Closure report on the project

Diagnosis and Immunopathogenesis of Dengue Virus Infections; a longitudinal investigation into genotype diversity, diagnosis and immune response to dengue infections in Assam, Northeast India

Sanctioned: Letter no.- O/o DG (TM)/81/48222/LSRB-301/BTB/2017,

Date- 21-07-2017

Project duration : 21-07-2017 to 31-03-21

Funded by- LSRB DRDO New Delhi, India

Principal Investigator- Dr Shashi Baruah

Professor

Department of Molecular Biology and Biotechnology

Tezpur University

Sonitpur, Assam-784028

DEPARTMENT OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY
TEZPUR UNIVERSITY
NAPAAM, TEZPUR-784 028 ASSAM, INDIA,

Tel: +91-3712-275408 (O)
S.Baruah **Fax: +91-3712-267005/6**
Professor **Email: sbaruah@tezu.ernet.in**

Member Secretary Date- 05-01-2022

Life Sciences Research Board

DRDO Bhawan

New Delhi- 110011

Sub- Submission of Project closure report and other documents for the project "Diagnosis and Immunopathogenesis of Dengue Virus Infections……. Northeast India".

Ref- Sanction order no.- O/o DG (TM)/81/48222/LSRB-301/BTB/2017

Dear Sir,

This is in reference to submission of closure documents for the above mentioned project. We have successfully completed the project. Kindly find the following documents enclosed with this letter. However equipment retention form is in process, we will submit as soon as it is done.

1. Detailed closure technical report
2. Format closure of the LSRB Project
3. Certificate from PI for completion of the project
4. Brief summary of achievements of the project
5. Grant Utilization (UC/SE) of the project
6. Equipment purchased form
7. Demand Draft for Rs 1,14,454.00, Sr No- 126055 dated 03-01-2022 for the unspent balance and interest earned.

We gratefully acknowledge LSRB, DRDO for their support.

Sincerely,

Dr. S Baruah

FORMAT FOR CLOSURE OF THE LSRB PROJECT

Title of the Project : **Diagnosis and Immunopathogenesis of Dengue Virus Infections; a longitudinal investigation into genotype diversity, diagnosis and immune response to dengue infections in Assam, Northeast India**

PI's Name and Address of the Institute : Dr Shashi Baruah
PDC of the Project : 3 years
Cost of the Project : Rs.61,59,727.00

(A) Objectives envisaged vis-à-vis achieved as under :-

Objectives/Aim of the project	**Achievement**
• Genotyping and serotyping of the prevalent dengue virus in study areas (Assam) by RT-PCR based assay.	• Our data suggests predominance of DENV1 and DENV2 during the period of sample collection.
• Screening of antibody in the sera for IgM, IgG isotypes using envelop and NS1 peptide by ELISA based assays.	• Predominant IgM response in absence of IgG response suggested recent exposure. • Immunodominant peptides of DENV Envelope protein and NS1 protein of DENV 1,2and 3 were identified.
• Polymorphisms of cytokine of the patient RNA and of DC-SIGN and FcγR (Fc receptor for IgG) will be studied by PCR-SSP approach.	• Two SNPS i.e., TNF-308G>A and IL1β-31 T>C were found to be differ in frequency between case and control. • Higher frequency of the variant C allele of SNP IL1β-31 T>C in DENV patient correlated with increased expression of IL1β.
• Characterize virus cell tropism, cytokine and chemokine storm and analyze differential regulation of key cytokine (s) /chemokine in DF and DHF/DSS and the associated cell populations	• Higher levels of proinflammatory cytokines IL-8, IL-1, IL-6 and chemokines CXCL9 , CXCL10 and CCL2 suggests activation on monocyte-neutrophil axis • Dendritic cell population appeared to be shift toward increased plasmacytoid DC (pDCs) population suggesting DENV is DC tropic. • Our study suggests high IL-1 β with low CCL5 as markers of DENV infection**.**

(B) Defence Relevance of the outcome of the project: -

Using the identified immunodominant peptides, we plan to develop a point of care diagnostic assay for Dengue virus infection. Considering high prevalence and co-circulation of multiple DENV serotypes in the country there is high chance of outbreak in the future. Specially in forest and rural areas with minimum facility of diagnosis, we hope use of peptide regions in DENV proteins identified by us in diagnostic kit will help the military soldiers in accurate and early diagnosis of DENV infection.

(C) The following equipments have been procured under the project :-

S.No.	Particulars	Cost of the equipment
(i)	Flow cytometer with Accessories	25,00,000

(D) Academic Achievements :-

- Journal papers (National) : NA
- Journal papers (International) : Manuscript under preparation.
- Conference presentation (National) : **Oral paper Presentation at Annual Conference of Indian Association of Medical Microbiologists, MICROCON 2021**
- Conference presentation (International) : NA
- Book Chapter : NA
- Bulletin : NA
- Popular article / news : NA
- Trainings : NA
- Success story : NA
- Radio talk : NA
- Patent (National) : NA
- Patent (International) : NA
- Participating DRDO lab : Defence Research Laboratory (DRL), Tezpur, Assam.
  Defence Research and Development Establishment (DRDE), Gwalior, Madhya Pradesh 474002, India

Title of the project- Diagnosis and Immunopathogenesis of Dengue Virus Infections; a longitudinal investigation into genotype diversity, diagnosis and immune response to dengue infections in Assam, Northeast India.

Summary of achievements of the project-

1. Based on the serotyping data and frequency of seropositivity, our data suggests predominance of DENV1 and DENV2 during the period of sample collection.
2. The predominant antibody isotype detected in NS1 positive samples was IgM (> 75%) In absence of concomitant IgG response in majority of the patients, its suggestive of recent infection.
3. A total of 9,5 and 8 Immunodominant peptides have been selected from Envelope protein peptide array of DENV1, DENV2 and DENV3 respectively and 6 each Immunodominant peptides have been selected from NS1 protein peptide array of DENV2 and DENV3 respectively.
4. Bio-informatics study confirms the identified immunodominant regions of DENV Envelope and NS1 proteins containing B cell epitopes. **These regions are shortlisted for serology based diagnosis of DENV infections.**
5. Two SNPS i.e., TNF-308G>A(rs1800629) and IL1β-31 T>C (rs1143627) were found to be differ in frequency between case and control. In IL1β-31 T>C (rs1143627), the C allele was seen at higher frequency in dengue patients while the T allele was higher in control participants. **Allele C also associates with high expression of IL1β which also corelates with our finding of high IL1β protein expression in Dengue patients.**
6. **Higher levels of proinflammatory cytokines IL-8, IL-1, IL-6 and chemokines CXCL9 , CXCL10 and CCL2 suggests activation on monocyte-neutrophil axis**.
7. **Levels of CCL5 positively correlated with platelet counts in studied patients**. **Our study suggests high IL-1 with low CCL5 as markers of DENV infection**. However, this needs to be validated in larger number of samples.
8. Monocyte population was predominantly CD14++ classical monocytes and the intermediate CD16+ CD14+. **The dendritic cell population appeared to be shift toward increased plasmacytoid DC (pDCs) population**, that are reported to secrete interferons during viral infections as well as proinflammatory cytokines and chemokines.

Dr. S Baruah
Dept of MBBT
Tezpur University, Assam

DEPARTMENT OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY
TEZPUR UNIVERSITY
NAPAAM, TEZPUR-784 028 ASSAM, INDIA,

S.Baruah
Professor

Tel: +91-3712-275408 (O)
Fax: +91-3712-267005/6
Email: sbaruah@tezu.ernet.in

CERTIFICATE OF SUCCESSFUL COMPLETION OF THE PROJECT

This is to certify that the project entitled "**Diagnosis and Immunopathogenesis of Dengue Virus Infections; a longitudinal investigation into genotype diversity, diagnosis and immune response to dengue infections in Assam, Northeast India"** funded by LSRB DRDO, New Delhi India has been successfully completed. The objectives listed in the project proposal were successfully met with and we are in the process of communicating the findings. During the course of project, the incidence of DENV fevers in Assam fell markedly, which was a constraint in the sample collection as was the disruption in work due to pandemic.

Some of the findings under the project were recently presented at the Annual Conference of Indian Association of Medical Microbiologists, MICROCON 2021and were shortlisted for presentation for the best paper in Immunology section.

We gratefully acknowledge LSRB, DRDO New Delhi for financial support as well as the support of mentors from DRDE, Gwalior.

Dr. S Baruah
Dept. of MBBT
Tezpur University
Assam

AUDITED/PROVISIONAL STATEMENT OF EXPENDUTURE ACCOUNTS

FOR THE FINANCIAL YEAR (1st January 2020 to 31st March 2020)

(a) Title of the Project: Diagnosis and Immunopathogenesis of Dengue Virus Infections; a longitudinal investigation into genotype diversity, diagnosis and immune response to dengue infections in Assam, Northeast India

(b) Sanctioned letter no. & date: Letter no.- O/o DG (TM)/81/48222/LSRB-301/BTB/2017, Date- 21-07-2017

(c) Principal Investigator : Dr. Shashi Baruah

(d) Date of Start of the Project: 23-10-2017

(e) Total Sanctioned cost of the Project: in Rs.61,59,727.00

(f) Grant received (Rs.) in **III yr** (upto 31st March 2020) -Rs 0.00

(g) Total Grants received so far: Rs. 53,75,259.00

S. No	Sanctioned Heads	Funds Sanctioned for the year	Funds released	Carried forward from Previous year	Funds available (iv+v)	Expenditure incurred during the FY	Balance (vi-vii)	Commitment	Total expenditure (vii+ix)
	Rs.	Rs.	Rs.	Rs.	Rs.	Rs.	Rs.	Rs.	Rs.
i	ii	iii	iv	v	vi	vii	viii	ix	x
(a)	Staff	5,16,000.00		1,22,258.00	1,22,258.00	95,000.00	4,436.00		95,000.00
(b)	Equipment	0.00		0.00	0.00	0.00	0.00		0.00
(c)	Operation &Maint.								
(d)	Expendables	1,93,702.00		-36,420.00	-36,420.00	0.00	0.00		0.00
(e)	Travel	45,000.00		-24,684.00	-24,684.00	0.00	0.00		0.00
(f)	Contingencies	22.641.00		201.00	201.00	0.00	0.00		0.00
(g)	Research Consultant								
(h)	Procured Service								
	Institutional over head	28,230.00		38,081.00	38,081.00	0.00	0.00		0.00
	Interest earned, if any								
	TOTAL	8.05,573.00		99,436.00	99,436.00	95,000.00	4,436.00		95,000.00

