"STUDIES ON THE ASSOCIATION OF FcγRIIa His131Arg POLYMORPHISM WITH DENGUE INFECTION: A HOSPITAL BASED CASE CONTROL STUDY"

By

Dr. SHAAMA GHUNGROO

Dissertation submitted to

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH,
(Deemed to be university)
TAMAKA, KOLAR, KARNATAKA

In partial fulfillment of the requirements for the award of degree of

DOCTOR OF MEDICINE

IN

GENERAL MEDICINE

Under the guidance of
Dr. PRABHAKAR K.MD
Professor
DEPARTMENT OF GENERAL MEDICINE
SRI DEVARAJ URS MEDICAL COLLEGE

And under the co-guidance of
Dr. SHARATH B.
Associate Professor
DEPARTMENT OF CELL BIOLOGY
AND MOLECULAR GENETICS

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH
TAMAKA, KOLAR, KARNATAKA

MAY 2019

SRI DEVARAJ URS MEDICAL COLLEGE
CONSTITUENT COLLEGE OF
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH,
TAMAKA, KOLAR, KARNATAKA

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "**Studies on the association of FcγRIIa His131Arg polymorphism with dengue infection : A Hospital Based case control Study**" is a bona-fide and genuine research work carried out by me under guidance of **Dr. PRABHAKAR K.** Professor, Department of General Medicine, Sri Devaraj Urs Medical College, Kolar and co-guidance of **DR.SHARATH B.** Assoc. Professor, Department of Cell Biology And Molecular Genetics of Sri Deveraj Urs Academy of Higher Education and Research, Kolar.

Date:

Place: Kolar **Dr. SHAAMA GHUNGROO**

SRI DEVARAJ URS MEDICAL COLLEGE
CONSTITUENT COLLEGE OF
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH,
TAMAKA, KOLAR, KARNATAKA

DECLARATION BY THE GUIDE

This is to certify that the dissertation entitled **"Studies on the association of FcγRIIa His131Arg polymorphism with dengue infection : A Hospital Based case control Study"** is a bona-fide and genuine research work carried out by **Dr. SHAAMA GHUNGROO** under my guidance in partial fulfillment of the requirement for the award of degree of **Doctor of Medicine in GENERAL MEDICINE .**

Date:
Place: Kolar

DR. PRABHAKAR K.
Professor
Department of General medicine
Sri Devaraj Urs Medical College
Tamaka, Kolar

SRI DEVARAJ URS MEDICAL COLLEGE
CONSTITUENT COLLEGE OF
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH,
TAMAKA, KOLAR, KARNATAKA

DECLARATION BY THE CO-GUIDE

This is to certify that the dissertation entitled **"Studies on the association of FcγRIIa His131Arg polymorphism with dengue infection : A Hospital Based case control Study"** is a bona-fide and genuine research work carried out by **Dr. SHAAMA GHUNGROO** under my co-guidance in partial fulfillment of the requirement for the award of degree of **Doctor of Medicine in GENERAL MEDICINE .**

Date:

Place: Kolar

DR.SHARATH B.
Associate Professor
Department of Cell Biology and Molecular Genetics
Sri Devaraj Urs Academy of
Higher Education and Research
Tamaka, Kolar.

SRI DEVARAJ URS MEDICAL COLLEGE
CONSTITUENT COLLEGE OF
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, TAMAKA, KOLAR, KARNATAKA

ENDORSEMENT BY THE HOD, PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"Studies on the association of FcγRIIa His131Arg polymorphism with dengue infection : A Hospital Based case control Study"** is a bona-fide and genuine research work done by **Dr. SHAAMA GHUNGROO** under guidance of **Dr. PRABHAKAR K. MD** Professor, Department of General Medicine, Sri Devaraj Urs Medical College, and **Dr. SHARATH B.** Associate Professor , Department of Cell Biology and Molecular Genetics, Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar.

Dr. RAVEESHA A.
Professor and HOD
Department of General Medicine
Sri Devaraj Urs Medical College
Tamaka, Kolar

Date:
Place: Kolar

Dr. HARENDRA KUMAR M.L.
Principal
Sri Devaraj Urs Medical College,
Tamaka, Kolar

Date:
Place: Kolar

SRI DEVARAJ URS MEDICAL COLLEGE
CONSTITUENT COLLEGE OF
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH,
TAMAKA, KOLAR, KARNATAKA

ETHICAL COMMITTEE CERTIFICATE

This is to certify that the Ethical Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has unanimously approved **Dr. SHAAMA GHUNGROO** Post-Graduate student in the subject of **GENERAL MEDICINE** at Sri Devaraj Urs Medical College, Kolar to take up the Dissertation work entitled **"Studies on the association of FcγRIIa His131Arg polymorphism with dengue infection : A Hospital Based case control Study"** to be submitted to the Sri Devaraj Urs Academy Of Higher Education And Research Centre, Tamaka, Kolar, Karnataka.

Date:

Place: Kolar

Member Secretary

Institutional Ethics Committee

Sri Devaraj Urs Medical College

Tamaka, Kolar-563101

SRI DEVARAJ URS MEDICAL COLLEGE
CONSTITUENT COLLEGE OF
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH,
TAMAKA, KOLAR, KARNATAKA

COPY RIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the Sri Devaraj Urs Academy of Higher Education and Research Centre, Kolar, Karnataka shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic /research purpose.

Date: **Signature of the Candidate**

Place: Kolar **Dr. SHAAMA GHUNGROO**

SRI DEVARAJ URS MEDICAL COLLEGE
CONSTITUENT COLLEGE OF
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH,
TAMAKA, KOLAR, KARNATAKA

ACKNOWLEDGEMENT

I am deeply indebted and grateful to my guide, ***Dr. PRABHAKAR K.*** *Professor, Department of General Medicine, Sri Devaraj Urs Medical College, for his able guidance, support, timely advice and constant encouragement throughout the period of the study.*

I would like to express my sincere gratitude to ***Dr. SHARATH B.*** *Associate Professor Cell Biology and Molecular Genetics, Sri Devaraj Urs Academy of Higher Education and Research, for his expert advice on the methodology, critical analysis of data of this study to get insight into molecular aspects of dengue fever.*

I would like to express my sincere gratitude to ***Dr. RAVEESHA A.*** *Prof. and Head of the Department of General Medicine, Sri Devaraj Urs Medical College for his expert advice and constant support during the preparation of this dissertation.*

I express my heartfelt gratitude to ***Dr. V.LAKSHMAIAH, Dr. B.N. RAGHAVENDRA PRASAD, Dr. VENKATA RATHNAMMA PN. Dr. VIDYASAGAR, Dr. SRINIVASA SV,*** *for their advice and encouragement throughout the study.*

I would like to thank all my teachers specially ***Dr. MAHESH K, and Dr. JAYAPRASAD,*** *from the Department of General Medicine for their valuable suggestions and support.*

I am evergreatful to ***Ms. Chaitra****, Junior Research Fellow, Dept. of Medicine for her unflinching support in the Molecular analysis of the DNA samples of patients. She has been*

exemplary with good knowledge about the methodology which enabled me to imbibe the analytical methods early.

I am thankful to my postgraduate colleagues especially ***Dr. MAYUR DAVE, Dr. ARATHI SG, Dr. SANKETH J, Dr. DWARAK S, Dr. TAMEEM I, Dr. MAHARAJ AND Dr. RAGHAVENDAR*** *for their motivation and for being a constant sources of strength.*

I am thankful to all my other ***seniors and dear juniors*** *for all their love, motivation and help.*

I would like to thank ***my friend, Mrs. HARSHITA KS,*** *for her patience and support throughout the preparation of this dissertation.*

I am also thankful to ***Mrs. ANURADHA B.M. And Mr. NATESH GOWDA K,*** *non-teaching staff for their invaluable help*

Date: **Dr. SHAAMA GHUNGROO**

Place: Kolar

SDUAHER

Sri Devaraj Urs Academy of Higher Education and Research

Certificate of Plagiarism Check

Author Name	Dr. SHAAMA GHUNGROO
Course of Study	Synopsis / Thesis / Dissertation
Name of Supervisor	Dr. PRABHAKAR .K.
Department	GENERAL MEDICINE .
Acceptable Maximum Limit	10%
Submitted By	librarian@sduu.ac.in
Paper Title	STUDIES ON THE ASSOCIATION OF FcÎ³RIIa His131Arg POLYMORPHISM WITH DENGUE INFECTION: A HOSPITAL BASED CASE CONTROL STUDY
Similarity	9 %
Paper ID	181129053459
Submission Date	2018-11-29 05:34:59

* This report has been generated by DrillBit Anti-Plagiarism Software

Shaama	
Signature of Student	Signature of Supervisor
Head of the Department	
29/11/18	
University Librarian	Post Graduate Director

ABSTRACT

INTRODUCTION:

Dengue is an acute mosquito-borne infection caused by dengue virus (DENV). Both innate and adaptive immune responses of the host are involved in the pathogenesis of dengue apart from genetic determents of the DENV. Antibodies generally neutralize the infection when bound in sufficiently large numbers to the dengue virus and prevent infection. Monocytes and macrophages are the key targets of DENV and the Fc receptor facilitates its entry into the cells. FcRIIa is a low affinity IgG receptor that binds to IgG 1-4. Functionality of FcRIIa is affected by genetic variations. One such genetic variation results in the substitution of histidine (His) amino acid with arginine (Arg) amino acid at position number 131 in the polypeptide chain. This substitution reduces the binding property of Fc fragment to the IgG class of antibodies.

OBJECTIVES:

1. To determine the spectrum of dengue and its clinical presentation.
2. To determine the frequency of FcγRIIa His131Arg gene polymorphism in dengue sero-positive patients.
3. To analyze by statistical methods the gene polymorphism data for association with susceptibility to dengue infection.

MATERIALS AND METHODS:

This was a hospital based case-control study involving 72 dengue sero-positive patients and 72 healthy controls. Genomic DNA was prepared from peripheral blood of the study participants and FcγRIIa His131Arg gene polymorphism was genotyped by PCR-RFLP method.

RESULTS:

Out of 72 patients included in the study, 49 patients were males and 23 were females. The age profile of the patients was 18-25 years (n=10), 26-45 years (n=51) and 46-60 years (n=11). The chief complaints in the patients were fever alone (n=10), fever and pain abdomen (n=33), fever and breathlessness (n=12), fever and vomiting (n=8), fever and myalgia (n=6) and fever with joint pains (n=4). Dengue serology profile was done, out of which most patients were NS1Ag positive (n=55), IgM positive (n=10), IgG positive in 7 cases.

The frequency of Arg allele was 45.1% in the patient group and 63.9 % in the control group. The frequency of the Arg allele was 1.4 times higher in the control group than in the patient group and the difference was statistically significant (p-value =0.002). The odds ratio for the difference was 0.46. Arg allele was therefore a protective factor. The frequency of His/His genotype was 30.5% in the patient group and 12.5% group in control group. Thus his allele was a risk factor to acquire severe dengue infection.

CONCLUSION:

FcγRIIa His131Arg gene polymorphism is associated with the susceptibility to dengue infection. This study underlines the importance of variability in the antibody-mediated immune response in dengue infection.

KEY WORDS: Dengue Fever, FcγRIIa gene polymorphism, ADE.

