in the area. Knowing the baseline prevalence and planning resources and services, might lead to better outcomes for women and infants. Therefore this study aimed to determine the seroprevalence and factors associated with HBV infection among pregnant women attending antenatal care (ANC) clinics in Karagwe District Council, Kagera region.

1.2 Statement of the Problem

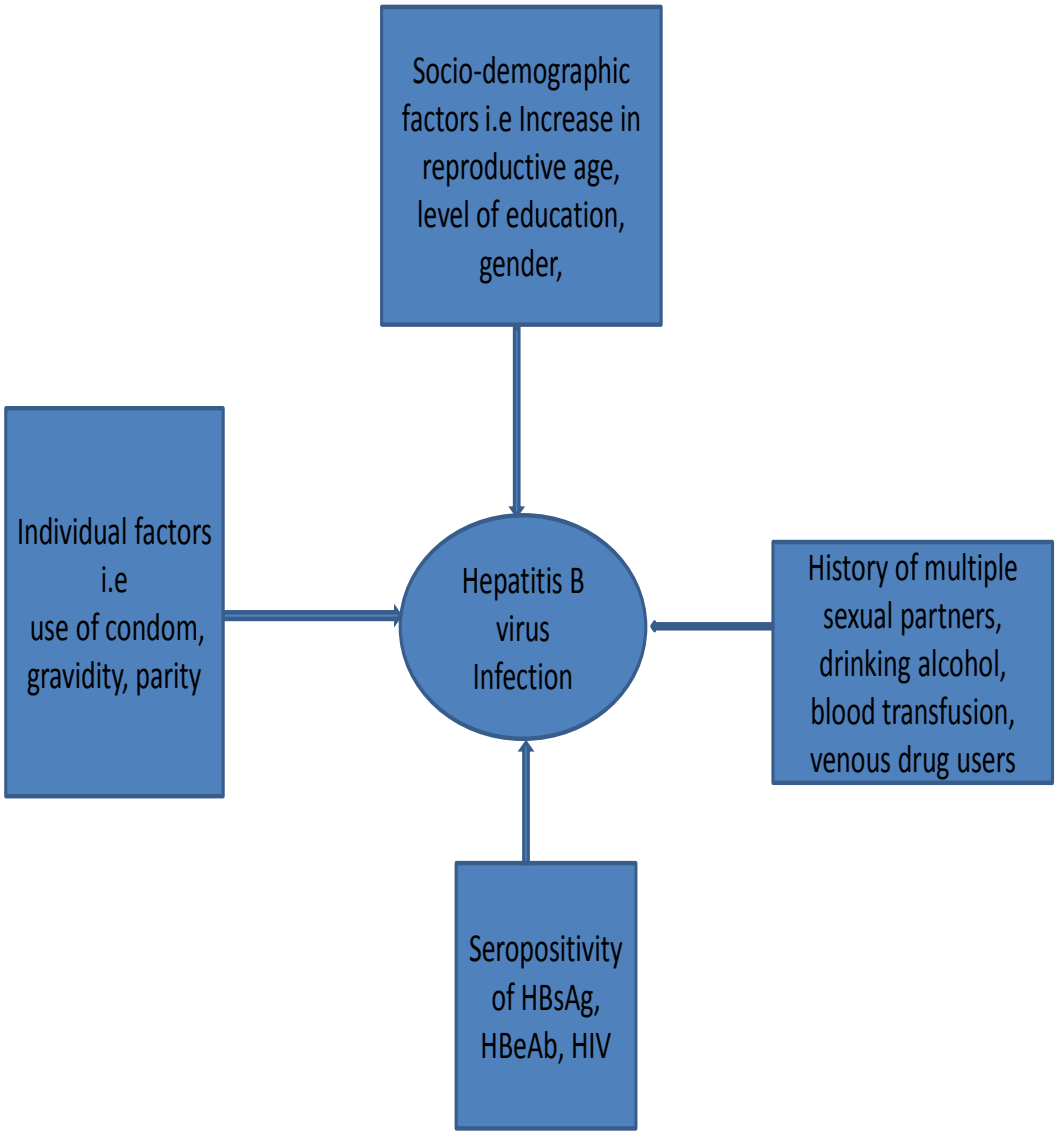
HBV infection is a common serious liver disease affecting millions of people throughout the world. HBV infection in pregnant women can result in maternal complications and poor neonatal or infant outcomes [1-5]. HBV infection in infancy increases the risk of developing liver cirrhosis and hepatocellular carcinoma [9]. HBV infection endemicity is associated with HIV infection since they share the common mode of transmission [9,10].

In Africa, the prevalence of HBV infection among pregnant women ranges from 3-17% [16-24] and in Tanzania ranges from 2-8% [25-27].

Karagwe District Council is considering plans to introduce immunization against HBV infection in women and infants at birth, though there is little information concerning seroprevalence of HBV infection in the area. Knowing the baseline prevalence and planning resources and services, might lead to better outcomes for women and infants. Therefore this study aimed to determine the seroprevalence and

factors associated with HBV infection among pregnant women attending antenatal care (ANC) clinics in Karagwe District Council, Kagera region.

1.3 Conceptual framework of factors associated with hepatitis B infection in pregnant women attending ANC clinic at Karagwe District Council.



1.4 Rationale

HBV infection is among the top ten public health problems with high morbidity and mortality rate in Tanzania [1, 5-6]. This infection can be prevented by vaccination hence vaccine is a cost effective way to prevent vaccine preventable disease only if there is information on its prevalence and associated risks [32]. The most affected group is pregnant women. Since the introduction of Expanded Program on Immunization in the country in 2002, needs information on status/prevalence of the disease thus, it is important to undertake study on prevalence and associated factors of HBV infection among pregnant women [33]. Karagwe District Council is considering plans to introduce immunization against HBV infection in women and infants at birth, though there is little information concerning seroprevalence of HBV infection in the area. Knowing the baseline prevalence and planning resources and services, might lead to better outcomes for women and infants. Data generated will inform on the magnitude and factors associated with HBV infection in Karagwe District. Also will influence policy, decision-making and best practices in implementing various interventions such as community screening for HBV infections, routine screening vulnerable group such as pregnant women and infants hence immunization to newborn babies.

1.5 Research Questions

i. What is the seroprevalence of HBsAg in pregnant women attending ANC clinic at Karagwe District Council?

ii. What is the seroprevalence of HBcAb in HBsAg positive pregnant women attending ANC clinic at Karagwe District Council?

iii. What is the seroprevalence of HBeAg in HBsAg positive pregnant women attending ANC clinic at Karagwe District Council?

iv. What are the socio-demographic and behavioural factors associated with HBsAg positivity in pregnant women attending ANC clinic at Karagwe District Council?

1.6. Research Objectives

1.6.1 Broad Objective

To determine seroprevalence and factors associated with HBV infection in pregnant women attending ANC clinic at Karagwe District Council in 2017.

1.6.2 Specific Objectives

i. To determine seroprevalence of HBsAg in pregnant women attending ANC clinic at Karagwe District Council.

ii. To determine seroprevalence of HBcAb in HBsAg positive pregnant women attending ANC clinic at Karagwe District Council.

iii. To determine seroprevalence of HBeAg in HBsAg positive pregnant women attending ANC clinic at Karagwe District Council.

iv. To determine the socio-demographic and behavioural factors associated with HBsAg positivity in pregnant women attending ANC clinic at Karagwe District Council.

