Progress Report for R&D Projects
[Final report, 2017]

Section-A: Project Details

A1. Project Title: : Systemic Lupus Erythematosus (SLE) - An investigation into diagnostics and disease pathogenesis/DBT-Twinning

A2. DBT Sanction Order No. & Date: No. DBT-NER/Health/38/2013 dated 06/05/2015

A3. Name of Principal Investigator: Dr. Shashi Baruah, Tezpur University, Tezpur
Name of Co-PI/Co-Investigator: Prof. Sanjeeb Kakati, Dr Anita Nadkarni, Dr. Vandana D. Pradhan

A4. Institutes: Tezpur University, Assam Medical College and Hospital & National Institute of Immunohaematology (ICMR), Mumbai

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A6. Total Cost: 207.24 lakhs

A7. Duration: 3 years

A8. Approved Objectives of the Project:

1. Establish an 'Autoimmune Diagnostic Centre' and SLE Registry, Serum and DNA bank (AMC&H, TU) at Tezpur University. Basic clinical diagnosis laboratory will be at Assam Medical College (AMC&H, NIIH, TU)*

2. To compare SLE clinical presentation, disease severity and immunological parameters (IC, Complement, ANA, anti-dsDNA, cytokines, macrophage phenotyping, CRP etc) among SLE patients of Eastern and Western parts of the country (NIIH, AMC&H, TU)*.

3. Determine markers for resolution between SLE related flares and active infections in SLE patients. (AMC&H,TU)*.

. **A9. Specific Recommendations made by the Task Force (if any):** NIL

Section-B: Scientific and Technical Progress

B1. Progress made against the Approved Objectives, Targets & Timelines during the Reporting Period

Our preliminary study on SLE at Rheumatology Clinic, AMC&H, Dibrugarh revealed that 30-40% of the total patients attending the clinic had SLE related symptoms. Majority (48%) of these patients were young girls of Tibeto-Burman population and reported to have kidney related complications. This was in contrast to reports on SLE patients from Western India where renal complications were observed to be more frequent in older patients. Our data suggested an early onset as well as complex disease pathogenesis in patients of SLE from Assam. Given this background and that Autoimmune Diagnostic facility and expertise in the region was minimal, Phase I of the project was conceived with the following objectives.

Progress against the Objectives:

Objective 1: Establish an 'Autoimmune Diagnostic Centre' and SLE Registry, Serum and DNA bank (AMC&H, TU) at Tezpur University. Basic clinical diagnosis laboratory will be at Assam Medical College (AMC&H, NIIH, TU)*.

Training and quality control:

NIIH Mumbai conducted hands on training and quality control programmes for clinical and research staff of AMC&H and TU as detailed in Table1.

Table 1: Details of training programmes organized

Sl. No.	Venue	Date
1.	AMC&H, Dibrugarh	29th September to 2nd October, 2015
2.	Tezpur University, Tezpur	7th June to 9th June, 2016
3.	NIIH, Mumbai	27th June to 1st July, 2016

The first training program was hands on for detection of Anti-nuclear antibodies (ANA) and anti-dsDNA antibody using IFA (Immuno-fluorescence Assay). This was followed by viewing of IFA Slides and interpretation of results. Identification of the respective auto-antibodies in ANA positive sera using ANA BLOT was also performed.

In the 2nd training, IFA of ANA and dsDNA antibody and BLOT were performed by the project staff of Tezpur University on the control sera provided by NIIH. The objective of this training was to assess the quality of performing these techniques as well as data interpretation by the project staff.

In the 3rd training, the project staff of Tezpur University received hands on training on nephelometer for quantification of C3, C4.

Samples and the SLE diagnosis interpretation data were exchanged between TU and NIIH to evaluate the quality of training and it was found to be satisfactory.

Establishment of diagnostic facility: An auto-immune diagnostic laboratory was established in AMC&H for diagnosis and enrolment of patients. Similar set up was also established in Department of MBBT, Tezpur University to extend diagnostic facilities to Tezpur Medical College and Hospital (TMC&H) and local clinicians of Tezpur.

DNA bank and SLE Registry: The registration of the 145 SLE patients in the SLE Registry of Tezpur University has been completed. DNA of all patients was extracted, catalogued and stored at -80°C.

The mean DNA yield was 6.68 ± 5.01 mg and DNA purity (A260/A280) was 1.6 ± 0.12.

