Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Public Health and Nursing

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Seroprevalence of Viral Hepatitis among Women in Urban Slums of Kathmandu, Nepal

A cross-sectional pilot study

Master's thesis in Global Health
Supervisor: Professor Jon Øyvind Odland
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Preface

This Master's thesis was conducted as a part of MSc. in Global Health at the Department of Public Health and Nursing, at the Norwegian University of Science and Technology (NTNU) during the fall semester of 2019 till spring semester of 2020. This thesis involves determination of seroprevalence of viral hepatitis among women who live in the slum areas of Kathmandu, Nepal; in collaboration with the health authorities of Kathmandu.

Trondheim, June 2020

Urusha Maharjan

Acknowledgement

I would like to express my special appreciation and gratitude to my supervisor Professor Jon Øyvind Odland, who is a tremendous mentor for me. His constructive input and expert advice have been invaluable throughout all stages of the work. I can never thank him enough for his support and encouragement which enabled successful completion of this project.

My special thanks to Dr. Tore Jarl Gutteberg (Professor emeritus at UiT The Arctic University of Norway) who was an important part during development of this thesis. His invaluable inputs and suggestions were crucial for progression of the project.

I am also thankful to my co-supervisor Manoj Pradhan (Department of Microbiology, NAIHS) for his huge contribution in conducting the project in Nepal. His suggestions and extended discussions have contributed greatly to the improvement of the project.

I would like to express my thankfulness to Norwegian University of Science and Technology for funding this project.

I am very grateful to the leaders of Society for Preservation of Shelters and Habitations in Nepal (SPOSH-Nepal). I am very thankful to Hukum Bahadur Lama (President of SPOSH-Nepal) also known as 'Grandfather of Slum' for trusting and supporting us to conduct the project in the slum communities of Kathmandu.

The thesis has also benefited from constructive feedbacks and suggestions made by Seema Das. The data collection team who were very patient and sincere during the work. I take this opportunity to thank them.

The person with the greatest contribution to this work is my mother Sharmila Maharjan. Thank you for always loving and believing in whatever I pursue. I also want to thank my mother and Lalitpur metropolitan for bringing us in contact to SPOSH-Nepal. I want to thank my father, brother and sister for their constant love and encouragement.

Urusha Maharjan
Trondheim, June 2020

Abstract

Background: Viral hepatitis is a worldwide public health problem. In Nepal, the prevalence of HAV, HBV and HCV are found to be low but the endemicity of HEV is found to be quite high in Kathmandu since many decades by various studies. But there isn't any study to explain prevalence of hepatitis infection in the vulnerable population of Nepal. This pilot study assessed the prevalence of viral hepatitis infection among women living in the slum areas of Kathmandu valley, Nepal.

Objective: To determine seroprevalence of viral hepatitis among women in the urban slums of Kathmandu, Nepal.

Methods: A community based cross-sectional study was conducted in three slum areas (Balkhu, Thapathali and Sankhamul) of Kathmandu, Nepal. A semi-structured questionnaire was used to collect socio-demographic and clinical data. Blood specimens of women between age group 18-40 were analysed by rapid diagnostic tests (RDTs) based on immunochromatographic assay for the qualitative detection of hepatitis A, B, C and E virus. The positive samples of HEV were further tested by enzyme linked immunosorbent assay (ELISA) method for the confirmation of virus.

Results: Three hundred and fourteen women were assessed in the study. The seroprevalence of HEV was 1.6 %, HBV was 0.3 % and none of the women were found positive for HAV and HCV.

Majority of the women were of age group 20-29 (43%) and 76.1% were married. The literacy rate was 76.1% and 35.7% were employed. More than 95% of women did not have a history of hepatitis infection and 62.4% have never received the vaccination of hepatitis B. Approximately 37% of women had scarce knowledge about hepatitis infection and 46% knew about jaundice.

Conclusion: In this pilot study, the infection rate of viral hepatitis is very low among women living in the slum areas of Kathmandu. The knowledge about hygiene, sanitation and purification of water among the women might be the reason for low prevalence of hepatitis infection. Further studies on a larger scale is necessary to understand the implications of hepatitis in the slum population.

Keywords: Women, Viral hepatitis, Urban slums, Nepal, Kathmandu

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Abbreviations

CHE	=	Current Health Expenditure
ELISA	=	Enzyme Linked Immunosorbent Assay
GDP	=	Gross Domestic Product
HDI	=	Human Development Index
HAV	=	Hepatitis A Virus
HBV	=	Hepatitis B Virus
HBsAg	=	Hepatitis B surface Antigen
HCV	=	Hepatitis C Virus
HEV	=	Hepatitis E Virus
HIV	=	Human Immunodeficiency Virus
IgG	=	Immunoglobulin G
IgM	=	Immunoglobulin M
LMIC	=	Low and Middle-Income Country
MDGs	=	Millennium Development Goals
NAIHS	=	Nepalese Army Institute of Health Sciences
NHRC	=	Nepal Health Research Council
OOP	=	Out-of-Pocket Payment
PCR	=	Polymerase Chain Reaction
PPP	=	Purchasing Power Parity
PWID	=	People Who Inject Drugs
RDTs	=	Rapid Diagnostic Tests
REK	=	Regional Committee for Medical and Health Research Norway
RNA	=	Ribonucleic Acid
RPM	=	Rotation Per Minute
SPOSH	=	Society for Preservation of Shelter and Habitations
SPSS	=	Statistical Package for Social Science
WHO	=	World Health Organisation

Chapter 1

Introduction

1.1 Background

1.1.1 Viral Hepatitis

Viral hepatitis is an infection that causes inflammation of the liver cells and damages the liver. The four major types of viruses i.e. hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis E virus (HEV) with different modes of transmission and outcomes are mainly responsible for the infection. The clinical outcome of viral hepatitis infection can be subclinical to fatal (Gupta et al., 2018). These major types of viral hepatitis along with their distinguishing characteristics are mentioned in Figure 1.1.

1.1.1.1 Hepatitis A Virus

Hepatitis A Virus (HAV) is a non-enveloped single-stranded RNA virus which belongs to the Hepatovirus genus of Picornaviridae family. HAV replicates in the liver cells and causes inflammation in liver inferring the liver function. Infection with any type of genotype provides lifelong immunity towards HAV (Cuthbert, 2001).

It is transmitted via faecal-oral route by ingestion of food or water contaminated by the infectious person. Young children usually have asymptomatic acute hepatitis A viral infection, but the adults may have mild disease or may develop major complications. Symptoms of HAV include fever, dark urine, light stool and jaundice. It does not cause chronic liver disease. However, the risk of the disease increases with age (Organization et al., 2010a).

1.1.1.2 Hepatitis B Virus

Hepatitis B Virus (HBV) is an enveloped double-stranded DNA virus which belongs to the Orthohepadnavirus genus of Hepadnaviridae family. HBV attacks the liver and can cause both acute and chronic disease. The immune response of host determines the course of

infection and degree of liver injury caused by the virus (Elizabeth W. Hwang, 2011).

It is transmitted via vertical transmission (infected mother to child) or horizontal transmission (sexual transmission and contaminated needles) as well through exposure to body fluids of infected person (Shrestha and Shrestha, 2012). In less than 5% of the cases, hepatitis B virus causes chronic hepatitis in adulthood; whereas in 95% of the cases, HBV causes chronic hepatitis in infancy or early childhood (Elizabeth W. Hwang, 2011). Symptoms of HBV include jaundice, dark urine, fatigue, nausea, vomiting and abdominal pain (Schweitzer et al., 2015). In some cases, chronic liver disease can develop into cirrhosis or liver cancer (Shrestha and Shrestha, 2012).

1.1.1.3 Hepatitis C Virus

Hepatitis C virus (HCV) is a small, enveloped, single-stranded RNA virus which belongs to the Hepacivirus genus of Flaviviridae family (Dubuisson and Cosset, 2014). It can cause both acute and chronic hepatitis, the degree of infection can range from mild to serious lifelong illness (Mohd Hanafiah et al., 2013).

It is a blood-borne virus and transmitted via direct contact with infected body fluids, mainly through injection drug use, unsafe health care and sexual contact. About 30% (15-45%) of the infected persons get rid of the virus within 6 months of infection without treatment while the remaining 70% (55-85%) develop chronic hepatitis C infection (Organisation, 2017). It is a leading cause of end-stage liver disease and hepatocellular carcinoma. Symptoms of HCV include fatigue, nausea, vomiting, dark urine, jaundice and joints pain (Chlibek et al., 2017). Those with chronic HCV infection can develop cirrhosis or liver cancer (Blach et al., 2017).

1.1.1.4 Hepatitis E Virus

Hepatitis E virus (HEV) is a single-stranded RNA virus. The virus released from the infected cell is quasi-enveloped but the one inside the cell is non-enveloped. It belongs to the Orthohepevirus and Piscihepevirus genera of Hepeviridae family (Khuroo et al., 2016b). The pathogenesis of HEV in liver is still not distinct however, the extrahepatic manifestations can also occur in acute or chronic HEV infections (Lhomme et al., 2016).

It is transmitted via faecal-oral route, mainly through contaminated water due to faecal contamination of drinking water. The poor sanitation and secretion of infected faeces into the drinking water supplies accounts for a high degree of clinical cases with HEV infection (Organization et al., 2010b). Beside faecal-oral route, other modes of transmission are also found to cause this infection such as ingestion of undercooked meat or meat products of infected animals, transfusion of infected blood products and vertical transmission (infected mother to child) (Khuroo et al., 2016b). More than 95% of the HEV cases are asymptomatic but the disease can be icteric. It usually follows an acute self-limiting course of illness but it can cause serious complications in pregnant women and immunocompromised people (Organization et al., 2010b). Symptoms of HEV include mild fever, nausea, vomiting, jaundice, dark urine, pale stool and hepatomegaly (enlarged liver). In some rare cases like pregnant women infected with HEV in the third trimester; 20-25% cases can develop into liver failure and consequential death (Corwin et al., 1996).

