SEROPREVALENCE OF HEPATITIS B SURFACE ANTIGEN AND ASSOCIATED FACTORS AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE CLINIC IN MOSHI MUNICIPALITY, KILIMANJARO REGION 2012

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Msc. Epidemiology and Laboratory Management Dissertation

Muhimbili University of Health and Allied Sciences

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By

Boniface Clemence Panga

"A Dissertation submitted in (partial) Fulfillment of the Requirements for The degree of Master of Science in 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 of Hepatitis B surface antigen and associated factors among pregnant women attending antenatal care clinic in Moshi Municipality Kilimanjaro region, 2012**" In fulfillment of the requirements for the degree of Masters of Science in Epidemiology and Laboratory Management of the Muhimbili University of Health and Allied Sciences

_____

Prof M. Matee

(Supervisor**)**

Date: _____

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Janneth Mghamba

(Supervisor)

Date: _____

DECLARATION AND COPYRIGHT

I**, Boniface Clemence Panga**, 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 Veronica, my children Damasi, Juliana and Ladislaus, my father Clemence and mother Veronica, and all the members of my family for their love, support, and encouragement during this process.

ABSTRACT

Background

Potential mother to child transmission of Hepatitis B virus (HBV) is a major concern, because of the associated long-term morbidity and mortality of these infections. Most chronic Hepatitis B Virus (HBV) infections occur during childhood, either from infected mother to child (perinatal transmission) or from one child to another (horizontal transmission). The presence of Hepatitis B e Antigen (HBeAg) among HBV positive mothers is an indicator of active infection and the potential for mother to child transmission of HBV infection. This study investigated HBeAg among Hepatitis B surface Antigen (HBsAg) positive among women attending antenatal to access the potential risk of mother to child transmission of the virus

Materials and Methodology

This was a cross- sectional study that was conducted among 346 pregnant women attending antenatal clinics at Moshi Municipality between January and March 2012. Participants were enrolled consecutively after consenting. Interviews were conducted to obtain information regarding potential risk factors. Blood was collected and screened for hepatitis B screening by Antigen Rapid test strip (HBsAg) and positive sample was subjected to ELFA (BIOMUREX-SA) for HBeAg detection. Syphilis was tested by rapid plasma reagin (RPR). HIV was tested by SD BIOLINE and Determine, discordant result was resolved with Unigold. IgG antibodies to HCV were detected by ONE STEP Ant-HCV Test technique (SD BIOLINE HCV). Data was coded, entered, cleaned, validated and analyzed using Epi Info version 3.5.1

Results

A total of 346 pregnant women were recruited. Their mean age was 24.7 (SD 5,4) years. About 36 pregnant women (10%) had serological evidence of infection with at least one pathogen and 3 (0.8%) had multiple infections. Overall the seroprevalence of HBV was 2.0%. The seroprevalence of HIV, HCV and syphilis were 4.9%, 0.6%, and 2.9%, respectively. One (14.3%) had HBV and syphilis co-infection. Among the seven women who were HBsAg seropositive one (14.3%) was positive for HBe antigen. The risk factors for HBV were age

group 30-34 yrs, mutiple partners and scarification (OR 4.9, 95%CI 1.01-24.13, OR 3.09, 95% CI 1.01-9.42, and OR 10.1, 95%CI 1.89-54.20 respectively)

Conclusion and Recommendations

Based on the prevalence of HBeAg among the HBsAg pregnant mothers there is a need of screening all pregnant women for HBsAg and for providing early immunization at birth to infants of HBeAg infected mothers.

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LIST OF ABBREVIATIONS

AFENET	African Field Epidemiology Network
ANC	Antenatal Clinic
CDC	Centre for Disease Control and prevention
DMO	District Medical Officer
DNA	Dinucleic Acid
DPT	Diphtheria-Tetanus-Pertussis (vaccine)
DUs	Drug users
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
EPI	Expanded Programme on Immunization
FTA	Fluorescent Treponema Antibody
HBc	Hepatitis B core
HBsAg	Hepatitis B Virus Surface Antigen
HBeAg	Hepatitis B Virus e Antigen
HBIG	Hepatitis B immune globulin
HCV	Hepatitis C Virus
HHV8	Human Herpesvirus type 8
HIV	Human immunodeficiency Virus
HTLV	Human T-Cell Leukemia Virus
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
KCMC	Kilimanjaro Christian Medical Centre
MUHAS	Muhimbili University of Health Sciences
MWW	Molecular Weight
NIMR	National Institute for Medical Research
OR	Odd Ratio
RCH	Reproductive and Child Health

RFV	Relative fluorescence value
RMO	Regional Medical Officer
RPR	Rapid Plasma Reagin
STIs	Sexually Transmitted Infections
TFENET	Tanzania Field Epidemiology Network
TPHA	Treponema Pallidum Hem Agglutination
USA	United State of America
UNAIDS	United Nations Programme on HIV/AIDS
UNICEF	United Nations Children's Fund
WHO	World Health Organization

OPERATIONAL DEFINITION / DEFINITION OF TERMS

Acute hepatitis B: 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 at sometime in the past)

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

Blood-borne pathogens: Infectious microorganisms present in blood that can cause disease 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 with Hepatitis B Virus.

Cirrhosis: Permanent liver damage (scarring).

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

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

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

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 *(http://www.icebergsand babies.org.uk/sti's.htm*)

CHAPTER ONE

1.0 Introduction

1.1Background

Hepatitis is the disease of a liver often caused by viruses, Hepatitis means inflammation of the liver, and there are different kinds of hepatitis caused by different hepatitis viruses. Hepatitis B virus is a small circular DNA virus within the family of six viruses and it was first characterized in 1965. Hepatitis D virus which only occurs when a patient has hepatitis B virus; Hepatitis E which is similar to hepatitis A and Hepatitis F which appears to produce hepatitis similar to Hepatitis C but scientists are not certain if it is a separate virus .Hepatitis G is a newly identified virus that is probably transmitted in similar fashion of hepatitis C in Africa.

In each case, the viruses once inside the body, begins to live in the liver cells, interferes with cells normal activities and then uses the cell to make new viruses, this disease hepatitis is characterized by high rate of viral persistence and potential to develop and of ever worsening chronic liver disease ranging from chronic hepatitis and occasionally hepatosoma carcinoma

Among the hepatitis virus, Hepatitis B virus remains a major public health problem in both developed and developing world, it is estimated globally that 350-400 million people are chronically infected with hepatitis B virus. In developing countries of Africa and Asia, the prevalence is > 8% and 2 billion have markers of current or past infection and 350 million have chronic infection. About 15-25% will die from chronic liver disease (liver cancer and cirrhosis) ie at least 1 million deaths per year. Young children who become infected with Hepatitis B virus are the most likely to develop chronic infection and moreover there is a 25% mortality in perinatal acquired disease. It has been found that, following childhood exposure, estimates of chronic HBV infection approach 20% in some African countries Hepatitis B associated hepatocellular carcinoma is also probably the most common tumor affecting males in sub-Saharan Africa. Of particular importance is the perinatal transmission. Potential mother to child transmission of Hepatitis B virus (HBV) is a major concern, because of the associated long-term morbidity and mortality of these infections

Most chronic Hepatitis B Virus infections occur during childhood, either from infected mother to child (perinatal transmission) or from one child to another (horizontal transmission) The prevalence of Hepatitis B Virus infection among an antenatal population may be a reliable indicator of hepatitis B virus prevalence rate in the general population. Hence, screening antenatal women for HBsAg can give a reliable prevalence of the disease in a population and provide an avenue for preventing mother to child transmission of the virus. While some countries have initiated screening, some are yet to implement.

