SEROEPIDEMIOLOGICAL STUDY OF DENGUE VIRUS INFECTION IN PARSA DISTRICT OF

NEPAL



A DissertationSubmitted to the Department of Microbiology Kantipur College of Medical Science (Affiliated to Tribhuvan University) In Partial Fulfillment of the Requirements for the Award of Degree of Master of Science in Microbiology (Medical)

By

SHRAWAN KUMAR SINGH Department of Microbiology Kantipur College of Medical Sciences Sitapaila, Kathmandu, Nepal

TU Registration No: 5-2-33-592-2007 Exam Roll No: 18773 2015

RECOMMENDATION

This is to certify that **Mr. Shrawan Kumar Singh** has completed this dissertation work entitled **"Seroepidemiological study of dengue virus infection in Parsa district of Nepal** "as a partial fulfillment of the requirements of M.Sc. degree in Microbiology (Medical) under our supervision. To **our** knowledge this work has not been submitted for any other degree.

•••••	•••••	•••••
Dr. Basu Dev Pandey	Mr. Nabaraj Adhikari	Mr. Upendra Thapa Shrestha
MD, Ph.D.		
Supervisor	Supervisor	Supervisor
Director	Program Co-ordinator	Head of Department
Everest International Clinic	Kantipur College of	Kantipur College of
and Research Center	Medical Science	Medical Science
Kalanki, Kathmandu	Sitapaila, Kathmandu	Sitapaila, Kathmadu

Date:

CERTIFICATE OF APPROVAL

On the recommendation of the supervisors **Dr. Basu Dev Pandey**, **Mr.Upendra Thapa Shrestha** and **Mr. Nabaraj Adhikari**, this dissertation work of **Mr. Shrawan Kumar Singh**, entitled **"Seroepidemiological study of dengue virus infection in Parsa district of Nepal**"has been approved for the examination and is submitted to Tribhuvan University in partial fulfillment of the requirement for M.Sc degree in Microbiology (**Medical**).

Mr.Upendra Thapa Shrestha Head Department of Microbiology Kantipur College of Medical Sciences (*Affiliated to Tribhuvan University*) Sitapaila, Kathmandu, Nepal

Date: -....

BOARD OF EXAMINERS

Recommended by:

••••••

Dr. Basu Dev Pandey, MD,Ph.D. Supervisor

•••••

Mr. Upendra Thapa Shrestha

Supervisor

••••••

Mr. Nabaraj Adhikari

Supervisor

Approved by:

••••••

Mr. Upendra Thapa Shrestha

Head of Department

Examined by:

.....

Ms. Binita Nepal

Lecturer Kantipur College of Medical Science Internal Examiner

••••••

Mr. Puspa Raj Dahal Teaching Assistant Trichandra Multiple Campus External Examiner

Date:

ACKNOWLEDGEMENTS

I am immensely pleased to express my heartfelt appreciation to all the people who helped me in completing my dissertation work.

I am indebted to my supervisors, **Dr. Basu Dev Pandey**, Director, Everest International Clinic and Research Center, Kathmandu, **Mr. Nabaraj Adhikari**, Programme Co-ordinator, Microbiology Department, Kantipur College of Medical Science, Tribhuvan University and **Mr.Upendra Thapa Shrestha**, Head, Department of Microbiology, Kantipur College of Medical Science, Tribhuvan University, for their expert guidance, supervision, constant encouragement and resource for the completion and accomplishment of this study.

I am profoundly thankful to Late **Prof. Dr. Shital Raj Basynat**, Former Head, Department of Microbiology for providing me constant support and suggestions in the entire work.

I would like to extend my gratitude towards **Mr. Kedarji Kadel**, Campus Chief, all the faculty members and entire staffs of Kantipur College of Medical Science, Tribhuvan University, Kathmandu, whose suggestions and guidance helped me to learn the fundamentals of carrying out this work.

Special thanks to Everest International Clinic and Research Center (EICRC) for providing laboratory facility. I would like to thank Mr. Biswas Neupane and Mr.Yogendra Shah for their guidance throughout my research period.

I would like to extend my heartfelt gratitude to Late Dr. Durga Datta Joshi, Chief of National Zoonoses, Food Hygiene and Research Center, Tahachal, Kathmandu for providing ELISA reader throughout the period.