LIST OF ABBREVIATIONS

ADE	Antibody dependant enhancement
Arg	Arginine
DENV	Dengue virus
DF	Dengue fever
DHF	Dengue hemorrhagic fever
DNA	Deoxyribonucleic acid
DSS	Dengue shock syndrome
EDTA	Ethylene diamine tetra acetic acid
EIP	Extrinsic incubation period
ELB	Erythrocyte lysis buffer
GAGS	Glycosaminoglycans
His	Histidine
HLA	Human leukocyte antigen
Ig	Immunoglobulin
IL	Interleukin
INF	Interferon
NS	Non-structural protein
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulphate
SNP	Single nucleotide polymorphism
TNF	Tumour necrosis factor

TABLE OF CONTENTS

SL.NO	TITLE	PAGE NO
1.	INTRODUCTION	1-2
2.	OBJECTIVES OF THE STUDY	3
3.	REVIEW OF LITERATURE	4-15
4.	MATERIALS AND METHODS	16-24
5.	RESULTS	25-32
6.	DISCUSSION	33-36
7.	SUMMARY	37-38
8.	CONCLUSION	39
9.	REFERENCES	40-49
10.	ANNEXURE I : PROFORMA	50-51
11.	ANNEXURE II : INFORMED CONSENT FORM	52
12.	ANNEXURE III : PATIENT INFORMATION SHEET	53-54
13.	ANNEXURE IV : MASTER CHART	55-57

LIST OF TABLES

TABLE NO	DESCRIPTION	PAGE NO
1	ATYPICAL MANIFESTATIONS	7
2	PRIMERS USED FOR THE PCR AMPLIFICATION	22
3	PROGRAM USED FOR PCR AMPLIFICATION	23
4	COMPOSITION OF THE PCR MIX	23
5	COMPOSITION OF RESTRICTION DIGESTION REACTION MIXTURE	24
6	GENDER PROFILE OF THE DENGUE PATIENTS	25
7	AGE RANGE OF THE DENGUE PATIENTS	26
8	CHIEF COMPLAINTS IN THE DENGUE PATIENTS	27
9	PROFILE OF DENGUE SEROLOGY IN THE PATIENTS	28
10	RFLP BAND PATTERN OF FcγRIIa His131Arg GENE POLYMORPHISM	30
11	ALLELE FREQUENCY OF FcγRIIa His131Arg GENE POLYMORPHISM IN THE PATIENT AND CONTROL GROUP	31
12	GENOTYPE FREQUENCY OF FcγRIIa His131Arg GENE POLYMORPHISM	32
13	COMPARISON OF GENOTYPIC AND ALLELIC FREQUENCIES OF FcγRIIa His131Arg GENE POLYMORPHISM ACROSS DIFFERENT STUDIES.	34

LIST OF FIGURES

FIGURE NO	DESCRIPTION	PAGE NO
1	CLINICAL CASE CLASSIFICATION	6
2	GENDER WISE DISTRIBUTION OF DENGUE PATEINTS	25
3	AGE WISE DISTRIBUTION OF DENGUE PATIENTS	26
4	SPECTRUM OF CLINICAL PRESENTATION IN DENGUE PATIENTS	27
5	PROFILE OF DENGUE SEROLOGY	28
6	REPRESENTATIVE AGAROSE GEL ELECTROPHORESIS IMAGE SHOWING PCR-RFLP PATTERN FOR FcγRIIa His131Arg GENE POLYMORPHISM	29

INTRODUCTION:

Dengue is a mosquito borne disease and is a major public health concern around the world. There has been a rapid increase in the infection rate and nearly 400 million people across the globe are at risk of dengue infection. [1, 18] This infection is caused by dengue virus (DENV) which is a member of the genus flavivirus of the family flaviviridae, and is a single - stranded positive sense RNA virus. There are four DENV serotypes (DENV 1 - 4) found in tropical and subtropical regions of the globe. [2] All 4 serotypes are capable of causing dengue infection and is now of endemic importance in India. DENV cycles between the vectors viz; Aedes aegypti ,Aedes albopictus and human beings. [3,4]

Clinically dengue can be classified into – mild, moderate and severe infection based on the signs and symptoms. The severity of dengue infection depends upon age, immune status and the viral serotypes. [5] Infection with any one of the DENV serotype could confer immunity to that specific serotype. It is established that all four serotypes exhibit reasonable antigenic similarity and are capable of eliciting cross protection for a very short period. However, it has been reported that subsequent infection by other serotypes leads to severe forms of dengue infection. [3] All the four forms of DENV serotypes are seen in India and among the four, DENV 1, 2 and 3 are wide spread.[6]

The mechanism by which the immune system of the host responds to DENV has been studied in great detail. [7] Both innate and adaptive immune responses are involved in the pathogenesis of dengue infection. Antibodies generally neutralize the infection when bound to dengue virus particles in sufficiently large numbers and protect against acquiring dengue. However, when the antibodies are below the threshold for neutralization, it promotes viral replication, and acquisition of dengue. [7]

Monocytes and macrophages are the principal target for the DENV. The candidate receptor molecules in the host cells include - Fc receptors, glycosaminoglycans (GAGS) and

lipopolysaccharide binding CD - 14 associated molecules. [8] Among the receptors the FcγR appears to be the most permissive for DENV entry into the cell. There are three distinct but closely related human FcγR classes - FcγRI (CD64) FcγRII (CD32) and FcγRIII (CD16) based on the affinity for IgG, and the location of the genes is mapped to chromosomes 1[9] FcγRIIa is a low affinity IgG receptor capable of binding IgG1-4 immunoglobulin subtypes. [10] It is the most widely distributed of the FcγR type, and is expressed in polymorphic forms on most of the hematopoietic cells. [9].

The functionality of FcγRIIa is affected by a genetic variation that affects the residue number 131 in the receptor protein molecule. The genetic variation results in the substitution of histidine (His) amino acid with arginine (Arg) amino acid. This genetic variation alters the affinity of the receptor for different subclasses of IgG such that the form having His interacts more efficiently with IgG2 while that with Arg shows higher affinity for binding IgG1 and IgG3 subclasses. [11, 12, 13] Thus, the genetic variations of the FcγRIIa due to polymorphisms could have differential binding properties. There are few studies done in different ethnic populations on the association of FcγRIIa polymorphisms and clinical outcomes of the dengue infection, with conflicting results. [14,15]

Therefore, adequately powered replicative studies are necessary. The dengue experience in India provides an excellent opportunity to study the host genetic determinants of this infection. Thus, this study is expected to contribute towards the existing knowledge and provide an insight into genetic susceptibility of DENV infection on account of FcγRIIa polymorphism in the South Indian population.

OBJECTIVES OF THE STUDY:

1. To determine the spectrum of dengue and its clinical presentation.
2. To determine the frequency of FcγRIIa His131Arg gene polymorphism in dengue sero-positive patients.
3. To analyze by statistical methods the gene polymorphism data for association with susceptibility to dengue infection.

REVIEW OF LITERATURE:

Dengue is the most common mosquito borne viral infection found in tropical and subtropical parts across the world. Each year approximately 400 million people are at risk of infection with dengue virus with one fourth of them developing clinical symptoms of infection and severe forms of this result in around 25,600 deaths. [4] This disease is caused by the DENV, which is a single stranded RNA virus of the flavivirus genus, family Flaviviridea. [16] There are four distinct serotypes of DENV namely, DENV1 - DENV4. Infection by any one of the serotypes could result in life long immunity against re-infection with the same DENV serotype. However, one could be vulnerable to infections with the other serotypes. [17, 18]

The number of dengue cases has increased significantly in the recent past and is an endemic in more than 130 countries world over including India. [1,19,20] The increase in spread of dengue in India has been attributed to unplanned urbanization, changes in environmental factors, host pathogen interaction and population immunological factors. In India, both Aedes aegypti and Aedes albopictus are the main vectors and inadequate control mechanisms also have been recorded as the cause for the increased infection rate. [4,21]

As per the Indian national guidelines for clinical management of dengue fever. [5] Dengue has to be considered as an infection with varied clinical presentations ranging from asymptomatic to severe which might lead to increased morbidity and mortality. [22]

Subjects infected with DENV can be classified as mild, moderate and severe dengue. All the symptoms and signs are manifested by capillary leakage, impairment of coagulation profile, bleeding or organ involvement. These three categories have been so defined thereby

making it easy for the clinician to decide whether the patient requires home management, close monitoring , hospitalization or referral to tertiary care hospital.[5]

MILD DENGUE INFECTION: is characterized by undifferentiated Dengue fever i.e fever without complications like bleeding, hypotension, organ involvement or any evidence of capillary leakage. [23] These patients can be typically managed at home.

MODERATE DENGUE INFECTION: These patients are classified into two groups

- Dengue infections with warning signs and symptoms
- Dengue infection with high risk and co-morbid conditions
  - Dengue Fever (DF) with warning signs and symptoms: This include features like recurrent vomiting, abdominal pain or tenderness, general weakness, lethargy, restlessness, minor bleeding, pleural effusion or ascites, hepatomegaly and increased hematocrit. The presence of any one or more of the above features is considered to be the indicator(s) of progression to severity and hence require close monitoring. [5] These also include patients having Dengue Hemorrhagic Fever without hypotension or shock. However, sometimes patients with minor bleeding may progress to severe plasma leakage and could lead to organ involvement, massive bleeding and shock and sometimes death.
  - Moderate dengue with high risk and comorbid conditions: Some dengue infected patients are likely to progress to severe manifestations in presence of high risk and comorbid conditions; which include - infants, old age, diabetes, hypertension, pregnancy, coronary artery disease, hemoglobinopathies, immunocompromised patients and patients on steroids, anticoagulants or immunosuppresants.

These patients need to be closely monitored or possibly hospitalized for further management as they can develop severe dengue manifestation due to abnormalities in metabolic conditions and plasma leakage. These patients contribute to the increasing mortality seen in dengue infected patients. [5,24]

SEVERE DENGUE INFECTION: These patients are recognized by the presence of shock, capillary leakage, significant bleeding, multiple organ involvement and severe metabolic abnormalities. They need immediate admission and intensive care management and should be thoroughly investigated for abnormalities in coagulation profile, complete blood count and organ function tests and may require prompt intravenous fluid, blood or platelet transfusion. [25,26,27]

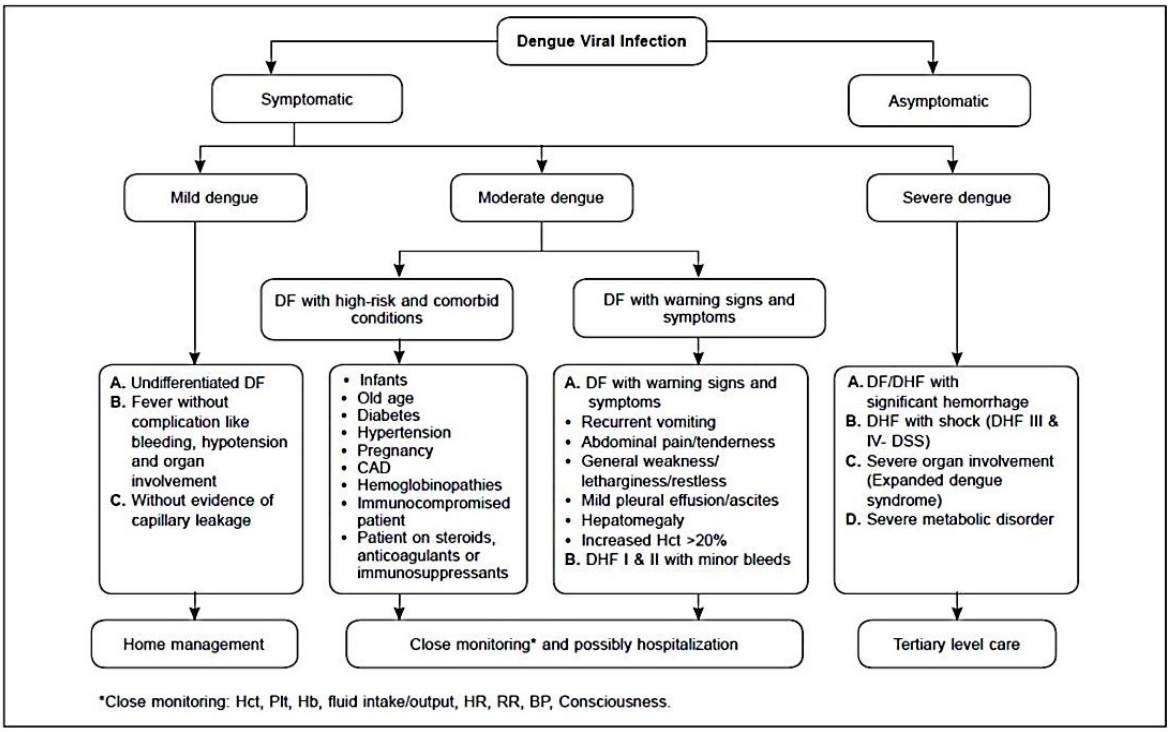

Figure 1: Clinical case classification according to the Indian national guidelines for clinical management of Dengue Fever. Journal of the Indian Medical Association. Vol.113, No 12, page no 198, December 2015.