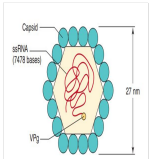
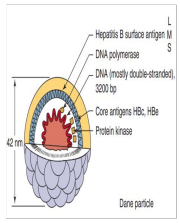
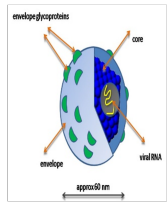
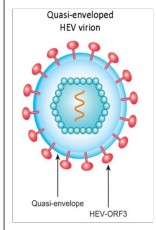
	Hepatitis A Virus (HAV)	Hepatitis B Virus (HBV)	Hepatitis C Virus (HCV)	Hepatitis E Virus (HEV)
Figure				
Classification	Picornavirus	Hepadnavirus	Flavivirus	Hepevirus
Viral structure	Picornavirus; non-enveloped	Hepadnavirus; enveloped	Flavivirus; enveloped	Calcivirus-like structure; non-enveloped
Viral genome	ssRNA	dsDNA	ssRNA	ssRNA
Route of Transmission	Fecal-oral	Parenteral, sexual	Parenteral, sexual	Fecal-oral
Incubation period	15-50	45-160	14-180+	15-50
Mortality	<0.5%	1%-2%	≈4%	Normal patients, 1%-2%; pregnant women, 20%
Prevalence	Worldwide	Worldwide	Probably worldwide	Developing countries

Figure 1.1: Viral hepatitis types: Comparative features. (Murray et al., 2016; GrahamColm, 2008; Nan et al., 2018)

1.1.2 Epidemiology of Viral Hepatitis in Developing and Developed Countries

Viral hepatitis is one of the major public health concerns. It is one of the world's eight leading causes of death responsible for an estimation of 1.4 million deaths per year, a toll comparable to that of HIV and tuberculosis in both developed and developing countries (Organization et al., 2016). According to WHO, the death from viral hepatitis has increased by 20% since 2000 and more than 80% of people infected with hepatitis don't have access to prevention, diagnosis, and treatment services (Organization et al., 2017a).

1.1.2.1 Epidemiology of Hepatitis A Virus

WHO estimates that about 11,000 deaths were caused worldwide by hepatitis A in 2015, which accounts to 0.8% of the mortality rate from viral hepatitis (Organization et al., 2017a). Only 1.5 million clinical cases are reported globally though the rate of infection is higher (Jefferies et al., 2018). HAV infections are more prevalent in economically underdeveloped countries (>90% of population) and least in developed countries (<50% of population) but America and some countries of the Eastern Mediterranean region have an exceptionally high prevalence rate of HAV (HHS, 2018; Behzadi et al., 2019). In most of the regions, the infection has occurred increasingly in adults rather than children, resulting in higher severity of the disease (Lemon and Walker, 2019).

1.1.2.2 Epidemiology of Hepatitis B Virus

It is estimated that among one-third of the world population infected with hepatitis B, 360 million people are the chronic carriers. It is considered that countries such as African and Asian, Amazon Basin and parts of the Middle East, and South East are highly endemic where the population is at the risk of more than 60% of getting hepatitis B infection; while the United States, Western Europe, and Australia have low endemicity (<1%) with <20% chance of getting the infection. Similarly, countries with intermediate endemicity (1-7%) such as Eastern and Southern Europe, Russia, Central and South America have 20-60% risk of getting HBV infection. In the areas with high endemicity, the most common mode of transmission is vertical transmission. Whereas, in the low endemic areas, the most common mode of transmission is horizontal transmission (Elizabeth W. Hwang, 2011).

1.1.2.3 Epidemiology of Hepatitis C Virus

Hepatitis C virus is endemic worldwide with wide variation in its geographical distribution. The global prevalence of hepatitis C is 3% with 170 million people infected worldwide (Kamal and Ghoraba, 2018). WHO estimates that people infected with hepatitis C may develop liver cirrhosis and cancer contributing to 399,000 deaths each year (Organization et al., 2017a). In developed countries such as Western Europe and North America, the prevalence rate of HBV is <0.1%. However, higher prevalence rates of >1% have been reported from France and Italy. In developing countries, high prevalence rates have been reported from South Asian countries such as China (0.29%-9.6%), Northern Thailand (3.8%-7.9%), Pakistan (5.9%) and African countries (0.8%- 13.8%). Egypt is also considered to have the highest prevalence of HCV in the world with prevalence rate of 10% in 2015 (Kamal and Ghoraba, 2018).

1.1.2.4 Epidemiology of Hepatitis E Virus

Every year, HEV is estimated to infect 20 million people worldwide. According to WHO, in 2015 about 44,000 deaths occurred by hepatitis E which accounted for 3.3% mortality due to viral hepatitis (Organization et al., 2019). Like HAV, it is also common in areas with limited access to clean drinking water, diagnosis and health services. In developing countries, it is responsible for repeated endemics and higher epidemics of jaundice. South-East Asia is highly endemic for HEV with an estimation of 12 million infected cases each

year (Jefferies et al., 2018). It has a higher case fatality rate in pregnant women. It is also hyperendemic in some countries of Central Asia, North Africa, East Africa, West Africa and North America (Mexico) (Khuroo et al., 2016a).

1.1.3 Nepal's Context

1.1.3.1 Country Profile

Nepal is a geographically and culturally diverse country located in South Asia. There are 126 ethnic groups, speaking 123 different languages and belonging to different indigenous groups. It is 48^{th} largest country by population with an estimated number of 29.7 million and 93^{rd} largest country by area covering 147,181 km^2.

Nepal is a federal republic with 7 provinces and 77 districts. There are 11 sub-metropolitan municipalities, 293 urban municipalities and 460 rural municipalities. The capital city of Nepal is Kathmandu.

Nepal is a developing nation, but it is one of the fastest growing economies in the world with an estimated GDP of $28.8 billion. Nepal is also predicted to halve the current poverty rate by 2020. But the political instability, poor infrastructure and increased foreign employment are the major factors hindering the development of this country (Sharma, 2006).

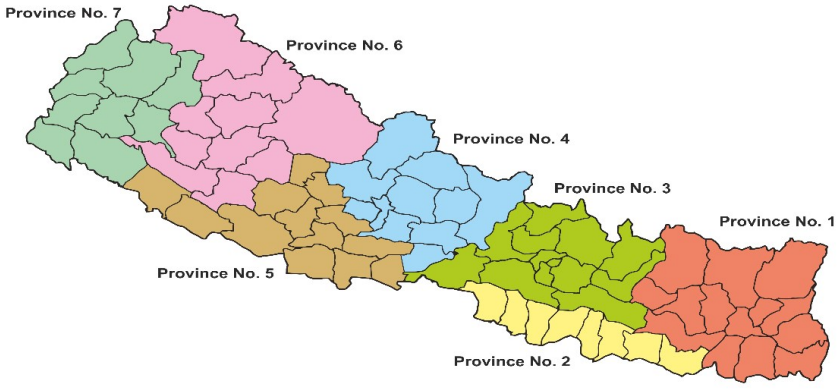


Figure 1.2: Map of Nepal showing the seven provinces (Commons, 2015).

Table 1.1: Demographic profile of Nepal (Mundi, 2019).

Total Population	29,717,587 (July 2018 est.)
Kathmandu population (Capital of Nepal)	1376108
Urban Population	Urban Population: 20.2% of total population (2019)
Human Development Index (HDI)	0.574 (2017 est.) Rank 149^{th}
GDP per capita	$10,10.95
Literacy rate	Male: 76.4% Female: 51.3% (2016 est.)
Life expectancy at birth (year)	71.3 (2019 est.)
Total fertility rate	186 deaths\100,000 live births
Total health expenditures	6.1% of GDP (2015 est.)

1.1.3.2 Health in Nepal

In Nepal, the public health and health care services are provided by both private and public sectors. The average life expectancy at birth is estimated to be 71 years. In rural areas of Nepal, the prevalence of disease is higher in comparison to other South Asian countries. The people residing in rural areas are at a high risk of infection and mortality by communicable diseases, malnutrition and other health-related events (Rai et al., 2001). Out of total current health expenditure (CHE), more than 60% is contributed by out-of-pocket payment (OOP). More than 3 million people have a burden of health expenditure and 1.67% of the total population has been thrust below the poverty line of PPP (purchasing power parity) $1.90 per capita per day due to out-of-pocket payment (Organization et al., 2017b). Despite such problems, Nepal has significantly improved the health of women and children. It has achieved Millennium Development Goals (MDGs) no.4 (to reduce child mortality) and on track to reach other goals as well (Rai et al., 2001).

1.1.3.3 Slum Settlement in Kathmandu, Nepal

UN-HABITAT defines slum as lacking at least access to safe water, sanitation, safe and secure tenure or durable housing structures. Overcrowd and economic deprivation aggravates the housing and health-related risks of slum dwellers. UN-HABITAT estimated that about 38% of LMIC population lives in slum and 433 million lives in Asia (Organization,

2018).

In context of Nepal, about 20.2% of the total Nepalese population lives in urban areas (Mundi, 2019). According to Millennium Development Goals Indicator, 54.3% of urban population of Nepal lives in slum areas (Bank, 2014).

The settlement started in 1950, when people from rural areas migrated to cities due to natural disaster, fleeing during Maoist insurgency and to obtain better employment and infrastructure facilities. Currently, there are more than 63 slum and squatter settlements in Kathmandu and their population is estimated to grow by 25% every year. These settlements are mostly located along the riverbanks of Bagmati, Bishnumati and Manohara river which are polluted due to mixing of drainage and sewage. The inhabitants of these areas have high level of health risks and problems due to deprivation of basic amenities such as clean water supply, less knowledge and access to health services, poor sanitation and nutrition which makes them more vulnerable towards viral infections (Oli et al., 2013; Khatun et al., 2012).

1.1.3.4 Epidemiology of Hepatitis A in Nepal

Hepatitis A was found to be responsible to cause acute hepatitis among young children and foreign visitors in Kathmandu. HAV accounted for 4% of acute hepatitis in adults of Kathmandu from 1986 to 2002. Almost all the children above 5 years of age had the presence of anti-HAV IgG. In 2013, it was observed that 40% of acute hepatitis in adults was caused by HAV (Shrestha, 2015). Also in 2016, HAV was one of the major infectious disease (food or waterborne disease) which caused high degree of health risk (Mundi, 2019).

