A COMPARATIVE STUDY OF IGM CAPTURE ELISA AND PARTICLE AGGLUTINATION ASSAY FOR THE DIAGNOSIS OF JAPANESE ENCEPHALITIS AMONG SOME NEPALESE PATIENTS

A

DISSERTATION

SUBMITTED TO THE CENTRAL DEPARTMENT OF MICROBIOLOGY

TRIBHUVAN UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER'S DEGREE OF SCIENCE IN MICROBIOLOGY (ENVIRONMENT AND PUBLIC HEALTH)

BY

SANTA RAJ KHANAL

CENTRAL DEPARTMENT OF MICROBIOLOGY

TRIBHUVAN UNIVERSITY

KIRTIPUR, KATHHUMANDU, NEPAL

2008

LETTER OF RECOMMENDATION

The thesis entitled "**A Comparative Study of IgM Capture ELISA and Particle Agglutination Assay for the Diagnosis of Japanese Encephalitis among Nepalese Patients"** has been completed by **Mr. Santa Raj Khanal** under my supervision. I hereby recommended this thesis for examination by the Thesis committee as a partial fulfillment for the Degree of **Master of Science in Microbiology (Environment and Public Health).**

Dr. Basu Dev Pandey, Ph.D.	**Miss Shaila Basnyat**
Senior Medical Officer	Assistant Professor
Sukra Raj Tropical and	Central Department of
Infectious Disease	Microbiology
Hospital Teku,	Tribhuvan University
Kathmandu, Nepal	Kirtipur, Kathmandu
Date:……...……..	Date: .....

CERTIFICATE OF APPROVAL

On the recommendation of **Miss Shaila Basnyat** and **Dr. Basudev Pandey,** this dissertation of **Mr. Santa Raj Khanal** is approved for the examination and is submitted to the Tribhuvan University in partial fulfillment of the requirements for **Master of Science in Microbiology (Environment and Public Health)**

.....

Dr. Anjana Singh, Ph.D.

Head

Central Department of Microbiology

Tribhuvan University

Kirtipur, Kathmandu,

Date:..... Nepal

BOARD OF EXAMINERS

Recommended by:

Dr. Basu Dev Pandey
Supervisor

Miss Shaila Basnyat
Supervisor

Approved by:

Dr. Anjana Singh
Head of Department

Examined by:

Dr. Shiba Kumar Rai
External Examiner

Mr. Binod Lekhak
Internal Examiner

Date: .....

ACKNOWLEDGEMENT

I would like to express my immense gratitude to my respected supervisors Dr. Basudev Pandey, Senior Medical Officer, Sukraraj Tropical Hospital, Teku and Miss Shaila Basnyat, Assistant Professor, Central Department of Microbiology, Tribhuvan University, Kirtipur for their continuous inspiration, full support and expert guidance.

I am very much thankful to all of the patients, who were included in my research without whose co-operation I would never have completed my research work.

I express my deepest gratitude to Associate Professor Dr. Anjana Singh, Head of Department of Microbiology, Kirtipur, Kathmandu. I am equally thankful to Professor Dr. Shital Raj Basnyat, Associate Professor Dr. Prakash Ghimire, Associate Professor Dr. Shreekant Adhikari, Associate Professor Dr. Dwij Raj Bhatta, Assistant Professor Mr. Binod Lekhak and all the respected teachers and staffs of Central Department of Microbiology for their full fledge support.

I express my heartfelt gratitude to Central Department of Microbiology for managing all necessary arrangements and facilities I need. I am very much indebted to Everest International Clinic and Research Center and its staff and Mr Kiran Pandey, in particular, for their material and logistics support.

I am very much grateful to Dr. Usha Shah, Medical Superintendent of Bheri Zonal Hospital, Nepalgunj, Mohuddin Kitdyai, Director of Nepalgunj Medical College, Nepalgunj, Mr. Govinda Paudel, Laboratory incharge, Bheri Zonal Hospital and other laboratory staffs for their co-operation during sample collection.

I would also like to express my sincere gratitude to Prof. Koichi Morita and all Japanese team for providing reagents as well as technical assistance.