EXPANDED DENGUE SYNDROME: At times there is severe organ involvement with atypical manifestations as elaborated in Table 1.

Table 1: Systems involved in expanded dengue syndrome as per the Indian National Guidelines for clinical management of dengue fever. [5]

System	Atypical manifestation
Central nervous system	Encephalopathy, encephalitis, seizures, intracranial bleed.
Gastrointestinal system	Acute/fulminant hepatitis, cholecystitis, cholangitis, acute pancreatitis.
Renal system	Acute renal failure, haemolytic uremic syndrome, acute tubular necrosis.
Cardiovascular system	Cardiomyopathy, cardiac arrhythmia, pericardial effusion.
Respiratory system	Acute respiratory distress syndrome, pulmonary edema, pleural effusion.
Eyes	Conjunctival bleed, macular haemorrhages, optic neuritis.

DENGUE VIRUS:

Dengue virus (DENV) is a small enveloped single-stranded positive sense RNA virus belong to the genus flavivirus, family flaviviridae. There are four serotypes DENV 1-4. The viral genome is approximately 11kb in length. [28] The virus particle has a diameter of 50nm enveloped with a lipid membrane and containing multiple copies of three structural proteins: Core (C), Membrane (M) associated and Envelope (E). The virus also contains seven nonstructural (NS) proteins – NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5, which are expressed both as membrane associated and secreted forms. [29]

NS1 is not a part of the virus but gets expressed on the surface of infected cells, this is a character true to dengue virus unlike other viral glycoproteins. Plasma levels of NS1 have a direct relation with viral titres, being lower in DF and higher in DHF. Also higher levels of NS1 in the first 72 hours of infection place the patient at a risk for DHF. It has also been observed that levels of NS1 have been higher in second time infection with DENV than the primary infection with DENV. Suggesting the role of NS1 to contribute to formation of circulating immune complexes which are known to have an important role in the pathogenesis of severe infection. The Envelope (E) glycoprotein is responsible for the main biological functions of the virus, it facilitates attachment to the receptor and fusion of virus envelope with the target cell membrane. It is also associated with heamagglutination of erythrocytes, induction of neutralizing antibodies and protective immune responses. [30,31,32, 33]

THE DENGUE VIRAL REPLICATION CYCLE:

Dengue virus infect a wide range of human and non-human cell types in vitro. The viral replication involves – attachment of virus particle to cell surface, entry of the virus particle into cytoplasm, translation of viral proteins, replication of the viral RNA genome, formation of virions and release from the cell.

The viral Envelope glycoprotein which mediates the entry of the virus into the cell is critical for infectivity, following the binding of the virus into the cell, the viral RNA enters the cytoplasm. The viral proteins are directly translated from the viral RNA as a single polyprotein molecule which is cleaved to yield the three structural and seven nonstructural proteins. The NS5 is the viral RNA dependent RNA polymerase, which assembles with several other viral proteins to form the replication complex. This complex transcribes the viral RNA to produce negative-strand viral RNA, which serves as the template for the production of the viral genomic RNA. [32]

VECTORS:

Mosquitoes are the vectors in dengue infection. Those belonging to the Aedes genus play an important part in the transmission of dengue virus to human beings. Aedes aegypti, Aedes albopictus and Aedes polynesiensis are the vectors of importance depending upon the geographic location. In India, the main vector is Aedes aegypti, however Aedes albopictus is also seen in South and South Eastern parts of India. [34]

Aedes aegypti, are day biting mosquitoes that thrive well both indoors, mainly in bedrooms and living rooms and also outdoors in small collections of polluted water such as

coconut shells and flower pots. Improper disposal of waste, unplanned urbanization and rainy season are mainly responsible for high mosquito density in endemic areas. [35]

It is the adult female mosquito that becomes infectious following a viremic blood meal from an infected human. The virus enters the midgut of the vector, which then spreads through the haemocoel and haemolymph to gain access to the different tissues of the vector. It finally reaches the salivary gland where the virus replicates and is capable of transmitting the virus to another human being via a skin bite. [36]

The extrinsic incubation period (EIP) is the time between this blood meal and the time that the vector takes to become infectious, which is an important determinant in the dengue transmission process. EIP approximately ranges between 8 to 10 days. [37]

HOST:

Infection by any one of the four serotypes of DENV can produce a wide spectrum of illnesses from mild, moderate to severe infection, which can occasionally be fatal. [5] The mechanisms contributing to this differential outcome are incompletely understood. However, it can be related to the interactions between the virus, host immune system and genetic factors. Amongst these, the host genetic factors have been shown to play a significant role in the disease susceptibility and outcomes. [38]

The host genetic factors include: blood type, human leukocyte antigens (HLA), compliments, cytokine mediated immunopathology, single nucleotide polymorphisms (SNP) of receptor (FcγR) especially FcγRIIa, vitamin D receptor, TNF-α, human platelet antigens and other factors like viral genetic determinants, prior dengue infection exposure

and antibody dependent enhancement (ADE), age, ethinicity as well as nutritional status relate to dengue virus susceptibility and infection manifestation and severity. [14,38]

The candidate receptor molecules in the host cells include - Fc receptors, glycosaminoglycans (GAGS) and lipopolysaccharide binding CD - 14 associated molecules [32]. Receptors for the Fc domain of IgG provide a critical link between specific humoral responses and cellular aspects of the immune system. There are three distinct but closely related human FcγR classes - FcγRI (CD64) FcγRII (CD32) and FcγRIII (CD16) based on the affinity for IgG and the location of the gene is mapped to chromosomes 1. [38]

FcγRIIa is a low affinity IgG receptor capable of binding IgG1-4 immunoglobulin subtypes.[9] It is the most widely distributed of the FcγR type, and is expressed in polymorphic forms on most of the hematopoietic cells. [38] The functionality of FcγRIIa is affected by a genetic variation that affects the residue number 131 in the receptor protein molecule. The genetic variation results in the substitution of histidine (His) amino acid with arginine (Arg) amino acid. The genetic variation alters the affinity of the receptor for different subclasses of IgG such that the form having His (H) interacts more efficiently with IgG2 while that with Arg (R) shows higher affinity for binding IgG1 and IgG3 subclasses. [39, 40, 41].

There are few studies done in different ethnic populations on the association of FcγR11a polymorphisms and clinical outcomes of the dengue infection, with conflicting results. The genotypic and allelic distribution of FcγR11a His131Arg polymorphism in subjects affected with DENV in a study conducted in Pakistan[14], showed that, the Arg/Arg homozygotes and His/Arg heterozygotes were significantly more likely to develop dengue

infection with an OR = 3.21, and His/His were noted to have protection from acquiring dengue.[14] Similar observations have been reported in a Cuban population[10], which reported that Arg/Arg and His/Arg contribute to acquiring dengue infection and His/His were noted to be protective, however findings from a Mexican[15] study were found to be contradictory where in the presence of His/His was found to be susceptible to acquire dengue infection and Arg/Arg was found to be protective.

PATHOGENESIS:

All infectious disease consists of the invasion of the microorganism followed by the host response, including disease manifestation and elimination of the organism. The DENV infection is unique in that pre-infection events may control the invasion of microorganism and in turn affect the severity of dengue manifestations and outcomes. Epidemiological and experimental studies carried out in humans and animals have provided valuable information on dengue virus infection.

One of the current hypotheses emphasize on the tropism of dengue virus, on the outcome of DENV infections. It has been shown that, three organ systems play an important role in the pathogenesis of DF, DHF and DSS. These are the immune system, the liver and the endothelial cells linings of the blood vessels. [42] The entry of the dengue virus due to mosquito bite into the blood stream, with spillover into the epidermis and dermis and infects the langerhans cells [43] and keratinocytes [44] It then enters into the lymph nodes through the lymphatic system, followed by a primary viremia and infection of several cells of the mononuclear lineage such as monocytes [45] myeloid dendritic cells [46,47] and splenic as well as liver macrophages. [48,49,50] Leukocytes are also shown to get infected with dengue virus in non-human primates. [51] During a secondary infection with a heterologous DENV the

DENV specific immunoglobulin IgG forms a complex which gets opsonized and are taken up by the mononuclear cells which in most occasions lead to death of the cells by apoptosis. [52,53] The bone marrow stromal cells also have been shown to be susceptible to the infection with DENV.[54,55]

DENV cell tropism can be understood from the studies utilizing in-situ hybridization, immunohistochemistry (IHC) and by the isolation of the virus utilizing PCR. Studies conducted on children who died of severe dengue infection have led to the identification of the following organ systems getting infected by DENV namely: skin [56] liver [57,58] spleen [59,60] lymph node [61] kidney [62] bone marrow [63] lung [64,65] thymus [66] and brain.[67] Among these, liver has been shown to be commonly involved in DENV infection leading to elevated liver enzyme levels and bleeding diathesis. [68,69]

Endothelial cells also play an important role in the dengue virus infection, though there are contrasting reports, the presence of dengue viral antigen has been shown in vascular endothelium. [70,71] The entry of the dengue virus into endothelial cells is thought to be by pinocytosis rather than FcR mediated entry of the opsonised virus. This clearly shows that there are alternate processes of entry of DENV into cell systems.

There are several studies suggesting vascular damage as one of the critical aspects in the pathogenesis of DHF and DSS [72] especially the pulmonary and abdominal vasculature. It has been shown that structural damage of the vascular cells is linked to NS1 of dengue virus.[73,74]

Viral virulence has also been shown to be responsible for differential dengue infection manifestations, the viral genetic variations have been shown to be associated with the virulence[75,76] epidemics with high incidences of DHF have been shown to be associated with a primary infection by DENV 1 followed by DENV 2 or DENV 3. [3,77] The observations of DHF and DSS on account of secondary infection are also seen in primary infection in a much lesser extent indicating that host factors must have some critical role in the manifestation of severe disease. [78]

As evident from the above, differences in the disease susceptibility could also be attributed to host genetic factors and this has been established by several epidemiological studies. This has been linked to certain host factors like deficiency of G6PD.[79] polymorphism in mannose binding lectin 2,[80] polymorphism in transporters associated with antigen presentation and human platelet antigen in developing DHF. Individuals who develop DHF or DSS but are otherwise healthy could serve as subjects to identify polymorphism single gene defects in developing severe form of dengue viral infection. [81]

In majority of the acute viral infection, antibodies both neutralizing and non-neutralizing levels have been shown to be associated with control, elimination and protection to the host. However, there are studies describing that subsequent to primary infection by one serotype the individuals are shown to exacerbate the infection when they get infected with another serotype for the second time. This has been shown to an affect termed as antibody dependant enhancement (ADE) of the disease. [39] There are several epidemiological studies in support of this, leading to pathogenesis of DHF.[82,83,84] There are reports which state that not all severe diseases are associated with ADE. In some cases, the DHF and DSS have been seen without much load of virus or viral RNA. [31]

Another, hypothesis is that the FcR mediated entry suppresses the anti-viral immune response. A study with rose river virus exhibited viral entry through the FcR pathway, could suppress anti-viral genes and enhance IL-10 production in macrophages.

Antigen presenting cells such as dendritic cells, monocytes and macrophages takes up opsonized virus through FcR mediated phagocytosis, this leads to antigen processing and presentation to effector cells of the immune system, ultimately leading to viral clearance. The integrity of FcγR functioning is critical in the handling of opsonized virus particle. Polymorphisms in the FcγR have been shown to significantly reduce the binding to immunoglobulins and opsonized virus.[14]

The aforementioned review of literature on dengue infection clearly indicates that, this infection is unique in many ways. The infection with DENV can be asymptomatic or could manifest in mild, moderate and severe forms. These events depend upon the viral virulence and host immune responses and importantly the host genetic factors. There are two types' infections possible with DENV- a primary infection which happens when the individual is exposed or infected with DENV for the first time. On the other hand, the secondary infection is in an individual who has had a contact with DENV earlier by any one of the serotypes of DENV 1-4.

Secondary infection leads to a more severe form of dengue and is explained through a process called ADE. This disease has been considered as an endemic in many countries of the world and people are at risk of acquiring this infection. Host genetic factors have been shown to be one of the primary determinants for the predisposition of the individual to the dengue infection. Therefore genetic analysis and studies gather momentum in dengue infection related research.