1.1.3.5 Epidemiology of Hepatitis B in Nepal

According to the world health rankings, Nepal is in 20^{th} position with a death rate of 2.28% due to hepatitis B. It is also ranked 40^{th} among the top 50 causes of deaths in Nepal (Rankings, 2017). Despite these rankings, a nationwide survey showed 0.9% prevalence of HBV (1.1% among male and 0.5% among female). It accounts for approximately 3% of sporadic acute hepatitis cases (Rankings, 2017). Nepal has the lowest prevalence rate in Asia and also among pregnant women because vertical transmission of HBV is very uncommon in this country (Shrestha and Shrestha, 2012). The prevalence of HBV is not uniform in all parts of the country, the variation is seen in different ethnic groups due to origin and socio-cultural practices. Various studies have shown a high prevalence of HBV ($\geq$ 8%) among the population in the mountain region of Nepal (Shedain et al., 2017). In Nepal, the markers of HBV infection has been seen in 46% of liver cirrhosis and 69% of hepatocellular carcinoma (Shrestha, 2015).

1.1.3.6 Epidemiology of Hepatitis C in Nepal

The seroprevalence of anti-HCV in the general population and intravenous drug users of Nepal is estimated to range between 0.3% to 1.7%. It accounts for 3% of acute hepatitis,

10% of chronic liver disease and 14% of hepatocellular carcinoma. HCV is the major cause for morbidity and mortality among intravenous drug users in Nepal (Shrestha, 2015).

1.1.3.7 Epidemiology of Hepatitis E in Nepal

Hepatitis E infection is the common cause of acute hepatic failure in Nepal (Shrestha, 2006). It is more prevalent in urban cities, mainly Kathmandu in comparison to rural areas. Five major outbreaks of HEV have occurred in Nepal during 1973, 1981 to 1982, 1987, 2006 to 2007 and 2014. The first four epidemics occurred in Kathmandu and the fifth one (2014) in Biratnagar city. During these epidemics, the attack rate of the general population was 1.4% to 4.6% and a higher mortality rate of about 25% among pregnant women. Complications such as acute liver failure, sub-acute hepatic failure and cholestasis (41% in older people) were seen during the epidemics (Shrestha, 2015). These epidemics are associated with faecal contamination of water and genotype 1 as the cause of acute hepatitis infection. HEV infection has been reported in foreign travellers of Nepal as well (Shrestha et al., 2016). In 2016 like HAV, it was also considered as one of the major health risks in Nepal (Mundi, 2019). However, the overall fatality rate of HEV infection is 0.4% in Nepal (Shrestha, 2015).

1.2 Rationale

1.2.1 Background of the Study

In Nepal, the viral hepatitis accounts for about 3% of liver disease and hepatocellular carcinoma. Numerous studies have been conducted to study the occurrence of viral hepatitis in the general population of Nepal. But the increase in asymptomatic or unreported cases underestimates the prevalence of the infection. Therefore, it is necessary to conduct studies in various population groups to estimate the true disease burden.

Kathmandu Valley is known to be an endemic region for HAV and HEV since many decades. Although the high endemicity of HAV and HEV is known, so far there is no study to explain the endemicity of these infections in the vulnerable population of Nepal, especially in the slum areas. The settlement of slums around the banks of rivers such as Bagmati river polluted with sewage and drainage put those inhabitants in constant threat towards the viral infections. During monsoon season, the slum area is flooded with polluted water of the river which increases their risk towards such infections. These inhabitants have high level of health risks due to insufficient water supply, less health knowledge, poor nutrition and sanitation, limited access to health services and poor economic status which might lead to increase in mortality rate (Oli et al., 2013).

Previous studies have also found higher risk of HEV infection among female in comparison to male and increased chances of mortality among pregnant women of Kathmandu (Khatun et al., 2012; Shrestha, 2015; for Disease Control, CDC et al.(1987; Krain et al., 2014). However, there is not enough information since much research hasn't been done on the magnitude of viral hepatitis infection especially among women of urban slums in Nepal. These women are more prone towards the infection due to engagement in house-

hold activities. Therefore, it is essential to investigate the prevalence of viral hepatitis in the women of these vulnerable areas and obtain information to upgrade the living standards and health of slum dwellers. These data might as well help to develop prevention, vaccination and treatment programs for viral infections.

1.2.2 Objectives of the Study

1.2.2.1 General Objective

To determine seroprevalence of viral hepatitis among women in the urban slums of Kathmandu, Nepal.

1.2.2.2 Specific Objectives

- To investigate prevalence rate of viral hepatitis among women in the urban slums of Kathmandu, Nepal.
- To investigate the presence of following parameters in the blood sample of study population through laboratory diagnosis:

  ⇒ Detection of Anti-HAV IgM antibodies to hepatitis A virus.

  ⇒ Detection of HBsAg (hepatitis B surface antigen) of hepatitis B virus.

  ⇒ Detection of Anti-HCV IgM antibodies to hepatitis C virus.

  ⇒ Detection of Anti-HEV IgM antibodies to hepatitis E virus.
- To study the socio-demographic characteristics of women living in the urban slums of Kathmandu.
- To study the medical history of women living in the urban slums of Kathmandu.
- To study the history of hepatitis and vaccination for HBV among women living in the urban slums of Kathmandu.
- To study the knowledge of hepatitis among women living in the urban slums of Kathmandu.

1.2.3 Research Question

- What is the seroprevalence of viral hepatitis among women in the urban slums of Kathmandu?

Chapter 2

Materials and Methods

2.1 Study Area

The study was conducted in three slum areas (Balkhu, Thapathali and Sankhamul) located inside Kathmandu Valley of Nepal. These settlements were purposely selected for the study because these are the biggest and oldest slums in Kathmandu valley situated at the banks of Bagmati river. A brief introduction to these settlements, conferring to the information from the study conducted is given in the following:

2.1.1 Balkhu Settlement

The settlement is 3.3 kilometres away from the centre of Kathmandu. It covers an area of 821,400 square feet where more than 1800 people are residing in 400 households. It is the biggest settlement in Kathmandu city.

2.1.2 Thapathali Settlement

The settlement was established 14 years ago. Currently there are more than 1000 people residing in 200 households in this settlement.

2.1.3 Sankhamul Settlement

The settlement was established 46 years ago which is one of the oldest slum areas in Kathmandu valley. It covers an area of $35,452m^2$ where more than 700 people are residing in around 108 households.

Figure 2.1: Slum area of Balkhu. The picture displays people performing their daily activities beside the Bagmati river.

(a) Street of Balkhu slum. The picture displays the condition of under construction street due to leakage of sewage pipe.

(b) Slum resident collecting drinking water from water tank supplier in Sankhamul slum.

Figure 2.2: Sanitation and water supply in slum settlements.

2.2 Study Period

The practical implementation was performed during autumn 2019.

2.3 Study Design

The study design is cross-sectional pilot study design.

2.4 Study Type

This study is quantitative study.

2.5 Sample Size and Sampling

There was no official information about the total population of slum dwellers due to which a sampling frame was not constructed. Therefore, a non-probability consecutive sampling was done. Based on existing literature, a sample size of 314 deemed to be adequate for this cross-sectional pilot study to detect the seroprevalence of viral hepatitis.

2.6 Study Population

The study population consisted of women aged between 18-40 years old from three slum areas (Balkhu, Thapathali and Sankhamul). A total of 314 women were assessed in this study.

2.7 Inclusion and Exclusion Criteria

The women of age group 18-40 years were eligible for the study. Women who couldn't donate blood samples due to a history of bleeding disorders and who weren't willing to participate were not included in the study.

2.8 Data Collection

2.8.1 Socio-demographic Data Collection

A semi-structured questionnaire was administered to the participants. The information related to general characteristics, medical history and knowledge about hepatitis and jaundice among the participants were recorded in the standard format of Microsoft Excel spreadsheet. The variables were selected based on literature review.

In this study, the independent variables were age, address, religion, marital status, number of children, occupation (women and their husband), education level, medical history such

as disease history, chronic illness, hepatitis history, vaccination of hepatitis B, number of doses taken, and knowledge of hepatitis and jaundice. These information were collected using a pre-structured questionnaire.

The data related to general characteristics of the participants were classified into different categories. The disease history, chronic illness, medication, blood transfusion, and knowledge of hepatitis and jaundice were self-reported by the participants. Later, the variables about knowledge of hepatitis and jaundice were categorized into “yes”, “heard” and “no” on the basis of the participant’s response. Other independent variables such as hepatitis history, vaccination of hepatitis B and number of doses taken were categorized into “yes”, “no” and “not sure” respectively.

The dependent variables HAV, HBV, HCV and HEV were entered as the binary variables. The women with and without HAV, HBV, HCV and HEV were categorised as “reactive” and “non-reactive” respectively. The laboratory screening test and confirmatory test on the blood samples of participants determined the presence and absence of these viruses.

2.8.2 Blood Specimen Collection

To determine the presence of HAV, HBV, HCV and HEV serological tests were performed on the blood samples of the participants. Approximately 3 ml blood sample was drawn from each participant by trained phlebotomists and collected in a blood collection tube containing clot activator and gel separator. The samples were transported from the collection site to the clinical laboratory, maintaining cold chain and centrifuged at 1500 rpm (rotation per minute). The serum was extracted from whole blood and then stored at -20 degree Celsius until further testing was performed.

The serological tests on the blood specimen were performed by skilled laboratory technologists; according to the manufacturer’s manual. First, a screening test was conducted using immunochromatography method on the serum sample to detect the presence of virus. Then, a confirmatory test (ELISA) was performed further on the sample which had positive results (presence of virus) in the screening test; for the purpose of confirmation and to avoid any false positive results (Figure 2.4). The results of the screening test and confirmatory test were entered in the Microsoft Excel spreadsheet.