CHAPTER TWO

LITERATURE REVIEW

2.1 Virology of HBV

Hepatitis B was recognized as a disease in ancient times, but its etiologic agent was only recently identified [17.29]. The first clue in unraveling this mystery was the discovery of an enigmatic serum protein named Australia antigen 50 years ago by Baruch Blumberg [5]. Some years later this was recognized to be the HBV surface antigen (HBsAg) 16]. Detection of HBsAg allowed for the first time screening of in apparently infected blood donors for a dangerous pathogen [1].

2.2 Epidemiology of HBV globally

Globally, it is estimated that 350-400 million people are chronically infected with HBV [2] with Africa and Asia bearing the greatest burden. In Africa and Asia, the prevalence is above 8% with approximately 2 billion people having markers of current or past infection and 350 million people having chronic infection [12].

2.3 Epidemiology of HBV in sub Saharan Africa

The prevalence of HBV infection in sub Saharan African countries among the general population ranged from 5% to 10% in a meta-analysis study of published data in 2015 [20] while that of HBV infection among pregnant women ranges from 3-17% [16-24] and in Tanzania ranges from 2-8% [25-27].

2.4 Epidemiology of HBV in East Africa.

In East Africa, the reported prevalence of HBsAg in Kenya was 9.3%, [22] and in Tanzania is 3.8% among pregnant women [25].

Tanzania was reported among the countries with intermediate endemicity levels of HBV infection according to the World Health organization [5].

2.5 Epidemiology of HBV in Tanzania

Pregnant women are not spared from HBV infection in which case the great concern is that their babies may also be affected. Indeed it is reported that 70%-90% of chronic HBV infections occur following the acute infections taking place during childhood [29]. Fortunately HBV vertical transmission can be prevented by immunization, and indeed universal immunization has been recommended by WHO [33]. In Tanzania universal immunization was introduced in 2002 whereby hepatitis B vaccine is given to infants in combination with Diphtheria, Pertussis and Tetanus (DPT) at 4,8 and 12 weeks of age [25, 32].

2.6 Factors associated with HBV infections

These factors associated with HBV infections include history of, multiple sexual partners or alcohol consumption, blood transfusion, scarification, non-use of condoms, increasing in age, male gender, HIV infection previous surgery, low level of education [8, 10, 20]. Among pregnant women high parity, polygamy, multiple sexual partners and previous history of sexually transmitted disease were shown to be among the significant risk factors for HBV infection in Nigeria [16, 36]. HIV/AIDS is a major global health problem. By the end of 2009, it was estimated

that 33.3 million people worldwide were living with HIV and AIDS. Sub-Saharan Africa is the world most severely affected region [7].

2.7 Immunity following HBV infections

Most persons who receive hepatitis B vaccine during infancy will have a level of antibody to hepatitis B surface antigen (anti-HBsAg) of <10 IU/liter 10 to 15 years later; however, most will demonstrate immune memory by an anamnestic response to a vaccine challenge dose [32].This is also true in most other countries where the immunization schedule of vaccination against hepatitis B is in combination with DPT vaccination and is given at 4^{th} week of life followed by 2 other doses given at a monthly interval [33]. The WHO has reported that this does not prevent perinatal transmission and hence advised on a 3 doses schedule or a 4 doses schedule [29]. According to the WHO, HBV vaccination for prevention of perinatal transmission needs the first dose to be given within 24 hours of delivery (before the mother has been discharged home) [29, 32]. Consequently, routine antenatal screening for HBsAg to all pregnant women, followed by vaccination of their babies at birth has been recommended by WHO [29]. However, in a previous study done in India, 2.25% of children under five were found to be positive for HBsAg despite immunization, and no statistically significant difference was observed between the age groups, suggesting that most of the infections occurred via vertical transmission [14].

Previous study done by Metodi et al in 2010 found that 69.3% of the immunized under five children had HBsAb level>10mIU/mL, a level considered protective [40]. On the other hand, 1.7% of the under-fives were found to be HBsAg positive

suggesting that vertical transmission occurred despite immunization [40]. The study recommended that a change in hepatitis B vaccination schedule including at birth dose would raise the level of immunity and thus offer protection [6]. Since a post exposure vaccination could break the chain of transmission, identifying children born to HBsAg mothers is very important [10].

Hepatitis B and co-infection with HIV in pregnant women could occur since the modes of transmission are shared [16]. A study done among pregnant women showed that one out of sixteen HIV infected had HBV infection as well. In this study HBV co-infection was also associated with low CD4 count [16].

2.8 Diagnosis of HBV

The diagnosis of HBV infection requires the evaluation of the patient's blood for hepatitis B surface antigen (HBsAg), hepatitis B envelope antigen (HBeAg), hepatitis B core antibody (HBcAb) and hepatitis B surface antibody. Although the presence of HBsAg indicates that the person is infectious, the presence of HBsAb indicates recovery and immunity from HBV infection or successful immunization against HBV. HBcAb appears at the onset of acute HBV infection, but may also indicate chronic HBV infection. The presence of hepatitis B envelope indicates transmission rate of HBV by contact i.e. vertical/congenital transmission, sexual intercourse etc. Interpretation of HBV immunologic markers is shown in table 1. HBV DNA sometimes may be the only marker present in early infections, this can be done either by rapid test strip that’s for HBsAg and HBcAb or using Abbott/AxSYM Germany machine that’s for HBeAg, obeying principle of an enzyme-linked fluorescent assay, microparticle enzymes immunoassay machine [13].

Table 1: Interpretation of HBV Immunologic Markers

HBsAg	**HBcAb**	**HBsAb**	**Interpretation**
Negative	Negative	Negative	Susceptible to HBV infection (should be vaccinated)
Negative	Negative	Positive	Immune because of vaccination
Negative	Positive	Positive	Immune because of natural HBV infection
Positive	Positive	Negative	Acute or chronic HBV infection
Negative	Positive	Negative	Interpretation unclear; four possibilities: 1. Resolved HBV infection (most common) 2. False-positive HBcAb, thus susceptible 3. "Low-level" chronic HBV infection 4. Resolving acute HBV infection

2.9 Management, prevention and control of HBV infection

HBV infection is prevented and controlled by different mechanisms such as screening blood before transfusion, using condom during sexual practices, obeying aseptic technique in medical practices like using sterile equipment [29]

Management of HBV infection is based on clinical signs and symptoms, also alpha interferon and lamivudine drugs are current used [29,32]

CHAPTER THREE

METHODOLOGY

3.1Study Area

The study was conducted at Karagwe District in Kagera Region. Karagwe District is one of the eight Districts in Kagera Region and covers an area of 4,630 Km^2 with 158 km^2 covered by water bodies. Karagwe borders Kyerwa District in Kagera region (Tanzania) and the Republics of Uganda and Rwanda in the North and West, respectively. Karagwe District also borders the Districts of Ngara and Biharamulo in the South and Muleba, Missenyi and Bukoba districts in the East.

Karagwe District is characterized by mountain ranges, which are reported to have swampy valley bottoms and wet lands. The attitudes range between 1500 - 1800 meters above sea level. The annual average temperature is 26^0c. Rainfall distribution is bi-modal with peak rains from September to December and from March to May. Most parts of District receive average rainfall between 800mm to 1000mm annually.

The population of Karagwe District has increased from 292,589 people in 2002 census to 332,020, in 2012 census with the sex composition of 49.4% male and 50.6% female. The dominant ethnic group is the Nyambo, though a lot of people are immigrants from neighboring Districts and from Uganda a neighboring country. A study was conducted in four health facilities which were; Kayanga, Nyaishozi, Nyakabanga and Nyakahanga health centres.