Name and Signature of
Principal Investigator
Date: 5/11/21

S. Baruah
Professor
Dept. of Mol. Bio. & Biotech
Tezpur University

Name and Signature of
Accounts Officer
Date:

Finance Officer
Tezpur University

Signature of Administrative
Date

Registrar
Tezpur University

UTILIZATION CERTIFICATE

FOR THE FINANCIAL YEAR 2017-18 (*From* 1st January 2020 to 31st March 2020)

1	Title of the Project / Scheme		Diagnosis and Immunopathogenesis of Dengue Virus Infections; a longitudinal investigation into genotype diversity, diagnosis and immune response to dengue infections in Assam, Northeast India
2	Name of the Institution		Tezpur University
3	Principal Investigator		Dr. Shashi Baruah
4	DRDO Letter No. and date of sanctioning the project Date of Start of the Project		Letter no.- O/o DG (TM)/81/48222/LSRB-301/BTB/2017, Date- 21-07-2017 Date of Start of Project- 23-10-2017
5	Head of account as given in the original sanction letter		**Major Head – 005** **Minor Head – 001**
6	Amount brought forward from the previous financial year quoting DRDO letter No. & date in which the authority to carry forward the said amount was given.		
7	Amount received during the financial year (Please give no. and date of DRDO sanction letter for the amount)		Rs. 0.00
8	Amount of interest accrued, if any, from the grants		NIL
9	Total amount that was available for expenditure (excluding commitments) during the financial year (SL. No 6 +7+8)		Rs. 99,436.00
10	Actual expenditure (excluding commitments) incurred during the financial year (upto 31st March 2019)		Rs. 95,000.00

11	Balance amount available at the end of the financial year.		Rs. 4,436.00
12	Unspent balance refunded, if any (Please give details of Cheque No. etc.)		
13	Amount allowed to be carried forward to the next financial year		Rs. 4,436.00

(contd6)

UTILIZATION CERTIFICATE

FY (*From* 1st January 2020 to 31st March 2020)

Certified that sum of Rs. 61,59,727.00 was sanctioned as grants-in-aid during the year 2017-18 in favour of Tezpur University .(Instt) vide DRD Letter no.- O/o DG (TM)/81/48222/LSRB-301/BTB/2017 dated 21-07-2017

A sum of Rs. 0.00 released and amount of Rs. NIL accrued as Interest during the year and Rs. 99,436.00 on account of unspent balance of the previous year, a sum of Rs. 95,000.00 has been utilized for the purpose for which it was sanctioned and that the balance of Rs. 4,436.00 remaining unutilized at the end of the year will be refunded/adjusted toward the grants-in-aid payable during the next year i.e 2020-21.

Principal Investigator

Accounts/Finance Officer

Finance Officer
Tezpur University

Administrative Authority

(with official seal)

Registrar
Tezpur University

2. Certified that I have satisfied myself that the conditions on which the grants in- aid was sanctioned have been fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Signature of Audit Authority of Grantee Institution

AUDITED/PROVISIONAL STATEMENT OF EXPENDUTURE ACCOUNTS

FOR THE FINANCIAL YEAR (1st April 2020 to 20th March 2021)

(a) Title of the Project: Diagnosis and Immunopathogenesis of Dengue Virus Infections; a longitudinal investigation into genotype diversity, diagnosis and immune response to dengue infections in Assam, Northeast India

(b) Sanctioned letter no. & date: Letter no.- O/o DG (TM)/81/48222/LSRB-301/BTB/2017, Date- 21-07-2017

(c) Principal Investigator : Dr. Shashi Baruah

(d) Date of Start of the Project: 23-10-2017

(e) Total Sanctioned cost of the Project: in Rs.61,59,727.00

(f) Grant received (Rs.) in **III yr** -Rs 7,84,468.00

(g) Total Grants received so far: Rs. 59,63,645.00

S. No	Sanctioned Heads	Funds Sanctioned for the year	Funds released	Interest	Carried forward from Previous year	Funds available (iv+v+vi)	Expenditure incurred during the FY	Balance (vii-viii)	Commitment	Total expenditure (ix+x)
	Rs.	Rs.	Rs.	Rs	Rs.	Rs.	Rs.	Rs.	Rs.	Rs.
i	ii	iii	iv	v	vi	vii	viii	ix	x	xi
(a)	Staff	5,16,000.00	4,33,742.00		4,436.00	4,38,178.00	4,35,967.00	2,211.00		4,35,967.00
(b)	Equipment	0.00	0.00		0.00	0.00	0.00	0.00		0.00
(c)	Operation &Maint.									
(d)	Expendables	1,93,702.00	2,30,122.00		0.00	2,30,122.00	1,98,633.00	31,489.00.		1,98,633.00
(e)	Travel	45,000.00	69,684.00		0.00	69,684.00	3,440.00	66,244		3,440.00
(f)	Contingencies	22.641.00	22,690		0.00	22,690	21,558.00	1,132.00		21,558.00
(g)	Research Consultant									
(h)	Procured Service									
	Institutional over head	28,230.00	28,230.00		0.00	28,230.00	17,644.00	10,586.00		17,644.00
	Interest earned, if any			2,792.00	-	2,792.00		2,792.00		
	TOTAL	8.05,573.00	7,84,468.00		4,436.00	7,91,696.00	6,77,242.00	1,14,454.00		6,77,242.00

Name and Signature of
Principal Investigator
Date: 5/11/21

S. Baruah
Professor
Dept. of Mol. Bio. & Biotech
Tezpur University

Name and Signature of
Accounts Officer
Date: 5/11/21

Finance Officer
Tezpur University

Signature of Administrative
Date

Registrar
Tezpur University

UTILIZATION CERTIFICATE

FOR THE FINANCIAL YEAR 2020-21 (*From* 1st April 2020 to 20th March 2021)

1	Title of the Project / Scheme		Diagnosis and Immunopathogenesis of Dengue Virus Infections; a longitudinal investigation into genotype diversity, diagnosis and immune response to dengue infections in Assam, Northeast India
2	Name of the Institution		Tezpur University
3	Principal Investigator		Dr. Shashi Baruah
4	DRDO Letter No. and date of sanctioning the project Date of Start of the Project		Letter no.- O/o DG (TM)/81/48222/LSRB-301/BTB/2017, Date- 21-07-2017 Date of Start of Project- 23-10-2017
5	Head of account as given in the original sanction letter		**Major Head – 005** **Minor Head – 001**
6	Amount brought forward from the previous financial year quoting DRDO letter No. & date in which the authority to carry forward the said amount was given.		Rs. 4,436.00
7	Amount received during the financial year (Please give no. and date of DRDO sanction letter for the amount)		Rs. 7,84,468.00.
8	Amount of interest accrued, if any, from the grants		Rs. 2,792.00
9	Total amount that was available for expenditure (excluding commitments) during the financial year (SL. No 6 +7+8)		Rs. 7,91,696.00
10	Actual expenditure (excluding commitments) incurred during the financial year (upto 31st March 2019)		Rs. 6,77,242.00

11	Balance amount available at the end of the financial year.		Rs. 1,14,454.00
12	Unspent balance refunded, if any (Please give details of Cheque No. etc.)		Rs. 1,14,454.00
13	Amount allowed to be carried forward to the next financial year		Rs.0.00

(contd6)

UTILIZATION CERTIFICATE

FY (*From* 1st April 2020 to 20th March 2021)

Certified that sum of Rs. 61,59,727.00 was sanctioned as grants-in-aid during the year 2017-18 in favour of Tezpur University .(Instt) vide DRD Letter no.- O/o DG (TM)/81/48222/LSRB-301/BTB/2017 dated 21-07-2017

A sum of Rs. 7,84,468.00 released and amount of Rs. 2,792.00 accrued as Interest during the year and Rs. 4,436.00 on account of unspent balance of the previous year, a sum of Rs. 6,77,242.00 has been utilized for the purpose for which it was sanctioned and that the balance of Rs. 1,14,454.00 remaining unutilized at the end of the year will be handed over to the grantee institution.

Principal Investigator.

Accounts/Finance Officer

Finance Officer
Tezpur University

Administrative Authority

(with official seal)

Registrar
Tezpur University

2. Certified that I have satisfied myself that the conditions on which the grants in- aid was sanctioned have been fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Signature of Audit Authority of Grantee Institution

FORM for Equipments Purchased

Assets acquired wholly for substantially out of government grants register maintained by grantee institution block account maintained by sanctioning authorities

Name of Sanctioning Authority: **Dte of ER&IPR,RBs** DRDO, Ministry of Defence,

Government of India, New Delhi.

S. No.	Name of Grantee Institution	No. and date of sanction	Amount of sanctioned grant	Brief purpose of the grant	Whether any condition regarding the right of ownership of Govt. in the property or assets acquired out of the grant was incorporated in the grant-in-aid sanction	List of equipments as per approved proposal	Particulars of assets/equipments actually created or acquired
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1	Tezpur University	No.- O/o DG (TM)/81/48222/LSRB-301/BTB/2017 Date- 21-07-2017	Rs. 25,00,000 (Rupees Twenty five Lakh only)	Purchase of Flow Cytometer and related accessories		BD Accuri C6 Plus System + Accessories	BD Accuri C6 Plus System + Accessories

Contd.....