2.8.3 Serology Test

The blood specimens of participants were diagnosed for IgM antibodies to HAV, HCV, HEV and surface antigen (HBsAg) of HBV in the screening test. Further, the positive samples of HEV obtained from the screening test were tested through a confirmatory test (ELISA) to confirm the presence of IgM antibodies in the sample. IgM antibodies and HBsAg antigens can diagnose the infection in an early stage.

2.8.3.1 Screening Test

Screening test was performed to diagnose the presence of HAV, HBV, HCV and HEV qualitatively in the serum specimen of sample population. The test was done on a rapid diagnostic kit using immunochromatography method as per the manufacturer's protocol (Hightop Biotech for HAV, HCV, HEV and Hepacard for HBV).

2.8.3.2 Screening Test for Hepatitis A Virus

This diagnostic method was performed for the qualitative detection of IgM antibodies to hepatitis A virus in serum. The test was done by immunochromatography method on a rapid diagnostic kit of Hightop Biotech HAV (Qingdao Hightop Biotech Co.,Ltd, China) as per Hightop Biotech protocol.

Note: The information regarding sensitivity and specificity of Hightop Biotech HAV and HEV could not be retrieved.

- **Test Procedure**

The rapid diagnostic kit was labelled with the participant's identification number. $1.5\mu l$ serum was added into the sample pad / membrane of sample well of the diagnostic kit with the help of micro pipette. Then, about 2 drops ($80\mu l$-$100\mu l$) of sample buffer (20mM phosphate buffer) was added into the sample well / buffer hole of the kit. The test results were observed immediately within 15-20 minutes (as shown in Figure 2.3); the result is invalid over 20 minutes. The kit was discarded immediately after reading the result at 20 minutes, considering it to be potentially infectious.

- **Result**

$\Rightarrow$ *Positive:* Two distinct red lines appear. One line should be in the control region (C) and another line should be in the test region (T).

$\Rightarrow$ *Negative:* One red line appears in the control region (C). No red or pink line appears in the test region.

$\Rightarrow$ *Invalid:* No red lines appear, or control lines fail to appear, indicating error or reagent failure.

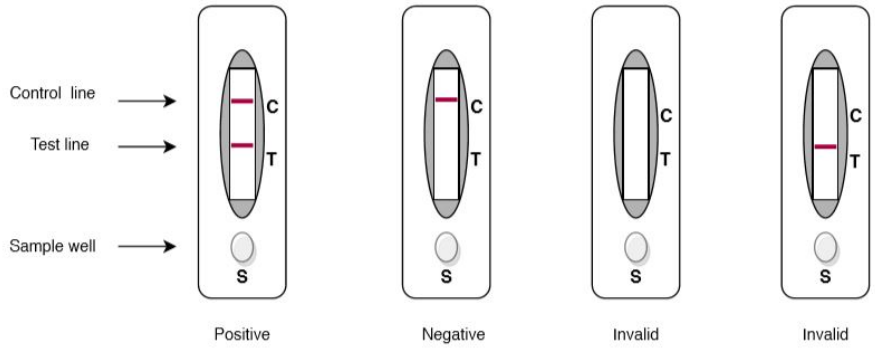


Figure 2.3: Diagram illustrating results of screening test in rapid diagnostic kits.

2.8.3.3 Screening Test for Hepatitis B Virus

This diagnostic method was performed for the qualitative detection of hepatitis B surface antigen (HBsAg) in serum. The test was done by immunochromatography method on a rapid diagnostic kit of Hepacard (J.Mitra & Co.Pvt.Ltd, India, Sensitivity 100% and Specificity 98.0% by WHO evaluation (Ltd, 2015)) as per Hepacard protocol.

- **Test Procedure**

The rapid diagnostic kit was labelled with participant's identification number. First, 2 drops ($70\mu l$) serum was added into the sample pad / membrane of sample well of the diagnostic kit using the dropper provided (separate dropper / microtip was used for each specimen). The test results were observed within 20 minutes (the result is invalid over 20 minutes). The kit was discarded immediately after reading the result at 20 minutes, considering it to be potentially infectious.

- **Result**

The method of observing result is similar to that of HAV screening test as illustrated in Figure 2.3.

2.8.3.4 Screening Test for Hepatitis C Virus

This diagnostic method was performed for the qualitative detection of IgM antibodies to hepatitis C virus in serum. The test was done by immunochromatography method on a rapid diagnostic kit of Hightop Biotech HCV (Qingdao Hightop Biotech Co.,Ltd, China, Sensitivity 69% & Specificity 80% (Waheed et al., 2019)) as per Hightop Biotech protocol.

Note: The information about sensitivity and specificity of Hightop Biotech HCV was obtained from a study conducted by Waheed et al (2019). However, the sensitivity and specificity of the test kit may vary according to the lot of manufacture.

- **Test Procedure**

The rapid diagnostic kit was labelled with participant's identification number. First, 1 drop ($25\mu l$) serum was added into the sample pad / membrane of the sample well of the diagnostic kit with the help of micro pipette. Then, about 2 drops ($80\mu l$-$100\mu l$) of sample buffer (20mM phosphate buffer) was added into the sample well / buffer hole of the kit. The test results were observed immediately within 10-20 minutes (the result is invalid over 20 minutes). The kit was discarded immediately after reading the result at 20 minutes, considering it to be potentially infectious.

- **Result**

The method of observing result is similar to that of HAV screening test as illustrated in Figure 2.3.

2.8.3.5 Screening Test for Hepatitis E Virus

This diagnostic method was performed for the qualitative detection of IgM antibodies to hepatitis E virus in serum. The test was done by immunochromatography method on a rapid diagnostic kit of Hightop Biotech HEV (Qingdao Hightop Biotech Co.,Ltd, China) as per Hightop Biotech protocol.

- **Test Procedure**

The rapid diagnostic kit was labelled with participant's identification number. First, $10\mu l$ serum was added vertically into the sample pad / membrane of the sample well of the diagnostic kit with the help of micro pipette. Then, about 2 drops ($80\mu l$-$100\mu l$) of sample buffer (20mM phosphate buffer) was added into the sample well / buffer hole of the kit. The test results were observed immediately within 15-20 minutes (the result is invalid over 20 minutes). The kit was discarded immediately after reading the result at 20 minutes, considering it to be potentially infectious.

- **Result**

The method of observing result is similar to that of HAV screening test as illustrated in Figure 2.3.

2.8.3.6 Confirmatory Test

Confirmatory test or ELISA (Enzyme linked immunosorbent assay) was performed in hepatitis E virus positive samples which was obtained after the screening test in rapid diagnostic kits. The ELISA test was conducted using a manual enzyme immunoassay with Euroimmun Anti-HEV ELISA (IgM) (EUROIMMUN Medizinische Labordiagnostika AG, Germany, Sensitivity 100% and Specificity 100% (AG, 2019)) as per Euroimmun Medizinische Labordiagnostika AG protocol.

The Anti-HEV ELISA(IgM) test provides semiquantitative in vitro determination of human antibodies of the immunoglobulin class IgM against hepatitis E virus antigens in serum or plasma to support the diagnosis of infections with HEV (AG, 2019).

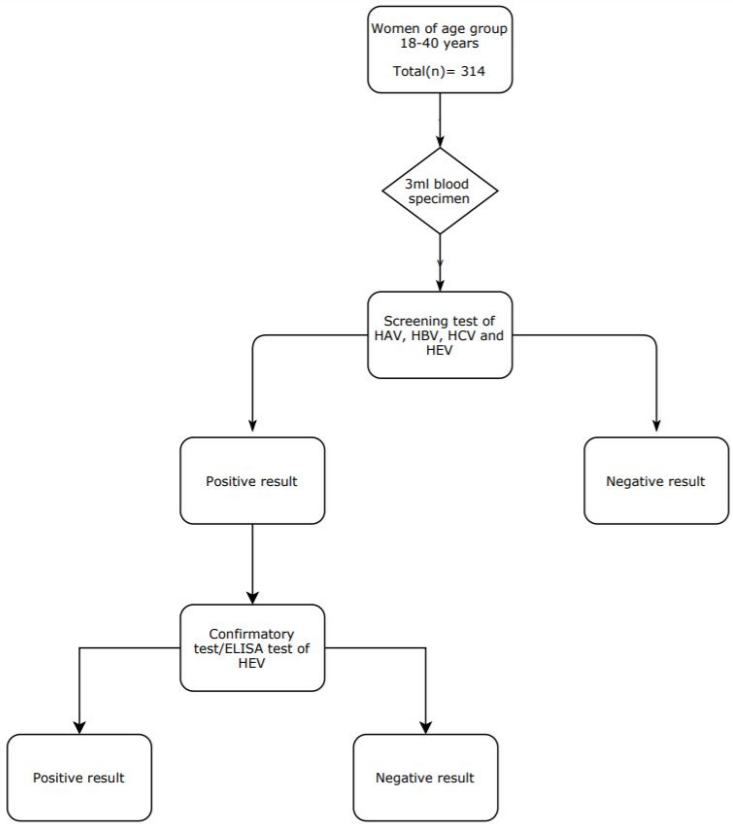


Figure 2.4: Laboratory diagnosis of study participants.

2.9 Ethical Approval

Ethical approval was obtained from Nepal Health Research Council (NHRC: Reg.no.241/ 2019) and the Regional Committee for Medical and Health Research Norway (REK-947070/2020), using anonymized and de-identifiable data released for this thesis. In addition to this, permission to conduct the study and collect blood samples was obtained from the Society for Preservation of Shelters and Habitations in Nepal (SPOSH-Nepal).

The verbal and written consent was taken from the participants before conducting the study. The consent for taking photos of these settlements were granted by the local people and the photo ethics were considered carefully while taking any photographs.

2.10 Statistical Analysis

The information obtained from semi-structured questionnaire and laboratory diagnosis were entered in the Microsoft Excel spreadsheet prior to further organization into SPSS. The information entered in the Microsoft Excel was checked, sorted and coded. The dependent or binary variables and independent variables as mentioned earlier in subsection 2.8.1 were categorized and coded. The response from open ended questions (knowledge about hepatitis and jaundice, medication and disease history) were later categorized and coded. Double data entry system was used to minimize errors in data entry.