In 1992, the world Health Assembly endorsed the recommendation issued by the Global Advisory Group of Expanded Program in Immunization (EPI). The Advisory group recommends that the HBV vaccine be integrated into the national immunization programs of the countries with hepatitis B carrier prevalence of 8% of greater by 1995 and in countries by 1997. According to study done in 2012 at Tanzania titled prevalence of hepatitis B co-infection and response to antiretroviral therapy among HIV-positive patients in urban Tanzania, the prevalence of Hepatitis B virus is 6.4% (73)) Tremendous progress have been made thereafter .Tanzania like many other countries, has also adopted the WHO recommendation by integrating hepatitis B vaccination into national immunization schedule. However, no effort has been made to integrate hepatitis B virus screening into reproductive and child health services as part of routine antenatal care. Only routine voluntary HIV screening and Syphilis is done for pregnant women. Routine screening for HBV infection has several potential benefits including: (i) offering prophylaxis to reduce perinatal transmission (ii) modifying management of pregnant women with Hepatitis B/HIV and HBV/HCV co-infections and (iii) estimating the potential for vertical transmission of the virus and hence aiding in correct decision towards the Hepatitis B vaccination among infants.

1.2. Statement of the problem

Globally, it's estimated that more than 2 billion persons are still living with hepatitis B virus infection. Over 350 million are believed to be chronically infected with the virus and are thought to be at a high risk of developing chronic hepatitis, cirrhosis, and primary hepatocellular carcinoma [36]. Despite efforts by many countries to reduce the HBV prevalence the burden is still high. Among the risk for acquiring the infection include

perinatal transmission from a pregnant woman. Other risk factors for getting the infection are well documented but they vary from one setting to the other due to differences in levels of prevention.

A study done at Muhimbili National Hospital among blood donors revealed that the Seroprevalence of HBV and HCV to be 8.8% and 1.5% respectively [31]. Studies done among pregnant women in Nigeria to determine the prevalence of hepatitis B and C viral infections among pregnant women attending the antenatal clinic of the University of Benin Teaching Hospital found that the prevalence of HBV was 12.5% while that of HCV was 3.6% [55]. Studies have also been done in Southern Tanzania to determine the Prevalence and mother-to-infant transmission of hepatitis viruses B, C, and E in Southern Tanzania by Menendez et.al, 1999[32] and it showed a high prevalence of Hepatitis B surfaces antigen and Hepatitis B e antigen being 6.3% and 24%, The findings suggest that 24% of the pregnant women screened had chance of over 85% of transmitting infection to the newborn. Similar findings have also been observed in other Tanzanian studies conducted among pregnant women showing the prevalence of HBV to range between 4.3% and 7.2% [39, 18], none of the previously mentioned Hepatitis B Virus studies conducted in Tanzania searched for the HBeAg, which is a marker of infectivity. As a consequence, the potential rate of mother to child transmission of Hepatitis B Virus in this country is unknown.

In an effort to reduce Hepatitis B infection, Tanzania like many other countries, has adopted the WHO recommendation by integrating hepatitis B vaccination into national immunization schedule. However, no effort has been made to integrate hepatitis B screening into reproductive and child health services as part of routine antenatal care. Only routine voluntary HIV screening is done for pregnant women. Routine screening for Hepatitis B Virus infection has several potential benefits including; (i) offering prophylaxis to reduce perinatal transmission (ii) modifying management of pregnant women with Hepatitis B combination with Human Immunodeficiency Virus and Hepatitis B Virus combination with Hepatitis C virus co-infections and (iii) estimating the potential for vertical transmission of the virus. As a consequence, it is difficult to identify mothers who are infected with hepatitis B virus, and institute appropriate preventive measures, including early administration of the vaccine than then recommended time to the exposed newborns.

. Due to shared modes of transmission it is logical to investigate other pathogens such as HIV, HCV and syphilis and determine their association if any.

1.3 Study rationale:

Among the infected mothers the risk of chronic HBV infection is about 70 to 90% among mothers who are Hepatitis B envelop Antigen (HBeAg)-positive compared to only5%-20% from those who are Hepatitis B envelop Antigen(HBeAg)-negative [28]. Furthermore, studies have shown that perinatal transmission of Hepatitis B Virus (HBV) occurs in infants born to mothers who are positive for hepatitis B surface antigen (HBsAg) and HBeAg [38].

Children born to HBeAg positive mothers are thus likely to be infected with HBV if immunization is not initiated early. According to the Tanzania Expanded Program on Immunization (EPI) the first dose of Hepatitis B vaccination is administered 4 weeks after delivery in combination with diphtheria, pertussis and tetanus (DPT) as pentavalent. The second dose and third dose are usually given at 8 weeks and 12 weeks respectively. The timing of the vaccination may be rather late for controlling of HBV infection among infants.

As mentioned previously there is paucity of information in this country regarding HBsAg and HBeAg co-occurrence, an information that is necessary to estimate the potential magnitude of mother with active viral infection and who are likely to cause mother to child transmission of HBV infection.

1.4 Research questions

1.4.1 What is the prevalence of HBsAg among pregnant women attending antenatal clinic at Majengo and Pasua Health centres

1.4.2 What is the prevalence of co infection of Hepatitis B Virus (HBV) with Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV) and Syphilis among pregnant women attending antenatal clinic

1.4.3 What are the risk factors associated with Hepatitis B virus among pregnant women attending antenatal clinic.

1.5 Broad objective:

Seroprevalence of Hepatitis B surface antigen and associated factors among pregnant women attending antenatal care clinic in Moshi Municipality

1.5.0 Specific objectives:

1.5.1 To determine the prevalence of HBsAg among pregnant women attending antenatal clinic.

1.5.2 To determine the prevalence of co infection of Hepatitis B Virus (HBV) with Human Immunodeficiency Virus, Hepatitis C Virus (HCV) and Syphilis among pregnant women attending antenatal clinic.

1.5.3 To determine the Risk factors associated with Hepatitis B virus among pregnant women attending antenatal clinic.

CHAPTER TWO

2.0 Literature review

2.1 Seroprevalence of Hepatitis B and Co infection

Hepatitis B virus (HBV) has been described as major public health, occurring endemically, in all areas of the world [25] Approximately 45% of the world population live in areas where chronic HBV infection is highly endemic (> 8% of the population are Hepatitis B surface Antigen-positive); 43% live in areas of intermediate endemicity (2-7% HBsAg-positive); and 12% live in areas of low endemicity (<2% HBsAg-positive) [60]. Infected individuals can remain asymptomatic for decades. However, more than 80% of them become chronic carriers which result in an increased risk of liver cirrhosis, liver cancer and liver failure 20 - 30 years later[49] .Hepatitis B virus (HBV) is endemic and poses a grave public health problem in Africa where it is mainly transmitted from mother to baby or during childhood.. Sexual transmission accounts for most adult HBV infections in the United States [67]. Approximately 25% of the regular sexual contacts of infected individuals will themselves become seropositive. [68], 10-20% of women seropositive for HBsAg transmit the virus to their neonates in the absence of immunoprophylaxis. In women who are seropositive for both HBsAg and HBeAg vertical transmission is approximately 90% [68]. In patients with acute hepatitis B vertical transmission occurs in up to 10% of neonates when infection occurs in the first trimester and in 80 -90% of neonates when acute infection occurs in the third trimester [68]. Chronic infection occurs in about 90% of infected infants, 30% of infected children aged <5 years, and 2%--6% of adults. Among persons with chronic HBV infection, the risk of death from cirrhosis or hepatocellular carcinoma is 15%--25%. [1]

There are various studies which have been done which demonstrate the existence of Hepatitis among pregnant mothers. According to the study done in Nigeria in 2004, approximately 63.3% of pregnant women were found to be positive for HBV core antigen while only 3% were HIV positive (17]. In another study done in Malawi, about 81% of young mothers under the age of 20 years were reported to have antibodies to HBV core antigen [1]. In another study done in Jos plateau state, in Nigeria, 10.3% of the subjects were HBsAg positive [48]. .A most recent study done in Nigeria to determine the

prevalence of hepatitis B and C viral infections among pregnant women attending the antenatal clinic of the University of Benin Teaching Hospital revealed a prevalence of 12.5% and3.6% respectively [55].