Figure 1: Map of Tanzania administrative showing Kagera region and Karagwe District.

Source; Tanzania administrative blog © 2010

3.2Study Design

This was a hospital based cross-sectional study that was conducted at the selected antenatal clinics in Karagwe District Council.

3.3Study Population

The study population was all pregnant women of different ages attending routine antenatal clinics for the first visit at Kayanga, Nyaishozi, Nyakabanga and Nyakahanga health centres in Karagwe District Council.

3.4Inclusion and Exclusion Criteria

3.4.1Inclusion criteria

1. All pregnant women attending antenatal clinic in Karagwe District Council for the first time and consent to participate in the study.

3.4.2Exclusion criteria

All pregnant women attending antenatal care clinic at Karagwe District Council health facilities

i. With age less than 16 years old,
ii. Who did not consent to participate in the study?
iii. Who came to the hospital for the second or more time?

3.4.3 Study Period

This study was carried out from March to April2017.

3.5 Sample Size

Sample size for this study was calculated by using the formula for proportion in cross-sectional study. Based on the prevalence of HBsAg infection in pregnant women the study done in DSM-Tanzania [9], the formula was:

$N= Z^2P (1-P)/ \varepsilon^2$

Where n =expected minimum sample

Z = standard, corresponding to 95% confidence; 1.96

P= prevalence of Hepatitis B surface antigen in pregnant women was 3.9% [9].

ε= maximum likely error taken as 2%

Hence, minimum sample size required was360 pregnant women but the sampling was involved clusters of hospital, the study encounters characters in the group which was not represent the true element of the population by applying design effect of 1.5. Thus the sample size, n = 540, with non-response rate of 10%, the sample size was 600. This sample size was increased the probability of detecting significant results if exist.

3.6 Sampling Technique

Multistage sampling was done to obtain a representative study sample. Karagwe District Council had34 health facilities where only four health facilities were providing services to pregnant women, thus, Nyakahanga designated district hospital, Nyaishozi, Kayanga and Nyakabanga dispensaries, and hence all those four were included in the study. In each selected health facility patient register book, a total number of pregnant women attended for two months before beginning of the study were counted and summed together to get the proportional of each health facility

which finally were used as the proportion of getting number of pregnant women required to be enrolled to get total of 600 pregnant women needed for the study. In each health facility, attendance report of pregnant women at ANC were taken for two months in calculating the proportion of 600 pregnant women needed for each health facility in data collection. A systematic random sampling method was adopted until the desired sample size of 600 was reached from all four health facilities. By using hospital records and staffs experiences, total number of pregnant women who attended ANC for two months (N) without repetition were calculated then divided by sample size required (n) to get sampling interval (k). Therefore first sampled pregnant women were selected by simple random sampling from equal or less to 'k' number from first visited pregnant women in the ANC clinic (by using ANC attendance register) followed by next pregnant women after k^{th} interval until desired sample size was reached in a given period of data collection.

$k=N/n$

Where,

k=sampling interval

N=Total number of pregnant women who attended the ANC clinic for two months of data collection period.

n=sample size for the study.

To determine the sample size per health facility, the formulae was used

Whereby, from the records of two months back before the study shows that;

Kayanga health centre was attending an average of 27 pregnant women per day hence a total of 810 pregnant women per month of 30 days.

- Nyaishozi dispensary was attending an average of three pregnant women per day hence a total of ninety pregnant women per month of 30 days.
- Nyakabanga dispensary was attending an average of six pregnant women per day hence a total of 180 pregnant women per month of 30 days, and
- Nyakahanga health centre was attending an average of 24 pregnant women per day hence a total of 720 pregnant women per month of 30 days.

  Hence their sample size was calculated as follows; -

  - Kayanga health centre = (810/1,800)*600)=270 out of total sample of 600
  - Nyaishozi dispensary = (90/1,800)*600=30 out of total sample of 600
  - Nyakabanga dispensary= (180/1,800)*600=60 out of total sample of 600.
  - Nyakahanga health centre=(720/1,800)*600=240 out of total sample of 600

3.7 Data Collection Methods

A Clinical Officer and medical laboratory professional performed data collection in each facility using a pretested and structured questionnaire. The questionnaire was translated from English to Swahili and back to English to ensure integrity of questions included. The Clinical Officer did face-to-face interviews with the enrolled pregnant woman in Swahili to collect information on social-demographic characteristics (age, sex, residence, marital status, level of education, and occupational status), previous history of blood transfusion, and number of sexual partners and parity.HIV and Syphilis status were recorded from documented ANC cards. Approximately 3millilitres of venous blood were collected into a plain tube. Blood samples were centrifuged, separated, and tested for HBsAg using SD Bioline

for HBsAg rapid test strips (VIDAS®HBe/Anti-HBe system Biomerieux, France). Those samples which were positive for HBsAg were stored at -20^{0}c until the end of data collection for shipping to Dar Es Salaam (Muhimbili National Hospital) for analysis of hepatitis B envelop antigen (HBeAg) and hepatitis B core antibody (HBcAb) using an enzyme-linked fluorescent assay (VIDAS®HBe/Anti-HBe system Biomerieux, France).

3.7.1 Laboratory Investigations

A venipuncture was performed and 5-ml of blood specimen was collected in a red top vacutainer tube. Serological testing for HBV was done in the selected health facility in Karagwe DC except for determination of HBcAb (IgG&IgM) and HBeAg which were sent to Muhimbili National Hospital for further testing.

3.7.2 Specimen Storage

Serum was separated after testing for HBsAg, aliquot in 1.8 ml cryogenic tubes and stored at -20°C at Karagwe District Hospital i.e. Kayanga health centre. Samples were then shipped in line with IATA regulations to the Department of Microbiology and Immunology laboratory at CPL Muhimbili National Hospital for further testing on HBcAb and HBeAg.

3.7.3 Detection of HbsAg

The Hepatitis B surface Antigen rapid test strip were used for the detection of hepatitis B surface antigen (HBsAg). The test with 99.6% sensitivity and 99.4% specificity were used when performed test according to the instructions of the manufacturer (Standard Diagnostic BIOLINE Biotech Ltd, Dar ford UK)

Briefly, the membrane was pre-coated with anti-HBsAg antibodies on the test line region of the strips. During testing the whole blood, serum and plasma specimens reacted with anti-HBsAg antibodies in the membrane and generate a colored line. The presence of this colored line in the test region indicates positive results, while its absence indicates a negative result. To serve as procedural control a colored line appeared on control line region indicating that proper volume of specimen has been added and membrane wicking occurred.

3.7.4 Detection of HbcAb

Enzyme linked Fluorescent Assay test (EIFA) (VIDAS HBc/Anti-HBc system Biomerieux) with 99.5%sensitivity and 99.7%specificity were used when performing test according to the instructions of the manufacturer. One milometer cubic of serum samples were put into the machine analyzer chamber. Then after ten minutes results came out.

3.7.5 Detection of HbeAg

This was performed using an enzyme-linked fluorescent assay (VIDAS®HBe/Anti-HBe system Biomerieux, France). The results were reported as positive or negative.
VIDAS HBe/Anti-HBe system was an automated qualitative test for use on the VIDAS family instruments, for the detection of hepatitis B e-antigen (HBe) in human serum or plasma using the ELFA technique (Enzymes Linked Fluorescent Assay).
It begins with a preliminary incubation step, which can be performed in a water bath, dry incubator or directly in the instruments.