The data was then organized and analysed further with SPSS version 25. Descriptive analysis was performed and reported to evaluate the distribution of variables. The data was organized into tabular formats, including descriptive statistic tables and the software's cross-tabulation tool was also used. The variables were assessed using the test of proportion (frequencies and percentage). The tabular formats were made to visually illustrate the variables and all the findings were cross-tabulated with three study sites (Balkhu, Thapathali and Sankhamul) using cross-tabulation tool, then expressed as frequency counts and percentages.

Chapter 3

Results

3.1 General Characteristics of the Sample Population

The general characteristics of the sample population is presented in Table 3.1. A total of 314 women were included in this study. The participation of women was lowest from Thapathali (T= 23.9%), followed by Sankhamul (S= 32.5%) and then Balkhu (B= 43.6%). Majority of the women were of age group 20-29 years (B= 43.8%, T= 45.3%, S= 40.2%). Most of the women were Hindu especially in Thapathali and Sankhamul (B= 41.6%, T= 78.8%, S= 78.4%) but the percentage of Buddhist participants was observed to be higher in Balkhu (38%) in comparison to remaining study sites. Approximately 70% of participants have been staying in these slum areas for more than 5 years (B= 69.3%, T= 70.7%, S= 74.5%).

The majority (75%) of women were married and the number of married women was almost similar in all three areas (B= 75.2%, T= 77.3%, S= 76.5%). More than 50% of women had less than 3 children (B= 55.5%, T= 53.3%, S= 58.5%). Most of the women (40.1%) had reported attending schooling of primary level or below and the proportion was parallel in all areas while the percentage of women with secondary level education was highest in Sankhamul (45.1%). In contrast, the overall percentage of illiterate women was approximately 21%; comparatively highest in Thapathali (30.7%).

Overall, the percentage of housewives was highest in Thapathali (56%), followed by Sankhamul (49%) and Balkhu (39.4%). 35.7% of the women were employed and most of them were reported from Balkhu. While more than 98% of the women's husbands were employed (B= 98.1%, T= 100%, S= 97.4%).

Table 3.1: General characteristics of the sample population

Variables	Count	Percentage (%)	Balkhu N=137 n (%)	Thapathali N=75 n (%)	Sankhamul N=102 n (%)
Age					
≥18	37	11.8	16(11.7)	11 (14.7)	10 (9.8)
20-29	135	43	60 (43.8)	34 (45.3)	41(40.2)
30-39	108	38.4	50 (36.5)	18 (24.0)	40 (39.2)
40	34	10.8	11 (8.0)	12 (16.0)	11 (10.8)
Religion					
Hindu	196	62.4	57 (41.6)	59 (78.8)	80 (78.4)
Muslim	5	1.6	5 (3.6)	0 (0.0)	0 (0.0)
Buddhist	45	14.3	52 (38)	11 (14.7)	4 (3.9)
Christian	67	21.3	22 (16.1)	5 (6.7)	18 (17.6)
None	1	0.3	1 (0.7)	0 (0.0)	0 (0.0)
Duration of stay in slum					
≤ 5 years	90	28.7	42 (30.7)	22 (29.3)	26 (25.5)
≥5 years	224	71.3	95 (69.3)	53 (70.7)	76 (74.5)
Marital status					
Single	70	22.3	32 (23.4)	16 (21.3)	22 (21.6)
Married	239	76.1	103 (75.2)	58 (77.3)	78 (76.5)
Divorced	3	1	1 (1.3)	1 (1.3)	2 (2.0)
Widowed	2	0.6	0 (0.0)	0 (0.0)	0 (0.0)
Number of children					
0	87	27.7	38 (27.7)	17 (22.7)	32 (31.4)
1-2	176	56.1	76 (55.5)	40 (53.3)	60 (58.8)
3-4	48	15.3	22 (16.1)	16 (21.3)	10 (9.8)
5	3	1	1 (0.7)	2 (2.7)	0 (0.0)
Education level					
Illiterate	67	21.3	33 (24.1)	23 (30.7)	11 (10.8)
Primary level and below	126	40.1	61 (44.5)	30 (40.0)	35 (34.3)
Secondary level	94	29.9	30 (21.9)	18 (24.0)	46 (45.1)
Higher level	27	8.6	13 (9.5)	4 (5.3)	10 (9.8)

Continued on next page

Occupation of women					
Student	31	9.9	11 (8.0)	5 (6.7)	15 (14.7)
Housewife	146	46.5	54 (39.4)	42 (56.0)	50 (49.0)
Employed	112	35.7	62 (45.3)	18 (24.0)	32 (31.4)
Unemployed	25	8	10 (7.3)	10 (13.3)	5 (4.9)
Occupation of women's husband					
Employed	235	98.3	101 (98.1)	58 (100)	76 (97.4)
Unemployed	4	1.7	2 (1.9)	0 (0.0)	2 (2.6)

3.2 Medical History

The participants were questioned about chronic illness, history of disease, medication and blood transfusion. Table 3.2 displays the information about medical history reported by all women during the interview. The percentage of women reporting about not having any chronic illness was 99.4% which was almost similar in all three slums (B= 99.3%, T= 98.7%, S= 100%). Only a single participant with a mental disorder and unsure about having a chronic illness was accounted for in Balkhu and Thapathali respectively.

Overall, the percentage of women without any history of disease was 59.9%. But among the women with disease, 8.3% had a history of jaundice (B= 10.9%, T= 6.7%, S= 5.9%). Most of the women were not taking any kind of medication (82.5%) and neither had a history of blood transfusion (90.1%).

Table 3.2: Medical history of the sample population

	Count	Percentage (%)	Balkhu N=137 n (%)	Thapathali N=75 n (%)	Sankhamul N=102 n (%)
Chronic illness					
Yes	1	0.3	1 (0.7)	0 (0.0)	0 (0.0)
No	312	99.4	136 (99.3)	74 (98.7)	102 (100)
Not Sure	1	0.3	0 (0.0)	1(1.3)	0 (0.0)
Disease history					
Jaundice	26	8.3	15 (10.9)	5 (6.7)	6 (5.9)
Hypotension	15	4.8	11 (8.0)	1 (1.3)	3 (2.9)
Hypertension	11	3.5	7 (5.1)	1 (1.3)	3 (2.9)
Thyroid problem	10	3.2	2 (1.5)	0 (0.0)	8 (7.8)
Others	65	40.1	25 (18.2)	17 (22.7)	17 (16.7)
No	188	59.9	76 (55.5)	49 (65.3)	63 (61.8)
Medication					
Yes	55	17.5	19 (13.9)	16 (21.3)	20 (19.6)
No	259	82.5	118 (86.1)	59 (78.7)	82 (80.4)
Blood transfusion					
Yes	30	9.6	9 (6.6)	6 (8.1)	15 (14.7)
No	283	90.1	128 (93.4)	68 (91.9)	87 (85.3)

3.3 Hepatitis and Vaccination History

Table 3.3 displays the history of hepatitis and vaccination taken for hepatitis B by participants. Among 314 women, only 2 of the participants reported about history of hepatitis B (one of each from Balkhu and Sankhamul) while the percentage of not having any history of all types of hepatitis were 95.5% (B= 98.5%, T= 90.7%, S= 95.1%) and that of women who weren't sure were 3.8%.

More than half of the participants (62.4%) had not taken vaccination for hepatitis B (B= 62%, T= 74.7%, S= 53.9%) and only 14% reported about taking the vaccine while about a quarter of women (21.2%) didn't know if they had ever received it.

Among the women receiving vaccination for hepatitis B, only 4.8% had taken three complete doses of the vaccine. The percentage of women who were unsure about receiving the complete dose was 27.7% and 67.5% of women reported about not taking the complete three doses of vaccination.

Table 3.3: Hepatitis and vaccination history of the sample population.

	Count	Percentage (%)	Balkhu N=137 n (%)	Thapathali N=75 n (%)	Sankhamul N=102 n (%)
Hepatitis history					
Yes (Hepatitis B)	2	0.6	1 (0.7)	0 (0.0)	1 (1.0)
No	300	95.5	135 (98.5)	68 (90.7)	97 (95.1)
Not Sure	12	3.8	1(0.7)	7 (9.3)	4 (3.9)
Vaccination of hepatitis B					
Yes	44	14	23 (16.8)	4 (5.3)	17 (16.7)
No	196	62.4	85 (62.0)	56 (74.7)	55 (53.9)
Not Sure	74	23.6	29 (21.2)	15 (20.0)	30 (29.4)
Three complete doses of vaccine					
Yes	15	4.8	8 (5.8)	2 (2.7)	5 (4.9)
No	212	67.5	93 (67.9)	58 (77.3)	69 (59.8)
Not Sure	87	27.7	36 (26.3)	15 (20.0)	36 (35.3)

3.4 Knowledge of Hepatitis and Jaundice

Of all the participants who were asked whether they knew about hepatitis 37.6% (B= 46.7%, T= 21.3%, S= 37.3%) could provide satisfactory answer and were categorised to have knowledge about hepatitis, 35.4% (B= 29.2%, T= 24%, S= 52%) had only heard about hepatitis and more than a quarter reported they didn't know about the disease (B= 29.2%, T= 54.7%, S= 10.8%).

The percentage of women who knew about jaundice was 46.2%, the percentage was highest in Sankhamul (60.8%) followed by Balkhu (46.7%) and then Thapathali (25.3%). The percentage of women who had an idea about jaundice was slightly higher than hepatitis (37.6%). However, the participants were familiar with clinical features of jaundice, but they were found unaware about causative agents, risk factors and transmission of hepatitis.