Finance Officer
Tezpur University

Contd.....

Value of the assets as on 30-11-2018	Purpose for which utilized at present	Encumbered or not	Reason if encumbered	Disposed of or not	Reasons & authority if any, for disposal	Amount realized on disposal	Remarks
(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
Rs. 25,65,919.00 (Rupees Twenty five lakh sixty five thousand nine hundred and nineteen only)	Characterization of cell tropism, cytokine and chemokine storm and analysis of key cytokine/chemokines.						

(Principal Investigator)

S. Baruah 11/12/18
Professor
Dept. of Mol. Bio. & Biotech
Tezpur University

Accounts Officer

Finance Officer
Tezpur University

13/12/18
Administrative Authority

Registrar
Tezpur University

भारतीय स्टेट बैंक
जारी करने वाली शाखा State Bank of India
Issuing Branch:TEZPUR UNIVERSITY
कोड क्र /CODE No: 14259
Tel No. 03712-267285

A/C Payee

मांगड्राफ्ट
DEMAND DRAFT

Key: YEHJEQ
Sr. No: 126055

D	D	M	M	Y	Y	Y	Y
0	3	0	1	2	0	2	2

या उनके आदेश पर
OR ORDER

मांगे जानेपर PCDA (R&D) **********

ON DEMAND PAY

रुपये RUPEES One Lakh Fourteen Thousand Four Hundred and Fifty Four Only

अदा करें ₹ 114454.00

IOI 000471535491
Name of Applicant

Key: YEHJEQ Sr. No: 126055
TEZPUR UNIVERSITY R& D

AMOUNT BELOW 114455(0/6)

मूल्य प्राप्त / VALUE RECEIVED

भारतीय स्टेट बैंक
STATE BANK OF INDIA
अदाकर्ता शाखा / DRAWEE BRANCH:NEW DELHI MAIN BRANCH
कोड क्र . /CODE No: 00691

प्राधिकृत हस्ताक्षरकर्ता
AUTHORISED SIGNATORY

शाखा प्रबंधक
BRANCH MANAGER

₹1,50,000/- एवं अधिक के लिखत दो अधिकारियों द्वारा हस्ताक्षरित होने पर ही वैध है।
INSTRUMENTS FOR ₹ 1,50,000/- & ABOVE ARE NOT VALID UNLESS SIGNED BY TWO OFFICERS

कम्प्यूटर द्वारा मुद्रित होने पर ही वैध
VALID ONLY IF COMPUTER PRINTED

केवल 3 महीने के लिए वैध
VALID FOR 3 MONTHS ONLY

सत्यजीत मजूमदार
SATYAJIT MAZUMDAR
M 13712

SESHAASAI(C) / CTS-2010

⑈535491⑈ 00000200⑆ 000471⑈ 16

1. About your Institute/Lab./University

Tezpur University was established by an Act of Parliament in 1994. The objects of this Central University as envisaged in the statutes are that it shall strive to offer employment oriented and interdisciplinary courses to meet the local and regional aspirations and the development needs of the state of Assam and also offer courses and promote research in areas which are of special and direct relevance to the region and in emerging areas in Science and Technology.

2. Project objectives

a. Genotyping and serotyping of the prevalent dengue virus in study areas (Assam) by RT-PCR based assay.
b. Screening of antibody in the sera for IgM, IgG and IgG isotypes using envelop and NS1 peptide by ELISA based assays.
c. Polymorphisms of cytokine of the patient RNA and of DC-SIGN and FcγR(Fc receptor for IgG) will be studied by PCR-SSP approach.
d. Characterize virus cell tropism, cytokine and chemokine storm and analyse differential regulation of key cytokine (s) /chemokine in DF and DHF/DSS and the associated cell populations. Cell tropism by flow cytometry/ fluorescent microscopy, cytokine and chemokine expression by RT-PCR and multiplex array methods. Cell populations specific cytokine secretion will be done by flow cytometry.
e. Identify the incriminated *Aedes* spp. by microscopic examination of morphological features and RT-PCR based confirmation of DENV infection.

3. Summary of Technical Achievements: {a, b, c……..}

The project was approved vide letter no. O/o DG (TM)/81/48222/LSRB-301/BTB/2017 Dated: 21/07/2017 and grant was released in the month of September, 2017.

a. Procurement of instruments and consumables

Procurement of flow cytometer and related accessories was completed. The procurement of chemicals was also completed.

b. Collaborations

Technical collaborations were established with local health authorities that included Directorate of Health Services, Sonitpur, Assam, Directorate of Health Services, Lakhimpur, Assam, Tezpur Medical College and Hospital (TMCH), Tezpur, Assam, Kanaklata Civil Hospital (KCH), Tezpur, Assam, 155 Base Hospital (BH), Tezpur, Assam, Primary Health Centres (PHCs) of Tezpur and adjacent areas.

c. Approvals

Ethical approval was obtained from collaborating hospital, TMCH, Tezpur, Assam.

d. Recruitment of manpower

Advertisement for the manpower was published by Tezpur University for one Project Assistant and one Junior Research Fellow (JRF).

4. Major Technical Achievements: {a, b, c……..}

Briefly,

1. Based on the serotyping data and frequency of seropositivity, our data suggests predominance of DENV1 and DENV2 during the period of sample collection.
2. The predominant antibody isotype detected in NS1 positive samples was IgM (> 75%) In absence of concomitant IgG response in majority of the patients, its suggestive of recent infection.
3. A total of 9,5 and 8 Immunodominant peptides have been selected from Envelope protein peptide array of DENV1, DENV2 and DENV3 respectively and 6 each Immunodominant peptides have been selected from NS1 protein peptide array of DENV2 and DENV3 respectively.
4. Bio-informatics study confirms the identified immunodominant regions of DENV Envelope and NS1 proteins containing B cell epitopes. These regions are shortlisted for serology-based diagnosis of DENV infections.
5. Two SNPS i.e., TNF-308G>A(rs1800629) and IL1β-31 T>C (rs1143627) were found to be differ in frequency between case and control. In IL1β-31 T>C (rs1143627), the C allele was seen at higher frequency in dengue patients while the T allele was higher in control participants. Allele C also associates with high expression of IL1β which also corelates with our finding of high IL1β protein expression in Dengue patients.
6. Higher levels of proinflammatory cytokines IL-8, IL-1, IL-6 and chemokines CXCL9 , CXCL10 and CCL2 suggests activation on monocyte-neutrophil axis.
7. Levels of CCL5 positively correlated with platelet counts in studied patients. Our study suggests high IL-1 with low CCL5 as markers of DENV infection. However, this needs to be validated in larger number of samples.
8. Monocyte population was predominantly CD14++ classical monocytes and the intermediate CD16+ CD14+. The dendritic cell population appeared to be shift toward increased plasmacytoid DC (pDCs) population, that are reported to secrete interferons during viral infections as well as proinflammatory cytokines and chemokines.

For detailed report kindly refer to Annexure I

5. Lessons learnt and best practices adopted

The project was sanctioned in the month of September, 2017 and sample collection could be initiated only in the month of November, 2017. Notably, during this period the dengue transmission was low and this led to the collection of small number of samples. Collaborations with the local health administrations enabled us to overcome issues like logistics and loss of road connectivity.

Eligibility of DRDO-JRF is NET/GATE, and this led to unavailability of suitable candidate at the first and second round of interview schedule. However, during the third interview, a suitable candidate was found who subsequently joined the project.

Essential regulatory guidelines were followed and best practices were adopted for the execution of this project.

Briefly,

a. Ethical approval was obtained from TMCH.
b. Informed consent form was signed and obtained.
c. Left over sample supplied by different hospitals was utilized for experimentation.

6. Economic impact (Funds released by LSRB and Expenditure made till date)

Please see Annexure- I

7. Defence relevance

The Northeastern region of India, which is a biodiversity hot spot, is highly prone to the incidence and transmission of mosquito-borne diseases. Thus military troops deployed in this area are susceptible to mosquito-borne diseases and Dengue virus infection. There is no specific treatment for dengue, only supportive treatments are provided. Moreover, the information on serotypes and genotypes circulating in the region is sketchy and demands in depth studies to determine the type and subtypes of the virus and its spatial and temporal variation if any. Besides, with concomitant Japanese Encephalitis virus circulation in the region, the diagnosis of Dengue infection is challenging. Thus our investigation is expected to benefit the civilian and military population at large**.** In this connection, we collaborated with 155 Base Hospital (BH), Tezpur, Assam. We obtained two dengue cases with high titre of NS1 and IgG antibody.

8. **Patents filed and Papers published with impact-factor :** Manuscript under preparation.

9. **Partnering and collaboration with other institutes**
   a. Directorate of Health Services, Sonitpur, Assam
   b. Directorate of Health Services, Lakhimpur, Assam
   c. Tezpur Medical College and Hospital (TMCH), Tezpur, Assam
   d. Kanaklata Civil Hospital (KCH), Tezpur, Assam
   e. 155 Base Hospital (BH), Tezpur, Assam
   f. Primary Health Centres (PHCs) adjacent to Tezpur.
   g. GMC&H, Guwahati

10. **Applications and civilian spin-off**

The dengue haemorrhagic fever which was unknown to the north east India has recently became a major heath care issues particularly the state of Assam. Persistent dengue outbreaks since last few years have been reported from several districts with an estimated 12,707 cases from 2015 to 2021 (http://nvbdcp.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715). Dengue virus infections can result in a range of clinical manifestations from asymptomatic infection to flu-like DF and the severe disease DHF/DSS. We investigated inflammatory response and the altered regulation of inflammation in DF to identify immunological markers of severe disease and potential therapeutic targets for early detection and clinical management of disease. Our study identified high IL-1ß and low CCL5 to be associated with disease. Further, our data suggests a role for plasmacytoid DC (pDCs) population, that are reported to secrete interferons during viral infections as well as proinflammatory cytokines and chemokines.