3.7.6 Preliminary incubation and principle of assay.

For each sample standards (S2) and control was tested; pipette.100ul of HBe (S1) standard was mixed with 100ul of sample, standard or control by using vortex and incubated at 37 ± 2^{0}C for one-hour ±5 minutes in the water bath. The test, standard, control positive and negative were identified by HBe code, S2, C3 and C2 respectively. After an assay was completed, the computer analyzed results automatically.

3.7.7 Data management and analysis

Hepatitis B virus (HBV) infection remains a global public health problem [1]. Approximately 350-400 million people are chronically infected with HBV [1-4]. The prevalence of HBV infection is categorized into three levels based on their endemicity, ranging from high (≥8%) to intermediate (2-7%) and low (<2%) [5,6]. It is most commonly transmitted through blood transfusions, sexual contact, from mother to child, and through open wound and other blood contact related activities [7,8].

HBV infection in pregnant women can result in maternal complications, including anemia, urinary tract infection, mental health conditions, hypertension, gestational diabetes mellitus, and obesity and weight gain, but may also lead to poor neonatal and infant outcomes, including very low birth weight and increased oxygen requirements [9,10]. HBV infection in infancy increases the risk of developing both liver cirrhosis and hepatocellular carcinoma [4,

11]. Health service provision may help improve outcomes in women and children with HBV infection through screening, management, and follow-up [12].

HBV infection is determined by screening for the presence of hepatitis B surface antigen (HBsAg). Prevalence of HBsAg varies geographically from<1% of the pregnant women in Germany [13] and India [14], to 2% in northern Turkey [15], to 4% in Logos Nigeria [16], to 11% in Yemen [17], and to 32% in South Korea [18]. In Africa, the prevalence of HBV infection among pregnant women ranges from 3-17% [16-24] and in Tanzania ranges from 2-8% [25-27].

Karagwe District Council is considering plans to introduce immunization against HBV infection in women and infants at birth, though there is little information concerning seroprevalence of HBV infection in the area. Knowing the baseline prevalence and planning resources and services, might lead to better outcomes for women and infants. Therefore this study aimed to determine the seroprevalence and factors associated with HBV infection among pregnant women attending antenatal care (ANC) clinics in Karagwe District Council, Kagera region.

3.8 Ethical Considerations

Ethical clearance was obtained from Muhimbili University of Health and Allied Sciences Senate Research and Publications Committee (SRPC). Permission to collect data was obtained from the District Medical Officer of Karagwe District Council. Written informed consent and verbal assent in Swahili language was obtained from each participant and parents for participants' age below 18 years old, by explaining the purpose of the study prior to the enrollment. Only members of the study team were allowed to handle the data to ensure confidentiality. Questionnaire forms and databases were stored in a locked cabinet and password protected electronic file on the main computer of the study team leader. Individual test results were

communicated with the attending physician and those who were positive for HBsAg were referred to Karagwe District hospital for further management. Blood samples were discarded after analysis.

CHAPTER FOUR

RESULTS

4.1 Social demographic characteristics of the study participants.

A total of 600 pregnant women were recruited in the study. The overall mean age of the study participants was 26.5 ± 5.8(range from 16 – 45) years, and the predominant age group of 20-24 years accounted for 32% of the study participants (Table 2). The majority of the study participants, 520 (86.7%), were married, 330 (55%) pregnant women had primary education and 291 (48.5%) were not employed.

Table2: The social-demographic characteristics of pregnant women attending antenatal clinic at Karagwe District Council, 2017 (N=600)

Variable	N	%
Age group, years		
<20	62	10.3
20-24	192	32.0
25-29	166	27.7
30-34	108	18.0
35-39	63	10.5
40+	9	1.5
Marital status		
Married	520	86.7
Separated	2	0.3
Divorced	2	0.3
Widowed	13	2.2
Single	63	10.5
Educational level		
Non formal	125	20.8
Primary	330	55.0
Secondary	123	20.5
Tertiary	22	3.7

Occupation status		
Self employed	242	40.3
Government employed	28	4.7
Private employed	39	6.5
Not employed	291	48.5

**Tertiary education means higher education level i.e. College and University education*

4.2 Health Facility

Many of the study participants were from Kayanga health centre (274, 46 %) and a few from Nyaishozi dispensary (33, 6%).

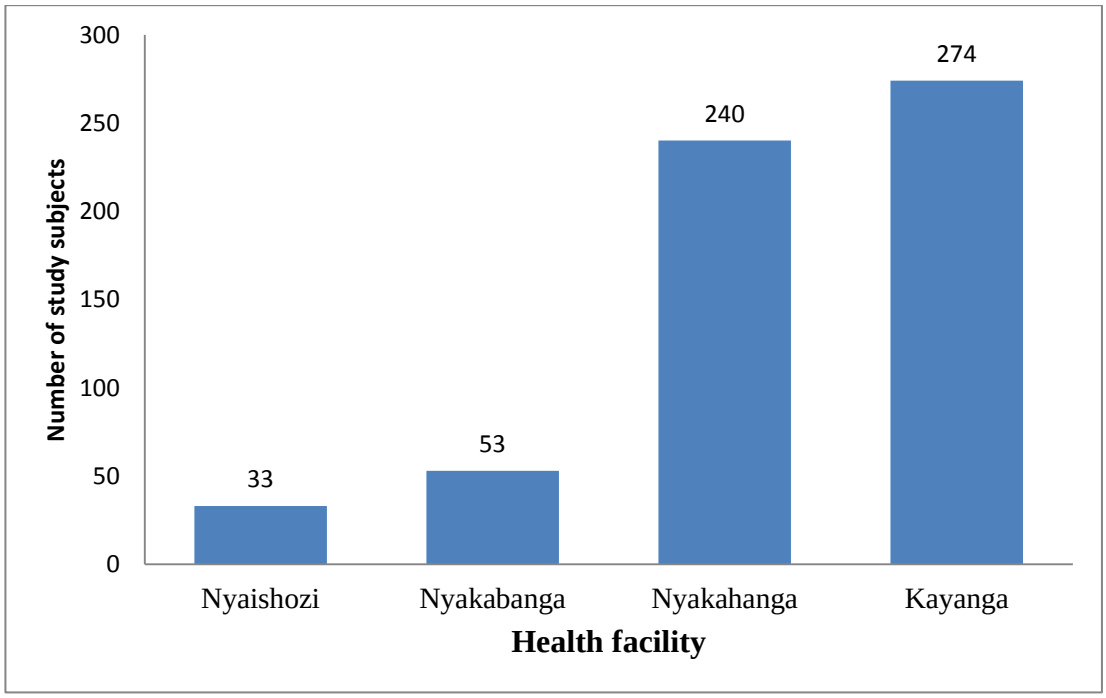


Figure 2: Distribution of study participants by health facility

4.3 Seroprevalenceand co-infection of HBV among pregnant women

Of 600 study participants enrolled, 31 tested positive for HBsAg giving a seroprevalence of 5.2% (95%CI: 3.6-7.3) as shown in figure 3 and table 4below. The highest prevalence rate was observed in the age group of35-39 years (7.9% CI: 2.6-17.6%). Study participants that were married had higher prevalence of HBsAg (5.4 95%CI: 3.7-7.8) than those who were unmarried (single, divorced, separated and widowed) and those with higher HBsAg had tertiary level of education. Those who were not employed had higher prevalence of HBsAg (6.9% CI: 4.2-10.4) compared to other group in occupational status. Three participants had co-infection of HIV and HBsAg (10.3% 95%CI: 2.2-27.4) while all study participants who had syphilis infection had negative HBsAg status (0.0% 95%CI: 0.0-97.5). Of the 31 HBsAg positive pregnant women, three (9.7%) had both HBeAg and HBcAb seropositive. Records generated from routine ANC visit shows that one pregnant woman (3.2%) out of 31 HBsAg seropositive mothers had co-infection of Syphilis and HBsAg.