MATERIALS AND METHODS:

Study Design: This was a case-control study. The study was started after obtaining ethical clearance from the Institutional Ethics Committee. Dengue patients who met the diagnostic and inclusion - exclusion criteria were enrolled in the study after obtaining informed consent (Annexure-II, III). Age and gender matched individuals without history of dengue infection were enrolled from the local population as controls. Standard of care was given to all the patients irrespective of their decision to enroll in the study. Clinical information was collected in a structured proforma (Annexure-I). 3 ml of peripheral blood sample was collected from both cases and controls and used for genetic analysis. FcγRIIa His131Arg gene polymorphism was determined by the PCR-RFLP method. The association of the polymorphism with susceptibility to dengue infection was determined by statistical analysis.

Study Population: Both inpatients **(**IP) and outpatients (OP) were enrolled from the Department of General Medicine, R. L. Jalappa Hospital and Research Centre the teaching Hospital of Sri Devaraj Urs Medical College, Tamaka Kolar. Age and gender matched individuals without history of dengue infection were enrolled from the local population as controls.

Sample Size: Sample size was calculated using Openepi web-tool using data from. [14] The number of samples required to achieve a power of 80 % with two-sided confidence level (1-alpha) of 95% was found to be 72 cases and 72 controls.

Study Period: November 2016- October 2017.

Inclusion Criteria:

1. Both male and female patients of age 18 years and above
2. Patients who meet the clinical criteria for dengue infection.
3. Patients with laboratory diagnosed co-infection.

Exclusion criteria:

Co-morbidities such as - hypertension, diabetes mellitus, hepatitis, chronic kidney disease, respiratory disorders like tuberculosis, chronic obstructive lung diseases, bronchial asthma , lung malignancies and gastrointestinal or any disorder with platelets less than 1,50,000 as an incidental finding.

Diagnostic criteria: According to WHO 2009 guidelines. [88]

1. Continuous fever lasting 2 to 7 days.
2. Haemorrhagic tendencies as shown by positive tourniquet test.
3. Thrombocytopenia (platelet <1,50,000).
4. Evidence of plasma leakage manifested by haematocrit >20%.
5. Confirmation by dengue serology.

GENETIC STUDY METHODS:

Composition of the Reagents and buffers used for DNA isolation and PCR-RFLP technique and the reagents and buffers were prepared with Analytical Grade chemical obtained from Merck, Qualigens, BDH, and Himedia.

A. Reagents for DNA extraction:

1. Tris-HCl 1.0M (pH 8.0)

121.1g of Tris-base was dissolved in 900ml of sterile Milli Q water and the pH was adjusted to 8.0 with hydrochloric acid. The final volume was made up to 1000ml with sterile MilliQ water and sterilized by autoclaving procedure.

2. EDTA di-sodium salt 0.5 M (pH -8.0)

18.6grams of di-sodium EDTA was dissolved in 80 ml of sterile Milli Q water and 15.20grams of sodium hydroxide pellets were added to increase the pH towards 8.0. When sodium hydroxide pellets dissolved completely, 1N HCl was added to bring pH to 8.0 the final volume was made up to 100ml and sterilized by autoclaving.

3. Sodium Chloride 5.0M

29.2g of sodium chloride was added to 80ml of sterile Milli Q water and warmed to assist dissolution. The final volume was made up to 100ml with double distilled water.

4. Sodium Dodecyl Sulphate (SDS 20%)

20g of SDS was dissolved in 80ml of sterile Milli Q water gently by slow mixing to avoid frothing. The solution was kept in water bath at 65^0C to assist complete dissolution. The final volume was made up to 100ml filtered and stored.

5. Proteinase K Solution

Stock Proteinase K solutions were prepared at a final concentration of 20mg/ml stored at -20^0C.

6. Isopropyl Alcohol

Commercially procured and stored under refrigerated condition.

7. Ethanol 80%

80 ml of ethanol was mixed with 20 ml of sterile Milli Q, stored at 4^0C.

8. Erythrocyte lysis buffer (ELB)

72 ml of 1.0 M NH_4CL solution mixed with a 0.5 ml of 1.0 M $KHCO_3$, these components were mixed with sterile Miili Q water to make up final volume of 500 ml.

9. 10 mM Tris- EDTA buffer (pH 8.0)

5 ml of Tris HCl 1mM (pH 8.0) mixed with 1ml of disodium EDTA 0.5 M dissolved in 496 ml of dH_2O to obtain 500ml of buffer to which final concentration of 10 mM Tris and 1 mM EDTA.

B. Reagents for PCR and RFLP

All the PCR fine chemicals were commercially procured from Bangalore Genei Bangalore. 10X DNA polymerase and 10X dNTP mix (10mM), Primers: Primers were obtained from Sigma Aldrich chemicals. Restriction enzyme BstuI were procured from New England Biolabs, USA.

C. Reagents for agarose gel electrophoresis:

1. Tris - Acetate EDTA (TAE) buffer 50X (pH 7.2)

Tris base and disodium EDTA (Tris base 2.0 M, glacial acetic acid 1.0 N and disodium EDTA (0.5 M) were dissolved in sterile Milli Q water. Using glacial acetic acid, pH was

adjusted to 7.2.The final volume was made up to 1000 ml and sterilized by autoclaving, stored at room temperature.

2. Ethidium bromide 10 mg/ml

To 1 ml of sterile Milli Q water, 10 mg of Ethidium bromide was added and mixed well for complete dissolution of the dye. The stock solution was stored in aliquots in air tight containers wrapped with aluminium foil, stored at room temperature.

3. DNA sample loading dye 6X

Ficoll-400 (6%): A synthetic high molecular weight (MW 400 000) polymer of sucrose and epichlorohydrin Ficoll – 400 which is readily soluble in water was made up to 6%. Bromophenol blue (0.12%), Xylene cyanol FF (0.12%), Tris –HCL 12mM (pH 7.5) and 120mM Disodium EDTA. H2O were mixed using sterile Milli Q and stored at Room temperature.

4. Agarose

Commercially available Agarose was prepared in TAE buffer to the required strength

METHODOLOGY:

A. DNA extraction from human blood:

Genomic DNA \was isolated from blood samples by salting out method (85) Blood samples were collected in sterile EDTA vacutainer and were stored at 4^0 C until processing. Genomic DNA was isolated from the peripheral blood by salting out method.

RBCs were removed by repeated osmotic shock treatment. To do this, 4 volumes of erythrocyte lyses buffer (ELB) was added to the blood and vortexed. The tubes were kept on ice for 30 min to facilitate hemolysis. The samples were then centrifuged at 3000 rpm for 10min and the supernatant was discarded. The pellet was re-suspended in 2.5 ml of ELB, vortexed and brought up to 10 ml with additional ELB. The sample was centrifuged at 3000 rpm for 10 min. ELB treatment was repeated two more times. The white pellet was suspended in 1.8 ml of ELB and vortexed again. To this ELB was added to make up the volume up to 5ml. 270µl of 20% SDS and 30 µl of proteinase K (10mg/ml) were added and mixed. Samples were incubated at 37^0C in waterbath overnight.

The next day, 500 µl of 5M NaCl followed by equal volume of isopropyl alcohol were added to the lysate. The tubes were swirled to force the resultant silky and mucoid threads of DNA into a globular mass. DNA precipitate was transferred to 0.5 ml of freshly prepared 80% ethanol and incubated at room temperature for 15 minutes. It was centrifuged at 12000 rpm for 5 minutes .the supernatant obtained was discarded, and this step was repeated for three times to obtain purified form of DNA. The DNA was then air dried and dissolved in 500 µl of Tris-EDTA buffer and then incubated at 65^0C for 30 minutes, and kept on rotator until it is dissolved in TE. The dissolved fraction was refrigerated at 4^0 C for one day and stored at -20^0C until use.

B. DNA quantification:

The quality and quantity of the DNA samples were assessed by spectrophotometric method by using Perkin Elmer Lambda 35 UV- visible spectrophotometer. 50µl of TE buffer was pipetted into quartz cuvette and subjected for auto zero correction. 48µl of TE buffer and 2µl of DNA sample were added in quartz cuvette, the absorbance was measured at 260 and 280 nm. The absorbance at 260nm gives DNA concentration and the ratio between 260/280 gives the purity of DNA. DNA samples with 260/280 absorbance ratio between 1.7-1.9 were considered for PCR procedure. However, DNA samples with absorbance ratio less than 1.7 were subjected for re-precipitation until the desired absorbance is obtained. Then after, DNA sample of expected purity was used for PCR procedure.

C. PCR amplification of FcγRIIa His131Arg gene polymorphism:

Genomic DNA was amplified by Polymerase Chain Reaction on Bio Rad C1000 Touch Thermal Cycler. The primer pairs used are given in Table 2: Before setting up the PCR, dNTP mix, genomic DNA and primers were diluted to their specific concentrations. 25µl of dNTP stock was diluted with 75µl of sterile Milli Q water.10µl of stock primer diluted with 90µl of sterile water respectively. PCR program for amplification is shown in Table 3: Composition of 25 µl reaction mixture shown in Table 4:

Table 2: Primers used for the PCR amplification

PRIMER	SEQUENCE
Forward primer	5' GGA AAA TCC CAG AAA TTC TCG C 3'
Reverse primer	5' TAC CTA TTA CCT GGG ACG TGA G 3'

PCR amplification was confirmed by 2 % Agarose gel electrophoresis. 100 bp DNA molecular weight marker was used to confirm the amplicon size. Electrophoresis was carried out at 100 V, for 1 hour and the gel was visualized in the gel documentation system.

Table 3: Program used for PCR amplification

Step	**Temperature (^{0}C)**	**Time**
Initial Denaturation	95	5 min
Cycle denaturation	95	30 sec
Annealing	59	30 sec
Cycle extension	72	30 sec
Repeat	--	30 X
Final extension	72	5 min

Table 4: Composition of the PCR mix

Component	**Final Concentration**
1X Taq Buffer with $MgCl_2$	1 x (1.5 mM)
dNTPs	0.2 mM
Forward primer	10 pM
Reverse primer	10 pM
Taq DNA Polymerase enzyme	1 U
Template DNA	100 ng
Sterile Milli Q water	As per requirement
Total Reaction Mixture Volume	**25 µl**

D. RFLP analysis of FcγRIIa His131Arg gene polymorphism:

The PCR product was digested with 5U of BstUI restriction enzyme and incubated for 8 hours at 37^0 C. Composition of the rection mixture for restriction Digestion is shown in Table 5. FcγRIIa His131Arg gene polymorphism creates a BstUI restriction site causing cleavage of the 348 bp fragment into 327 bp and 21 bp fragments. The digested products were separated on 3% Agarose gel, stained with ethidium bromide and visualization under UV illumination in a Gel Doc System.

Table 5: Composition of the restriction digestion reaction:

Component	Volume
PCR product	8.5 µl
10 X reaction buffer	1 µl
BstUI Enzyme (10 U/ µl)	0.5 µl
Total Reaction Volume	**10 µl**

E. Statistical analysis:

Statistical analysis was done using the Statistical Packages for Social Sciences software (SPSS, version 22, SPSS Inc., and Chicago, IL, USA). Allele and genotype frequencies of the two groups were compared using relevant contingency tables. Difference between the groups was determined by calculating Dominant and Recessive genetic model using Fisher's exact. P-values <0.05 were considered as statistically significant. The study population was tested for conformity to Hardy-Weinberg equilibrium using the web program by Rodriguez and coworkers. [86]

RESULTS:

1) Patient profile analysis:

This study was carried out at R. L. Jalappa Hospital and Research Centre, a tertiary care teaching hospital of Sri Devaraj Urs Medical College. The study included a total of 144 subjects which was divided into two groups, group 1 included 72 patients suffering from dengue infection fitting into the inclusion criteria and group 2 included 72 normal controls. Among the 72 dengue patients, 49 were males and 23 were females Table 6 and Figure 2, falling in the age range of 18 to 65 years, with the bulk of them falling in the range between 25 to 45 years Table 7 and Figure 3. The clinical presentations included fever alone, fever with pain abdomen, fever with breathlessness, fever with vomiting, fever with myalgia, fever with joint pain. The distribution of these presentations is depicted in Table 8 and Figure 4.