11. **Conference Presentation :** Oral paper presentation at Annual Conference of Indian Association of Medical Microbiologists,MICROCON 2021.

12. **Research Degree: [Ph.D. registered and awarded to JRF/SRF working in project]:** Sushmita Singha, JRF, and Registered for Ph.D.

13. Participating DRDO Lab

Defence Research Laboratory (DRL), Tezpur, Assam.

Defence Research and Development Establishment (DRDE), Gwalior, Madhya Pradesh 474002, India.

Technical Plan/ Programme of Research

Laboratory Investigations

1. **Dengue infection incidence**

Dengue case in Assam was first recorded in the year 2010 however, a large-scale outbreak of dengue in the state occurred in the year 2016. A total of 6157 dengue cases with 4 deaths has been recorded in 2016 which is still the highest number in the history of Assam. Gradual decrease in incidence rate was seen in the state from the following year and only 55 dengue cases has been reported this year.

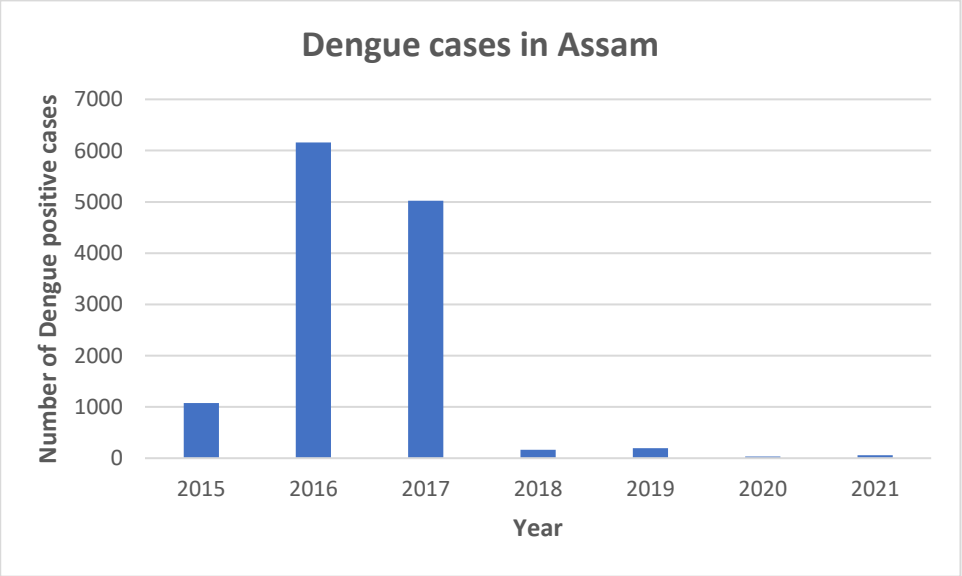


Fig 1: Dengue statistics in Assam (2015- October 2021) www.nvbdcp.gov.in.

2. **Study participants**

For the study, ELISA based method for dengue positivity was employed. The patients showing positive result were enrolled for the study.

3. **Inclusion criteria**

Patients showing positive dengue infection as confirmed by Dengue rapid detection kit.

4. **Exclusion criteria**

   Dengue infection along with other flavivirus infections. Other vulnerable patients like seriously or terminally ill.

5. **Towards objective 1**

   Genotyping and serotyping of the prevalent dengue virus in study areas (Assam) by RT-PCR based assay.

Table 1: Sample collected

Collected from	Number	Year	Sample type	Sample Nature	Storage condition
TMCH	1	2019	Whole blood		-20°C
GMCH	15	2019	Whole blood		-20°C
GMCH	6	2020	Serum	Cloudy	-20°C
Times Hospital	1	2019	Whole blood		-20°C
BK memorial	7	2018	Serum	Cloudy	4°C
Base hospital	6	2018, 2019	Serum	Cloudy	4°C
Civil Hospital Tezpur	59	2018	Serum	Cloudy	4°C

Viral RNA extraction was performed in 10 dengue positive samples that had been stored at -20 for less than week. In other samples viral RNA extraction failed which could be attributed to low stability of the RNA and improper or prolonged storage of samples. It may be noted here that most of our collected samples were previously stored in the collaborating institutes. Therefore, long-term storage may have degraded the viral RNA in the samples. Hence, genotyping of all samples was not successfully accomplished and alternative approach of serotyping was adopted using peptide array.

6. Towards objective 2

Screening of antibody in the sera for IgM, IgG and IgG isotypes using envelop and NS1 peptide by ELISA based assays

6.1. Antibody isotyping

A one step rapid test was used for detecting infection with dengue virus. Isotyping for IgG and IgM were performed in all samples collected and the data is represented in Table 2.

Table 2: Antibody Isotyping of Samples

Hospital/DHS	NS1 Positive Sample	IgG Positive Sample	IgM Positive Sample
GMCH	13	1	12
TMCH	2	1	2
*CH	59	1	
BH	1	1	
DHS	1		

The predominant IgM response in absence of IgG response suggests recent exposure.

* Samples collected from Civil Hospital were all NS1 positive, however due to long term storage, the condition of the samples were not good enough so antibody isotyping in those samples could not be done. Further, IgG isotyping was not done as number of samples positive for IgG were less than five.

6.2. Identification of immunodominant peptides of viral proteins Envelope and NS1 using ELISA

To determine immunodominant regions of the viral envelope (E) and non-structural (NS1) proteins, peptide array of DENV proteins of DENV1-4 were acquired. ELISA were performed with patient serum to detect antibodies against viral proteins. The criteria used for short listing of the peptides were frequency of positivity and the antibody titre.

a) DENV 1 - Envelope protein

DENV1 Envelope protein peptide array consists of 84 peptide array of 13-18 mers with 11 or 12 overlaps. Using ELISA, based on positive reaction of patient sera of 1.5 fold or higher above cut off with the peptide, 19 peptides were selected from the array (Fig2).

The titre in mean arbitrary units of these patient sera with selected 19 peptides was determined and is presented in Fig3.

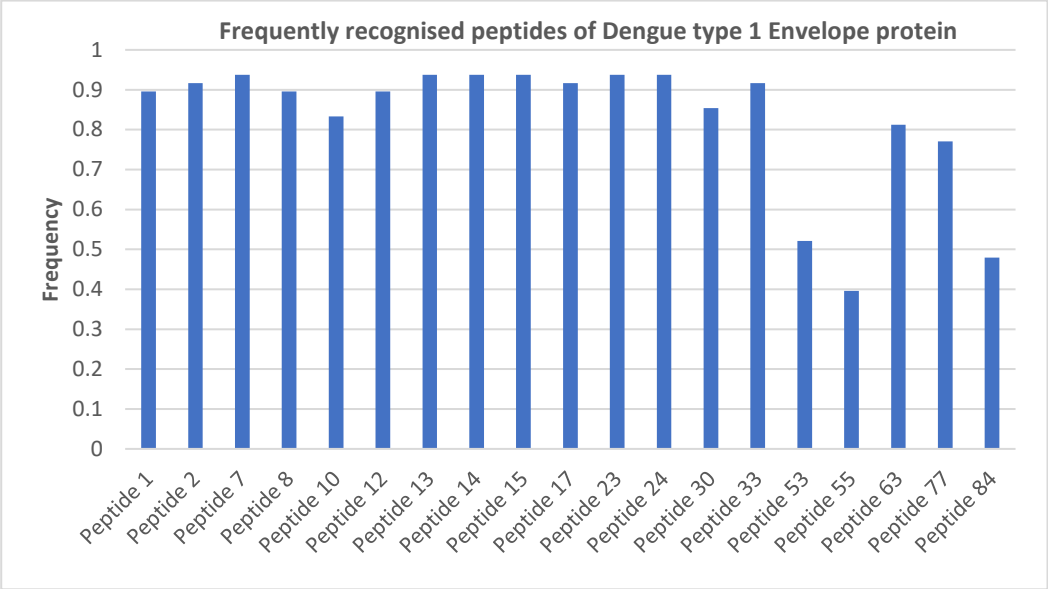


Fig 2: Frequency of patients recognizing the peptides with mean titre (AU) greater than 1.5 fold of cut off value.

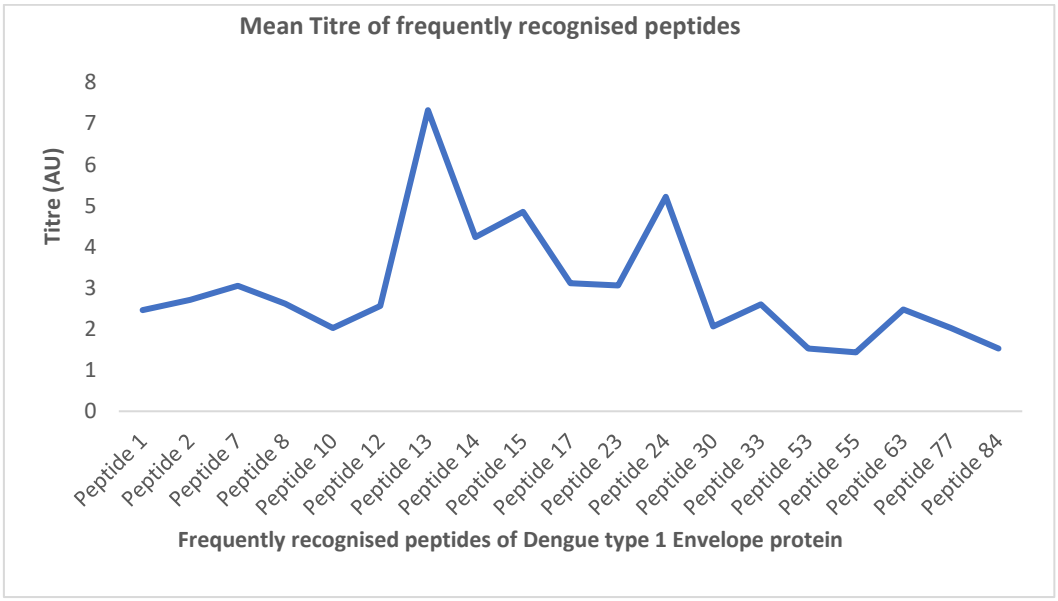


Fig 3: Mean titre in arbitary unit (AU) of 19 frequently recognised peptides of Dengue type 1 Envelope protein.

b) Dengue type 2 - Envelope- protein

Using ELISA ,24 peptides were selected from a panel of 67 peptide array that spans the Dengue type 2 Envelope - protein. Patient immunoglobulins showed higher positivity with these 24 peptides and were considered as most frequently identified peptides (Fig4). The

titre in mean arbitrary units of these patient sera with selected 24 peptides was determined and is presented in Fig5.