Studies have shown that, STIs and other blood-borne infections are significant contributors of maternal and prenatal morbidity and mortality in Africa [35, 58, 12, and 50]. Syphilis is the commonest STIs among pregnant women, for instance studies show that between 4–15% of pregnant women are believed to be infected with syphilis in sub-Saharan Africa [9]. Active infection with syphilis in pregnant women leads to foetal or infant death or disability for 50–80% of affected pregnancies, and is a major cause of adult morbidity as well [10] Syphilis is also a systemic disease caused by *Treponema pallidum* which can be spread by sexual contact, blood transfusion and via vertical transmission[33]. In sub-Saharan Africa, syphilis remains a serious public health problem. The prevalence of active syphilis infection among East African countries in 2001 was 12.8% in Tanzania [54], and 3.8% in Kenya [46].Furthermore, literature shows that Sexually Transmitted Infections (STIs) contribute to a variety of obstetric and gynecologic complications in women, including increased risk of tubal infertility and pelvic inflammatory disease, and has been associated with chronic pelvic pain [16]). Sexually Transmitted Infections (STIs) significantly associated with adverse pregnancy outcomes such as spontaneous abortion, preterm delivery, ectopic pregnancy, premature rupture of membranes, intrauterine infection of the fetus, and low birth weight in infants[11,29]

Hepatitis has also been found to co exist with other STI among pregnant women. One study done in Bukina Faso to determine the Seroprevalence of immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), human T-cell leukemia virus (HTLV), human herpesvirus type 8 (HHV-8), and dengue virus among pregnant women and blood donors, revealed the following prevalence values for Nouna (rural area) or Ouagadougou (urban area), respectively: HIV 3.6/4.6, anti-HBV core (anti-HBc) 69.6/76.4, HBV surface antigen (HBsAg)14.3/17.3, HCV 2.2/1.5, HTLV 1.4/0.5, HHV-8 11.5/13.5, dengue virus 26.3/36.5[7].

Another retrospective study done in Abidjan, Côte d'Ivoire to estimate the prevalence of HBV and HCV among pregnant women found that HBsAg was in a similar proportion among HIV-positive (9.0%) and HIV-negative (8.0%) women. Also, the diagnosis of

chronic hepatitis B, based on HBV DNA positive results, was more frequent in HIV-positive women (26.7%), compared to HIV-negative women (9.4%) [42]. The prevalence of hepatitis C infection was about 1%, both in HIV-positive (1.2%) and HIV-negative (0.8%) women[42].These studies demonstrate that there are different seroprevalence values for blood borne infections and STIs in different settings. According to a recent national serosurvey in Uganda, the prevalence of chronic HBV infection was 10%, with regional variation from 18–24% in northern and West Nile regions to 4% in southwestern Uganda[25]

Although there are limited data, lower chronic Hepatitis B Virus (HBV) prevalence in this region also appears consistent for at least 20 years. One study conducted in 1986 at two hospitals in Southwest Uganda, showed a 5% prevalence of chronic HBV[25]. Sero-surveys in Tanzania showed similar prevalence of HBV as 5-9% [14] and 2.4% in Rwanda [40].

2.2 Risk factors for Hepatitis B virus

In Africa, most HBV infections are traditionally thought to occur in young children through close contact with household contacts, ritual scarification and other mechanisms, with exposure to Hepatitis B Virus (HBV) occurring before the age of sexual debut [2] Sexual transmission has also been suggested to play a role in East Africa.

Sexual transmission in adulthood was found to be the most likely risk factor for adult Hepatitis B Virus transmission in Rakai, Uganda.[25] In Southwestern Uganda, another study found low rates of scarification[24]. In study conducted by Mark et al 2008 results suggested that younger age of sexual initiation and multiple sex partners are significant risk factors for the acquisition of Hepatitis B Virus (HBV).[24]

CHAPTER THREE

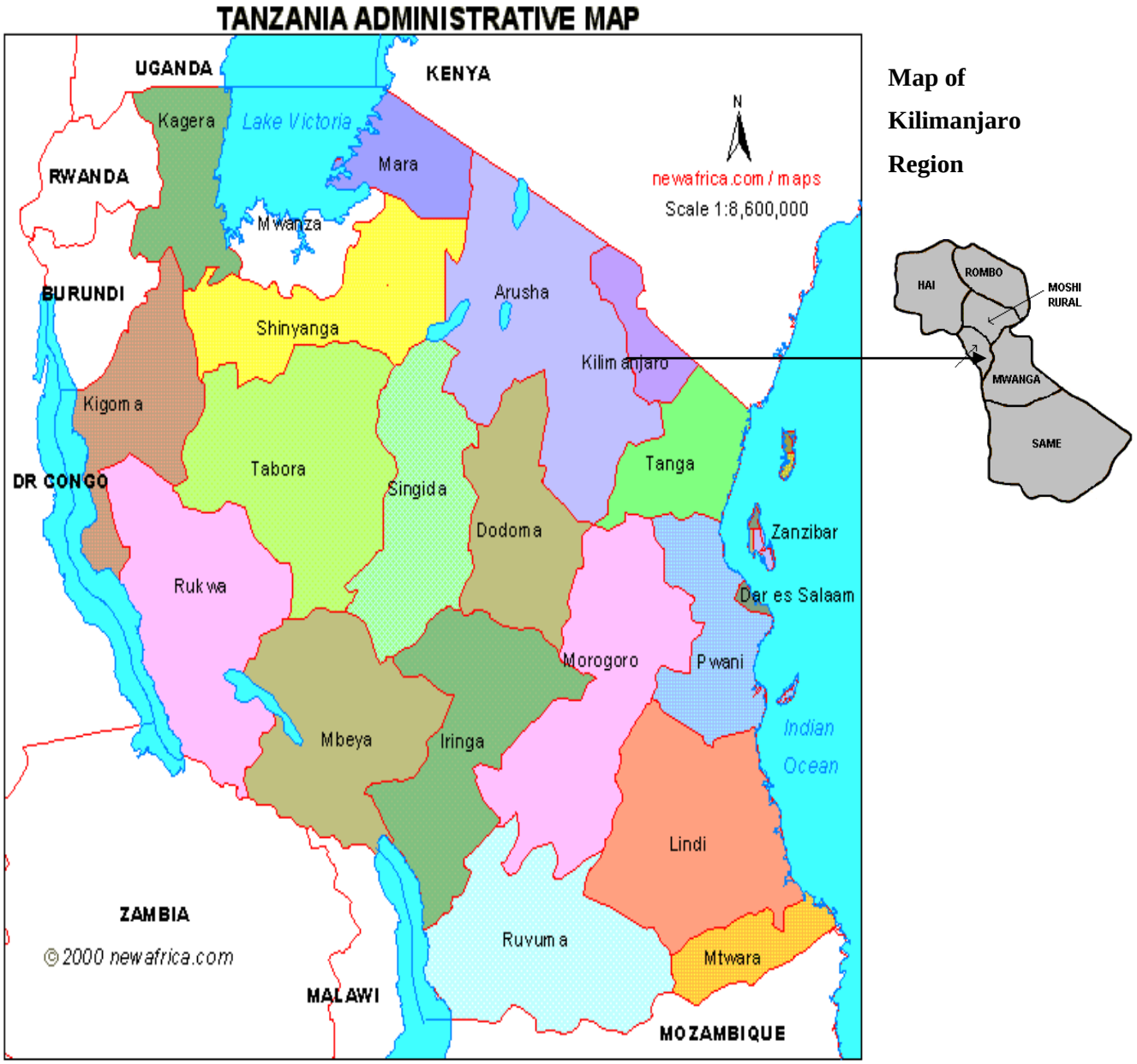
3.0 Methodology

3.1 Study design and study area

This was a cross- sectional study that was conducted among 346 pregnant women attending antenatal clinics at Moshi Municipality between January and March 2012. Kilimanjaro region is the tourist, administrative and commercial centre of Kilimanjaro Region in Northern Tanzania; the municipality borders Moshi Council in North and Hai District to the West and has an estimated population of approximately 230,000 people [56].The economy of Moshi depends on commerce, industry, small-scale cultivation and tourism. These are the areas where the basic services like education, health facility, security and commercial activities are found. The Municipal has a good access to other cities through tarmac roads and international airport.