Table 6: Gender profile of the dengue patients

No of patients	Male	Female
72	49	23

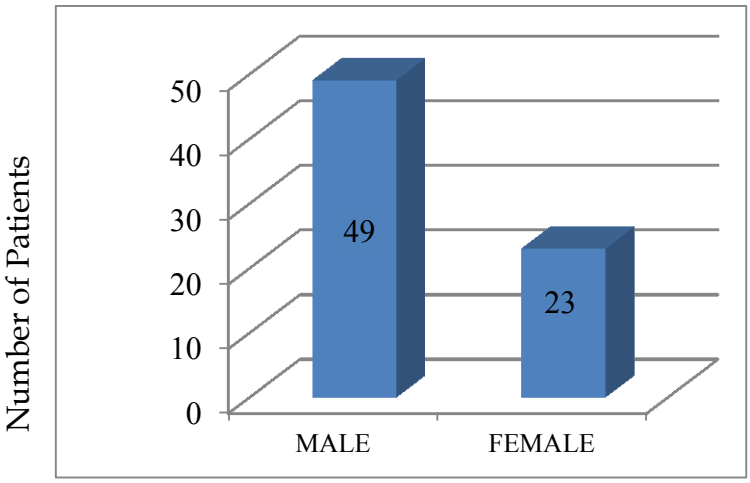

Figure 2: Gender wise distribution of dengue patients

Table 7: Age range of the dengue patients

Age range (yrs)	Male	Female	Total
18-25	10	0	10
26-45	30	21	51
46-65	9	2	11
Total	**49**	**23**	**72**

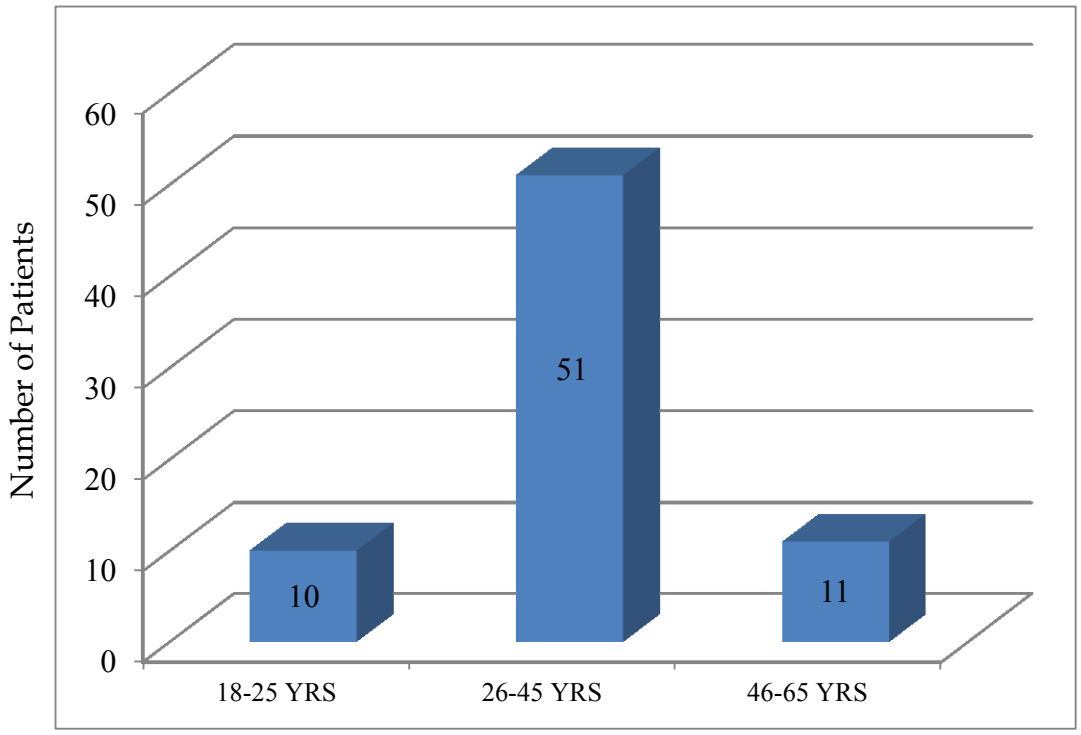

Figure 3: Distribution of patients based on age range

Table 8: Chief complaints in the dengue patients

Chief complaints	Male	Female	Total
Fever alone	8	2	10
Fever + pain abdomen	24	8	32
Fever + breathlessness	8	4	12
Fever + vomiting	7	1	8
Fever + myalgia	1	5	6
Fever + joint pain	1	3	4
Total	**49**	**23**	**72**

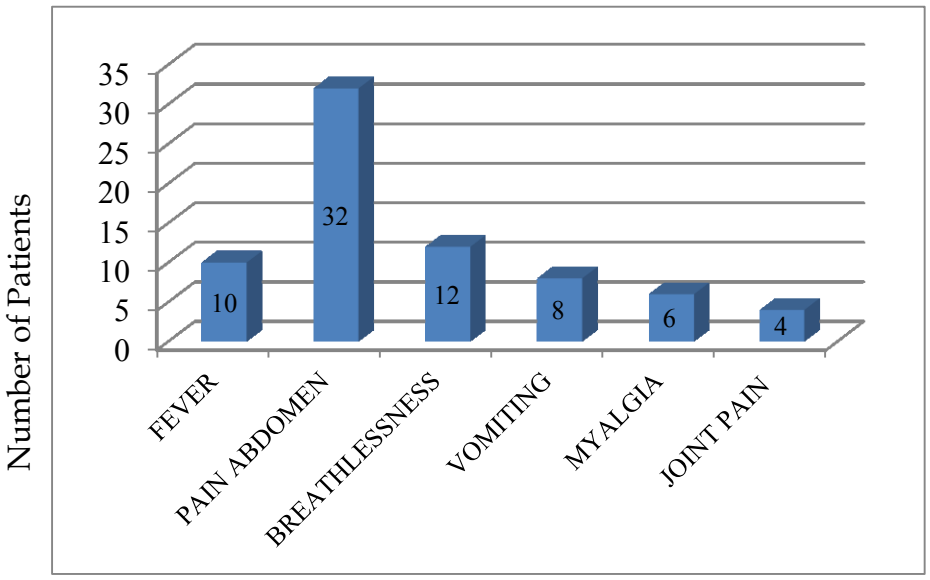

Figure 4: Spectrum of clinical presentation observed in the patients.

The dengue serology was carried out in the Central Diagnostic Laboratory of R. L. Jalappa Hospital and Research Center, Kolar by ELISA method. The results indicated that among the 72 patients studied, NS1Ag group was the highest (n = 55), followed by IgM (n = 10) and IgG (n = 7) groups Table 9 and Figure 5.

Table 9: Serological profile of the dengue patients

Serology	Male	Female	Total
NS1Ag	35	20	55
IgM	8	2	10
IgG	6	1	7
Total	**49**	**23**	**72**

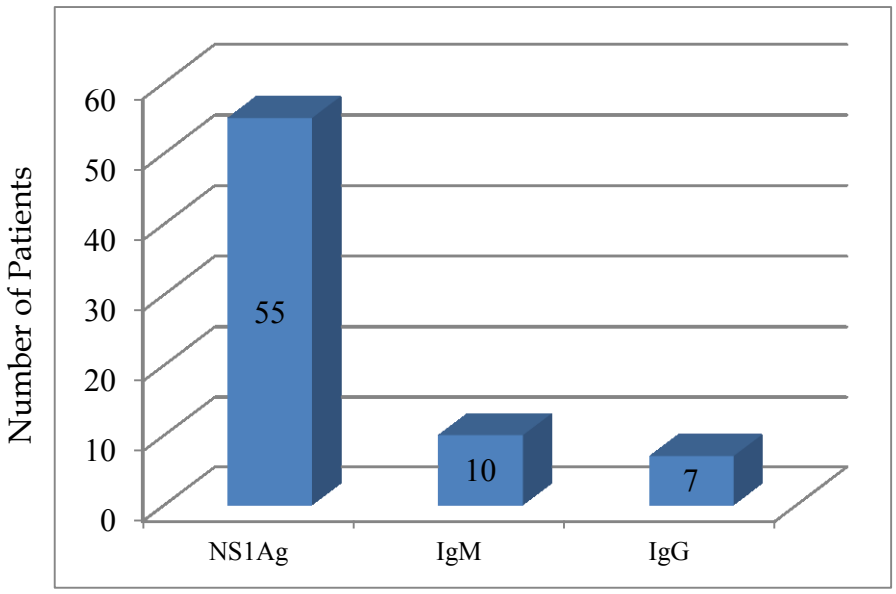

Figure 5: Profile of dengue serology in the patients.

Genetic analysis:

Genomic DNA isolated from both the patient and control groups and the genotype of FcγRIIa His131Arg gene polymorphism was analyzed by PCR-RFLP method. The details of the patient wise and control wise genotypes are represented in the Master chart (Annexure -IV) The genotype was interpreted on the basis of the PCR-RFLP band pattern on agarose gel electrophoresis, a representative sample of which is depicted in Figure 6. The RFLP band pattern is given in Table 10. The homozygous His/His genotype was represented by bands with the size of 327 and 21 bp, the homozygous Arg/Arg genotype was represented with a band of size 348 bp and the heterozygous His/Arg was represented by bands of sizes 348, 327 and 21 bp.

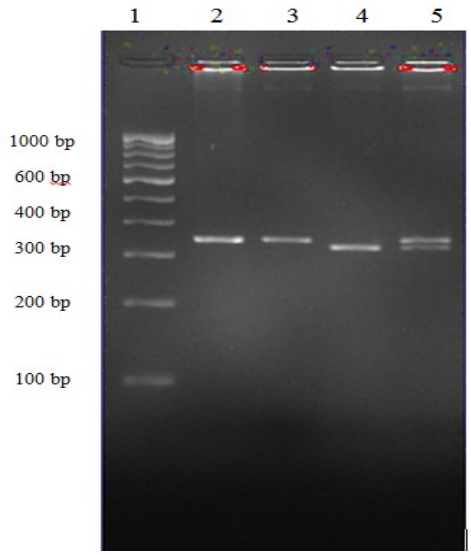

Figure 6: Representative Agarose gel electrophoresis image showing PCR amplicon and RFLP pattern for FcγRIIa His131Arg gene polymorphism. Lane 1, 100bp DNA ladder; Lane 2, PCR amplicon (348 bp); Lane 3: His/His genotype (348 bp); Lane 4: Arg/Arg genotype (327 + 21 bp); Lane 5: His/Arg genotype (348 + 327 + 21 bp).

Table 10: RFLP band pattern of FcγRIIa His131Arg gene polymorphism

PCR product (bp)	Homozygous condition		Heterozygous condition
	His/His (bp)	Arg/Arg (bp)	His/Arg (bp)
348	327 + 21	348	348 + 327 + 21

Frequency of FcγRIIa His131Arg alleles in the study groups:

The frequencies of both the alleles among the patients and controls are summarized in Table 11. The frequency of His allele was 54.9 % in the patient group and 36.1% in the control group. The frequency of the His allele was therefore 0.6 times lower in the control group than in the patient group. The frequency of Arg allele was 45.1% in the patient group and 63.9 % in the control group. The frequency of the Arg allele was therefore 1.4 times higher in the control group than in the patient group. The difference in the frequency of the two alleles in the patient and control groups was compared by Chi-square test to check if it was statistically significant. The P-value was 0.002. The P-value is less than 0.05, therefore, the difference in the frequency of the alleles between the patient and control groups is statistically significant. The odds ratio for the difference was 0.46 (0.95 CI: 0.29 - 0.75). Since the odds ratio is less than 1, Arg allele is a protective factor.