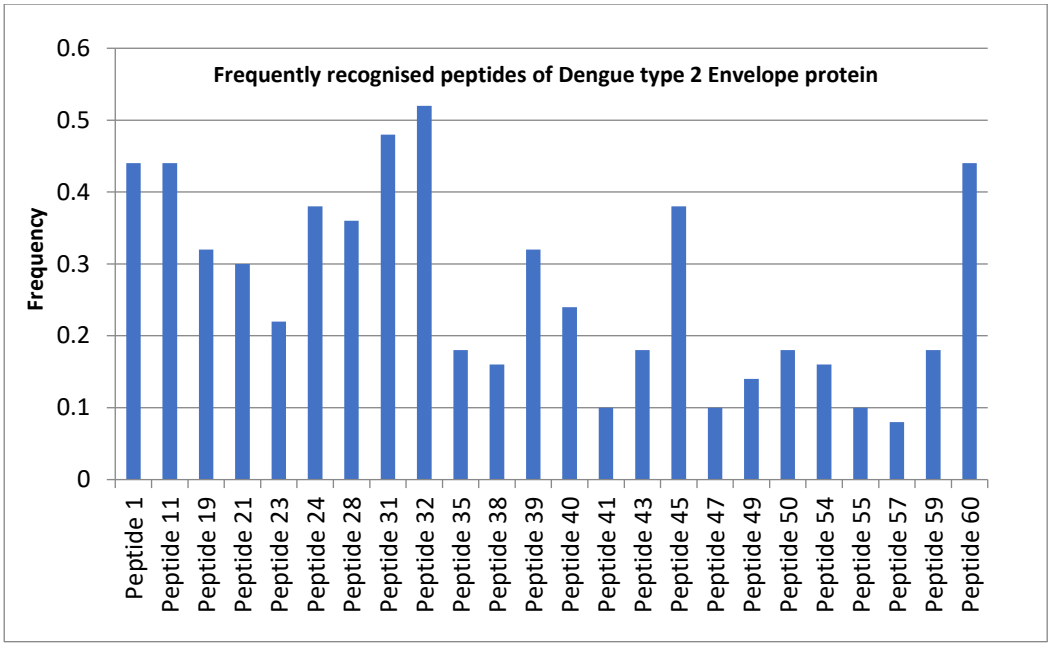


Fig 4: Frequency of patients recognizing the peptides with mean titre (AU)greater than 1.5 fold of cut off.

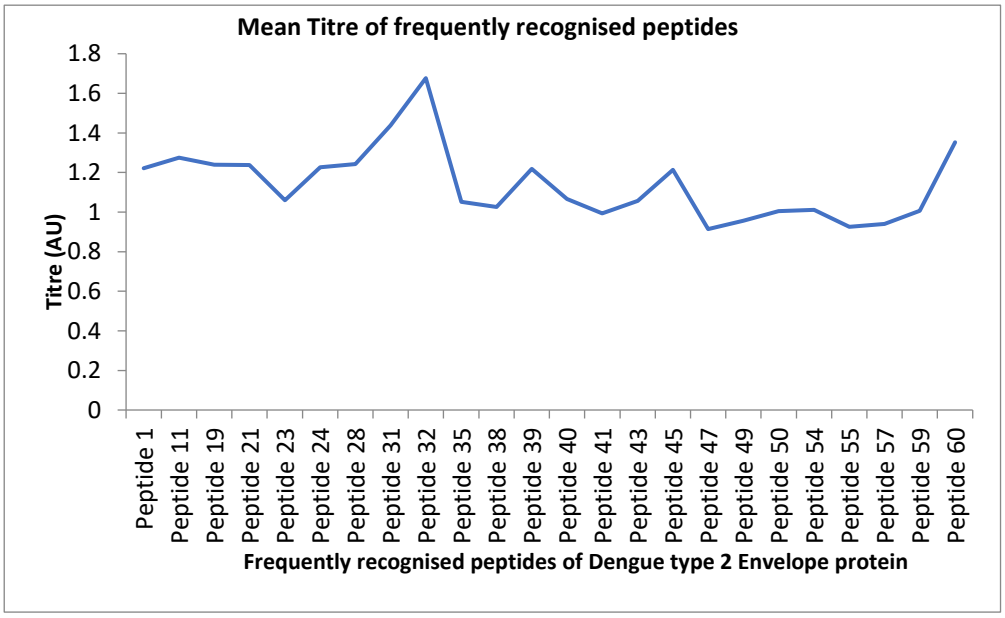


Fig 5: Mean titre in arbitary unit (AU) of 24 frequently recognised peptides of Dengue type 2 of E-protein.

c) Dengue Type 2 - NS 1 protein

Using ELISA ,20 peptides were selected from a panel of 47 peptide array that spans the Dengue type 2 NS1 - protein. Patient immunoglobulins showed higher positivity to these

20 peptides and were considered as most frequently identified peptides (Fig6). The titre in mean arbitrary units of these patient sera with selected 20 peptides was determined and is presented in Fig7.

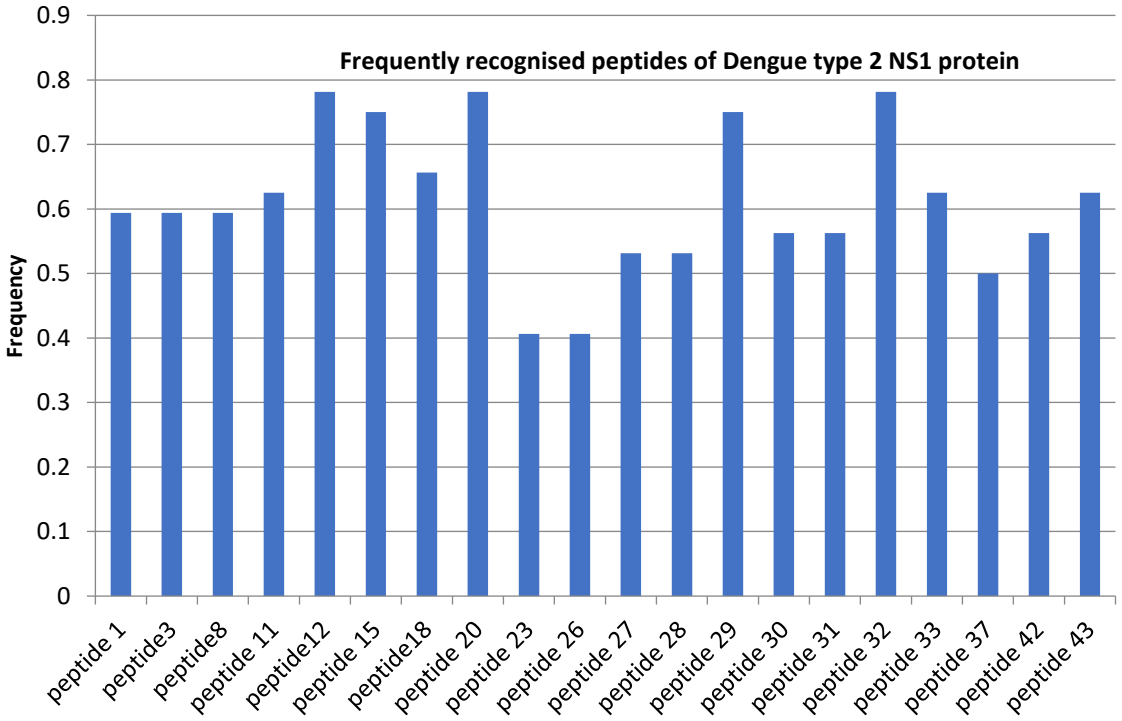


Fig 6: Frequency of patients recognizing the peptides with mean titre (AU)greater than 1.5 fold of cut off.

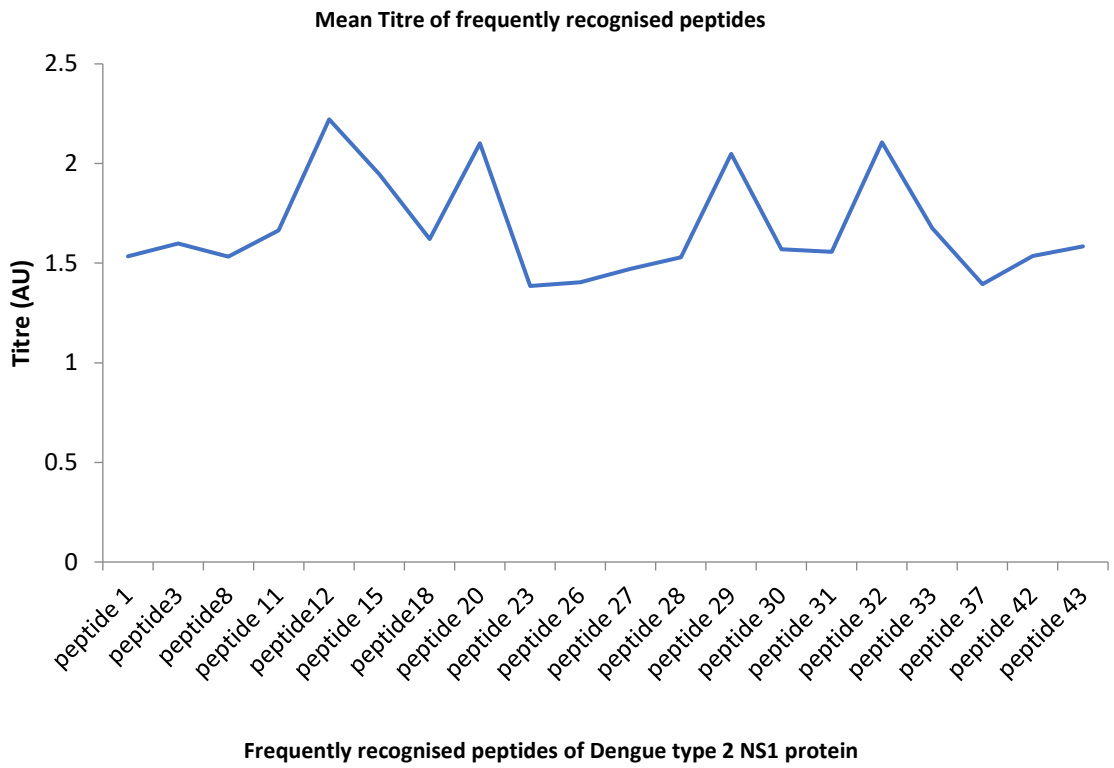


Fig 7: Mean titre in arbitary unit of 20 frequent peptides of Dengue type 2 NS1 protein.

d) **Dengue Type 3 Envelope protein**.

