

**SEROPREVALENCE OF HEPATITIS B VIRUS AMONG BLOOD
DONORS IN JHAPA, NEPAL**

A

Dissertation

**Submitted to the Central Department of Microbiology
Tribhuvan University**

**In Partial Fulfilment for the Award of the Degree of
Master of Science in Microbiology
(Medical)**

By

Hemanta Khanal

**Central Department of Microbiology
Tribhuvan University
Kirtipur, Kathmandu, Nepal**

2009

RECOMMENDATION

This is to certify that **Mr. Hemanta Khanal** has completed this dissertation work entitled “**SEROPREVALENCE OF HEPATITIS B VIRUS AMONG BLOOD DONORS IN JHAPA, NEPAL**” as a partial fulfilment of M. Sc. Degree in Microbiology under our supervision. To the best of our knowledge, this is an original research work of his and has not been submitted for any other degree.

Dr. Dwij Raj Bhatta

Assistant Professor,
Head of Department,
Central Department of Microbiology,
Tribhuvan University,
Kirtipur, Kathmandu

Date:

Mr. Bishnu Raj Tiwari

Quality Officer/ Microbiologist,
Nepal Red Cross Society,
Central Blood Transfusion Service,
Exhibition Road, Kathmandu

Date:

CERTIFICATE OF APPROVAL

On the recommendation of **Dr. Dwij Raj Bhatta** and **Mr. Bishnu Raj Tiwari**, this dissertation of **Mr. Hemanta Khanal** entitled “**SEROPREVALENCE OF HEPATITIS B VIRUS AMONG BLOOD DONORS IN JHAPA, NEPAL**” has been approved for the examination and is submitted to Tribhuvan University in partial fulfilment of requirements for M. Sc. degree in Microbiology (Medical).

Dr. Dwij Raj Bhatta

Head of Department,
Central Department of Microbiology,
Tribhuvan University,
Kirtipur, Kathmandu, Nepal

Date:

BOARD F EXAMINER

Recommended by:

Mr. Bishnu raj Tiwari
Supervisor

Dr. Dwij Raj Bhatta
Supervisor

Approved by:

Dr. Dwij Raj Bhatta
Head of Department

Examined By:

Prof. Dr. Bharat Mani Pokhrel
External Examiner

Mr. Dev Raj Joshi
Internal Examiner

Date .../.../.....

ACKNOWLEDGEMENT

I gratefully pay my sincere gratitude to my supervisors Mr. Bishnu Raj Tiwari, Microbiologist, NRCS, CBTS and Asst. Prof. Dr. Dwij Raj Bhatta for their expert scientific guidance, technical inputs and arrangement of laboratory facilities during the entire period of the dissertation.

I highly acknowledge to Head of Department, Asst. Prof. Dr. Dwij Raj Bhatta, Central Department of Microbiology, and Tribhuvan University for providing me an opportunity to work in this challenging field.

I would like to honour faculties members Prof. Dr. Shreekant Adhikari and Prof. Dr. Anjana Singh, Central Department of Microbiology for special directions.

I would like to thank Mr. Torna Bikram Karki, Director and Mr. Raj Kumar Bhujel, Technician of NRCS, Blood Transfusion Service, Jhapa Branch and all the staffs of Nepal Red Cross Society, Damak Sub-branch for their technical and logistic support during the entire period of research.

Those blood donors by whom the work could not be started and completed, my heartfelt thanks go to all of them who have brought smile to thousands of families.

I would also like to thank my parents, my Brothers and sisters Mr. Raju Rijal, Ms. Barsha Khanal, Ghanendra Khanal and Mausam Khanal for their kind promotion to my work.

April, 2009

.....
Hemanta Khanal

ABSTRACT

During the study period from July 2008 to April 2009, a total blood samples of 2277 blood donors with modal age 31 – 40 years at Nepal Red Cross Society, Blood Transfusion Service (NRCS, BTS) of Bhadrapur and Damak were screened for HBsAg using rapid diagnostic tests kits (HEPACARD manufactured by Biomed Industries). Serum was separated from each collected blood samples and analyzed in respective laboratory of NRCS of Bhadrapur and Damak.