Kilimanjaro has seven districts. Moshi urban district was chosen conveniently so as to be able to compare with various studies done on HIV and other STI in the same place some years back. The Moshi Municipality has 16 wards and 7 Government primary health care clinics namely Majengo, Pasua, Bondeni, Karanga, Mabogini, Msaranga, and Uchira. Among the 7 government health facilities, Majengo and Pasua primary health care clinics were chosen because they are the largest primary health care centres in Moshi Municipality and they were receiving pregnant women from 13 out of 16 wards serving a catchment population of approximately 170,000 people.

Figure 1: Map of Tanzania Showing Kilimanjaro Region:



3.2 Study population.

The target population was all pregnant women of different ages attending routine antenatal clinic for the first visit for any pregnancy at Majengo and Pasua Health centres.

3.3 Inclusion criteria

Pregnant women attending antenatal clinic for the first time and consent

3.4 Exclusion criteria

Pregnant women who had prior history of Hepatitis B Vaccination and attending the clinic for the first visit

Pregnant women who didn't consent to the study

3.5 Sample size

This sample size was estimated using the formular for calculating sample size for cross sectional study as described below.

$$N = \frac{Z^2 P (100-P)}{E^2}$$

The following assumptions were made during sample size calculation.

z =Standard deviation of the normal distribution = 1.96 (confidence level at 95%)

p = prevalence =3.6 %(prevalence of HBsAg in serological survey in Tanzania [39]

100-P = pregnant women who not exposed

E = Tolerable error / level of significance = 2%.

n=Minimum sample size

Samples collected during the study was 346

3.6 Sampling technique

Probability proportion to size was used to allocate the calculated samples size to the two health facilities within Moshi municipality. The following procedure was used:

The monthly uptake of the two Health facilities was determined by the following formulae

Majengo Health Centre: Z x 30days =M where Z=the number of client/day and M total number/month

Pasua Health centre: Z x 30 days =P.

Z for Pasua Health centre was 6 pregnant women per day, and Z for Majengo Health centre was 7 pregnant women per day

Total = 390 (pregnant women)

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

Pasua Health Centre = (180/390)* 346 = 160 out of total sample of 346

Majengo Health Centre = (210/390)*346 = 186 out of total sample of 346

Pregnant women were recruited from a list of new attendees each time they register in the clinic., sampling was not feasible as such study participants were recruited in each clinic until respective required samples were obtained (160 for Pasua and 186 for Majengo).

3.7 Ethical issues

The ethical clearance was sought from Muhimbili University of Health and Allied Sciences. Permission to collect data was sought from The Regional Medical Officer and the Medical officer of municipal. Written informed consent was applied when collecting data from pregnant women attending routine antenatal clinics. During testing no names were used in the data collection process only unique identity numbers were used. All study respondents found to be positive for HBsAg, HCV and HBeAg were referred to Kilimanjaro Christian Medical Centre for further management. Pregnant women positive for syphilis were treated at the clinic, also the couples were tested and treated in the clinic. Those who are positive for HIV were counseled and referred to Care and Treatment Clinic for further management Confidentiality was the first concern during data management and all information collected was treated as strictly confidential. All information collected was kept confidential and for this study only

3.8 Data collection procedures

The data for the study was derived from serological testing and questionnaires. Socio demographic and risk factor data was collected using a standard structured questionnaire. Hepatitis B virus, HIV and Syphilis serological data by testing, an informed consent was obtained from the participants using a standard consent form designed for this study

Participants were interviewed using a questionnaire

3.9 Blood sample collection

The study subjects were enrolled in a special log book and assigned a unique identification number. After consenting, interview was done to collect soci demographic information and information on factors associated with Hepatitis B Virus. 5mls of venous blood was taken aseptically by vacuntainer needle in tube, sample was left at room temperature for 20

minutes to clot, sample was centrifuged at 3000rpm for 5 minutes to get clear serum for serological analysis of HIV, syphilis, HCV and HBV, the specimen were shipped in a cool box to Kilimanjaro Christian Medical Centre and stored in Deep freeze at (-20°C) before analysis was done at KCMC. The analysis for HBV e Antigen testing was done at TMJ Hospital at Dar-es-salaam. All samples were kept at -20^{o}C

3.9.1 Specimen Storage

Serum were secondarily aliquated in nunc tubes and stored at -20°C awaiting air shipment to MUHAS microbiology laboratory for Hepatitis B Virus e Antigen testing. Repeated freezing and thawing was avoided. All International Air Regulation was strictly followed during parking and transportation.

3.9.2 Detection of Hepatitis B surface antigen (HBsAg)

The Hepatitis B surface Antigen rapid test strip was used for the detection of hepatitis B surface antigen (HBsAg).The test has sensitivity and specificity of approximately 99.7% and 99.3%, respectively when performed according to the instructions of the manufacturer (BIOLINE Biotech Ltd, Dart ford, UK)

3.9.3 Detection of Hepatitis B e Antigen (HBE/HBET) (Enzyme linked Fluorescent Assay) the test has sensitivity and specificity of approximately 99.8% and 99.4%, respectively when performed according to the instructions of the manufacturer (BIOMUREX –SA)

3.9.4 Hepatitis C Virus serology

IgG antibodies to HCV were detected using ONE STEP Ant-HCV Test technique (SD BIOLINE HCV) The test has sensitivity and specificity of approximately 100% and 99.4%, respectively when performed according to the instructions of the manufacturer (STANDARD DIAGNOSTICS INC.Kyonggi-do,Korea.

3.9.5 Syphilis serology

Syphilis was diagnosed using Rapid Plasma Reagin (BIOLINE Diagnostics, Kent, and UK). Active syphilis was diagnosed if an individual's Serum became positive. The test has sensitivity and specificity of approximately 99.3% and 99.5%, respectively when

performed according to the instructions of the manufacturer (SD BIOLINE Netherland) (Appendix 7.3)

3.9.6 Human Immunodeficiency Virus Serology

HIV status was determined by SD Bioline 3.0 (Abbott Laboratories, IL, USA,the Netherlands) and reactive samples were retested by Determine HIV1/2 (Abbott Laboratories, IL, and US), assays detect both HIV-1/2 infections. Samples reactive on both tests were considered to be positive for IgG anti HIV antibodies.

3.9.7 Data management and analysis

Data were entered in Epi Info soft ware version 3.5.1. To minimize error data cleaning and validation was done.The seroprevalence of HIV,HCV, HBsAg and syphilis was expressed in percentages for the entire study group. Logistic regression was used to determine the associations between the occurrence of HBsAg and risk factors .The associations are presented as odds ratio (OR) together

Data validation includes how to run frequency during analysis and abnormal findings

Descriptive analysis was carried out by calculating the mean, standard deviation, 95% confidence interval and frequencies of different variables using Epi Info software version 3.5.1.

Bivariate analysis was performed to identify the possible risk factor for getting hepatitis. . Odds Ratio was used to compare sero-positivity of hepatitis B infection with history of exposure to risk factors. Risk factor variables with $p<0.05$ were considered as having significant association with hepatitis B Virus. Any factor with a p-value <0.05 was significant and was included in logistic regression model. Risk factors with less than 10% level of probability ($p<0.1$) in univariate analysis were entered into unconditional logistic regression model. Stepwise backward elimination logistic regression was used to come up with the final model. `

Dependent variables: In this study the dependent variable is the seropositive for Hepatitis B virus surface Antigen and HBeAg.