Table 11: Allele frequency of FcγRIIa His131Arg gene polymorphism in the patient and control groups

Allele	Cases (n = 72)	Control (n = 72)	P #
His	79 (54.9 %)	52 (36.1 %)	0.002 *
Arg	65 (45.1 %)	92 (63.9 %)	OR: 0.46 (0.95 CI: 0.29 – 0.75)

* Significant (P < 0.05)

Chi-squared test–Fisher's exact

OR: Odds ratio; CI: Confidence interval

Frequency of FcγRIIa His131Arg genotypes in the study groups:

The frequencies of the three genotypes in the patient and control groups is summarized Table 12. The frequency of His/His genotype was 30.5 % in the patient group and 12.5 % in the control group. Thus, the frequency was 0.4 times lower in the control group than the patient group. The frequency of His/Arg genotype was 48.7 % in the patient group and 47.2 % in the control group. Thus, the frequency was similar in both the control group than the patient group. The frequency of Arg/Arg genotype was 40.2 % in the patient group and 20.8 % in the control group. Thus, the frequency was 1.9 times higher in the control group than the patient group. The difference in the frequency of the three genotypes in the patient and control groups was compared by Chi-square test to check if it was statistically significant. The P-value was 0.007 The P-value is less than 0.05, therefore, the difference in the frequency of the genotypes between the patient and control groups is statistically significant.

Table 12: Genotype frequency of FcγRIIa His131Arg gene polymorphism in the patient and control groups

Genotype	Cases (n = 72)	Control (n = 72)	P #
His/His	22 (30.5%)	9 (12.5%)	0.007 *
His/Arg	35 (48.7%)	34 (47.2%)	
Arg/Arg	15 (20.8%)	29 (40.2%)	

* Significant ($P < 0.05$)

Chi-squared test – Fisher's exact

DISCUSSION:

The aim of this study was to determine the genetic link between antibody-mediated immune response and susceptibility to dengue infection. This was accomplished by evaluating the association between FcγRIIa His131Arg gene polymorphism and dengue fever. His131Arg was genotyped in 72 dengue patients and 72 healthy control and the allele frequencies were compared for differences. The major findings of this study are: (i) the frequency of His131 allele was comparatively higher in patients, (ii) the frequency of Arg131 allele was comparatively higher in healthy controls, and (iii) the differences in the distribution of His131 and Arg131 alleles was statistically significant. These results together indicate that FcγRIIa His131Arg gene polymorphism is associated with the susceptibility to dengue fever with Arg131 being a protective factor.

Prior to this study, four studies were carried out on the association of FcγRIIa is131Arg gene polymorphism with the susceptibility to dengue in various parts of the worlds. The results of these studies are summarised in Table no 13. Overall, all the four studies [10,14,15,87] found association between FcγRIIa His131Arg gene polymorphism and susceptibility to dengue fever. However, the direction of the association was not same in all the studies. The studies from Pakistan [14] Cuba [10] and Vietnam [87] found that the Arg131 allele was a risk factor in the susceptibility to dengue fever. In contrast, the studies from Mexico [15] found that the Arg131 allele to be a protective factor in the susceptibility to dengue fever. The result of this study is in agreement with protective role found in the studies from Mexico. [15]

Table 13: Comparison of genotypic and allelic frequencies of FcγRIIa His131Arg gene polymorphism across different studies

Population	Arg/Arg genotype frequency (%)	Arg Allele frequency (%)	OR with 95% CI for His/His and His/Arg vs. Arg/Arg	P value	References
Pakistani (n=110)	23.63	52.27	3.90 (1.13-13.37)	0.024	Mohsin et al. [14]
Cuban (n=139)	28.77	52.51	2.36 (0.79-7.03)	0.119	Garcia et al.[10]
Mexican (n=248)	23	49.2	0.45 (0.21-0.96)	0.026	Noecker et al.[15]
Vietnamese (n=540)	8.1	27.77	1.74 (0.93-3.25)	0.075	Loke et al. [87]
This study (n=72)	15	65	0.39 (0.19–0.82)	0.018	NA

(NA: not applicable)

The two alleles of FcγRIIa differ in their strength of binding to the Fc fragment of neutralizing antibodies. His131 allele shows weaker binding while Arg131 shows stronger binding. As a result, Arg131would be expected to support stronger antibody-mediated immunity in the clearance of the dengue virus. The results of this study are in agreement

with the functional implication of the Arg131allele wherein the allele was found to be a protective factor i.e., present at a higher frequency in the healthy controls than in dengue patients.

FcγRIIa His131Arg gene polymorphism belongs to a class of genetic variations called as single nucleotide polymorphism. Genetic variations are classified into two groups as mutations and polymorphisms. The two groups differ mainly in their impact on the protein coded by the corresponding gene. Mutation results in massive alteration in the function of the encoded protein. In contrast, polymorphism results in a marginal alteration in the function of the protein. The mutation of a gene causes massive molecular and physiological aberrations leading to genetic disorders like phenylketonuria, cystic fibrosis, sickle cell anaemia etc. In contrast, the minor alterations that arise due to polymorphism do not cause any disease. However, a polymorphism can work summatively along with other factors to manifest multifactorial diseases like infection, cancer, cardiometabolic syndromes etc. The frequency of a mutation in the general population is very low (< 1%) since they cause genetic disorder and reduce the reproductive fitness of the carrier. In contrast, the frequency of the polymorphism is relatively higher in the general population since they directly do not cause any major disease. In this study, the frequency of His131 was 36.1 % and that of Arg131 was 63.9 % in the healthy controls.

Current research across the world in the interdisciplinary areas of genetics and medicine is focused on the role of gene polymorphisms in multifactorial diseases. The purpose of these studies are to understand the molecular etiology of diseases which would provide rationale on the host genetic variations, drug development and personalized medicine. The results of this study adds to the understanding of the molecular etiology of

dengue fever. The observation of the presence of higher frequency of Arg131 allele in the healthy controls reported in this study, provides information on the protective capabilities of the Arg allele from acquiring dengue infection. Thus, the potential application of the outcomes of this study are directly suggestive of the need for inclusion of FcγRIIa gene in the molecular screening studies of populations at risk for dengue infection.

SUMMARY:

Dengue is a mosquito borne infection caused by flavivirus belonging to four well defined serotypes DENV 1-4.

Dengue infection presents with a spectrum of clinically distinct features which include asymptomatic infection to potentially life-threatening forms. 5-10% progress to dengue hemorrhagic fever (DHF) and Dengue Shock Syndrome (DSS).

Both host immunal and genetic factors apart from viral genetic determinants have been shown to play critical role in the differential clinical presentation in the dengue infected patients.

Dengue viruses have been shown to be neutralized by the cells expressing Fcγ receptors. They are important in the clearance of DENV opsonized with neutralizing level of antibodies.

However, cross reactive or subneutralizing levels of antibodies could result in severe form of dengue due to a process termed as Antibody Dependent Enhancement (ADE) of the infection.

The host genetic back ground with varying single nucleotide polymorphism has been shown to be associated with susceptibility to infection.

A single nucleotide polymorphism in the FcγRIIa has been implicated in the altered affinity of the receptor for different subclasses of immunoglobulin G which is key component in the determining the susceptibility of protection from severe clinical dengue manifestations.

Thus, this was a case control study, designed to investigate the association of the FcγRIIa polymorphism in the clinical outcome of dengue infection in an endemic setting for dengue infection with 72 patients presenting with of dengue infection.

The DNA from the patients and healthy controls were isolated and subjected to PCR-RFLP studies.

The results of the study revealed that the frequency of Arg-Arg genotype of FcγRIIa was 40.2% which provided protection from Dengue Infection while the frequency of His / His genotype of FcγRIIa was 30.5% which exhibited susceptibility to the infection and these observations were statistically significant.

This study demonstrates that FcγRIIa His131Arg polymorphism is associated with the susceptibility to dengue fever. Thus underlying the importance of antibody mediated immunity in the etiology of dengue fever.

CONCLUSION:

The results of the current study shows that FcγRIIa His131Arg polymorphism is associated with the susceptibility to dengue fever, reiterating the importance of antibody mediated immunity in the etiology of dengue fever.

The observation of the presence of higher frequency of Arg131 allele in the healthy controls reported in this study, provides information on the protective capabilities of the Arg allele from acquiring dengue infection.

The potential application of the outcomes of this study are directly suggestive of the need for inclusion of FcγRIIa gene in the molecular screening studies of populations at risk for dengue infection.

REFERENCES:

1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CI. *et al.* The global distribution and burden of dengue. Nature 2013 Apr; 496(7446):504 -7.
2. Guha-Sapir D, Schimmer B. Dengue fever: new paradigms for a changing epidemiology. Emerg themes Epidemiol 2005 Dec;2(1):1.
3. Halstad SB. Dengue. Lancet 2007;370: 1644-52.
4. Mutheneni SR, Morse AP, Caminade C, Upadhyayula SM. Dengue burden in India: recent trends and importance of climatic parameters. Emerging microbes & infections 2017 Aug;6(8):e70.
5. Biswas A, Pangtey G, Devgan V, Singla P, Murthy P, Dhariwal AC, *et al.* Indian National Guidelines for clinical management of Dengue fever. J Indian Med Assoc 2015;113(12):196-206.
6. Pandya G. Prevalence of Dengue infection in India. Def Sci J 1982;32(4):359-70.
7. Diamond MS, Pierson TC. Molecular insight into dengue virus pathogenesis and its implications for disease control. Cell 2015 Jul 30;162(3):488-92.
8. Bielefeldt-Ohmann H, Meyer M, Fitzpatrick DR, Mackenzie JS. Dengue virus binding to human leukocyte cell lines: receptor usage differs between cell types and virus strains. Virus research 2001 Jan 1;73(1):81-9.
9. van de Winkel JG, Capel PJ. Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. Immunology today 1993 May 1;14(5):215-21.
10. Garcia G, Sierra B, Perez AB, Aguirre E, Rosado I, Gonzalez N, *et al*. Asymptomatic Dengue Infection in a Cuban population confirms the Protective Role of the RR variant of the Fc gamma RIIa Polymorphism. Am J Trop Med Hyg 2010;82:1153-6.
11. Winkel JG and Capel PJ. Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. Immunol today 1993;14:215-21.

12. Clark MR, Stuart SG, Kim berly RP, Ory PA, Goldstein IM. A single amino acid distinguishes the high-responder from the low-responder form of Fe receptor II on human monocytes. Eur J Immunol 1991;21:1911-6.
13. Warmerdam PA, Van de Winkel JG, Vlug A, Westerdaal NA, Capel PJ. A single amino acid in the second Ig-like domain of the human Fc gamma receptor II is critical for human IgG2 binding. J Immunol 1991;147:1338-43.
14. Mohsin SN, Mahmood S, Amar A, Ghafoor F, Raza SM, Saleem M. Association of FcγRIIa polymorphism with clinical outcome of dengue infection: First insight from Pakistan. Am J Trop Med Hyg 2015 Oct 7;93(4):691-6.
15. Noecker CA, Larios IYA, Hernandez MG, Castaneda JR, and Vega RAM. Contrasting associations of polymorphisms in FcyRIIa and DC-SIGN with the clinical presentation of dengue infections in a Mexican population . Acta Trop 2014;138:15-22.
16. Flipse J, Wilschut J, Smit JM. Molecular mechanisms involved in antibody-dependent enhancement of dengue virus infection in humans. Traffic 2013 Jan;14(1):25-35.
17. Halstead, S B. Pathogenesis of dengue: Challenges to molecular biology. Science 1988; 239(4839): 476-81.
18. Kurane I. Dengue hemorrhagic fever with special emphasis on immune pathogenesis. Comparitive Immunology. Microbiol Infect Dis 2007;30:329-40.
19. Lam SK, Burke D, Gubler D. Call for a World Dengue Day. Lancet 2012;379:411-2.
20. Brady OJ, Gething PW, Bhatt S, *et al*. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLOS Negl Trop Dis 2012;6:e1760.
21. Gulber DJ. Dengue and dengue hemorrhagic fever. Clin Microbial Rev1998;11(3):480-96.
22. Dash AP, Bhatia R, Kalra NL. Dengue in South-East Asia: an appraisal of case management and vector control.Dengue Bull 2012;36:1-3.