Patient follow-up details: Serum and RNA samples of the enrolled patients were received upto four follow-ups as detailed in Table 2.

Table 2: Details of samples

	No. of patients (n)				
	F0	F1	F2	F3	F4
Total	145	76	62	55	33
Serum	145	65	60	53	33
RNA sample	80	62	57	53	33
Clinical data	145	76	62	54	33
Blood R/E	127	60	58	45	31

Urine R/E Biochemical analysis	118 140	64 74	48 61	52 54	30 33
Cumulative mortality		8	12	15	16

The mean± SD age of mortality of the patients was 25±8.8 years. In majority of the cases mortality was associated with infection (33.3%) and renal complications (25%). In 33.3% cases, renal complications along with infection were found to be associated with mortality.

Objective 2: To compare SLE clinical presentation, disease severity and immunological parameters (IC, Complement, ANA, anti-dsDNA, cytokines, macrophage phenotyping, CRP etc) among SLE patients of Eastern and Western parts of the country (NIIH, AMC&H, TU)*.

Patient characteristics: A total of 145 patients were enrolled at AMC&H which serves the districts of Upper Assam region and a few places of Eastern Arunachal during the period of September 2015 to June 2017.

Table 3: Characteristics of SLE patients from Assam

Patient Characteristics	
No. of patients	145
Age at evaluation (Mean)	24 Yr. (12-49Yrs)
Disease duration (Median)	1Yr. (0-15 Yrs)
Male :Female ratio	1:28
Linguistic groups Indo-Aryan Tibeto-Burman Austro-Asiatic	 33.8% 59.3% 6.9%
SLEDAI Median Range Mild	 12 0-31 25.5%

Moderate	54.5%
Severe	20%

No of Tibeto-Burman patients was high in the patient group as this is the dominant population of the catchment area. Majority of them had school level education while only a few were graduates. 35.9% of the patients belonged to lower socio-economic group. Most patients enrolled in the study had a history of disease and had been seeking various treatments. Only 13.7% patient was newly diagnosed during the time of enrolment.

Clinical manifestations: Clinical examinations and laboratory investigations of the patients were done at the time of enrolment and during each follow -up. The findings were recorded in predesigned questionnaires/profoma. SLEDAI (SLE Disease Activity Index) was calculated as per ACR (American College of Rheumatology) criteria. Incidences of mucocutaneous complications (Skin rash, photosensitivity, hyperpigmetation and oral/nasal ulcer) and arthritis of the enrolled patients were found to decrease in follow-ups (Fig.1). However, occurrence of renal and haematological complications remained consistent during the follow-ups, suggesting renal and haematological manifestations to be the major SLE related complications in our population.