I would like to thank doctors, medical technicians and patients of Narayani Sub Regional Hospital and Bhawani Hospital and Research Centre, Birgunj,Parsa for their valuable suggestion,and co-operation during the sample collection.

I am especially thankful to my friends Deepak Awasthi, Rojina Shrestha, Ram Sharma and Bipin Chaurasiya for providing me necessary assistance, valuable suggestions and support to do this work successfully.

Finally, my deepest and heartiest gratitude is to my parents who gave me strength in my career and for their everlasting support and encouragement.

Date:

Shrawan Kumar Singh

ABSTRACT

Dengue fever (DF) is an emerging mosquito borne viral disease and important public health problem in low land of Terai region which is also expanding to hilly region of Nepal. The study aims to shed light on the clinical, epidemiological and serological aspects associated with dengue virus infections (DVI) and its implications in future diagnosis, management, prevention and control of the disease in Nepal. Two hundred sixty one serum samples were collected from patients suspected of dengue virus infection visiting hospitals of Parsa districts during July- December of 2013 and tested by IgM Capture Enzyme linked immunosorbent assay (Standard Diagnostic INC., Korea) and Dengue IgM/IgG Rapid immunochromatographic test kit(Panbio, Australia). The anti-dengue IgM positivity was found to be 18.8% and 15.3% by IgM capture Enzyme linked immunosorbent assay and Rapid immunochromatographic test respectively. Among 49 anti-dengue IgM positive cases, the highest numbers 40(15.3%) cases were observed in age group of 15-50 years. Student was the most commonly affected with the highest number of positive cases 16(6.2%). Patients with Joint pain, retroorbital pain and Skin rash as clinical symptoms were more likely to be diagnosed as anti-dengue IgM positive. The highest numbers 41(20.6%) of cases have duration of fever more than 5 days among IgM positive cases. Knowledge of dengue was found in 20(11.6%) of anti-dengue IgM positive cases. Water logging 15(10.9%) and Travel to endemic area 10(37%) were found as the more likely risk factors in anti-dengue IgM positive cases. Flower pot was found as the most likely breeding place with the highest number of positive cases 19(36.5%). Use of net 87.3% and change stored water 85.8% was the most likely used preventive measures respectively. The diagnostic accuracy of RDT for the detection of IgM antibody is low. So it would be better to use ELISA test than RDT whenever possible.

Keywords: DVI, IgM Capture ELISA, RDT

CONTENTS

Page No.

Title	Ι
Recommendation	II
Certificate of Approval	III
Board of Examiners	IV
Acknowledgement	V
Abstract	VI
Contents	VII-IX
List of Tables	Х
List of Figures	XI
List of Photographs	XII
Appendices	XIII
Abbreviations	XIV
CHAPTER I: INTRODUCTION AND OBJECTIVES	1-4
1.1 Introduction	1
1.2 Objectives	4
1.2.1 General objective	
1.2.2 Specific objectives	
CHAPTER II: LITERATURE REVIEW	5-21
2.1 Historical review	5
2.2 Dengue the disease	5
2.3 Dengue virus	6
2.3.1 Morphology and genome structure	6
2.3.2 Replication	7
2.4 The vector	8
2.5 Host	9
2.6 Transmission of dengue virus	10
2.7 Immune response	
2.8 Dengue burden	12

2.8.1 Global senerio	12
2.8.2 Dengue situation in Nepal	14
2.9 Clinical diagnosis	15
2.9.1 Clinical symptoms	15
2.9.2 Grading the severity of dengue infection	16
2.10. Laboratory diagnosis	16
2.10.1 Serological detection	17
2.10.2 Enzyme linked immune sorbent assay (ELISA)	18
2.10.3 Rapid Immunochromatographic strip test	20
2.11 Risk factor and Breeding place of DVI	20
2.12 Prevention and control of DVI	21
CHAPTER III: MATERIALS AND METHODS	22-27
3.1 Materials	22
3.2 Methods	22
3.2.1 Case inclusion criteria	22
3.2.2 Case-exclusion criteria	22
3.2.3 Ethical clearance	22
3.2.4 Sample collection, storage and transport	23
3.2.5 Clinical Profile	23
3.2.6 Laboratory Tests	23
3.2.6.1 Detection of anti-dengue IgM by RDT	23
3.2.6.2 Detection of anti-dengue IgM-Capture ELISA	24
3.2.7 Interpretation of the result	25
3.2.7.1 Rapid test result interpretation	25
3.2.7.2 Elisa result interpretation	26
3.2.8 Quality Control	26
3.2.9 Statistical analysis	22
CHAPTER IV: RESULTS	28-36
4.1 Socio-Demographic Study of Suspected Dengue Cases	28