Independency variables: Age, parity, education, scarification, multiple sexual partners and history of drinking alcohol

CHAPTER FOUR

4.0. Results

4.1 Socio demographic characteristics

A total of 346 pregnant women were recruited during the study, the mean age of the study subjects was 24.7 years (SD= 5.4). The youngest study respondent was 16 years with the oldest being 41 years. All pregnant women recruited in the study got only tetanus vaccination.

Table 1: The socio demographic characteristics of pregnant women attending antenatal clinic at Moshi Municipal, 2012

Age group	n	Percentage
<20	89	25.7%
20-24yrs	103	29.8%
25-29yrs	88	25.4%
30-34yrs	45	13%
35-39yrs	17	4.9%
40+yrs	4	1.2%
Marital status		
Single	52	15%
Married	293	84.75%
Separated	1	0.3%
Educational Level		
No formal education	4	1.2%
Primary education	273	78.9%
Secondary education	60	17.3%
Tertiary	9	2.6%
Occupation		
Government employed	8	2.3%
notemployed	91	26.3%
private employed	23	6.6%
self employed	224	64.7%
Pregnancy status;		
First pregnancy	130	37.6%
Second pregnancy	110	31.8%
Third pregnancy	58	16.8%
Above Four pregnancy	48	13.9%

Of the 346 respondents, majority (29.8%) were aged between 20 and 24 years , 84.7% (293) were married, 97% had attained primary education, 62.4% (216) were multi-gravidae and 64.7% were self employed.

4.2 Seroprevalence and co infection of Hepatitis B Virus among study participants

Of 346 study respondent who were recruited 7 tested positive for Hepatitis B surface antigen giving a seroprevalence of 2%. The Seroprevalence of HIV, HCV and syphilis was 4.9%, 0.6%, and 2.9%, respectively. . One out of seven (14.3%) HBsAg seropositive mothers had syphilis co-infection.

Table 2: Seroprevalence of Hepatitis B surface Antigen in relation to socio demographic characteristics among pregnant women attending antennal clinic at Moshi Municipal, 2012

Varibles	Frequency	No of HbsAg positive	Prevalence (95%CI)
age group			
<20	89	0	0 (0-4.1)
20-24yrs	103	3	2.9 (0.6-8.3)
25-29yrs	88	1	1.1 (0-6.2)
30-34yrs	45	3	6.7 (1.4-18.3)
35-39yrs	17	0	0 (0.0 -19.5)
40+yrs	4	0	0 (0-60.2)
Marital status			
Single	52	0	0 (0-68)
Married	293	7	2.4(1-4.9)
Separated	1	0	0 (0-97.5)
Educational Level			
No formal education	4	0	0 (0-60.2)
Primary education	273	6	2.2 (0.8-4.7)
Secondary education	60	1	1.7 (0.-8.9)
Tertiary	9	0	0 (0-33.6)
Occupation			
Self employed	224	5	2.2 (0.7-5.1)
Govenment employed	8	1	12.5 (0.3-52.7)
Private employed	23	0	0 (0.0-14.8)
Not employed	91	1	1.1 (0.0-6.0)
Pregnancy status;			
First pregnancy	130	1	0.8 (0.0-4.2)
Second pregnancy	110	2	1.8 (0.2-6.4)
Third pregnancy	58	2	3.4 (0.4-11.9)
Above Four pregnancy	48	2	4.2 (0.5-14.3)

In this study the study respondent from Majengo were 179, and 167 were attending Pasua health centre. The prevalence of HBsAg among the primgravidae (n =129) was 0.8%, while among the multgravidae (n =217) the prevalence was 2.8%. Respondents who were unemployed had a HBsAg seropositivity of 5%.

Table 3: Frequency distribution of risk factors for Hepatitis B surface antigen

Risk factor	n (%)
Blood transfusion	8(2.3)
Drinking Alcohol	116(33.5)
Multiple sexual partners	126(36.4)
Scarification	60(17.3)
Ear piercing	284(82.1)
Tattooing	5(1.4)
Unsafe injection	10(2.9)
Hospital admission	100(28.9)
Tooth extraction	96(27.7)
Surgery	31(9.0)
Catheterization	21(6.1)
Abortion miscarriage	32(9.2)

Regarding behavioral factors, 8(2.3%) of the study respondent indicated that they had history of blood transfusion, about 33.5 % (116) reported history of history of drinking alcohol,36.4% (126) reported having more than one sexual partner and. Those who reported scarification were 60 (17.3%).

Table 4: Bivariate analysis of soci demographic risk factors among pregnant women attending antenatal clinic at Moshi Municipal, 2012

Varibles	No of HBsAg positive N=7	No of HBsAg Negative N=339	OR	95%CI
age group				
20-24yrs	3	100	2.6	0.3-25.5
30-34yrs	3	42	0.6	0.6-61.5
25-29 yrs**	1	87	Reference	
Educational Level				
Primary education	6	267	0.5	0.1-5.9
Secondary education**	1	59		
Occupation				
Formal employment	1	7	7	0.8-74.1
Informal employment	0	23	0.0	0.0-10.1
Pregnancy status;				
First pregnancy	1	129	Reference	
pregnancy two	2	108	0.9	0.16-4.48
Third pregnancy	2	56	2.0	0.38-10.6
Above Four pregnancy	2	46	0.0	Und-60.6
Number of delivaries				
≤1 pregnancy	2	137	Reference	
>1 pregnancy	3	103	1.7	0.4-7.8
Gravidity				
Primigravidae	1	128	2.0	0.2-20.6
Multigravidae	6	211	0.4	0.1-4.9

- Fisher Exact was used for value < 5 in the cells
- A formal employment includes Government employees while other employments
- Included Private employee, not employed and self employed. ** Reference value

Table 5: Bivariate analysis of behavioral risk factors among pregnant women attending antenatal clinic at Moshi Municipal, 2012

	Frequency	**No_HBsAg positive N=7**	No of HBSAg Negative **N=339**	Crude Odds Ratio	95%CI
Scarification					
YES	70	5(7.1)	65(92.9)	10.5	1.99-55
NO	276	2(0.7)	274(99.3)		
Drinking alcohol					
YES	116	4(3.4)	112(96.6)	2.6	0.59-12.2
NO	230	3(3.1)	227(98.7)		
Multiple sexual partners					
YES	126	2(1.6)	124(98.4)	4.5	1.5-13.1
NO	220	5(2.3)	215(97.7)		
Blod transfusion					
YES	8	0(0)	8(100)	0.0000	Undefined
NO	338	7(2.1)	331(98.0)		
Tooth extraction					
YES	96	1(1.0)	95(99)	0.37	0.05-3.6
NO	250	6(2.4)	244(97.6)		
Ear piercing					
YES	284	7(2.5)	277(97.5)	Unde	0.40- Unde
NO	62	0(0)	62(100)		
Unsafe injection					
YES	10	0(0)	7(2.1)	0.0000	Undefined
NO	336	(10)	329(97.9)		
Tattooing					
YES	5	0(0)	5(1.5)	0.0000	Undefined
NO	341	5(100)	334(98.5)		

The identified risk factors from both soci demographic and behavioral; found to be associated with hepatitis B included scarification ($p<0.005$), age group 30-34yrs and multiple sexual partners ($p<0.005$)

All factor with a p value<0.1 were put into unconditional logistic regression model. Age group 30-34 years (aOR 4.9, 95%CI 1.01-24.13, p value 0.047), scarification (aOR 10.1, 95% CI 1.89-54.2 p value 0.006) and multiple sexual partners (aOR 3.09, 95%, CI 1.01-9.42, p value 0.04) remained to be significant

Table 7: Multivariate analysis of Risk factors for HBsAg among pregnant women attending antenatal clinic at Moshi Municipal, 2012