I would like to express my gratitude to Mr. Bal Ram Adhikari for his co-operation during sample collection and valuable suggestions.

My special thanks go to Mr. Shyam Prakash Dumre, Medical Microbiologist, National Public Health Laboratory, Teku Kathmandu for his help during my thesis work.

I would like to acknowledge to all of my colleagues for their valuable suggestions and help to complete my research work.

And, I ought to record my heartfelt gratitude to my parents for their unending supply of blessings and all round assistance in my work.

_____

Date: ..... **Santa Raj Khanal**

ABSTRACT

Japanese encephalitis (JE) is one of the serious public health problems particularly in the Terai regions of Nepal. At present, diagnosis of JE in Nepal is particularly based in the clinical diagnosis. Laboratory diagnosis is made with IgM-capture ELISA in few referral centres, which require sophisticated equipment, costly and time consuming. Recently, a simple particle agglutination assay (PA) had been developed to diagnose JE, which can be done anywhere, does not require sophisticated equipment and can be done at room temperature. This study was conducted to evaluate the particle agglutination assay as a diagnostic method to detect anti-JEV antibodies in human serum samples.

In the study, a total of 279 serum samples were collected. PA was compared with IgM-capture ELISA in 263 serum samples of suspects of Acute Encephalitis Syndrome (AES) and viral fever. Of these 138(52.5%) serum samples showed positive result for JE by PA among which 106 serum samples (77% of PA positive) were also positive by IgM-capture ELISA method. Among 263 serum samples, three of the serum samples were JE negative by PA while positive by IgM-capture ELISA. One hundred twenty two samples were JE negative by both PA and IgM-capture ELISA.

PA showed 97% sensitivity and 79% specificity in comparision with IgM-capture ELISA for diagnosis of JE. A positive predictive value of 0.77 and negative predictive value of 0.98 was observed in comparison with IgM-capture ELISA. The result was compatible in comparison with IgM-capture ELISA. To know the cross reactivity, sixteen laboratory confirmed samples of Dengue, Malaria and other infectious diseases were examined for JE by PA. Maximum cross reactivity against dengue was observed with qualitative assay by PA. However with quantitative assay for JE and dengue by PA far greater titre was observed with dengue. Both PA and IgM-capture ELISA showed cross reactivity with Malaria. Since IgM-capture ELISA also showed cross reactivity with Malaria, there is a chance of mixed infection of JE with Malaria. This study showed that particle agglutination assay could be used in rural areas of Nepal for laboratory diagnosis of JE.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ABC	Avidin biotin system
AES	Acute encephalitis syndrome
AFP	Acute flaccid paralysis
Cx.	*Culex*
CDC	Centre for Disease Control and Prevention
CF	Complement fixation
CFR	Case fatality rate
CI	Case incidence
CNS	Central nervous system
CSF	Cerebrospinal fluid
DoHS	Department of Health Services
E	Envelope protein
EDCD	Epidemiology and Disease Control Division
ELISA	Enzyme Linked Immunosorbent Assay
Ha-Ny beads	Hydroxyapatite- coated nylon beads
HI	Haemagglutination Inhibition
hMDF	Human macrophage derived factor
HMIS	Health management Information System
IFA	Indirect fluorescent antibody
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IPD	Immunization preventable diseases

JE	Japanese encephalitis
JEV	Japanese encephalitis virus
MAb	Monoclonal antibody
MAC-ELISA	IgM antibody capture ELISA
MoHP	Ministry of Health and Population
NS	Non-structural protein
NTA	Neutralization assay
PA	Particle agglutination assay
PCR	Polymerase chain Reaction
PHK	Primary Hamster Kidney
PrM	Pre-membrane protein
PRNT	Plaque reduction neutralization test
RT-PCR	Reverse transcriptase polymerase chain reaction
SEARO	South East Asian Regional Office
SLE	St. Louis encephalitis
UTR	Untranslated region
WDR	Western Development Region
WHO	World Health Organization
WNV	West Nile Virus

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOGRAPHS

LIST OF APPENDICES