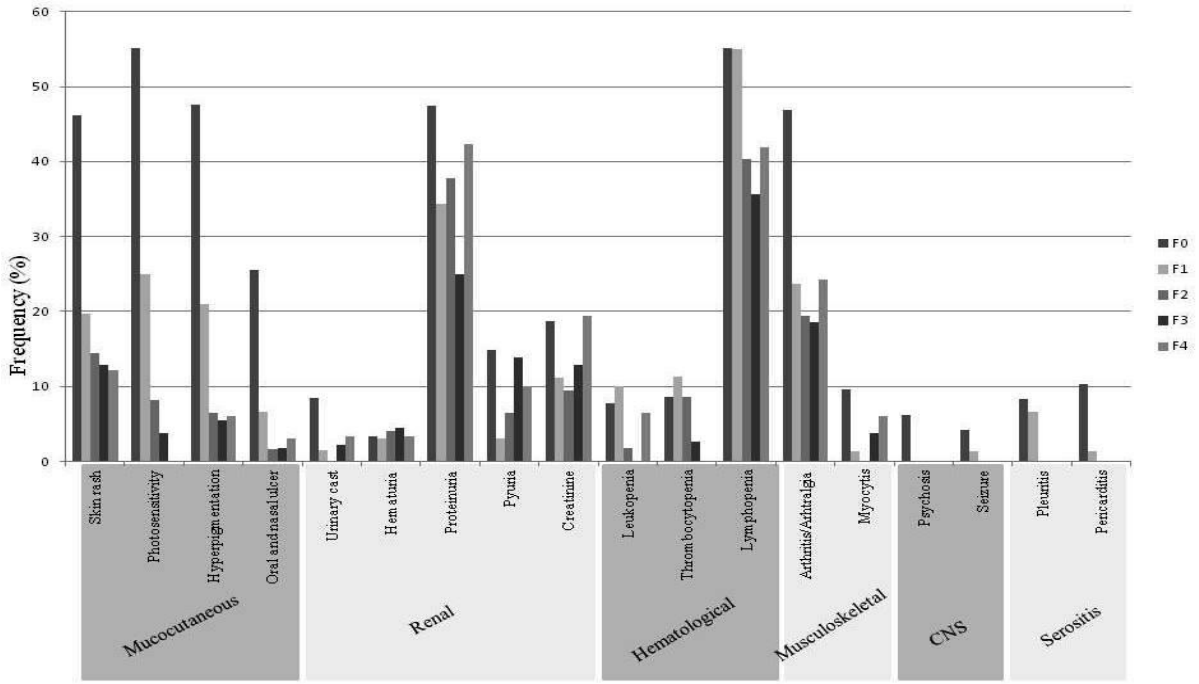
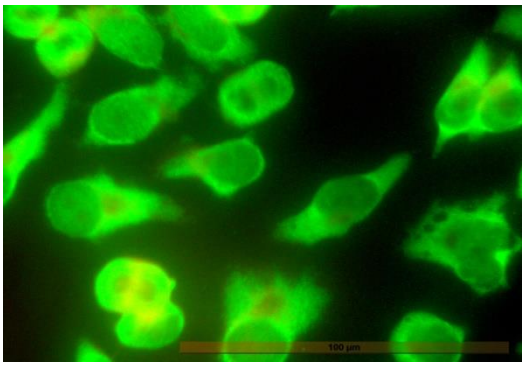
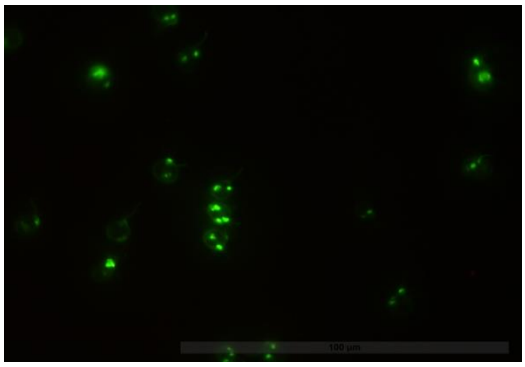


Fig.1: Frequencies of clinical manifestations of SLE patients from Assam during enrolment and up to 4 follow-ups

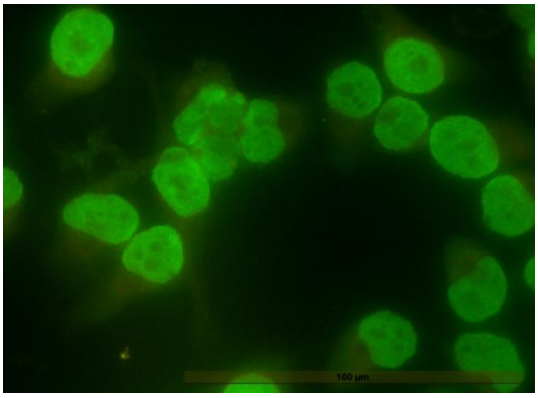
Anti-nuclear autoantibodies (ANAs): The patients were screened for Antinuclear Antibody (ANA) positivity using Immuno-florescence assay (Fig.2). Different ANA patterns of each individual were identified using ANA-BLOT. ANA-IFA patterns are presented in Fig.2.



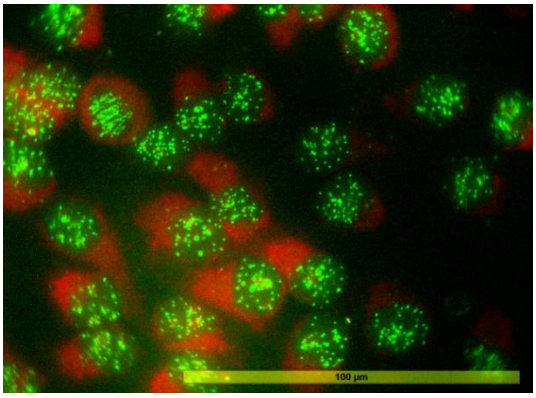
a. Homogenous pattern



b. ds-DNA positive



c. Coarse Speckled pattern



d. Centromeric pattern

Fig.2: Representative ANA-IFA pattern of the samples

All the patients were positive for ANA-IFA. The titer of ANA-IFA was lower than or equal to 1:160 dilution in 61.3% cases, which is considered low as 1:80 dilution taken as the cut-off.