Variable	aOR	95%CI	P value
Age group 30-34yrs	4.9	1.01-24.13	**0.047**
Age 35-39	2	0.16-14.8	0.7
Age < 20	6	0.037-3.399	0.370
Multiple sexual partners	3.09	1.01-9.42	**0.046**
Multigravidity	4	undefined	0.98
Scarification	10.1	1.89-54.2	**0.006**
Drinking alcohol	4	0.537-5.078	0.380
Primigravidity	2	0.000->1.0E1	0.983

4.6 Serologic marker of Hepatitis B Virus detected

Of the 346 sample study respondents who are recruited 7 tested positive for Hepatitis B infection .To assess the risk of perinatal transmission the seven positive blood samples were subjected to HBe Antigen, where 14.3%(1/7) tested positive for both HBsAg and HBeAg,

CHAPTER FIVE

5.0 Discussion

This study found that about 10% of the pregnant women have serological evidence of infection with at least one pathogen ie HBsAg, HCV, HIV or Syphilis. Only one pregnant woman had co infection of HBsAg and Syphilis. The seroprevalence of Hepatitis B surface antigen among pregnant women was 2%. There was a risk for perinatal infection among those HBsAg positive pregnant women, 14.3% were positive for HBe antigen. Identified risk factors for HBsAg included age group 30-34 years, having multiple sexual partners and scarification.

Perinatal transmission of Hepatitis B virus

In this study the prevalence of hepatitis B surface Antigen was 2% among pregnant women attending antenatal clinic at Moshi Municipal. Among those positive HbsAg pregant womenThis places Tanzania, specifically the Kilimanjaro region, as a medium endemic area (2-7 %) of HBsAg positive for HBV infection according to WHO criteria [65]. The seroprevalance of HBsAg among pregnant women found in this study is a bit lower than that done in 1994 in Dar es salaam where it was found to be 4.3 % [39]. Although the difference could be attributed by the different geographical locations, this findings could also mimic the HIV seroprelance declining trend which has also been observed in recent years. HIV and Hepatitis B share common risk factors as such Hepatitis seroprevalance trend is likely to simulate HIV declining trend. However, the seroprevalance for HBeAg was also found to be as high as 10% in this Dar es salaam study[39].

Perinatal transmission from mothers infected with HBV, i.e. positive for hepatitis B surface antigen (HBsAg) to their newborn infants has been shown to be a major source of HBV infections in many countries [38].Studies have shown that infants born to mothers positive for both HBsAg and HBeAg have a 70-90% chance of acquiring perinatal Hepatitis B Virus (HBV) infection, and 85-90% of infected infants become chronic carriers [72]. This means that one child born by mother who is positive for both HBsAg and HBeAg will have a chance of acquiring HBV infection and six children born by HBsAg positive mothers will become chronic carriers if early immunization at birth is not initiated [72]. Although Tanzania is already providing Hepatitis B vaccine as a pentavalent vaccine (Diptheria, Tetatuns, Pertusis, Heamophilus, Hepatitis) 4 weeks post delivery. The study

findings indicate there is a likehood of perinatal trasmission and if early immuzation at birth is not initated, the current immunization will not ensure infants born to be infection free in future. Our study was done in Moshi and this finding if extrapolated show that more that 46,000 people in a population of 230,000 of Moshi urban in Kilimanjaro region could thus be infected with hepatitis B

Seroprevalance of Hepatitis B and co infection

This study found only one pregnant women had co infection of HBsAg and Syphilis. This study found HBsAg/Syphilis coinfection but no HbsAg/HIV or HbsAg/Hepatitis C coinfection. This is suprising as it has been postulated that because of shared routes of transmission, HBV an HCV infections could occur frequently among HIV infected patients,this could be due to low prevalence of HbsAg among pregnant women at Moshi Municipality. Studies have shown that prevalence of these co-infections ie Hepatits B Virus /Human Immuno Virus (HBV/HIV) can be critical for the appropriate management of these patients since co-infected individuals are at increased risk of liver-related mortality[30]

Risk factors for Hepatitis B virus

This study found among the risk factors studied to be associated with Hepatitis included age, scarification and mutiple sexual partners. Other risk factors like blood transfusion, , tooth extraction,unsafe injection,ear piercing and tattooing were not associated with Hepatitis B Virus infection.. Studies have also showed exposures to Hepatitis B Virus occurred before the age of sexual debut[2]. In study conducted by Mark et al 2008 results suggested that younger age of sexual initiation and multiple sex partners are significant risk factors for the acquisition of Hepatitis B Virus. In a study done in Ethiopia, the age group infected with HbsAg was also 30-34 years which is similar to our study [61].

Sexual transmission has also been suggested to play a role in East Africa as demonstrated in the Rakai Ugandan study [25]. The finding that scarification is a risk factor for acquiring Hepatitis B is however different from a Southwestern Uganda study which found low rates of scarification.[24]. This might be due to variation of culture of underlying population studied between the two areas.

Limitations

The study has some limitations. This study was done in one region only and moreover one district and this could explained the low seroprevalance obtained in this study compared to the other studies. We however still feel that our findings might protray the reality when we compare the current declining seroprevalance trend of HIV which also shares the same risk factors like Hepatitis

CHAPTER SIX

6.0 Conclusion and Recommendations

6.1 Conclusion:

This study found that Kilimanjaro region in Tanzania has the seroprevalence of hepatitis B surface antigen among pregnant women lies within the intermediate endemic area (2-7 %) of Hepatitis B infection based World Health Organization criteria for endemicity. There was a high infectivity rate and this poses a risk for perinatal infection among those HBsAg positive pregnant women to their infants. Identified risk factors included having more than one sexual partner, history of scarification and age group between 30-34 yrs.

6.2 Recommendations

1. Based on the prevalence of HBsAg pregnant mothers in Moshi Municipality there is a need of free screening all pregnant women for HBsAg, Knowing hepatitis B infection status of expectant mothers attending ANC will be an important aspect in for early initiation of immunization at birth to infants of HbeAg infected mothers and this will protect the infants born to hepatitis B positive mothers from getting the infection..

2. Advocate for a bigger study to be done which will involve more regions in Tanzania.

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8.0 Appendices

8.1: Informed consent form English Version

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
DIRECTORATE OF RESEARCH AND PUBLICATIONS, MUHAS

I, (Full Name) ……...……
Of ……...……..
Here by voluntarily authorize the researcher, **Mr. Boniface Clemence Panga** to interview me and consent to him obtaining information that is relevant to his research on "**Seroprevalence of HBeAg among HBsAg positive pregnant women and factors associated with hepatitis B virus in Moshi Municipality, in Kilimanjaro Region, 2012**"
I understand that as a participant, my privacy will be maintained and that the information obtained in this research will be used in a manner that protects guaranteed confidentiality, respect and personal rights.
I am aware that participating in this study is voluntary and I do not have to answer every question asked for me, and that I may withdraw my consent at any time without disadvantage to myself or others. I am informed that information collected in this study will be strictly confidential and for this study only.

Signature of Participant …… Dated……..

Signature of Researcher…… Date……

8.*2* Kiswahili Version

CHUO KIKUU CHA AFYA NA SAYANSI YA TIBA CHA MUHIMBILI

KURUGENZI YA UTAFITI NA UCHAPISHAJI

FOMU YA RIDHAA

Ridhaa ya kushiriki utafiti

FOMU YA RIDHAA

Majina ya mshiriki----------

Hujambo!

Ninaitwa ---------- mwanafunzi wa digrii ya uzamili katika magonjwa ya milipuko katika Chuo Kikuu cha Afya cha Muhimbili (MUHAS)

Madhumuni ya utafiti

Utafiti huu unalenga kuainisha ni kwa kiasi gani akina mama wajawazito wamepata maambukizi ya magonjwa ya zinaa kama Virusi Vya Ukimwi, kaswende na ugonjwa wa ini.