Table 3.4: Knowledge of hepatitis and jaundice in the sample population

	Count	Percentage (%)	Balkhu N=137 n (%)	Thapathali N=75 n (%)	Sankhamul N=102 n (%)
Knowledge of hepatitis					
Yes	118	37.6	64 (46.7)	16 (21.3)	38 (37.3)
Heard	111	35.4	40 (29.2)	18 (24.0)	53 (52.0)
No	85	27	40 (29.2)	41 (54.7)	11 (10.8)
Knowledge of jaundice					
Yes	145	46.2	64 (46.7)	19 (25.3)	62 (60.8)
Heard	156	49.7	63 (46.0)	56 (74.7)	37 (36.3)
No	13	4.1	10 (7.3)	0 (0.0)	3 (2.9)

3.5 Result of Diagnostic Test

The laboratory test was performed on blood samples collected from 314 women to diagnose HAV, HBV, HCV and HEV.

None of the participants from three slums were diagnosed with the presence of Anti-HAV IgM antibodies to hepatitis A virus and Anti-HCV IgM antibodies to hepatitis C virus. Only 1 (0.3%) participant from Thapathali was diagnosed with presence of surface antigen (HbsAg) for hepatitis B virus and 5 (1.6%) participants were diagnosed with presence of Anti-HEV IgM antibodies to hepatitis E virus among which 3 (2.2%) of them belonged to Balkhu and remaining 2 (2.7%) were from Thapathali (Table 3.5).

The presence of HEV was confirmed by ELISA method on positive samples of hepatitis E infection obtained from screening test.

Table 3.5: Result of diagnostic test for viral hepatitis in the sample population.

Viral Hepatitis	Count	Percentage (%)	Balkhu N=137 n (%)	Thapathali N=75 n (%)	Sankhamul N=102 n (%)
Hepatitis A Virus					
Non-Reactive	314	100	137 (100)	75 (100)	102 (100)
Reactive	0	0	0 (0.0)	0 (0.0)	0 (0.0))
Hepatitis B Virus					
Non-Reactive	313	99.7	137 (100)	75 (100)	101 (99.0)
Reactive	1	0.3	0 (0.0)	0 (0.0)	1 (1.0)
Hepatitis C Virus					
Non-Reactive	314	100	137 (100)	75 (100)	102 (100)
Reactive	0	0	0 (0.0)	0 (0.0)	0 (0.0)
Hepatitis E Virus					
Non-Reactive	308	98.4	134(97.8)	73 (97.3)	102 (100)
Reactive	5	1.6	3 (2.2)	2 (2.7)	0 (0.0)

In summary, among 314 women only 1 of the participants from Sankhamul was diagnosed with hepatitis B infection and 5 participants with hepatitis E infection; 2 from Thapathali and 3 from Balkhu. But the presence of hepatitis A and hepatitis C infection was not diagnosed in any of the participants. The overall number of viral hepatitis cases found in the study population is presented in Figure 3.1.

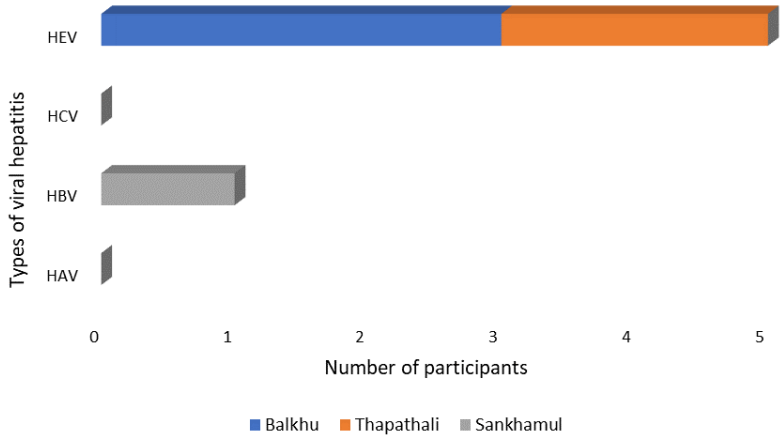


Figure 3.1: Bar graph showing the total number of viral hepatitis cases in the study population.

Chapter 4

Discussion

4.1 Main Findings

This descriptive cross-sectional study was conducted to assess the seroprevalence of viral hepatitis among women residing in the slum communities. To the best of our knowledge, this is the first study to determine the seroprevalence of viral hepatitis among women of slum areas in Nepal and especially in Kathmandu valley. A total of 314 women were included in this study, among them 1.6% had hepatitis E virus and 0.3% had hepatitis B virus while none of them had hepatitis A virus (0%) and hepatitis C virus (0%).

4.2 Interpretation and Comparison to Past Literature

In 2015, WHO estimated that global mortality from hepatitis is on an increasing trend unlike HIV, tuberculosis and malaria (Organization et al., 2017a). However, in this study, we found very low prevalence of all four types of hepatitis in the slum population. A hospital-based study conducted among jaundice patients in Kathmandu had a similar finding of low prevalence in case of hepatitis C virus (1.9%) but had a high prevalence of hepatitis A virus (15.3%) and hepatitis B virus (13.4%) while significantly higher prevalence of hepatitis E virus (69.2%). According to their findings, Kathmandu is still an endemic region for hepatitis infection, especially for hepatitis E virus which is very contrasting in this study (Gupta et al., 2018).

4.2.1 Prevalence of Hepatitis A Virus

Hepatitis A infection accounted for 40% of acute hepatitis among Nepalese adults in 2013. In the past, occurrence of hepatitis A infection was very common in children (Shrestha, 2015). Although there is not sufficient research, studies suggest hepatitis A infects almost all the Nepalese by the age of 5 years and develop life-long immunity against the infection (Shrestha, 1986). In fact, most of the children under 5 years of South Asia get exposed to

the hepatitis A and acquire immunity during adolescent (Organization et al., 2010a).

The lower seroprevalence of hepatitis A in this study corresponds to the countries of the high-income Asia pacific region such as Brunei, Japan, Republic of Korea and Singapore. Studies have shown low endemicity of HAV infection in recent decades and possibility of high-risk of the infection, if outbreaks occur in these countries (Organization et al., 2010a; Goh et al., 1987; Heng et al., 1994; KIYOHARA et al., 1997; Lee et al., 2008). Many studies conducted in 2000s across India and China, the neighbouring countries of Nepal have found decrease in incidence of HAV infections along with socioeconomic development and initiation of immunization programs. The epidemiology of the hepatitis A infection in these countries particularly in East Asia is shifting from high to intermediate endemicity due to improved hygiene, drinking water and living standards (Organization et al., 2010a; Gadgil et al., 2008). The similar epidemiological shift is also experienced in African region (Patterson et al., 2019).

The increase in outbreaks of hepatitis A around 30 states of the United States contradicts with the prevalence of HAV in comparison to other regions. According to HHS (U.S. Department of Health and Human Services) the outbreaks of hepatitis A infection are taking place mostly among people who use drugs and the ones who are homeless (HHS, 2018). Some countries of the Eastern Mediterranean region have also reported higher prevalence of HAV (Afghanistan(99%), Iraq(96%), Palestine(93.7%) and Southern Iran(93.2%)). The recent study conducted by Behzadi et al(2019) in Southern Iran suggests that the higher prevalence of HAV might be due to contaminated drinking water and improper disposal system (Behzadi et al., 2019).

4.2.2 Prevalence of Hepatitis B Virus

In this study, the prevalence of HBV and HCV is very low whereas other studies have found higher prevalence in South-Asian countries. A similar study conducted by Uppal et al(2009) in an urban slum of Northern India reported lower seroprevalence of HCV (1.15%) but higher prevalence of HBV (10.38%) among the male population (Bibhabati and Singh, 2009).

China has a higher intermediate prevalence of HBV (5-7.99%) among 20-39 aged individuals and the prevalence is found to be higher among male than female (Wang et al., 2019). Likewise, studies also show that the Philippines is a hyperendemic country for hepatitis B infection among adults with seroprevalence ranging between 4% to 18% since many decades (Wang et al., 2019). In Vietnam, the estimated prevalence of hepatitis B is 10.8% in the general population, accounting for 46% of deaths from liver cancer in this country (Hang Pham et al., 2019).

It is estimated approximately 45% of individuals infected with HBV live in highly endemic areas (Southeast Asia, China, Taiwan, Alaska and Sub-Saharan countries), 43% live in intermediate endemic areas (Mediterranean countries, Eastern Europe, Central Asia, Middle East and South America) and remaining 12% reside in low endemic areas (USA, Western Europe, Australia and Japan) (Papastergiou et al., 2015).

Shrestha et al(2012) indicated in a study that low prevalence of HBV in Nepal was due

to horizontal transmission during adolescence and the prevalence of HBV was low among pregnant women due to lack of vertical transmission resulting in the absence of infection during early childhood (Shrestha and Shrestha, 2012). However, many studies have found a high prevalence of hepatitis B infection among women in the mountain region of Nepal (Shedain et al., 2017). Various hospital-based studies have also found higher prevalence of hepatitis B in comparison to community-based studies (Organization et al., 2017a). According to WHO, the worldwide use of hepatitis B vaccine has significantly reduced the incidence of new chronic HBV infections (Elizabeth W. Hwang, 2011). Though in this study, 95% of respondents did not have a history of hepatitis while one of the interesting issues is that more than 60% of respondents have never received vaccination for hepatitis B virus.

4.2.3 Prevalence of Hepatitis C Virus

Studies have reported increase in prevalence of hepatitis C virus in the Southeast Asia and Indian subcontinents ranging from 1.5% to 9% and higher in Northern and Central Africa, Eastern Mediterranean and Ukraine ranging from 2% to 14% while lower (0.3% to 1.2%) in the United States, Northern Europe and Australia. Hepatitis C is found to be more prevalent than hepatitis B virus in Western Europe and the United States (Brown Jr and Gaglio, 2003).

Studies suggest that China has the world's largest burden of hepatitis C with an estimation of 8.9 million people living with chronic HCV infection and the prevalence is found to be higher among people who use drugs (Liu et al., 2019). Seroprevalence of HCV is also found to be higher among PWID (people who inject drug) or individuals receiving blood or blood products in Western Africa (15.69%) whereas Central (16.26%) and Southern Africa (6.40%) had highest seroprevalence in the general population (Sonderup et al., 2017). Egypt is also considered the country with the highest prevalence of HCV in the world where 14.7% carry HCV antibodies and 9.8% have active infection (Esmat, 2013).