DENV3 Envelope protein peptide array consists of 61 peptide array of 13-17mers with 11 or 12 overlaps. Using ELISA, 27 peptides has been selected from the array. These selected peptides showed higher positivity by patient immunoglobulin (Fig8). The titre in mean arbitrary units of these patient sera with selected 27 peptides was determined and is presented in Fig9.

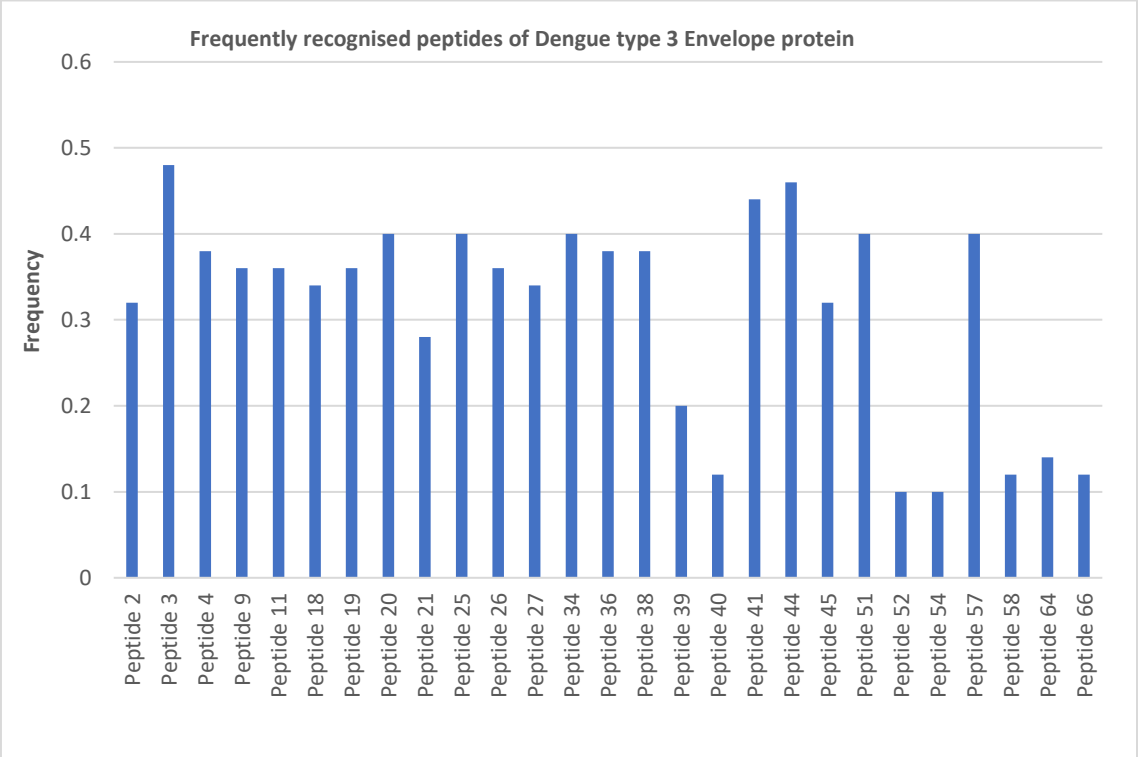


Fig 8: Frequency of patients recognizing the peptides with mean titre (AU)greater than 1.5 fold of cut off.

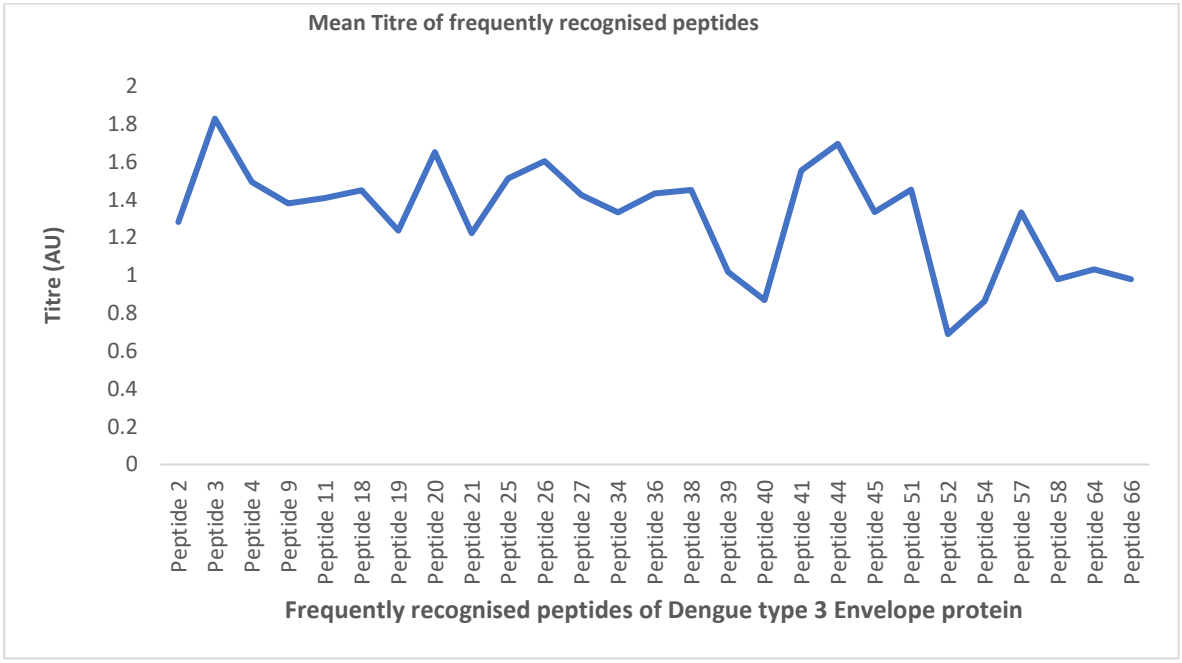


Fig 9: Mean titer in arbitary unit of 27 frequent peptides of Dengue type 3 Envelope protein.

e) Dengue Type 3 NS1 protein.

DENV3 NS1 peptide array consists of 60 peptide array of 13-17mers with 11 or 12 overlaps. Using ELISA, 16 peptides has been selected from the array on basis of frequency of positivity (Fig10). The titre in mean arbitrary units of these patient sera with selected 16 peptides was determined and is presented in Fig11.

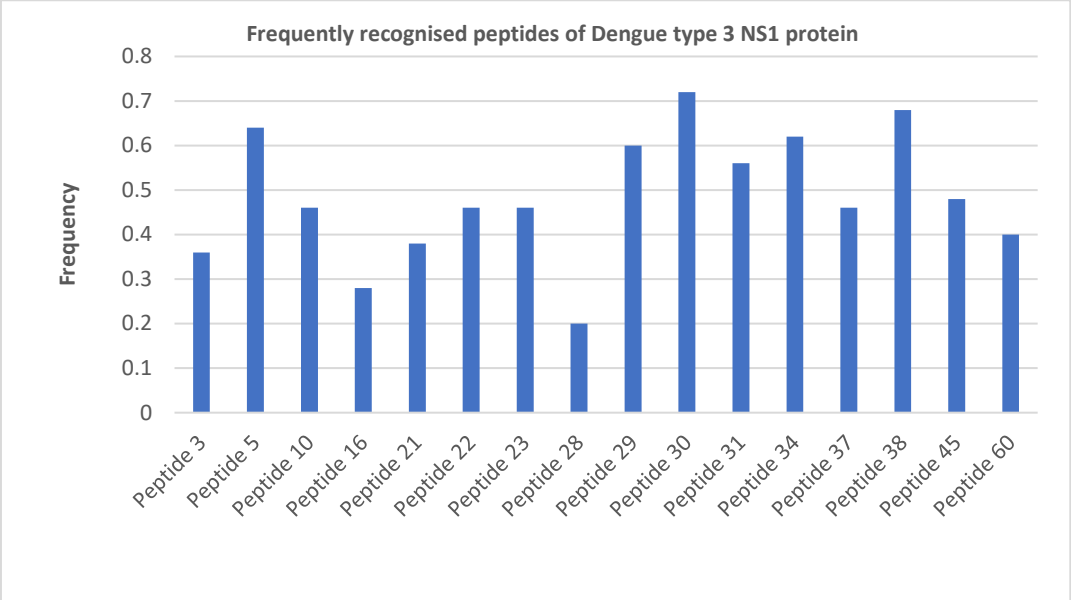


Fig-10: Frequency of patients recognizing the peptides with mean titre (AU)greater than 1.5 fold of cut off.