Nini kinahitajika ili kushiriki

Ili kushiriki katika utafiti huu inabidi kukubali na kujiunga kwa kujibu maswali yaliyotungwa kwa ajili ya utafiti huu.

Usiri

Taarifa zitakazokusanywa na dodoso hili zitaingizwa kwenye ngamizi kwa kutumia namba za utambulisho za siri

Hatari

Hakuna hatari yoyote itakayojitokeza kwa kushiriki

Haki ya kujitoa au vinginevyo

Ushiriki katika utafiti huu niwa hiari,.kutoshiriki au kujitoa kutoka kwenye utafiti hakutakuwa na adhabu yeyote na hutapoteza stahili zako.

Faida

Kama utakapokubali kushiriki kwenye utafiti huu itakuwa ni faraja kwa vile ina nia ya kuchangia uboreshaji wa utoaji wa huduma kwa kubaini ukubwa akina mama wajawazito walioathirika na magonjwa ya ugonjwa wa ini, Virusi vya Ukimwi na kaswende

Nani wa kuwasiliana naye

Kama una maswali kuhusiana na utafiti huu itakubidi kuwasiliana na msimamizi mkuu wa utafiti

Boniface Clemence Panga **(0754269629**)

Sahihi_____

Je umekubali/

Mshiriki umekubali _____ Mshiriki hajakubali_____

Mimi _____ nimesoma maelezo ya fomu hii.maswali yangu yamejibiwa.Nakubali kushiriki katika utafiti huu

Sahihi ya mshiriki _____

Sahihi ya mtafiti msaidizi _____

Tarehe ya kutia sahihi ya kushiriki_____

8.3 Questionnaire English version

Questionnaire for "Seroprevalence of HBeAg among HBsAg positive pregnant women and factors associated with hepatitis B virus in Moshi Municipality, in Kilimanjaro Region, 2012"

Questionnaire Number_____ **Date of interview**_____

Interviewer Name_____

Name of Health Centre_____

A**. Socio-demographic information (TICK**)

Participant's Name_____ (Optional)

Pregnant registration number_____

Residence (Street/Village)_____Ward_____

Age (in years)_____

Marital status ;(TICK)

Single ☐

Married ☐

Widowed ☐

Separated ☐

Divorced ☐

☐

Education Level (TICK)

No formal education at all ☐

Primary education ☐

Secondary education ☐

College level ☐

☐

University level

What is your occupational status? (TICK)

Self employed ☐

Government employed ☐

Private employed ☐

Not employed ☐

B. Risk factors of Hepatitis B and C (Please tick)

Did you involved in any of the following practices?

Unsafe injection (intravenous drugs user I)	☐	NO	☐	YES
Multiple sexual partners	☐	NO	☐	YES
Scarification	☐	NO	☐	YES
Ear piercing	☐	NO	☐	YES
Tattooing	☐	NO	☐	YES

Did you receive any of the following treatment?

Blood transfusion	☐	NO	☐	YES
Hospital admission	☐	NO	☐	YES
Tooth extraction	☐	NO	☐	YES
Surgery	☐	NO	☐	YES
Catheterization	☐	NO	☐	YES

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

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

Yes ☐

No ☐

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

Yes ☐

No ☐

Are you drinking alcohol? (Circle)

Yes ☐

No ☐

D. Health and pregnant/gynecological history (TICK)

Pregnancy status;

First pregnancy ☐

Second pregnancy ☐

Third pregnancy ☐

Above Four pregnancy ☐

Number of deliveries_____

Number of live babies (Parity)_____

4. Gravidity

Primgravidae ☐

Multgravidae ☐

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

Yes ☐

No ☐

6 .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) ……

7. 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 diseases ☐

History of jaundice

Other problems (mention).....

Vaccination status (circle)

Tetanus Yes / No

Hepatitis (B or C) Yes/No

D. Serology (Laboratory findings) (TICK);

1.0 HIV-1/2

Positive ☐

Negative ☐

2.0 Hepatitis B Virus (HBV) ☐

Positive

Negative ☐

3.0 Hepatitis C Virus (HBV)

Positive ☐

Negative ☐

4.0 Hepatitis Be/Ant-HBE Virus ☐

Positive ☐

Negative ☐

5.0 Active syphilis; Specify type of test: SD Bioline/ RPR

Positive ☐

Negative ☐

8.4 Laboratory diagnosis

8.4.1 Treponema Pallidum detection

Active syphilis was diagnosed by positive results by the SD Bioline (Becton Dickinson, MD, USA). The test was done at Majengo and Pasua Health centres

The SD Bioline Syphilis 3.0 test is a solid phase immunochromatographic assay for the qualitative detection of antibodies of all isotypes (IgG, IgM, and IgA) against Treponema pallidum. (TP) The test device was removed from foil pouch and placed it on a flat, dry surface (using capillary pipette) then add 10ul of serum specimen into the sample well and this was followed by adding 4 drops (about 120ul) of assay diluents into sample well(S).

As the test begins to work a purple color move across the result window in the centre of the test device, the test was interpreted within 5-20minute

When a color band appears in the left section of the result window that showed that the test was working properly; that band was the control band.

The right section of the result window indicated the test results. When another color band appeared in the right section of the result window, the band was the test band

The presence of only one purple color band within the result window indicated a negative result while the presence of two color bands (T band and C band) within the result window, no matter which band appeared first, indicated a positive result for Treponema pallidum antibodies and if the purple color band was not visible within the result window after performing the test, the test result was considered invalid and this can be due to failure in following direction or test may have deteriorate

The test is intended for professional use as an aid on the diagnosis of Syphilis

Treponema pallidum (TP0 is the causative agent of the veneral disease syphilis, Syphilis is a disease caused by the spirochetal bacterium Treponema Pallidum (TP) .Clinical diagnosis issues related to Syphilis are the detection of Syphilis antibodies in human blood by the immunoassay. Among existing the existing immunological method ,the confirmatory Treponema tests are the agglutination format such as the Treponema Pallidum hem agglutination assay (TPHA) and the Immunostaining analysis by Fluorescent Treponema antibody adsorption test (FTA,ABS),Recently the ELISA format and immunochromatographic format (rapid to detect antibody of T. Pallidum are available. Since even highly purified antigen from inoculated TP may contain a certain amount of

contaminating materials such as flagella of TP, native TP Antigen may cause a non-specific reaction in the assay of the test serum samples ,and this may results in lower sensitivity and poor reproducibility .To circumvent these potential problems in immunoassays ,researches has constructed TP genes for the expression of recombinant antigens in bacterium systems such as E.Coli and focused on TP membrane protein which are definitely immunogenic .The major immunoreactive antigens of these membrane proteins have been reported to have a MW 47,42,17, and 15KDa based on western blot analysis

Principle of the test

The SD BIOLINE Syphilis 3.0 contain a membrane strip ,which is pre-coated with recombinant Treponema pallidum antigens (17,15KDa) on test band region. The recombinant Treponema pallidum antigens-colloid gold conjugate (17,15KDa),patient sample and sample diluents moves along the membrane chromatographically to test region (T) and form a visible line as the antigen –antibody gold particles complex together forms. Therefore ,the formation of visible line in the test region (T) indicates a positive result for the detection of Treponema pallidum specific antibodies (IgG,IgM,IgA).When the Treponema pallidum specific antibodies (IgG,IgM,IgA) are absent in the sample ,no visible color band in the test region(T)

Precaution/storage and Kit stability

The SD BIOLINE Syphilis 3.0 test device was stored at room temperature

The test device is sensitive to humidity and as well as to heat hence the test was performed immediately after removing test device from the foil pouch

The kit used will expire on January 2012

Specimen collection, storage and precaution

The collection was done aseptically by venipuncture in the Clinic where 5mls of whole blood was collected into the vacuntainer tube (Not containing anticoagulants such as EDTA, Heparin and sodium citrate) the sample was left at room temperature (18-25°C) for 30 minutes for blood coagulation. The clotted Blood was centrifuged at 3000rpm for 5 minutes to get serum specimen at supernatant. If serum specimens are not tested immediately, we refrigerated at 2-8°C. For storage period longer than 2 weeks, freezing is recommended

Procedure of test

The test device was removed from foil pouch and placed it on a flat, dry surface (using capillary pipette) then add 10ul of serum specimen into the sample well this was followed by adding 4 drops (about 120ul) of assay diluents into sample well(S).