Table 3: Seroprevalence of Hepatitis B Surface Antigen in Relation to Social-demographic and Obstetric Characteristics among pregnant women attending antenatal clinic at Karagwe District Council, 2017.

Variables	**Frequency (N=600)**	**HBsAg positive (n=31)**	**Prevalence (95%CI)**	**P value**
Age group (yr.)				0.68
<20	62	4	6.5(1.8-15.7)	
20-24	192	11	5.7(2.9-10.0)	
25-29	166	8	4.8(2.1-9.3)	
30-34	108	3	2.8(0.6-7.9)	
35-39	63	5	7.9(2.6-17.6)	
40+	9	0	0.0(0.0-2.0)	
Marital status				0.05
Married	520	28	5.4(3.7-7.8)	
Separated	2	0	0.0(0.0-84.2)	
Divorced	2	1	50(1.3-98.7)	
Widowed	13	0	0.0(0.0-24.7)	
Single	63	2	3.2(0.4-11.0)	
Educational level				0.04
Non formal	125	5	4.0(1.3-9.1)	
Primary	330	20	6.1(3.8-9.4)	
Secondary	123	4	3.3(0.9-8.1)	

Tertiary	22	2	10.0(0.1-24.9)	
Occupation status				0.21
Self employed	242	10	4.1(2.0-7.5)	
Government employed	28	1	3.6(0.1-18.3)	
Private employed	39	0	0.0(0.0-9.0)	
Not employed	291	20	6.9(4.2-10.4)	

Table 3: Continued...

HIV status				0.18
Yes	29	3	10.3(2.2-27.4)	
No	571	28	4.9(3.3-7.1)	
Syphilis status				0.95
Yes	1	0	0.0(0.0-97.5)	
No	599	31	5.2(3.6-7.3)	
Gravidity				0.06
Primgravidae	215	7	3.3(1.3-6.6)	
Multigravidae	385	24	6.2(4.1-9.3)	

4.4 Social demographic factors associated with Hepatitis B virus infection among pregnant women attending antenatal clinic at Karagwe District Council, 2017.

There were no differences in the HBV infection in pregnant women by age, marital status, level of education, occupation, HIV serostatus, syphilis, and gravidity (Table 4).

Table 4: Bivariate analysis of social demographic factors associated with Hepatitis B virus infection among pregnant women attending antenatal clinic at Karagwe District Council, 2017

Variables	**HBsAg positive (n=31)**	**HBsAg negative (n=569)**	**OR**	**CI (95%CI)**
Age group (yr.)				
<20***	4	58	Reference	
20-24	11	181	0.9	0.27-2.87
25-29	8	158	0.7	0.21-2.53
30-34	3	105	0.4	0.09-1.92
35-39	5	58	1.3	0.32-4.89
40+	0	9	0.0	0.00-1.00
Marital status				
Married	28	492	1.5	0.43-4.92
Separated***	3	77	Reference	
Educational level				
Non formal education	5	120	0.8	0.09-7.15
Primary education	20	310	1.2	0.16-9.63
Secondary education	4	119	0.6	0.07-6.02
Tertiary education***	2	20	Reference	

Occupation status				
Self employed	10	232	1.2	0.14-9.45
Not employed	20	271	2.0	0.28-15.4
Private employed	0	39	0.0	0.00-1.00
Government employed***	1	27	Reference	

Table 4: Continued...

HIV status				
Yes	3	26	0.4	0.13-1.57
No***	28	543	Reference	
Syphilis status				
Yes	0	1	0.0	Undefined
No***	31	568	Reference	
Number of deliveries				
≤1pregnancy	24	389	1.6	0.67-3.75
>1pregnancy	7	180	Reference	

- Fisher exact test was used for value less than 5 in the cells
- Separated in marital status was including; Divorced, widowed, single and separated status.

- Tertiary education in Education level was including; College and university education, and *** mean reference value.

4.5: Behavioral risk factors among pregnant women attending antenatal clinics in Karagwe District Council, 2017.

Pregnant women who had history of multiple sexual partners were about five times more likely to test positive for Hepatitis B surface antigen (OR=4.5, 95%CI: 2.09-9.67) compared to those who had no multiple sexual partners. Those who were transfused blood were almost five times more likely to test positive for Hepatitis B surface antigen (OR=4.5, 95%CI: 1.70-11.74) compared to those who were not transfused blood. Alcohol drinking behavioral were around four more likely to test positive for Hepatitis B surface antigen (OR=3.8, 95%CI: 1.02-6.66) compared to those who were not drinking alcohol. Pregnant women who had scarification were three times more likely to test positive for Hepatitis B surface antigen (OR=3.0, 95%CI: 1.17-7.75) compared to those who not scarified. Other factors like ear piercing, tattooing, hospital admission, tooth extraction, surgery abortion/miscarriage, unsafe injection and catherisation were not associated with Hepatitis B surface antigen positivity in the study participants.

Table 5: Bivariate analysis of behavioral associated factors among pregnant women (N=600) attending antenatal clinic at Karagwe District Council, 2017

Associated factors	**Frequency**	**HBsAg positive n=31**	**HBsAg negative n=569**	**OR**	**95%CI**	**P- value**

Multiple sexual partners						
YES	41	12(29.3)	70(70.7)	4.5	2.09-9.67	<**0.01**
NO	559	19(4.3)	499(95.7)			
Blood transfusion						
YES	31	6(19.4)	31(80.6)	4.5	1.70-11.74	<**0.01**
NO	569	25(4.4)	569(95.6)			
History of Drinking alcohol						
YES	52	8(15.4)	48(84.6)	3.8	1.02-6.66	<**0.01**
NO	548	23(4.2)	521(95.8)			
Scarification						
YES	48	6(12.5)	42(87.5)	3.0	1.17-7.75	**0.02**
NO	552	25(4.5)	527(95.5)			
Ear piercing						
YES	232	14(6.0)	218(94)	1.3	0.64-2.74	0.2
NO	368	17(4.6)	351(95.4)			
Tattooing						
YES	11	2(18.2)	9(81.8)	4.3	0.87-20.77	0.1

Table 5: Continued...

NO	589	29(4.9)	560(95.1)			

Hospital admission						
YES	196	10(5.1)	186(94.9)	1.0	0.45-2.12	0.5
NO	404	21(5.2)	383(94.8)			
Tooth extraction						
YES	111	2(1.8)	109(98.2)	0.3	0.07-1.24	0.05
NO	489	29(5.9)	460(94.1)			
Surgery						
YES	37	4(10.8)	33(89.2)	2.4	0.80-7.28	0.1
NO	563	27(4.8)	536(95.2)			
Catheterization						
YES	1	0(0.0)	1(100.0)	Unde fined	0.00-1.00	0.9
NO	599	31(5.2)	568(94.8)			
Abortion/miscarriage						
YES	34	2(5.9)	32(94.1)	1.2	0.26-5.07	0.5
NO	566	29(5.1)	537(94.9)			
Unsafe injection						
YES	66	2(3.0)	64(97.0)	0.5	0.13-2.33	0.3
NO	534	29(5.4)	505(94.6)			

4.6: Factors associated with HBsAg among pregnant women attending antenatal clinic at Karagwe District Council, 2017.

In multivariate analysis, History of multiple sexual partners and drinking alcohol were found to be significant predictors of HBV infection in pregnant women (Table 6).