The incidence of Hepatitis C infection is expected to increase further due to its high mortality and treatment cost though new infections are decreasing (Chlibek et al., 2017). In developed countries, HCV is transmitted due to illicit drug injection while in developing countries, most of the cases are related to medical procedures such as blood transfusion and vaccination needles (Kamal and Ghoraba, 2018).

The findings of HCV in this study is like other studies which reported lower prevalence (4%) of hepatitis C infection in the general population of Nepal. Also, the percentage of respondents who had a history of low blood transfusion was very low (9.6%) in this study. However, some studies have found a prevalence rate up to 19% among intravenous drug users and HIV infected individuals. Even though, there is not enough research but the lack of proper screening during blood transfusion and unsterile injections are predicted to cause the transmission in Nepal (Gupta et al., 2018).

4.2.4 Prevalence of Hepatitis E Virus

In 2017, the surveillance report of ECDC (European Centre for Disease Prevention and Control) reported an increase in HEV cases since 2005 with three times increase between 2011 and 2015 in EU/EEA countries. About 75% of the reported cases were mostly from Germany, France and the United Kingdom (Aspinall et al., 2017). A seroprevalence study conducted in Ghana by Yeboah et al(2018) reported 12.3% of HEV prevalence rate in pregnant women indicating higher risk of maternal and neonatal mortality and morbidity (Obiri-Yeboah et al., 2018).

According to seroepidemiology study conducted in 1999 to 2000, Kathmandu valley is a hyper endemic area for hepatitis E with seroprevalence of 38% (Shrestha, 2006). Past studies have found the outbreaks of HAV and HEV in Kathmandu were related to poor sanitation and hygiene transmitted by faecal-oral route. The prevalence of HEV infection was higher among female than male which might increase the chances of mortality in pregnant women (Gupta et al., 2018). The finding of our study is distinct from other studies with a lower prevalence rate of 1.6% among women.

In 1987 epidemics of hepatitis E, almost half of the infected population were migrants from rural areas and in 1985 sporadic in Central jail only the people from other areas than Kathmandu were affected by the infection. The major reason for the spread of this disease was consumption of contaminated water and increase in susceptible population by internal migration.

The study showed the past epidemics of hepatitis E infection in Kathmandu coincided with increase in internal migration during Maoist insurgency and the non-immune migrants from rural areas were mostly infected than inhabitants of Kathmandu (Shrestha, 2006). In our study, almost all participants were migrants from rural areas but most of them have lived in these slum areas for more than 5 years and might have developed immunity towards this infection.

Southeast Asia is a hyper-endemic region for hepatitis E where numerous outbreaks have occurred frequently. These recurrent epidemics are known to be associated with usage of water from sewage mixed river for household purposes and the leakage in water pipes passing through soil contaminated with sewage causing outbreaks in underdeveloped urban areas (Organization et al., 2010b). A study conducted by Khatun et al(2012) in urban slums of Bangladesh reported acute failure by viral hepatitis accounted for 11% of maternal deaths. During their study, they countered the outbreak of hepatitis E due to faecal contamination of drinking water (Khatun et al., 2012). Another study conducted by Behzadi et al(2019) also found high prevalence of HEV (15.8%) in Southern Iran due to consumption of contaminated drinking water and poor sanitation (Behzadi et al., 2019).

In this study, the participants were found to use water for drinking and other household purposes from a water tank supplier which delivered 6000 litres of drinking water every week for the whole slum community (as shown in Figure 2.2b). The water was found to be untested before delivery and suspected to be contaminated with coliform bacteria (Shrestha and Shukla, 2018). However, most of the slum dwellers reported about drinking the supplied water after treatment such as boiling, using chemicals or filtration.

4.3 Population Characteristics

In this study, only less than a quarter (21.3%) of women were illiterate while the adult female illiteracy rate of Nepal is 67.8% (2015) which is increasing in average annual rate of 4.13% (Atlas, 2015). Similarly, more than 35% of women and about 98% of their husbands were employed which indicates a better living standard of this slum population. The study conducted in Bangladesh by Mahejabin et al(2015) had also highlighted the relationship between education and income of slum dwellers with their health seeking behaviour (Mahejabin et al., 2015). Approximately 95% of women did not have a history of hepatitis and more than 30% had scarce ideas about hepatitis and jaundice; the knowledge about risk factors and aetiology of disease was found to be inadequate among the participants. Many women reported experiencing jaundice during their childhood or family member, especially that their child has suffered from jaundice at the age of less than 5 years.

Despite living beside the sewage mixed river and being exposed to the polluted water due to flooding inside their house during heavy rainfall, the prevalence of the hepatitis A and E infection was found to be quite low. Likewise, the prevalence of hepatitis B and C was also very low in our study population. One particular reason for the quite good situation might be that the people were well trained or educated about personal hygiene such as handwashing and using different water types for daily household works and drinking purposes. It was found that almost 70% of households used only commercially available treated water for drinking and rest used mechanical filters and boiling for sterilisation of water. On the other hand, they used tap water and water from tank suppliers for household purposes.

4.4 Strengths and Limitations of the Study

4.4.1 Strengths

This is the first known cross-sectional pilot study conducted in urban slums of Nepal, to determine the seroprevalence of hepatitis virus. Slum dwellers are suspected to be vulnerable towards viral infections due to inaccessibility to health facilities and basic amenities. Therefore, it is necessary to study the prevalence of viral infection in slum communities.

The data collection was done in each community hall to ensure access and coverage of the survey in those three settlements. Participants were informed about the study through leaders of SPOSH-Nepal (Society for Preservation of Shelters and Habitations in Nepal), community leaders and local people. We assume that there was no participant bias as the study population was informed through social networks. Prior to conducting study, those leaders went door-to-door to spread the information and collect the general information such as name, age and contact number. There was no disease bias, as most of the cases of hepatitis infection are asymptomatic and also, all the participants who were willing to participate were included. To determine the prevalence of HAV and HEV the data was collected during monsoon season when the settlements were flooded with polluted water.

4.4.2 Limitations

4.4.2.1 Sample Size Calculation

There was no official information about the total population of slum dwellers due to which a sampling frame was not constructed. Therefore, consecutive sampling was done for the study. The sample size is also relatively smaller in this pilot study.

4.4.2.2 Generalizability

Though the study was conducted in the biggest slums of Kathmandu, the results cannot be generalized to the whole slum population of Nepal. Because in this study, non-probability consecutive sampling was done and also, only female population were included. Therefore, the results obtained can also not be generalized to the male population of slum areas. The factors associated with low prevalence of hepatitis infection were not studied which hinders the establishment of causal relationship.

4.4.2.3 Selection Bias

Only women in the age group 18-40 years were included in the study. Also, the women from all the slum areas of Kathmandu valley were not addressed in this study.

4.4.2.4 Information Bias

The participants weren't asked about information related to maintenance of basic hygiene, sanitation and methods adopted for sterilization of water. Therefore, it was documented on the basis of the observation during slum visits and information obtained from the community leaders of the study area.

4.4.2.5 Laboratory Diagnosis

The IgM antibodies to viral hepatitis (hepatitis A, C and E) were only diagnosed in the sample population. Also, ELISA test or any confirmatory test was not performed on the positive sample of hepatitis B virus because the participant had reported a history of hepatitis B infection.

The seropositivity of the blood specimen is better evaluated through diagnosis of IgM antibodies along with IgG antibodies (in case of HAV, HCV and HEV) and PCR (Polymerase Chain Reaction) method. PCR method is used for the detection of genetic material (DNA and RNA) of hepatitis virus. But in our study, diagnosis of IgG antibodies and PCR method was not possible due to resource constraints.

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

The results obtained from current study reflect that the prevalence of viral hepatitis is very low among the women of urban slums in Kathmandu. It suggests lower risk of hepatitis infection and associated liver problems in this slum population.

The women of this study population were found to be aware about sanitation and hygiene. Though they live beside polluted river and the surrounding is not clean, the cleanliness inside the houses of slum dwellers were found to be very well maintained. They were quite aware and educated about basic hygiene such as handwashing and used different types of water for drinking and other household purposes after proper treatment.

Our study highlights the lower prevalence of viral hepatitis might be due to mostly literate and sanitation conscious women who contribute towards a healthy family of urban slums in Kathmandu. The slum population might have also acquired immunity towards hepatitis A and E infection after living there for more than 5 years. This study also addressed the necessity of hepatitis B vaccination in the slum population for prevention from the infection in the future.

Despite awareness related to hygiene, there is a lack of basic amenities such as proper shelter, safe drinking water and clean environment in these settlements. The unmanaged sewage system, polluted river and deprivation from health facilities put these individuals in constant risk of viral infections and other health problems.

The information collected in this study is limited in size and diagnostic method. Further larger-scale research with gold standard test is necessary to verify the presumption and confirm the preliminary results to understand the implications of viral hepatitis in the slum population. However, this pilot study can still contribute in understanding the prevalence and knowledge of hepatitis in the slum population.

5.2 Recommendations

- There isn't enough research done on seroprevalence of viral hepatitis of Nepal; especially in the slum population and the present study only provides information about the women in the urban slum population of Kathmandu. Therefore, further research must be conducted with large sample size including other slum areas of Nepal and the male population as well.

- Educational and awareness programs related to transmission, prevention and treatment of viral hepatitis infection is recommended in the slum population.

- It is recommended that the health authorities and policy makers should implement vaccination programs especially for the children and women of the slum population.

- It is recommended to create awareness among slum dwellers about sanitation and hygiene regarding maintaining cleanliness in the surrounding and proper waste management.

- It is recommended to teach different cost-effective methods to treat the water especially obtained from water tank suppliers and other sources before drinking and using for other household purposes.