On the study HBsAg seroprevalence were observed 7 (0.52%) among 1340 samples of Bhadrapur and on Damak blood donors were 6 (0.64%) among 937 blood samples. Overall sero-prevalence on Jhapa blood donors observed were 13 (0.57%) out of 2277. Male blood donors of Bhadrapur had lower seroprevalence 0.51% (7 /1172) than the male blood donors of Damak 0.64% (6 /760) and overall seroprevalence on Jhapa male blood donors was 0.62% (12 /1932). On the other hand female blood donors at Damak were not observed for HBsAg positive whereas at Bhadrapur a single female out of 168 was positive with 0.59% of seroprevalence and overall seroprevalence of only female at Jhapa blood donors was 0.29% (1 /345). On age wise distribution highest HBsAg seroprevalence was observed on Bhadrapur male blood donors of age group 51- 60 years - 14.2% (1/ 7) and others attributes of same age group of Damak and Bhadrapur were negative. Similarly only 2 (0.91%) male blood donors of age group 41 - 50 years at Bhadrapur were positive. Male blood donors of Bhadrapur, Damak and both sites with the age 31 - 40 had 0.40% (2/ 462), 1.51% (3 /198) and 0.75% (5 /660) seroprevalence respectively. Similarly 0.25% (1/ 395), 0.80% (3 /374) and 0.52% (4/ 769) are the prevalence of only male blood donors of age group 21 – 30 of Bhadrapur, Damak and overall of same age respectively but females of this group were not screened positive. The age group of 18 - 20 years, only a single female at Bhadrapur were screened with seroprevalence of 3.3% (1/ 30) and the males were not observed positive.

Key words: HBsAg, Sero-prevalence, Blood donors.

TABLE OF CONTENTS

Title Page	i
Recommendation	ii
Certificate of Approval	iii
Board of Examiners	iv
Acknowledgement	v
Abstract	vi
Table of Contents	vi
List of Abbreviations	x
List of Tables	xi
List of Figures	xii
List of Photographs	xiii
List of Appendices	xiv
1. <u>CHAPTER I: INTRODUCTION</u>	1
2. <u>CHAPTER II: OBJECTIVES</u>	6
2.1 General Objective	6
2.2 Specific Objectives	6
3. <u>CHAPTER III: LITERATURE REVIEW</u>	7
3.1 Hepatitis B Virus	7
3.2 Epidemiology	7
3.3 Classification	8
3.4 Structure	9
3.5 Pathogenesis	10
3.5.1 Portal of Entry	11
3.5.2 Entry of virus into the cell	12

3.5.3	Completion of viral Genome	13
3.5.4	Integration of the provirus into host Cell DNA	13
3.5.5	Transcription of viral RNA	13
3.5.6	Translation assembly and maturation of integrated viral DNA Sequences	14
3.5.7	Clinical Pathogenesis/Symptoms/Diseases	15
3.5.7.1	Acute Hepatitis	16
3.5.7.2	Chronic Hepatitis	16
3.5.7.3	Hepatocellular Carcinoma	17
3.5.7.4	Fulminant Hepatitis B	18
3.6	Lab Diagnosis	18
3.6.1	HBsAg Rapid Screening Tests	19
3.6.2	Determination of HBV antigens and antibodies By Rapid Tests	20
3.6.3	Hepatitis B virus DNA Testing	25
3.6.4	Other Tests of Medical Importance	26
3.6.4.1	Liver Biopsy Tests	26
3.6.4.2	Biochemical Tests	26
3.7	Reactivation of HBV	27
3.8	Prevention	27
3.9	Treatment of Hepatitis B	29
3.9.1	Interferon	29
3.9.2	Lamivudine for the treatment of chronic hepatitis B	30
3.9.3	Interferon and Lamivudine in Combination	31
4.	<u>CHAPTER IV: MATERIALS AND METHODS</u>	32
4.1	Equipment and Others	32
4.2	Research Site and Population	32
4.3	Type of Study	33
4.4	Donor Screening	33
4.5	Transport	33
4.6	Procedure for Lab Tests	33