4.1.1 Sex wise distribution of Suspected Dengue Cases284.1.2 Age wise distribution of Suspected Dengue Cases28

4.1.3 Profession wise distribution of Suspected Dengue Cases	29
4.2 Diagnostic Tests	30
4.3 Comparison between RDT and IgM-capture ELISA	30
4.4 Age wise Distribution of IgM Positive Cases	31
4.5 Sex wise Distribution of IgM Positive Cases	31
4.6 Profession wise Distribution of IgM Positive Cases	32
4.7 Clinical Features of Anti-Dengue IgM Positive Patients	32
4.8Relation between Duration febrile illness and IgM Detection	33
4.9 Relation between knowledge of dengue and IgM detection	34
4.10 Relation between Risk factor and IgM Detection	35
4.11 Relation between Breeding place and IgM Detection	35
4.12 Relation between Use of preventive measures and IgM Detection	36

CHAPTER V: DISCUSSION	37-44
5.1 Discussion	37
CHAPTER VI: CONCLUSIONN	45-46
6.1 Conclusion	45
6.2 Recommendations	46
REFERENCES	47-54

LIST OF TABLE

Table 1: Diagnostic test

Table 2: Comparison between RDT and IgM-capture ELISA

Table 3: Age -wise distribution of IgM Positive Cases

Table 4: Sex-wise distribution of IgM Positive Cases

Table 5: Profession wise Distribution of IgM Positive Cases

Table 6: Clinical manifestation in anti-dengue IgM positive cases

Table 7: Duration of fever in relation to IgM Detection

Table 8: Relation between knowledge of dengue and IgM detection

Table 9: Relation between Risk factor and IgM Detection

Table 10: Relation between Breeding place and IgM Detection

Table 11: Relation between use of preventive measures and IgM Detection

Table 12: Grading the severity of dengue infection

Table 13: Summary of characterstic of dengue diagnostic method

Table 14: Calculation of comparison between RDT and IgM-capture ELISA Assay

LIST OF FIGURES

- Figure 1: Morphology of dengue virus
- Figure 2: Sylvatic and Urban Dengue Transmissions Cycles
- Figure 3: Map of Nepal showing the Dengue detected districts
- Figure 4: Diagnostic methods for dengue
- Figure 5: Age wise Distribution of Suspected Dengue Cases
- Figure 6: Sex wise Distribution of Suspected Dengue Cases
- Figure 7: Profession wise Distribution of Suspected Dengue case

LIST OF PHOTOGRAPHS

- Photograph 1: Microtiter wells after addition of stop solution (ELISA)
- Photograph 2: RDT kit 15 minutes after addition of buffer

LIST OF APPENDICES

Appendix A:	Materials
Appendix B:	Grading the severity of dengue infection
Appendix C:	Summary of characteristics of dengue diagnostic methods
Appendix D:	Calculation
Appendix E:	Dengue case details and Lab form
Appendix F:	SD Dengue IgM Capture ELISA
Appendix G:	Panbio Dengue Duo Cassette

ABBREBRATION

ADE	Antibody Dependent Enhancement
APCs	Antigen Presenting Cells
Den(1-4)	Dengue (1-4)
DF	Dengue fever
DHF	Dengue Hemorrhagic fever
DSS	Dengue Shock Syndrome
DV	Dengue Virus
DVI	Dengue Virus infection
EC	Endothelial Cells
EDCD	Epidemiology and Disease Control Division
EICRC	Everest International Clinic and Research Centre
ELISA	Enzyme linked Immuno Sorbent Assay
HLA	Human Leukocyte Antigen
HRP	Horse reddish peroxidase
IL	Interleukin
JE	Japanese Encephilitis
MAB	Monoclonal Antibody
NHRC	Nepal Health Research Council
РАНО	Pan American Health Organization
PCR	Polymerase chain Reaction
RDT	Rapid Diagnostic Test
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase PCR
SEARO	South East Asian Region
TAE	Tris-acetate EDTA
TMB	Tetramethylbenzidine
WHO	World Health Organization