Table 6: Multivariate analysis of associated factors for HBsAg among pregnant women attending antenatal clinic at Karagwe District Council, 2017

Variable	AOR	95% CI	P value
History of multiple sexual partners	9.1	4.1-20.1	<0.01
History of Drinking alcohol	2.8	1.1-7.0	0.03

CHAPTER FIVE

DISCUSSION

According to WHO classification criteria, HBsAg seroprevalence among pregnant women in Karagwe District Council is intermediate (5.2%). In this prevalence category, WHO recommends immunization of all new born or adolescents (or both) for HBV [10, 29]. In this study, higher rates of HBsAg positivity were most strongly associated with a history of multiple sexual partners and alcohol consumption.

Our prevalence agreed with studies from Mwanza-Tanzania (3.8%) [26], Nigeria (4.2%) [16], Ethiopia (4.3%) [30], and Tanzania (5.7%) [27]. However, it is in lower than in Mali (8.0%) [19], Makele-Ethiopia (8.1%) [20], Cameroon (10.2%) [21], and Honiara Solomon Islands (13.8%) [31]. These differences may be explained by vaccination coverage, rates of safe sexual practices, alcohol consumption, access to healthcare (e.g., screening and treatment for HBV infection), as well as the risk of transmission through healthcare related exposures. Scaling up ANC services, including HBV immunization programs may reduce this burden by reducing mother to child transmission, which is a common means of HBV transmission [1,32]. About half of new infections result from mother to child transmission during pregnancy thus vaccinating infants of mothers positive for HBsAg and HBeAg are important in HBV prevention and control programs [33]. In countries where the Expanded Program for Immunization of hepatitis B vaccine is well established, prevalence of HBV infection is as low as 0.48% in Germany [13] and India [14,34].

Multiple sexual partners may increase the number of times a non-infected person is exposed to an infected person [35]. We found that pregnant women were five times more likely to have HBV infection if they reported a history of multiple sexual partners. This finding is in agreement with other findings from Northern Ethiopia [30], and Nigeria [36] but in contrast with study reported in Tanzania [25] and Nigeria [37]. Programs to reduce sexual transmission of HBV, and other sexually transmitted diseases, including increasing access to condoms, education on sexual transmission of disease, screening and treatment programs, could help in lowering this burden.

Alcohol consumption may reduce decision-making capacity. We found that pregnant women were about four times more likely to have HBV infections if they reported a history of alcohol drinking, similar to a study done in Ethiopia [38]. We are unsure the causal relationship between alcohol and HBV infection, however, alcohol consumption may be related to other risky behaviors, like unprotected sexual intercourse, that may put individuals at risk. Programs to address alcohol consumption may reduce HBV infection rates.

This study has some limitations. We did not quantify the number of sexual partners or the amount of alcohol consumption, which makes it difficult to assess the dose-response of these factors on development of HBV infection. More information on use of condoms might also influence how we understand the relationship between multiple sexual partners and subsequent HBV infection. Recall bias during data collection was reduced by asking questions on latest events. Also there was reporting

bias on sensitive topics since people are very likely to under-report stigmatized behaviors like multiple sex partners and alcohol consumption.

Blood transfusion, a known risk factor for blood borne pathogens like HBV, was not significantly associated with HBsAg seropositivity in the present study. This finding corroborates the report from Nigeria [37], however, blood transfusion has been associated with transmission of HBV in a number of studies [21, 38]. Improving the quality of the laboratory screening of blood for HBV is one of the components in reducing the risk for transfusion-transmitted HBV. The possible explanation for absence of significant association between HBV infection and blood transfusion might be because of improved screening for HBV infection from blood donors before transfusion in Tanzania.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Seroprevalence of HBsAg in pregnant women in Karagwe District Council was intermediate (5.2%) according to the WHO classification. History of multiple sexual partners and drinking alcohol predicted significantly HBV infection in pregnant women.

6.2 Recommendations

An integrated HBV prevention program should be considered to address safer sex and behaviors that may impair decision-making capacity such as excessive alcohol consumption. Though a known risk factor for HBV, we did not find an association between blood transfusion and HBV infection among pregnant women suggesting that blood safety programs are working to reduce HBV transmission through blood transfusion. Screening of pregnant women for HBsAg infection should be part of antenatal visits followed by at birth-hepatitis B vaccination of new born babies of pregnant mothers who tested positive for HBsAg so as to reduce and prevent the spread of HBV infection.

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APPENDICES

Appendices 1a: **INFORMED CONSENT FORM- English Version**

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES - DIRECTORATE OF RESEARCH & PUBLICATIONS.

ID-NO _____

Consent to participate in this study

Greetings, my name is …..... from Muhimbili University of Health and Allied Sciences, Dar es Salaam. We are carrying out a study to determine factors associated with hepatitis B virus infection in pregnant women attending ANC care at Karagwe District Council, in Kagera region, Tanzania.

Purpose of the study

This study has the purpose of collecting information on factors associated with hepatitis B virus infection in pregnant women attending ANC care at Karagwe District Council, in Kagera region. You are being asked to participate in this study because you are a pregnant woman who fit for this study hence may be important to the study.

What participation involved

If you agree to participate in this study, you will be required to answer series of questions that have been prepared for the study through interviewing in order to obtain the intended information regarding hepatitis B virus infection in pregnant women attending ANC care at Karagwe District Council, in Kagera region

Confidentiality

I assure you that all the information collected from you will be kept confidential. Only people working in this research will have access to the information. We will be compiling a report, which will contain responses from four selected health facilities in Karagwe District Council. We will not put your name or other identifying information on the records of the information you provide.

Risks

You will be asked questions about factors that are associated with hepatitis B virus infection in pregnant women. Some questions could potentially make you feel uncomfortable. You may refuse to answer any particular question and stop the interview at any time. We do not expect any harm to happen to you because of participation in this study.

Right to withdraw and alternatives

Taking part in this study is completely your choice. If you choose not to participate in the study or if you decide to stop participating in the study you will not get any harm. You can stop participating in this study at any time, even if you have already given your consent. Refusal to participate or withdrawal from the study will not involve penalty or loss of any benefits to which you are otherwise entitled.

Benefits

The information you provide will help to increase our understanding on factors associated with hepatitis B virus infection in pregnant women attending ANC care at Karagwe District Council, in Kagera region and communicate the findings to policy makers in the council and ministry for improvement of control and preventive measures in the district as well as in the country.

Responsibility of Investigator

In the case where the pregnant woman will be positive for HBeAg and HBcAb will be linked to Karagwe District hospital for further care and be advised to visit the health facility for regular follow-up services.

In case of injury

We do not anticipate that any harm will occur to you or your unborn baby as well as your family as a result of participation in this study.

Who to contact

If you have questions about this study, please don't hesitate to contact:

Denis Kapinga, The **Principal Investigator** Muhimbili University of Health and Allied Sciences (MUHAS), P.O. Box 65001, Dar es Salaam (Tel. no. 0763806082 or 0652110723).

Dr. Joyce Masalu, Chairperson, Senate Research and Publications Committee P. O. Box, 65001, Dar es Salaam. My supervisors; Prof. **Said Aboud** Tel: 0754301692and Ms Loveness Urio, (Tel. no. 0784322835).

Signature

Do you agree?

Participant agrees…… Participant does not agree…….