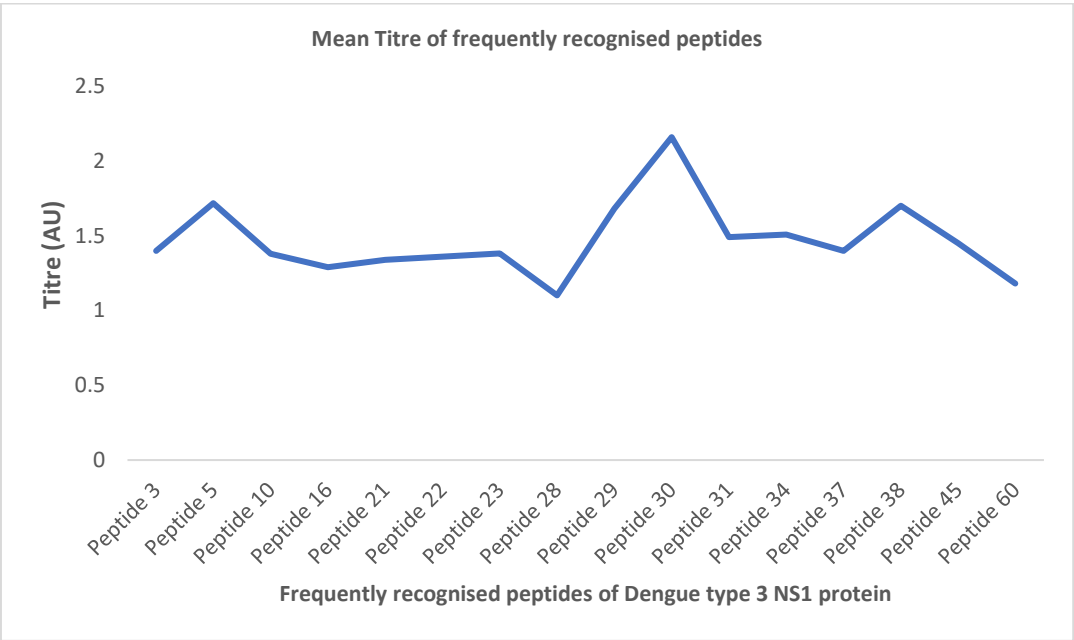


Fig 11: Titre in mean arbitary unit of 16 frequent peptides of Dengue type 3 NS1 protein.

Based on the serotyping data and frequency of seropositivity, our data suggests predominance of DENV1 and DENV2 during the period of sample collection Analysis of peptide sequences that showed seropositivity with high titres, immunodominant regions of E and NS1 of DENV2 and of DENV1 were determined. Bioinformatic analysis revealed the identified amino acid sequences to contain the predicted B cell epitopes.

7. **Towards objective 3**

Polymorphisms of cytokine of the patient RNA and of DC-SIGN and FcγR (Fc receptor for IgG) will be studied by PCR-SSP approach.

To study polymorphism, pro-inflammatory and anti-inflammatory cytokines associated with dengue infection were selected. SNPs associated with dengue infection or other infectious diseases were selected to study the polymorphism of these cytokines. Most of the selected SNPs were in the promoter region of the gene.

Blood samples were collected from dengue positive and healthy controls. Genomic DNA was isolated using QIAamp DNA Blood Mini Kit. ARMS-PCR was employed for the study. SNPs were typed in 29 Dengue positive and 100 control samples.

Table 3: SNPs of some selected cytokines.

Gene name	SNP	Product Size
IL 10 -819	T/C	233/234
IL 10 -1082	A/G	192/193
TNFα -308	G/A	185/185
IL 1β -31	T/C	103/103
IL8 -251	A/T	120/120
TGF β1-509	C/T	350/350

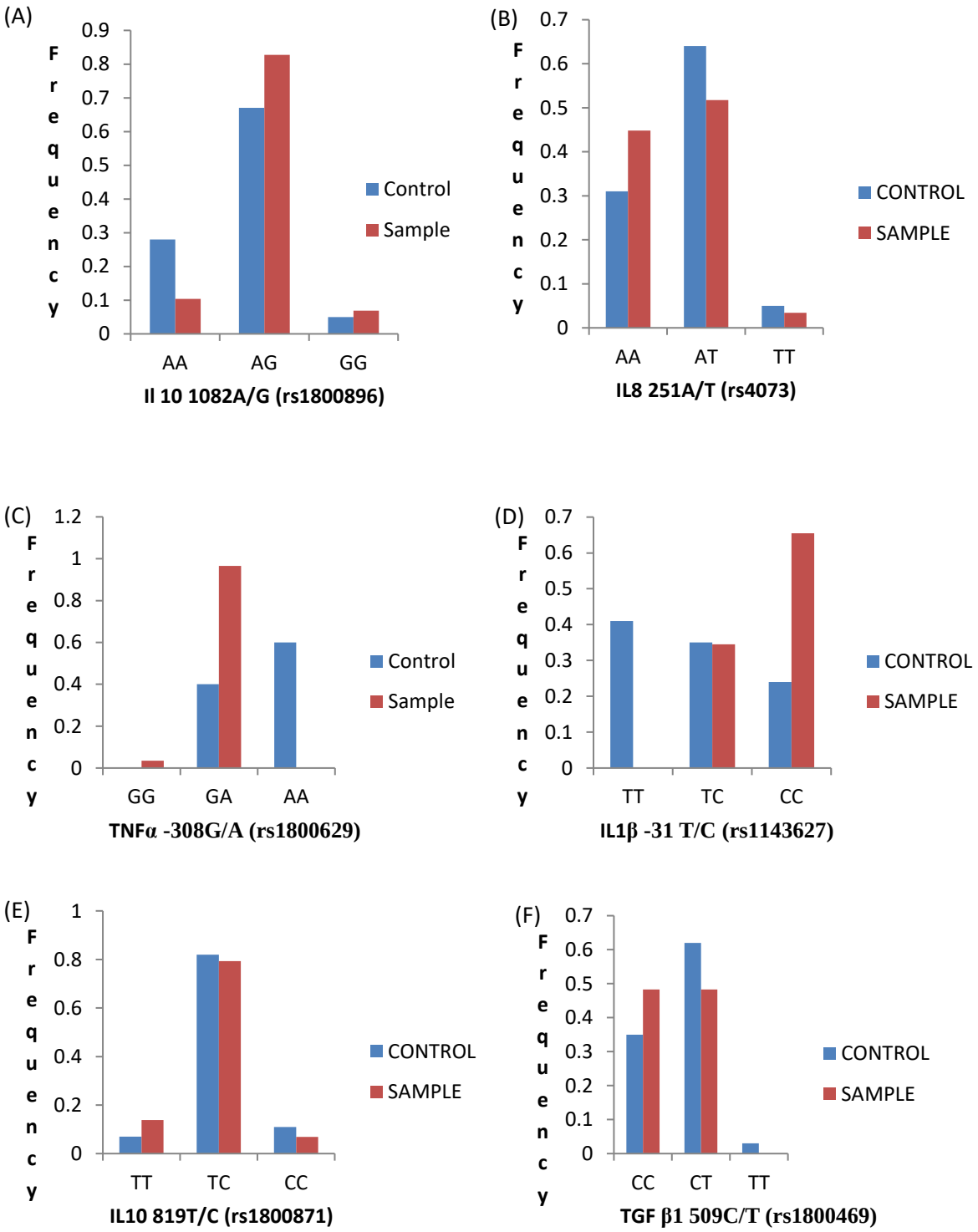


Fig-12 (A-F) : Genotype frequency of polymorphisms studied in the population**.**

Of the six SNPs studied, two SNPS i.e., TNF-308G>A(rs1800629) and IL1β-31 T>C (rs1143627)) were found to be differ in frequency between case and control (p-values <0.0001). In TNF-308G>A(rs1800629), the G allele was seen at higher frequency in cases while the A allele was at higher frequency in control participants. Similarly, in IL1β-31 T>C

(rs1143627), the C allele was seen at higher frequency in dengue patients while the T allele was higher in control participants. Notably the variant C allele is reported to result in higher levels of IL1β.

8. Towards objective 4

a) Determination of Cytokine and Chemokine expression in Dengue patients

I . Inflammatory Cytokine

Expression of inflammatory cytokines were studied with the help of Human Inflammatory Cytokine Cytometric Bead Array Kit (CBA, BD Bioscience) using the bench top flowcytometry system, BD Accuri C6 Plus (BD Biosciences). Mean levels of cytokines in case (n=30) and control serum (n=5) were compared (Fig-13). Significant increase in the levels of interleukin 8 (IL8), interleukin 6 (IL6) and interleukin 1β (IL1β) is suggestive of monocyte- neutrophil based activation of the innate immune system.

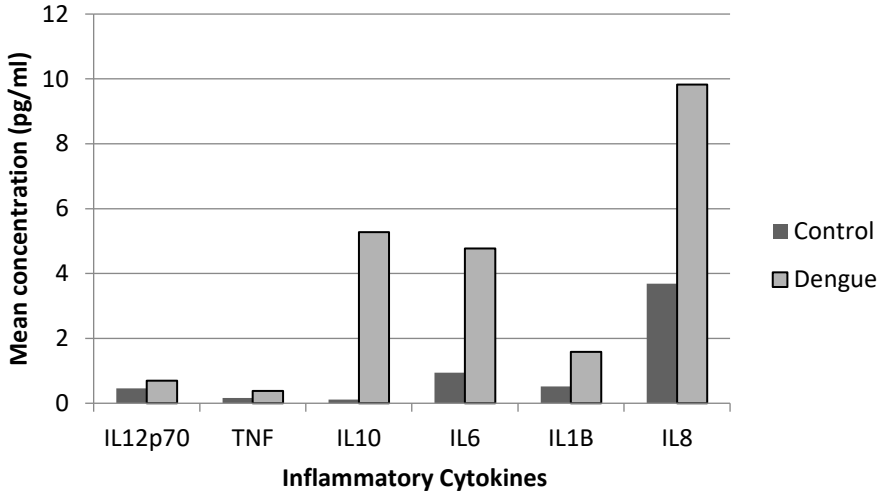


Fig-13: Comparative analysis of protein expression profiles of Inflammatory Cytokines between dengue cases and healthy controls.