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Appendix A: NHRC Approval

Government of Nepal

Nepal Health Research Council (NHRC)

Estd. 1991

Ref. No.: 3504

9 July 2019

Ms. Urusha Maharjan
Principal Investigator
Norwegian University of Science and Technology
Norway

Ref: **Approval of thesis proposal** entitled **Seroprevalence of Viral Hepatitis among women in Urban Slum of Kathmandu- a community based cross-sectional study**

Dear Ms. Maharjan,

It is my pleasure to inform you that the above-mentioned proposal submitted on **20 April 2019** (**Reg. no. 241/2019)** please use this Reg. No. during further correspondence) has been approved by Nepal Health Research Council (NHRC) Ethical Review Board on **3 July 2019**.

As per NHRC rules and regulations, the investigator has to strictly follow the protocol stipulated in the proposal. Any change in objective(s), problem statement, research question or hypothesis, methodology, implementation procedure, data management and budget that may be necessary in course of the implementation of the research proposal can only be made so and implemented after prior approval from this council. Thus, it is compulsory to submit the detail of such changes intended or desired with justification prior to actual change in the protocol. Expiration date of this proposal is **June 2020.**

If the researcher requires transfer of the bio samples to other countries, the investigator should apply to the NHRC for the permission. The researchers will not be allowed to ship any raw/crude human biomaterial outside the country; only extracted and amplified samples can be taken to labs outside of Nepal for further study, as per the protocol submitted and approved by the NHRC. The remaining samples of the lab should be destroyed as per standard operating procedure, the process documented, and the NHRC informed.

Further, the researchers are directed to strictly abide by the National Ethical Guidelines published by NHRC during the implementation of their project proposal and **submit progress report in between and full or summary report upon completion.**

As per your thesis proposal, the total research amount is **$ 2,240** and accordingly the processing fee amounts to **$ 100.** It is acknowledged that the above-mentioned processing fee has been received at NHRC.

If you have any questions, please contact the Ethical Review M & E Section at NHRC.

Thanking you,

Prof. Dr. Anjani Kumar Jha
Executive Chairperson

Tel: +977 1 4254220, Fax: +977 1 4262469, Ramshah Path, PO Box: 7626, Kathmandu, Nepal
Website: http://www.nhrc.gov.np, E-mail: nhrc@nhrc.gov.np

Appendix B: Consent form

Informed Consent form

(For participants)

This consent form is written for individuals interested to participate in the study: "Seroprevalence of Viral Hepatitis among Women in Urban Slums of Kathmandu, Nepal- a cross-sectional pilot study". This Informed Consent Form has two parts:

- Information Sheet (to share information about the research with you)
- Certificate of Consent (for signatures if you agree to take part)
  You will be given a copy of the full Informed Consent Form

PART I: Information Sheet

Introduction

I am Urusha Maharjan, a Master Student at Norwegian University of Science and Technology, Norway. I am a student of Global Health and doing research on viral hepatitis among women living in urban slum of Nepal. I am going to give you information and invite you to be part of this research. You do not have to decide now whether you will participate in the research. Please use time to consider participating in this study. You might consult any other people if you want to before you reach a decision. There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain.

Purpose of the research

The inhabitants of urban slum areas have high levels of health risks and problems.
This research is being conducted to determine the occurrence of viral hepatitis infection among women in areas with poor amenities and health awareness

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. You may change your mind later and stop participating even if you agreed earlier.

Procedures and Protocol

Taking part in this study, we request you to answer questions I will be asking you regarding your health. We will take about 3 milliliters of blood from your arm using a syringe and a needle. The blood sample will be sent to laboratory for analysis. All your identity will be confidential. I will contact you on your address if you would like to know some of the results.

Risks/Benefits of participation

Except for your time, this study will incur no considerable physical risks as such in any way. I will use a syringe to draw the blood sample and you can expect that it goes as smoothly as it did with your blood donation. It takes less than a minute to tap 3 ml of blood. We will not ask you for any kinds of payments for the blood tests and will report the results to you if you wish to be informed of or if they are important for your treatment. You will be offered snacks during the interview. You may feel uneasy because you may be reminded of past mis-happenings. We will do our best to not overwhelm you and help you in that case. If you have questions regarding your problem which you might like to ask will be addressed at the end of the session.

Reimbursements

The investigation that we will perform in your blood sample will incur no cost on your behalf.

Confidentiality

We will not be sharing the identity of those participating in the research. The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one, but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is, and we will lock that information up. It will not be shared or given to anyone outside our research team.

Sharing the Results

The knowledge that we get from doing this research will be shared with you. I will discuss with you regarding the outcome of the study. The results will be shared in the scientific community, but confidential information will not be shared. After these meetings, we will publish the results avoiding any identifiable data of any of the research participants, in order that other interested people may learn from our research.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so and refusing to participate will not affect you in any way. You may stop participating in the research at any time that you wish.

Who to Contact

If you have any questions you may ask now or later, even after the study has started. If you wish to ask questions later, you may contact me at the following:

Urusha Maharjan
Address: Sankhamul, Lalitpur.
Tel: 5548172, Mobile: 00977 9860086472/9841894998,
Email: urusham@stud.ntnu.no , Urusha.maharjan@yahoo.com

This proposal has been reviewed and approved by the national Research ethics committee in Norway and Nepal. These committee's' task is to make sure that the research participants are protected from harm.

You can ask me any more questions about any part of the research study if you wish to. Do you have any questions?

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked to have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Name of Participant_____ Choose to be contacted for further research: Yes/No

Signature of Participant _____Telephone Number 1) _____

Date _____ 2) _____

Day/month/year

<u>If illiterate</u>

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ Thumb print of participant

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done: 1. Interview

2. Blood sample collection

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily. A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

(Adapted from WHO format for Informed Consent form for Clinical research, a Nepali translation for the same will be used in practice.)

Appendix C: Questionnaire

Questionnaire

"Seroprevalence of Viral Hepatitis among Women in Urban Slums of Kathmandu, Nepal- a cross-sectional pilot study".

Date:

Name:

Participant ID:

A. Personal and Social data:

1. What is your age? _____

2. Please check (√) your religion.
- Hindu
- Muslim
- Christian
- Buddhist

3. Please check (√) your marital status.
- Single
- Married
- Divorced
- Widowed

4. How many children do you have? _____

5. Please check (√) your education level?
- No education (Illiterate)
- Primary level and below
- Secondary level
- Higher level

6. What is your occupation?

   _____

7. What is your husband's occupation?

   _____

8. How long have you stayed in slum?

   _____

B. Personal medical data

9. Are you on any medication?
   _____

10. Do you have any history of blood transfusion?
   _____

11. Do you have any chronic illness?
   _____

12. Do you know about hepatitis?
   _____

13. Do you know about jaundice?
   _____

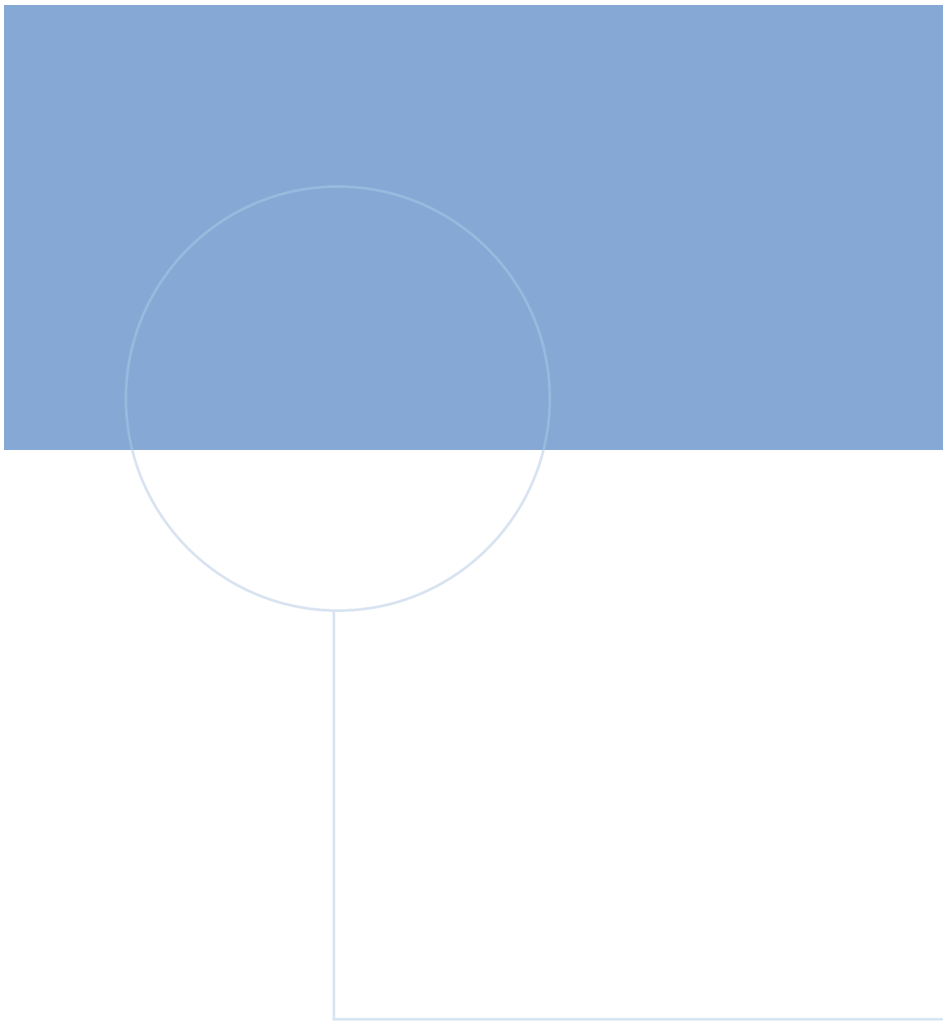
14. Did you have hepatitis before?
- ☐ Yes (Which: ___)
- ☐ No
- ☐ Not sure

15. Did you receive hepatitis B vaccine before?
- ☐ Yes
- ☐ No
- ☐ Not sure

16. Did you receive the complete doses of the vaccine?
- ☐ Yes
- ☐ No
- ☐ Not sure

…..…… *Thank you for your valuable time*……

Signature of research enumerator: _____



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