I …….have read the content in this form, my questions have been answered, I agree to participate in this study

Signature of Participant…….……

Signature of Research assistant……

Date of signed consent……

Appendix 1b: **INFORMED CONSENT FORM- Swahili version**

CHUO KIKUU CHA AFYA NA SAYANSI YA TIBA CHA MUHIMBILI

KURUGENZI YA UTAFITI NA UCHAPISHAJI

FOMU YA RIDHAA

ID-NO _____

Ridhaa ya kushiriki

Hujambo? Ninaitwa……. Ninafanya kazi ya kutafiti sababu zinazoleta maambukizi ya homa ya ini kwa mama wajawazito wanaohudhuria kliniki kwenye vituo vya afya katika halmashauri ya wilaya ya Karagwe, mkoani Kagera.

Madhumuni ya utafiti

Utafiti huu unakusudia kuchunguza sababu zinazoleta maambukizi ya homa ya ini kwa mama wajawazito wanaohudhuria kliniki kwenye vituo vya afya katika halmashauri ya wilaya ya Karagwe, mkoani Kagera. Unaombwa kushiriki kwenye Utafiti huu kwa sababu wewe ni miongoni mwa mama wajawazito na ushiriki wako ni muhimu sana kwenye huu utafiti.

Nini kinahitajika ili kushiriki?

Ili ushiriki katika Utafiti huu inabidi kukubali na kujiunga kwa kujibu maswali toka kwenye dodoso kwa ajili ya utafitihuu.

Usiri

Ninakuhakikishia kuwa taarifa zitakazokusanywa kutoka kwako kupitia dodoso hili zitakua siri na hakuna mtu yeyote ambaye hafanyikazi kwenye Utafiti huu atakayeambiwa ulichosema. Itaadaliwa taarifa ya Utafiti huu ambao hautamtaja mshiriki yeyote. Jina lako wala utambulisho mwingine wowote hautawekwa kwenye taarifa unazozitoa. Taarifa zako zitaingizwa kwenye matuamizi kwa kutumia namba za utambulisho.

Hatari

Hakuna hatari yoyote itakayotokea kwako kutokana na ushiriki wako kwenye Utafiti huu.

Haki ya kujitoa au vinginevyo

Ushiriki katika Utafiti huu ni hiari. Kutokushiriki au kujitoa kutoka kwenye Utafiti hakutakua na adhabu yoyote na hutapoteza stahili zako endapo utaona ni vema kufanya hivyo.

Faida

Kama utakubali kushiriki kwenye utafiti huu itakua ni fanaka kwa vile Utafiti huu unalengo la kugundua sababu zinazoleta maambukizi ya homa ya ini kwa mama wajawazito wanaohudhuria kliniki kwenye vituo vya afya katika halmashauri ya wilaya ya Karagwe, mkoani Kagera.

Waajibu wa mtafiti

Endapo mama mjamzito atakutwa na maambukizi ya homa ya ini HBeAg atashauriwa kuonana na madaktari katika hospitali ya wilaya kwa ushauri na matibabu ya kiafya.

Endapo utapata madhara au la

Hatutegemei kupata madhara yoyote kutokana na ushiriki wako katika Utafiti huu.

Nani wakuwasiliana naye

Kama kuna swali kuhusiana na Utafiti huu itakubidi kuwasiliana na mtafiti mkuu **Denis Kapinga** wa Chuo Kikuu cha Afya na Sayansi ya Tiba Muhimbili, S.L.P. 65001 DSM. Simu (Tel: 0763806082). Kama una maswali zaidi unaweza kuwasiliana na **Joyce Masalu**, Mkurugenzi wa Kamati ya Utafiti na Uchapishaji, Chuo Kikuu cha Muhimbili, au **Profesa S. Aboud**(0754301692) au **Loveness Urio** (0784322835) ambao wote ni wasimamizi wa Utafiti huu.

Je, Umekubali?

Mshiriki amekubali…… Mshiriki hajakubali……

Mimi……...nimesoma maelezo ya fomu hii, maswali yangu yamejibiwa na nimeridhika. Nakubali kushiriki katika Utafiti huu.

Sahihi ya Mshiriki……

Sahihi ya mtafiti msaidizi……

Tarehe ya kuweka sahihi ya kushiriki……

Appendices2: **Questionnaire English version**

Questionnaire for Determination of seroprevalence and factors associated with Hepatitis B infection in pregnant women attending ANC clinic at Karagwe District Council in 2017.

1. Questionnaire Number_____
2. Date of interview_____
3. Interviewer Name_____
4. Name of Health Centre_____

A. Socio-demographic information (TICK)

5. Pregnant registration number_____

6. Residence (Street/Village)_____Ward_____

7. Age (in years)_____

8. Marital status ;(TICK)

Single ( )

Married ( )

Widowed ( )

Separated ( )

Divorced ( )

9. Education Level (TICK)

No formal education at all

Primary education

Secondary education

College level

University level

10. What is your occupational status? (TICK)

Self employed

Government employed

Private employed

Not employed

B. Risk factors of Hepatitis B (Please tick)

Did you involved in any of the following practices?

11. Unsafe injection (intravenous drugs user I)

NO ( ) YES ( )

12. Multiple sexual partners

NO ( ) YES ( )

13. Scarification

NO ( ) YES ( )

14. Ear piercing

NO ( ) YES ( )

15. Tattooing

NO ( ) YES ( )

Did you receive any of the following treatment?

16. Blood transfusion

NO ( ) YES ( )

17. Hospital admission

NO ( ) YES ( )

18. Tooth extraction

NO ( ) YES ( )

19. Surgery

NO ( ) YES ( )

20. Catheterization

NO ( ) YES ( )

C.History of sexual partners characteristics (STI, HBV and HBV)

21. Did you have any history of liver disease? (Circle)

Yes ( ) No ( )

22. Did you have the history of sexually transmitted diseases (circle?)

Yes ( ) No ( )

23. Did you have the history of drinking alcohol? (Circle)

Yes ( ) No ( )

D. Health and pregnant/gynecological history (TICK)

24. Pregnancy status;

First pregnancy ( )

Second pregnancy ( )

Third pregnancy ( )

Above Four pregnancy ( )

25. Number of deliveries_____

26. Number of live babies (Parity)_____

27. Gravidity

Primgravidae ( ) Multgravidae ( )

28. Have you experienced yourself any pregnant related problems in life?

Yes ( ) No ( )

29. If YES what pregnant problems? (TICK)

Abortion/miscarriage ( )

Still birth ( )

Sickle cell anemia ( )

Epilepsy ( )

Obstructed labour( )

Ectopic pregnancy ( )

Caesarian section/ Vacuum ( )

Death of a baby while in uterus ( )

Oedema ( )

Others (mention) .....

30. Have you experienced any other health problems like (TICK?)

Severe bleeding after delivery/Anemia/ Hemorrhage ( )

Remained placenta in the uterus ( )

Heart attack/ High blood pressure/ Hypertension ( )

Diabetes ( )

Tuberculosis ( )

Syphilis ( )

Liver disease ( )

History of jaundice ( )

Other problems (mention).....

31. Vaccination status (circle)

Tetanus Yes ( ) No ( )

Hepatitis B Yes ( ) No ( )

D. Serology (Laboratory findings) (TICK);

32. HIV-1/2

Positive ( ) Negative ( )

33. Hepatitis B surface antigen (HBsAg)

Positive ( ) Negative ( )

34. Hepatitis Be antigen (HBeAg)

Positive ( ) Negative ( )

35. Hepatitis Bc antibody

Positive ( ) Negative ( )

Appendices 3;Laboratory investigation Standard Operating Procedures (SOP's).