As the test begins to work a purple color move across the result window in the centre of the test device, the test was interpreted within 5-20 minutes

Interpretation of the test

When a color band appears in the left section of the result window that showed that the test was working properly; that band was the control band.

The right section of the result window indicated the test results. When another color band appeared in the right section of the result window, the band was the test band

Negative result

The presence of only one purple color band within the result window indicated a negative result

Positive result

The presence of two color bands (T band and C band) within the result window, no matter which band appeared first, indicated a positive result for Treponema pallidum antibodies

Invalid results

If the purple color band was not visible within the result window after performing the test, the test result was considered invalid and this can be due to failure in following direction or test may have deteriorated

Internal quality control

The SD BIOLINE 3.0 test device has a letter of T and C as test line and control line on the surface of the case .Both the test line and control line in result window are not visible before applying any samples. The control line was used for procedural control.

Control should always appear if the test procedure was performed properly and the test reagents of control line are working

Performance characteristics

The SD BIOLINE Syphilis 3.0 test has tested with positive and negative clinical samples tested by a leading commercial TPHA syphilis test. The result shows that the SDBIOLINE Syphilis 3.0 test is very accurate to TPHA with relative sensitivity of 99.3% (152/153) and specificity of 99.5% (209/210) the overall accuracy is greater or equal to 99.0%.

8.4.2 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 4 drops (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

8.4.3. Detection of Human Immuno Virus (HIV)

HIV was diagnosed by a positive result on both the third generation of ONE STEP antibodies to HIV-1/HIV-2 test. SD Bioline 1/2 (Abbott Laboratories, IL,

Principle of the test

The SD BIOLINE HIV-1/2 3.0 kit is a rapid, qualitative test for detection of antibodies to all isotypes (IgG,IgM,IgA) specific to HIV -1 including subtype o and HIV -2 simultaneously in human serum, plasma or whole blood.

The SD BIOLINE HIV -1/2 3.0 test contains a membrane strip ,which is procoated with recombinant HIV-1 capture antigen (gp41,p24) on test band 1 region and with recombinant HIV-2 Capture antigen(gp41p24,and gp36)-colloid gold conjugate and the specimen samples move along the membrane chromatographically to test region (T) and form a visible line as the antigen-antibody- antigen gold particle complex forms with high degree of sensitivity and specificity .This test device has a letter of 1,2 and C as Test line 1 (HIV-1),Test line 2(HIV-2) and control line on the surface of the test device .Both the test lines and control line in result window are not visible before applying any sample .The control line is used for procedural control. Control line should always appear if the test procedural is performed properly and the test reagents of control line are working.

Precaution /kit storage and stability

The test device was stored at -30°C.and not stored at Refrigerator

The test device is sensitive to humidity as well as to heat, so the test was performed immediately after removing the test device from foil pouch

The test KIT used will expire on Feb 2013, and therefore it is not recommended to use the expire kit

Specimen collection, storage and precaution

The collection was done aseptically by venipuncture in the Clinic where 5mls of whole blood was collected into the vacuntainer tube (Not containing anticoagulants such as EDTA, Heparin and sodium citrate) the sample was left at room temperature (18-25°C) for 30 Minutes for blood coagulation. The clotted Blood was centrifuged at 3000rpm for 5 minutes to get serum specimen at supernatant. If serum specimens are not tested immediately, we refrigerated at 2-8°C. For storage period longer than 2 weeks, freezing is recommended

Procedure of the test:

The test device was removed from foil pouch and 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 4 drops (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

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 results

Interpretation of the test

A color band will appear in the left section of the result window to show that the test is working properly. This band is control line (C)

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)

Negative result

The presence of only control line (C) within the result window indicates a negative result

Positive result

The presence of two lines as control line (C) and test line 1 (1) within the result window indicate a positive result for HIV-1

The presence of two lines as control line (C) and test line 2 (2) within the result window indicate a positive result for HIV-1

The presence of three lines as control line (C) and test line 1 (1) and test line 2(2) within the result window indicate a positive result for HIV-1 and /or HIV-

If the color intensity of the test line 1 is darker than one of the test line 2 in the window, you can interpret the result as HIV-1 positive

If the color intensity of the test line 2 is darker than one of the test line 1 in the window, you can interpret the result as HIV-2 positive

If the sample is positive for SD BIOLINE 3.0 Then according to National Rapid HIV Allogarithm the sample was retested with Determine as second test

Invalid Result

No presence of control line (C) within the result window indicates an invalid result. The direction may not have been followed correctly or the test may have deteriorated it is

recommended that the specimen be re-tested. HIV status was determined by HIV-1/HIV-2 test. SD Bioline 1/2 (Abbott Laboratories, IL, USA) and Determine HIV 1/2. Discordant results will be resolved by Uni-Gold as tie-breaker test, (Abbott Laboratories)

These assays detect both HIV-1/2 infections. Samples reactive on both tests were considered to be positive for IgG anti HIV antibodies.

8.4.4 The Determine HIV-1/2:

This is aninvitro, visually read, qualitative immunoassay for the detection of antibodies to HIV-1 and HIV-2 in human serum, plasma or whole blood. The test is intended as an aid to detect antibodies to HIV-1/HIV-2 from infected individual

8.4.5 Detection of Hepatitis C Virus.

Hepatitis C virus (HCV) now is recognized as a major agent of chronic hepatitis, transfusion acquired non-A, non B hepatitis and liver disease throughout the world.HCV is an enveloped positive sense, single stranded RNA virus. A clinical diagnostic issue related to HCV is the detection of HCV antibodies in human serum, plasma or whole blood by immunoassay. We have constructed HCV Genes for the expression of recombinant antigens in bacterium systems such as E.Coli and focused on structural and non-structural regions of HCV –encoded polyprotein, which are definetly immunogenic.The major immunoreactive antigens of these proteins have been reported as core, NS3, NS4 and NS5 Regions of HCV genome, which are known to be highly immunodominant regions. For diagnosis of HCV infection, these recombinant proteins were used as capture materials of a immunochromatographic (rapid)test. Compared to the first generation HCV test using single recombinant antigens, multiple antigens using recombinant proteins have been added in new serologic test to avoid non specific cross-activity and to increase the sensitivity of the HCV antibody test.

The SD BIOLINEHCV test is a immunochromatographic test for qualitative detection antibodies specific to HCV, in human serum, plasma or whole blood.

The SD BIOLINE HCV test contains a membrane strip ,which is pre-coated with recombinant HCV capture antigen on test band region .The protein A-colloid gold conjugate and serum sample moves along the membrane chromatographically to the test region(T) and forms a visible line as the antigen-antibody-protein A gold particle complex from with high degrees of sensitivity and specificity .This test device has letter of T and C

as Test line and control Line on the surface of the case .Both the Test line and control line in result window are not visible before applying any samples. The control line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working

Procedure of the test

1. The test device was removed from foil pouch and places it on flat, dry surface.
2. Using a micropipette, 10ul of serum was added into sample well(s)
3. four drops of assay diluent was added into sample well(s)
4. As the test begins to work, a purple color move across the results window in the centre of the test device.
5. The test was interpreted within 5-20 minutes

8.4.6 Detection of HBV by the use of HBVe Antibodies Kit

VIDAS HBe/Ant-HBe: Vidas HBe/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 VIDAS HBE 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.