4.6.1	Separation of Serum/Plasma	33
4.6.2	HBsAg Testing	33
4.6.3	Recording and Reporting of Test Results	35
4.6.4	Statistical Analysis	35
5.	<u>CHAPTER V: RESULTS</u>	36
5.1	Study Population	36
5.2	Seroprevalence of HBsAg	36
5.2.1	Overall Seroprevalence	36
5.2.2	Gender wise Overall seropravalence	37
5.2.3	Age group and Gender wise seroprevalence of Blood Donors of Jhapa	38
6.	<u>CHAPTER VI: DISCUSSION AND CONCLUSION</u>	40
6.1	Discussion	40
6.2	Conclusion	41
7.	<u>CHAPTER VII: SUMMARY AND RECOMMENDATION</u>	43
7.1	Summary	43
7.2	Recommendation	44
8.	REFERENCES	45
9.	APPENDICES	

ABBREVIATIONS

HBV:	Hepatitis B Virus
ALT:	Alanine Aminotransferase
AST:	Aspartate Aminotransferase
EIA:	Enzyme Immuno Assay
ELISA:	Enzyme Linked Immunosorbent Assay
HBsAg:	Hepatitis B Surface Antigen
HBcAg:	Hepatitis B Core Antigen
HBeAg:	Hepatitis B e Antigen
HCC:	Hepatocellular Carcinoma
WHO:	World Health Organization
ORFs:	Open Reading Frames
CTLs	Cytotoxic T Lymphocytes
PCR:	Polymerase Chain Reaction
NRCS:	Nepal Red Cross Society
BTS:	Blood Transfusion Service
NRCS:	Nepal Red Cross Society
BTS:	Central Blood Transfusion Service
CDC:	Centers for Disease Control and Prevention

LIST OF TABLES

Table: 3.1 Interpretation of hepatitis B virus in blood

Table: 5.2.1 HBsAg prevalence and Blood Donors of Bhadrapur and Damak.

Table: 5.2.2 Gender wise seroprevalence of HBsAg at Bhadrapur and Damak

Table: 5.2.3 Distribution of HBsAg on Blood donors of Jhapa Age group wise and Gender Wise

LIST OF FIGURES

Figure: 3.1 Structure of Hepatitis B viruses (A simplified drawing of the HBV particle and surface antigen)

Figure: 3.2 Hepatitis B viruses replication in liver

Figure: 3.3 Progression of HBV infections acquired as an adult

Figure: 3.4 Timeline of detection hepatitis B infection (no late seroconversion) hepatitis B viral antigens and antibodies detectable in the blood of a acute chronically infected person

Figure: 3.5 Detectable Hepatitis B viral antigens and antibodies, in the blood following acute infection

Figure: 3.6 Important Serological diagnosis of HBV

Figure: 3.7 Symptoms and elevated level of ALT observed after months of exposure in patients with acute HBV infection

Figure: 4.1 Flow chart of HBsAg testing method

Figure: 5.1 Group of blood donors as studied population

LIST OF PHOTOGRAPHS

Photograph 1: HEPACARD Immunochromatographic Test Kits

Photograph 2: Test Kit and its Cover

LIST OF APPENDICES

Appendix I	Donors Screening Form
Appendix II	Literature of Test Kits
Appendix III	HBsAg Test (For Research purpose, not for Laboratory Test).
Appendix IV	Evaluation of tests Kits
Appendix V	Co-relation between the different attributes among the blood donors
Appendix VI	Seropositivity rate of HBsAg (Nation wide and in Kathmandu