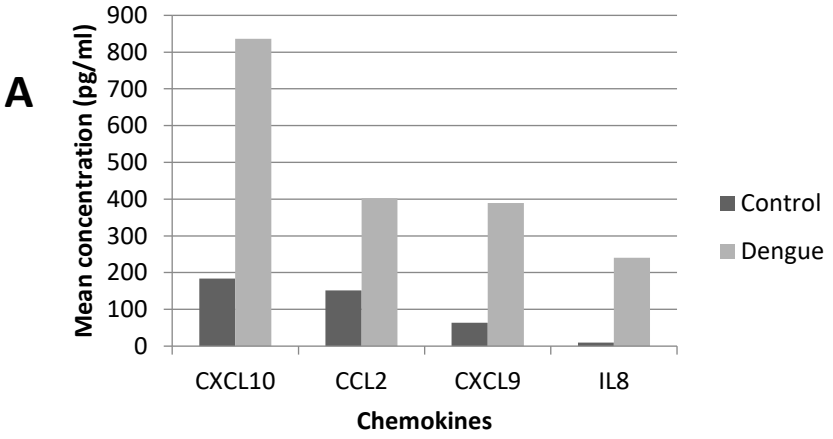
Correlation analysis between the levels of inflammatory cytokines in patient serum revealed a significant positive correlation between IL12p70 and TNF, IL6, IL1β and IL8 whereas no correlation was seen between IL10 and any of the studied cytokines. Expression level of IL8, IL6 and IL1β showed significant correlation with p -value <0.0001, TNF and IL12p70 expression showed significant correlation with p-value <0.0005.

Table 4: Inflammatory Cytokine Expression Correlation Matrix (Pearson)

Variables	IL12p70	TNF	IL10	IL8	IL6	IL1B
IL12p70	**1**	**0.619**	0.144	-0.110	-0.087	-0.125
TNF	**0.619**	**1**	0.223	-0.041	-0.010	-0.034
IL10	0.144	0.223	**1**	0.330	0.218	0.129
IL8	-0.110	-0.041	0.330	**1**	**0.986**	**0.969**
IL6	-0.087	-0.010	0.218	**0.986**	**1**	**0.994**
IL1B	-0.125	-0.034	0.129	**0.969**	**0.994**	**1**

II . Chemokine

Expression of chemokines were studied with the help of Human Chemokine Cytometric Bead Array Kit (CBA, BD Bioscience) using the bench top flow cytometry system, BD Accuri C6 Plus (BD Biosciences). Mean levels of chemokine in case (n=30) and control serum (n=5) were compared (Fig-14).



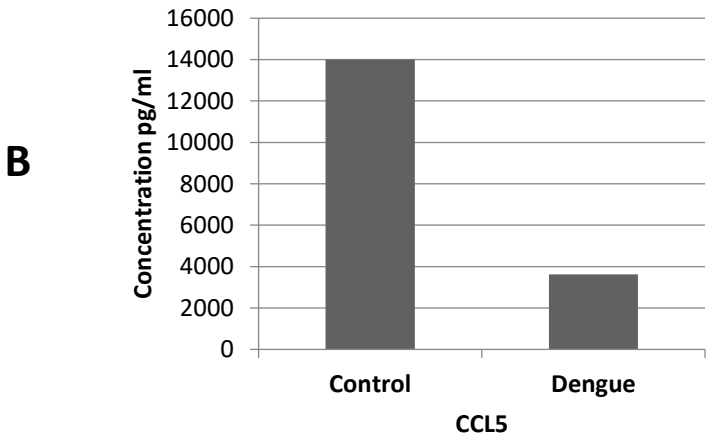


Fig-14: Comparative analysis of protein expression profiles of (A) Chemokines (CXCL10, CCL2, CXCL9 and IL8) between dengue cases and healthy controls. (B) CCL5 between DENV cases and healthy controls

High expression levels of CXCL10, CXCL9, CCL2 and IL8 were seen in the dengue patients, whereas expression levels CCL5 were significantly lower in dengue patients. High levels of CXCL10, CXCL9, CCL2 and IL8 suggests activation of the inflammatory axis as well as recruitment and activation of NK and T cells of adaptive immune response.

Correlation analysis between the levels of chemokines in patient serum revealed a significant positive correlation between CXCL10 and CXCL9, CCL2 and IL8 whereas no correlation was seen between CCL5 and any of the studied chemokine (Table 6). Expression level of CXCL10 and CXCL9 showed significant correlation with p -value <0.0001, TNF and IL12p70 expression showed significant correlation with p-value <0.0005 .

Table 6: Chemokine Expression Correlation Matrix (Pearson)

Variables	CXCL10	CXCL9	CCL5	CCL2	IL8
CXCL10	**1**	**0.535**	-0.032	-0.050	0.016
CXCL9	**0.535**	**1**	0.113	0.035	0.063
CCL5	-0.032	0.113	**1**	0.233	0.173
CCL2	-0.050	0.035	0.233	**1**	**0.985**
IL8	0.016	0.063	0.173	**0.985**	**1**

Our data with higher levels of proinflammatory cytokines and chemokines suggests activation on monocyte-neutrophil axis (IL-8, IL-1, IL-6) with activation of innate immune responses. Upregulation of IFN-gamma inducible chemokines CXCL9, CXCL10, suggests involvement and polarization of T cells to TH1 phenotype. However, given low levels of IL-12 , it is probable that these chemokines are upregulated by Type I interferons. As none of the recruited patients exhibited severe DENV disease, determination of markers of disease severity could not be established.

b) Determination of Monocyte and Dendritic cells population by Flow cytometry

Analysis of differential Monocyte (Classical, Intermediate and Non-Classical) and Dendritic (Classical and Plasmacytoid) cell (DC) population in dengue virus infected patients' blood has been studied using monocyte specific cell surface marker such as CD14 and CD16 and Dendritic cell specific surface marker like CD123, CD11c and CD1c. Cell population were analysed using the Table top Flow Cytometer, BD Accuri C6 plus table-top Flow-Cytometer (BD Biosciences).

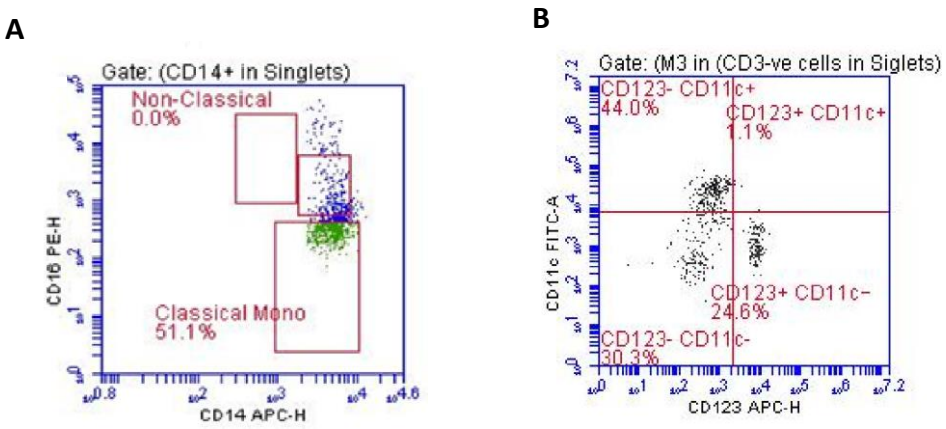


Fig-15 : Flow Cytometric Analysis of A) Differential Monocyte population in blood sample of dengue patient B) Differential Dendritic Cell population in blood sample of dengue patient

Differential cell population has been studied in 12 dengue patient samples. Monocyte population was predominantly CD14++ classical monocytes and the intermediate CD16+ CD14+. The classical monocyte population exhibits phagocytic function and secretion of proinflammatory cytokines, while intermediate population is reported to have a role in antigen presentation and secretion of cytokines, suggesting activation of inflammatory responses and probable priming of T cells for adaptive immune responses. The dendritic cell population appeared to be shift toward increased plasmacytoid DC (pDCs) population, that are reported to secrete interferons during viral infections as well as proinflammatory cytokines and chemokines.

Conclusion:

Higher levels of proinflammatory cytokines together with higher levels of monocyte-neutrophil recruiting chemokines and proinflammatory phenotype of peripheral monocytes and dendritic cells suggests of a role for monocyte-neutrophil axis to play a major role during dengue virus infection mediated inflammation. Increased plasmacytoid DC (pDCs) population, that are reported to secrete interferons during viral infections as well as proinflammatory cytokines and chemokines suggests the virus to be tropic for dendritic cells.

Constraints of the study

Peripheral PHC, CHC collected serum and we were informed that samples were sent to RMRC FOR TYPING, hence it was difficult to perform assays that required whole blood. Besides, the peripheral PHC, CHC were storing serum at 4 degrees centigrade, which is not appropriate for RNA stability. Secondly, this project was sanctioned in the month of September 2017 and sample collection could only be initiated in the month of November/December when transmission of DENV was negligible So only 2-3 few fresh samples could be collected in the same year from TMC&H. Majority of the samples was collected in 2017-2018 were mainly stored samples of previous year as transmission was low in 2018.With decrease in incidence rate in the state and also due to the SARS-CoV2 pandemic, sample collection was difficult. However, we could manage to collect 6 new positive serum samples in 2020.

During lock downs due to SARS-CoV-2 pandemic that affected functioning of academic institutes, project work was affected both with respect to sample collection and laboratory work.

It was not possible to determine markers of DHF as all DENV positive hospitalised patients were identified not to suffer from DHF.