23. Dengue guidelines for diagnosis, treatment, prevention and control. Geneva World Health Organization, Special Programme for Research and Training in Tropical Diseases (TDR); 2009.
24. Dengue Case Management - Report of an informal expert consultation Colombo, Sri Lanka, World Health Organization, South East Asian Region August 2013.
25. Clinical guidelines for management of DF/DHF/DSS. Directorate of National Vector Borne Diseases control Programme, Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India; 2007.
26. Dutta AK, Biswas A, Baruah K, Dhariwal AC. National Guidelines for diagnosis and management of Dengue Fever/Dengue Hemorrhagic fever and Dengue Shock Syndrome. J Indian Med Assoc 2011;109(1):30-5.
27. Bandyopadhyay S, Lum LC, Kroger A. Classifying dengue: a review of the difficulties in using the WHO case classification for dengue hemorrhagic fever. Trop Med Int Health 2006;11(8):1238-55.
28. Guzman MG, Kouri G. Dengue: an update. Lancet infect Dis 2002;2(1):33-42.
29. Guzman MG, Kouri G. Advances in dengue diagnosis. Clin Diagn Lab Immunol 1996;3: 621-7.
30. Young PR, Hilditch PA, Blechly C, *et al*. An antigen capture and enzyme linked immunosorbent essay reveals high levels of dengue virus protein NSI in the sera of infected patients. J Clin Microbol 2000;38(3):1053-7.
31. Libraty DH, Young PR, Pickering D, *et al*. High circulating levels of the dengue virus nonstructural protein NS 1 early in dengue illness correlate with the development of Dengue hemorrhagic fever. J infect Dis 2002;186(8):1165-8.
32. Seema, Jain SK. Molecular mechanism of pathogenesis of dengue virus: entry and fusion with target cell. Indian J Clin Biochem 2005 Jul 1;20(2):92-103.

33. Cardosa MJ, Wang SM, Sum MS, *et al*. Antibodies against PrN protein distinguish between previous infection with dengue and Japanese Encephalitis virus. BMC Microbial 2002;2(9)2-9.
34. World Health Organization. Prevention and control of dengue and dengue hemorrhagic fever: comprehensive guidelines. WHO Regional publication, SEARO. No 29:1999.
35. Perich MU, Davila G, Turner A, *et al*. Behavior of resting Aades aegypti (culicidae: diptera) and its relation to ultra-low volume aldulticide efficacy in Panamacity, panama. J. Med entomol 2000;37(4):541-6.
36. Malavige G.N, Fernando S, Fernando DJ, Seneviratne SL. Dengue viral infections. Postgrad Med J 2004;80(948):588-601.
37. Bancraft T.L. On the etiology of dengue fever. Aust Med Gaz 1906;25:17-8.
38. Coffey LL, Mertens E, Brehin AC, Fernandez-Garcia MD, Amara A, Despres P, Sakuntabhai A. Human genetic determinants of dengue virus susceptibility. Microbes and Infection 2009;11(2):143-56.
39. Halstead SB, Nimmannitya S, Cohen SN. Observations related to pathogenesis of dengue hemorrhagic fever. Relation of disease severity to antibody response and virus recovered. Yale J Biol Med 1970;42:311-28.
40. Burke DS, Nisalak A, Johnson DE, Scott RM. A prospective study of dengue infections in Bangkok. Am J Trop Med Hyg 1988;38:172-80.
41. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, et al . Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis 2000;181:2-9.
42. Martina BE, Koraka P, Osterhaus AD. Dengue virus pathogenesis: an integrated view. Clin Microbiol Rev 2009 Oct 1;22(4):564-81.

43. Wu SJ, Grouard-Vogel G, Sun W, Mascola JR, Brachtel E, Putvatana R, Louder MK, Filgueira L, Marovich MA, Wong HK, Blauvelt A. Human skin Langerhans cells are targets of dengue virus infection. Nature 2000 Jul;6(7):816.

44. Limon-Flores AY, Perez-Tapia M, Estrada-Garcia I, Vaughan G, Escobar-Gutierrez A, Calderon-Amador J, et al. Dengue virus inoculation to human skin explants: an effective approach to assess in situ the early infection and the effects on cutaneous dendritic cells. Int J Exp Pathol 2005 Oct;86(5):323-34.

45. Durbin AP, Vargas MJ, Wanionek K, Hammond SN, Gordon A, Rocha C, et al. Phenotyping of peripheral blood mononuclear cells during acute dengue illness demonstrates infection and increased activation of monocytes in severe cases compared to classic dengue fever. Virology 2008 Jul 5;376(2):429-35.

46. Ho LJ, Shaio MF, Chang DM, Liao CL, Lai JH. Infection of human dendritic cells by dengue virus activates and primes T cells towards Th-like phenotype producing both Th1 and Th2 cytokines. Immunol Inv 2004 Jan 1;33(4):423-37.

47. Ho LJ, Wang JJ, Shaio MF, Kao CL, Chang DM, Han SW, et al. Infection of human dendritic cells by dengue virus causes cell maturation and cytokine production. J Immunol 2001 Feb 1;166(3):1499-506.

48. Blackley S, Kou Z, Chen H, Quinn M, Rose RC, Schlesinger JJ, et al. Primary human splenic macrophages, but not T or B cells, are the principal target cells for dengue virus infection in vitro. J Virol. 2007 Dec 15;81(24):133-4.

49. Huerre MR, Lan NT, Marianneau P, Hue NB, Khun H, Hung NT,et al. Liver histopathology and biological correlates in five cases of fatal dengue fever in Vietnamese children. Virchows Archiv 2001 Feb 1;438(2):107-15.

50. Jessie K, Fong MY, Devi S, Lam SK, Wong KT. Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and in situ hybridization. J Infect Dis 2004 Apr 15;189(8):1411-8.
51. Marchette NJ, Halstead SB, Falkler Jr WA, Stenhouse A, Nash D. Studies on the pathogenesis of dengue infection in monkeys III. Sequential distribution of virus in primary and heterologous infections. J Infect Dis 1973 Jul 1;128(1):23-30.
52. Espina LM, Valero NJ, Hernandez JM, Mosquera JA. Increased apoptosis and expression of tumor necrosis factor-α caused by infection of cultured human monocytes with dengue virus. Am J Trop Med Hyg 2003 Jan 1;68(1):48-53.
53. Palmer DR, Sun P, Celluzzi C, Bisbing J, Pang S, Sun W, Marovich MA, Burgess T. Differential effects of dengue virus on infected and bystander dendritic cells. J Virol 2005 Feb 15;79(4):2432-9.
54. Kyle JL, Beatty PR, Harris E. Dengue virus infects macrophages and dendritic cells in a mouse model of infection. J Infect Dis 2007 Jun 15;195(12):1808-17.
55. Nakao S, Lai CJ, Young NS. Dengue virus, a flavivirus, propagates in human bone marrow progenitors and hematopoietic cell lines. Blood 1989 Sep 1;74(4):1235-40.
56. Kangwanpong D, Bhamarapravati N, Lucia HL. Diagnosing dengue virus infection in archived autopsy tissues by means of the in situ PCR method: a case report. J Clin Virol 1995 Feb 1;3(2):165-72.
57. Couvelard A, Marianneau P, Bedel C, Drouet MT, Vachon F, Hénin D, et al. Report of a fatal case of dengue infection with hepatitis: demonstration of dengue antigens in hepatocytes and liver apoptosis. Hum pathol 1999 Sep 1;30(9):1106-10.
58. Huerre MR, Lan NT, Marianneau P, Hue NB, Khun H, Hung NT, et al. Liver histopathology and biological correlates in five cases of fatal dengue fever in Vietnamese children. Virchows Archiv 2001 Feb 1;438(2):107-15.

59. Balsitis SJ, Coloma J, Castro G, Alava A, Flores D, McKerrow JH,et al. Tropism of dengue virus in mice and humans defined by viral nonstructural protein 3-specific immunostaining. The Am J Trop Med Hyg 2009 Mar 1;80(3):416-24.

60. Basílio-de-Oliveira CA, Aguiar GR, Baldanza MS, Barth OM, Eyer-Silva WA, Paes MV. Pathologic study of a fatal case of dengue-3 virus infection in Rio de Janeiro, Brazil. Braz J Infect Dis 2005 Aug;9(4):341-7.

61. Jessie K, Fong MY, Devi S, Lam SK, Wong KT. Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and in situ hybridization. J Infect Dis 2004 Apr 15;189(8):1411-8.

62. Guzmán MG, Alvarez M, Rodriguez R, Rosario D, Vázquez S, Valdés L,et al. Fatal dengue hemorrhagic fever in Cuba, 1997. Int J Infect Dis 1999 Mar 1;3(3):130-5.

63. Nisalak A, Halstead SB, Singharaj P, Udomsakdi S, Nye SW, Vinijchaikul K. Observations related to pathogenesis of dengue hemorrhagic fever: Virologic studies of fatal disease. Yale J Biol. Med 1970;42**:**293-310.

64. Limonta D, Capó V, Torres G, Pérez AB, Guzmán MG. Apoptosis in tissues from fatal dengue shock syndrome. J Clin Virol 2007 Sep 1;40(1):50-4.

65. Miagostovich MP, Ramos RG, Nicol AF, Nogueira RM, Cuzzi-Maya T, Oliveira AV, et al. Retrospective study on dengue fatal cases. Clin Neuro Pathol 1997;16(4):204-8.

66. Killen H, O'Sullivan MA. Detection of dengue virus by in situ hybridization. J Virol Methods 1993;41**:**135-46.

67. Miagostovich MP, Ramos RG, Nicol AF, Nogueira RM. Retrospective study on dengue hemorrhagic cases. Clin Pathol 1997;16(4):20-8.

68. Kyle JL, Beatty PR, Harris E. Dengue virus infects macrophages and dendritic cells in a mouse model of infection. J Infect Dis 2007 Jun 15;195(12):1808-17.

69. Wichmann O, Gascon J, Schunk M, Puente S, Siikamaki H, Gj I, Lopez-Velez R, Clerinx J, Peyerl-Hoffmann G, Sundøy A, Genton B. Severe dengue virus infection in travelers: risk factors and laboratory indicators. J Infect Dis 2007 Apr 15;195(8):1089-96.

70. Sahaphong, S, Riengrojpitak S, Bhamarapravati N, Chirachariyavej T. Electron microscopic study of the vascular endothelial cell in dengue hemorrhagic fever. S Asian J Trop Med 1980; 11:194-204.

71. Jessie K, Fong MY, Devi S, Lam SK, Wong KT. Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and in situ hybridization. J Infect Dis 2004 Apr 15;189(8):14-8.

72. Murgue B, Cassar O, Deparis X. Plasma concentrations of sVCAM-1 and severity of dengue infections. J Med Virol 2001 Sep;65(1):97-104.

73. Limonta D, Capó V, Torres G, Pérez AB, Guzmán MG. Apoptosis in tissues from fatal dengue shock syndrome. J Clin Virol 2007 Sep 1;40(1):50-4.

74. Avirutnan P, Zhang L, Punyadee N, Manuyakorn A, Puttikhunt C, Kasinrerk W,et al. Secreted NS1 of dengue virus attaches to the surface of cells via interactions with heparan sulfate and chondroitin sulfate E. PLoS 2007 Nov 30;3(11):e183.

75. Cologna R, Rico-Hesse R. American genotype structures decrease dengue virus output from human monocytes and dendritic cells. J virol 2003 Apr 1;77(7):3929-38.

76. Leitmeyer KC, Vaughn DW, Watts DM, Salas R, Villalobos I, Ramos C, et al. Dengue virus structural differences that correlate with pathogenesis. J Virol 1999 Jun 1;73(6):4738-47.

77. Guzman MG, Kouri G. Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. J Clin Virol 2003 May 1;27(1):1-3.

78. Casadevall A, Pirofski LA. Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity. Infect Imm1999 Aug 1;67(8):3703-13.

79. Nkhoma ET, Poole C, Vannappagari V, Hall SA, Beutler E. The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. Blood cell Mol Dis 2009 May 1;42(3):267-78.

80. Thiel S, Frederiksen PD, Jensenius JC. Clinical manifestations of mannan-binding lectin deficiency. Mol Immunol 2006 Jan 1;43(1-2):86-96.

81. Soundravally R, Hoti SL. Immunopathogenesis of dengue hemorrhagic fever and shock syndrome: role of TAP and HPA gene polymorphism. Hum Immunol 2007 Dec 1;68(12):973-9.