Hepatitis B virus surface antigen detection by Rapid test

Bioline HBsAg One Test:-

The Bioline HBsAg One Test is a qualitative, solid phase, two-site sandwich immunoassay for the detection of HBsAg in serum or plasma. The membrane is pre-coated with anti-HBsAg antibodies on the test band region and anti-mouse antibodies on the control band region. During testing, the serum sample reacts with the dye conjugate (mouse anti-HBsAg antibody colloidal gold conjugate) that has been coated in the test strip. The mixture then by capillary action reacts with anti-HBsAg antibodies on the Membrane and generates a red band. Presence of this red band indicates a positive result while its absence indicates a negative result. Regardless of the presence of HBsAg, as the mixture continues to migrate across the membrane to the immobilized goat anti-mouse region a red band at the control band region will always appear. The presence of this red band serves as verification for sufficient sample volume and proper flow and as a control for the reagents

Briefly, the procedure is as follows:

1) The Bioline HBsAg test strip was removed from foil pouch.

2) The test strip in the serum samples was immersed with printed sample pointing toward the serum or plasma.

3) Then waited for the red bands to appear. The test was read after approximately 5 minutes. Results after 30 minutes were not interpreted.

Interpretation of the test:

Positive -Two distinct red bands appear, one in test region and another in the control region.

Negative -A single red band appears in the control region. No apparent red or pink band appears in the test region.

Invalid -Control band fails to appear which means improper testing procedure or deterioration of reagents probably.

Accuracy of the Bioline HBsAg strip

Bioline HBsAg was compared with a leading commercial Radio immunoassay (RIA) and an Enzyme immunoassay (EIA) test for Hepatitis B. There was 98% overall agreement between RIA and Bioline HBsAg and 97% between EIA and Bioline HBsAg. Bioline HBsAg will detect any level of HBsAg in serum higher than 5ng/ml within 10 min. However, to detect concentrations below 5ng/ml and to confirm negative results, the test should be read at the end of 15 to 20 min. All ten HBsAg subtypes(ayw1, ayw2, ayw3, ayw4, aayr, adw2, adw4, adrg +adr and adr) produce a positive result in HBsAg assay [.64]

The HBsAg Hepatitis B surface Antigen Rapid test strip is a rapid test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HBsAg in whole blood, plasma and serum

Rapid diagnostic test kits was used to screen for Hepatitis B surface antigen (HBsAg).The HBsAg Hepatitis surface Antigen Rapid test strip is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B Surface Antigen.

Summary

Viral Hepatitis is a systemic disease primarily involving the liver, most cases of acute viral Hepatitis are caused by Hepatitis A Virus, Hepatitis B virus (HBV) or Hepatitis C virus. The complex antigen found on the surface of HBV is called HBsAg. The presence of HBsAg in whole blood, serum or plasma is an indication of an active Hepatitis B infection, either acute or chronic. In a typical Hepatitis B infection, HBsAg will be detected two to four weeks before the ALT level becomes abnormal and 3 to 5 weeks before symptoms or jaundice develop. HBsAg has four principle subtype adw, ayw, adr, and ayr. Because of Antigenic heterogeneity of the determinant, there are 10 major serotypes of HBV.

The HBsAg Hepatitis B surface Antigen Rapid test strip is a rapid test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HBsAg in whole blood, plasma and serum

Principle of the test

The HBs Ag Hepatitis B Surface Antigen Rapid test strip (whole blood, serum and plasma) is a qualitative solid phase. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the strip. During testing the whole blood, serum and plasma specimens reacts with ant –HBsAg antibodies conjugated particles? The mixture migrates upward on the membrane chromatographically by capillary action to react with ant-HBsAg antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive results, while its absence indicates a negative result. To serve as procedural control, a colored line appeared in the control line region indicating that proper volume of specimen has been added and membrane wicking occurred.

Storage and stability

The test kit can be stored at Room temperature or Refrigerated (2-30°C).The test strip is stable through the expiration date printed on the sealed pouch .The test strip must remain in the sealed pouch until the expiration date.

Test procedure.

The test device was removed from foil pouch, placed it on a flat dry surface and add 10ul of serum into the sample well (S) using micropipette and this was followed by adding 4drops (about 120ul) of assay diluent into sample well. When test begins to work, purple color moved across the result window in the centre of the test device a color band will appear in the left section of the result window to show that the test is working properly. Color bands will appear in the middle and right section of the result window. These bands are test line 2 and test line 1(2, 1) The results were interpreted within 5-20 minutes according to standard operating procedure (SOPs) and instruction from the manufacturer. During the study it was not recommended to read the results after 20 minutes since reading too late gives false result

Detection of HBV by the use of HBVe Antibodies Kit

VIDAS HBe/Ant-HBe: VidasHBe/Anti-HBe is an automated qualitative test for use on the VIDAS family instruments, for the detection of the hepatitis B e–antigen (HBe) in human serum or plasma using the ELFA technique (Enzyme Linked Fluorescent Assay).

Summary and explanation

Approximately 5%of the world population is infected by the hepatitis B virus which causes a necro inflammatory liver disease of variable duration and severity. Chronically infected people with active liver disease carry a high risk of developing

cirrhosis or hepatocellular carcinoma. The immuno response to HBV-encoded antigen is responsible both for viral clearance and for disease pathogenesis during this infection. Hepatitis B can be transmitted through sexual contact, exposure to blood products, or perinatally.

Perinatal transmission can be as high as 90% in women who are chronically infected with HBV, in highly endemic areas or in regions with no systematic testing of pregnant women. The child becomes a chronic carrier of HBsAg in 90% of cases

Anti-HBe assay procedure

The VIDAS Anti-HBe assay begins with a preliminary incubation step which can be performed in a water bath, dry incubator, or directly in the instrument

Preliminary incubation

1.Mix the SI and S2 standards, the C2 and C3 controls and the sample using a vortex type mixture.

2. For each sample standard (S2) and control to be tested: pipette 100ul of HBe (S1) standard into a glass or plastic tube or into the sample well of the VIDASHBE strip. Add 100ul of sample, standard or control. Cover and mix the tubes using a vortex type mixer by pippeting several times in the sample well. Cover incubates at 37+_2OC for 1 hour +-5 minutes in a water bath, dry incubator if tubes are used, or in the instrument if preliminary incubation is performed in the strips.

Testing with the instrument

3. Remove the required the required reagents from the refrigerator 30 minutes before the end of preliminary incubation to allow them to room temperature

4. The test is identified by the HBE code on the instrument, the standard, must be identified by S2 and tested in duplicate. If positive control is to be tested, it should be

identify by C3.If the negative control needs to be tested , it should be identified by C2

5. If preliminary incubation is not performed in the strip, pipette 150ul of the mixture into the sample well and controls will be tested singly and the standard in duplicate 5b.If preliminary incubation has been performed in the instrument, remember to insert the SPRs.

6. Check to make sure that the color labels with the assay code on the HBE, SPRs and the HBE reagents match.

7. Initiate the assay as directed in the user manual .All the assay steps are performed automatically by the instrument.

The assay will be completed within approximately 90 minutes .After the analysis the analysis is completed, remove the SPRs and the strip from the instrument.

8. Dispose of the used SPRs and the strips into an appropriate recipient

Results and interpretation of HBe test

Once the assay is completed, results are analyzed automatically by the computer Fluorescence is measured twice in the reagents strips reading cuvette for each test. The first reading is a background reading of the substrate cuvette before the SPRs is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPRs .the RFV (Relative fluorescence value) is calculated by subtracting the background reading from the final result. The index value is calculated by dividing the sample or control RFV by standard Relative Fluorescence value.