82. Burke DS, Kliks S. Antibody-dependent enhancement in dengue virus infections. J Infect Dis 2006 Feb 15;193(4):601-3.

83. Guzmán MG, Kouri G, Bravo J, Valdes L, Susana V, Halstead SB. Effect of age on outcome of secondary dengue infections. Int J Infect Dis 2002 Jun 1;6(2):118-24.

84. Guzmán MG, Kouri G, Valdes L, Bravo J, Alvarez M, Vazques S, Delgado I, Halstead SB. Epidemiologic studies on Dengue in Santiago de Cuba, 1997. Am J Epidemiol 2000 Nov 1;152(9):793-9.

85. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells.Nucleic Acids Res 1988; 16(3):1215-6.

86. Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am J Epidemiol 2009;169:505-14.

87. Loke H, Bethel D, Phuong Cxt, Day N, White N, Farrar J, et al. Susceptibility to dengue hemorrhagic fever in Veitanam : evidence of an association with variation in the Vitamin D receptor IIa genes. Am J Trop Hyg 2002;67:102-6.

88. World Health Organization, Special Programme for Research, Training in Tropical Disease, World Health Organization. Department of Control of Neglected Tropical Diseases, World Health Organization. Epidemic, Pandemic Alert. Dengue: guidelines for diagnosis, treatment, prevention and control. World Health Organization;2009.

ANNEXURE-I

PROFORMA

TITLE: "STUDIES ON THE ASSOCIATION OF FcγRIIa His131Arg POLYMORPHISM WITH DENGUE INFECTION: A HOSPITAL BASED CASE CONTROL STUDY"

Name: Age:

Sex: Address:

Date of Admission: Date of Discharge:

History:

Bleeding Manifestation:

Polyserositis:

Co-morbidities:

Co-infections:

Treatment:

Blood transfusions:

Laboratory findings:

Platelet count:

Hematocrit:

Dengue serology:

Did the patient improve:

Death:

ANNEXURE-II:

INFORMED CONSENT FORM

TITLE: "STUDIES ON THE ASSOCIATION OF FcγRIIa His131Arg POLYMORPHISM WITH DENGUE INFECTION: A HOSPITAL BASED CASE CONTROL STUDY"

PATIENTS NAME: HOSPITAL NUMBER: AGE:

If you agree to participate in the study we will collect information (as per proforma) from you or a person responsible for you or both. We will collect the treatment and relevant details from your hospital record. This information collected will be used for only dissertation and publication. This study has been reviewed by the institutional ethical committee. The care you will get will not change if you don't wish to participate. You are required to sign/ provide thumb impression only if you voluntarily agree to participate in this study.

I understand that I remain free to withdraw from the study at any time and this will not change my future care. I have read or have been read to me and understood the purpose of the study, the procedure that will be used, the risk and benefits associated with my involvement in the study and the nature of information that will be collected and disclosed during the study. I have had the opportunity to ask my questions regarding various aspects of the study and my questions are answered to my satisfaction. I, the undersigned agree to participate in this study and authorize the collection and disclosure of my personal information for dissertation.

Subject name: (Parents / Guardians name)

DATE: **SIGNATURE /THUMB**

ANNEXURE-III:

PATIENT INFORMATION SHEET

TITLE: "STUDIES ON THE ASSOCIATION OF FcγRIIa His131Arg POLYMORPHISM WITH DENGUE INFECTION: A HOSPITAL BASED CASE CONTROL STUDY"

Principal Investigator: Dr Shaama Ghungroo

Study site: R.L Jalappa Hospital and Research Center attached to Sri Devaraj Urs Medical College, Tamaka, Kolar.

Aim of the study: The aim of the study is to assess the association between risk of FcγRIIa His131Arg polymorphism with Dengue infection. Dengue is a type of fever caused due to a virus that is carried by mosquitoes.

Voluntary Participation: Your participation in this study is entirely voluntary. There is no compulsion to participate in this study. You will be no way affected if you do not wish to participate in the study. You are required to sign only if you voluntarily agree to participate in this study. Further you are at a liberty to withdraw from the study at any time. We assured that your withdrawal will not affect your treatment by the concerned physician in any way.

Procedure: We will take 3ml of blood from your arm with a syringe. This blood will be tested forFcγRIIaHis131Arg polymorphism.

Confidentiality: All information collected from you will be strictly confidential and will not be disclosed to anyone except if it is required by the law. This information collected will be used only for research. This information will not reveal your identity.

We would not compel you any time during this process; also we would greatly appreciate your cooperation to the study. We would like to get your consent to participate in the study. For any information you are free to contact investigator. This study has been approved by

the InstitutionalEthics Committee & has been started only after their formal approval. The sample collected will be stored in the institute and I request you to permit us to store and use this sample for any future study.

Subject name: (Parents / Guardians name)

DATE: **SIGNATURE /THUMB**

ANNEXURE-IV:

MASTER CHART

Sl. No	Case	Sex	Age	Chief Complaint	Serology	Genotype	Control	Sex	Genotype
1.	DS1	M	18	Pain abdomen	NS1Ag	His/His	C1	M	Arg/His
2.	DS2	M	25	Pain abdomen	NS1Ag	Arg/His	C2	M	Arg/His
3.	DS3	M	28	Pain abdomen	NS1Ag	His/His	C3	M	His/His
4.	DS4	F	29	Pain abdomen	NS1Ag	Arg/Arg	C4	F	Arg/Arg
5.	DS5	F	33	Myalgia	NS1Ag	Arg/Arg	C5	F	His/His
6.	DS6	M	22	Pain abdomen	NS1Ag	Arg/His	C6	M	Arg/Arg
7.	DS7	M	31	Pain abdomen	MS1Ag	His/His	C7	M	Arg/Arg
8.	DS8	M	27	Fever	NS1Ag	Arg/His	C8	M	Arg/Arg
9.	DS9	F	34	Pain abdomen	NS1Ag	Arg/Arg	C9	F	Arg/Arg
10.	DS10	F	34	Breathlessness	NS1Ag	Arg/His	C10	F	Arg/Arg
11.	DS11	F	48	Joint pain	NS1Ag	Arg/His	C11	F	His/His
12.	DS12	M	40	Fever	NS1Ag	His/His	C12	M	Arg/Arg
13.	DS13	M	35	Pain abdomen	NS1Ag	His/His	C13	M	Arg/Arg
14.	DS14	F	26	Pain abdomen	NS1Ag	Arg/His	C14	F	Arg/Arg
15.	DS15	F	32	Pain abdomen	NS1Ag	Arg/His	C15	F	Arg/His
16.	DS16	M	65	Joint pain	IgG	Arg/His	C16	M	Arg/His
17.	DS17	M	52	Vomiting	IgG	Arg/Arg	C17	M	Arg/His
18.	DS18	M	58	Vomiting	IgG	Arg/His	C18	M	Arg/Arg
19.	DS19	F	45	Joint pain	IgM	Arg/Arg	C19	F	Arg/His
20.	DS20	F	27	Pain abdomen	NS1Ag	His/His	C20	F	Arg/His
21.	DS21	M	44	Breathlessness	IgM	His/His	C21	M	Arg/His
22.	DS22	M	51	Vomiting	IgM	Arg/His	C22	M	His/His

23.	DS23	M	51	Vomiting	IgG	Arg/His	C23	M	Arg/Arg
24.	DS24	M	52	Myalgia	IgG	Arg/His	C24	M	Arg/Arg
25.	DS25	M	38	Joint pain	NS1Ag	Arg/His	C25	M	Arg/Arg
26.	DS26	F	46	Joint pain	IgG	Arg/His	C26	F	Arg/Arg
27.	DS27	F	39	Myalgia	NS1Ag	Arg/His	C27	F	Arg/Arg
28.	DS28	F	34	Vomiting	NS1Ag	Arg/His	C28	F	Arg/Arg
29.	DS29	M	18	Pain abdomen	NS1Ag	His/His	C29	M	Arg/His
30.	DS30	M	37	Pain abdomen	NS1Ag	His/His	C30	M	Arg/His
31.	DS31	M	27	Fever	NS1Ag	Arg/His	C31	M	Arg/His
32.	DS32	M	42	Breathlessness	NS1Ag	Arg/Arg	C32	M	His/His
33.	DS33	M	28	Pain abdomen	NS1Ag	His/His	C33	M	His/His
34.	DS34	M	23	Pain abdomen	NS1Ag	His/His	C34	M	His/His
35.	DS35	M	33	Pain abdomen	NS1Ag	Arg/His	C35	M	Arg/His
36.	DS36	F	36	Myalgia	NS1Ag	Arg/His	C36	F	Arg/Arg
37.	DS37	M	22	Pain abdomen	NS1Ag	His/His	C37	M	Arg/His
38.	DS38	M	24	Pain abdomen	NS1Ag	His/His	C38	M	Arg/His
39.	DS39	M	34	Pain abdomen	NS1Ag	Arg/His	C39	M	Arg/His
40.	DS40	F	29	Pain abdomen	NS1Ag	Arg/His	C40	F	Arg/His
41.	DS41	F	38	Myalgia	NS1Ag	Arg/His	C41	F	Arg/His
42.	DS42	M	25	Pain abdomen	NS1Ag	Arg/Arg	C42	M	Arg/Arg
43.	DS43	M	26	Pain abdomen	NS1Ag	His/His	C43	M	Arg/Arg
44.	DS44	M	28	Breathlessness	IgM	Arg/His	C44	M	Arg/His
45.	DS45	M	52	Vomiting	IgM	Arg/Arg	C45	M	Arg/His
46.	DS46	F	30	Breathlessness	NS1Ag	His/His	C46	F	Arg/Arg
47.	DS47	M	52	Vomiting	IgG	His/His	C47	M	Arg/His
48.	DS48	M	39	Fever	NS1Ag	Arg/His	C48	M	His/His

49.	DS49	M	26	Fever	NS1Ag	Arg/Arg	C49	M	Arg/His
50.	DS50	M	25	Pain abdomen	NS1Ag	Arg/Arg	C50	M	Arg/His
51.	DS51	M	29	Fever	NS1Ag	Arg/His	C51	M	Arg/His
52.	DS52	M	33	Breathlessness	IgM	Arg/His	C52	M	Arg/His
53.	DS53	M	29	Pain abdomen	NS1Ag	Arg/Arg	C53	M	Arg/His
54.	DS54	F	36	Myalgia	NS1Ag	Arg/His	C54	F	Arg/Arg
55.	DS55	M	25	Pain abdomen	NS1Ag	His/His	C55	M	Arg/Arg
56.	DS56	M	26	Pain abdomen	NS1Ag	His/His	C56	M	Arg/Arg
57.	DS57	M	26	Pain abdomen	NS1Ag	Arg/Arg	C57	M	Arg/Arg
58.	DS58	F	34	Breathlessness	NS1Ag	Arg/His	C58	F	Arg/His
59.	DS59	M	28	Pain abdomen	NS1Ag	His/His	C59	M	Arg/Arg
60.	DS60	M	29	Pain abdomen	NS1Ag	His/His	C60	M	Arg/His
61.	DS61	M	32	Pain abdomen	NS1Ag	Arg/His	C61	M	Arg/His
62.	DS62	M	29	Fever	NS1Ag	Arg/His	C62	M	Arg/His
63.	DS63	M	41	Breathlessness	NS1Ag	His/His	C63	M	Arg/Arg
64.	DS64	M	43	Breathlessness	NS1Ag	Arg/His	C64	M	Arg/Arg
65.	DS65	M	45	Breathlessness	IgM	Arg/Arg	C65	M	His/His
66.	DS66	M	29	Breathlessness	IgM	Arg/His	C66	M	Arg/His
67.	DS67	M	52	Vomiting	IgM	His/His	C67	M	Arg/His
68.	DS68	F	32	Breathlessness	NS1Ag	Arg/His	C68	F	Arg/His
69.	DS69	F	33	Pain abdomen	NS1Ag	Arg/His	C69	F	Arg/His
70.	DS70	F	30	Fever	NS1Ag	Arg/Arg	C70	F	Arg/Arg
71.	DS71	F	34	Pain abdomen	NS1Ag	Arg/Arg	C71	F	Arg/Arg
72.	DS72	F	32	Fever	NS1Ag	Arg/His	C72	F	Arg/Arg