Table 4: ANA-IFA titre of SLE patients from Assam

Auto-antibody	Frequencies (%)
ANA titer	
1:80	30.3
1:160	31.0
1:320	22.8
≥1:640	15.9

Analysis of ANA-Blot results showed anti-dsDNA antibody was the most frequent auto-antibody followed by anti-SSA antibody in the study cohort (Fig. 3). Correlation of disease activity with presence of anti-dsDNA (**r=0.221, p=0.008**), anti-nucleosome (**r=0.301, p=0.0002**) and anti-histone antibody (**r=0.225, p=0.006**) was observed suggesting possibility of using these antibodies as marker of active disease.

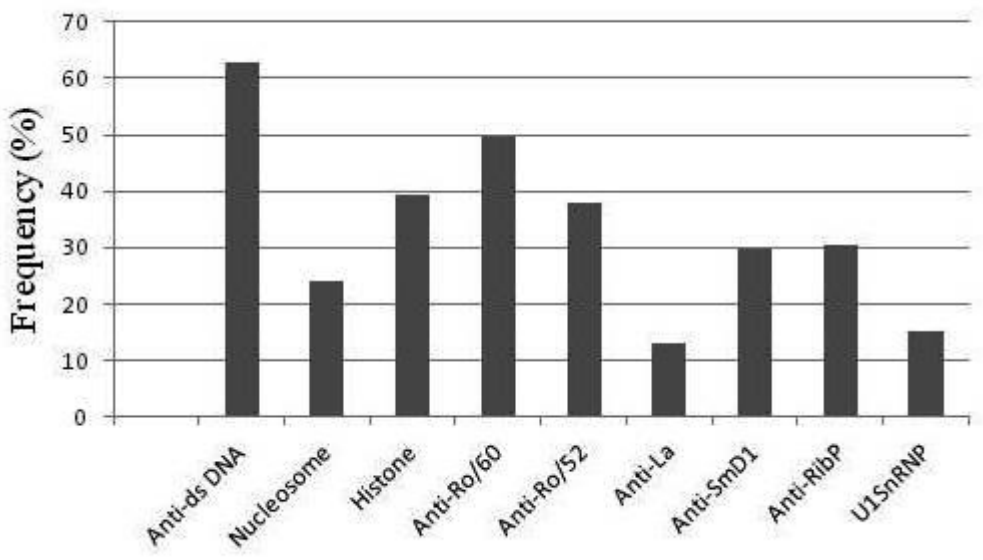
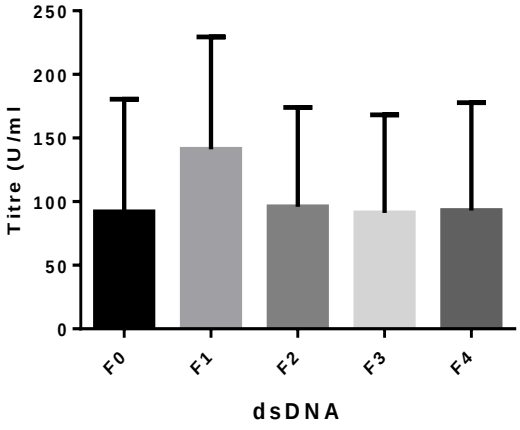
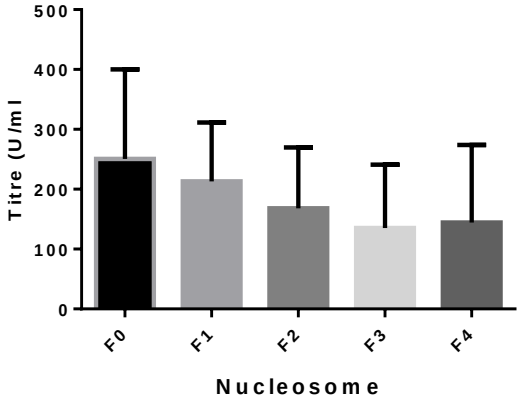


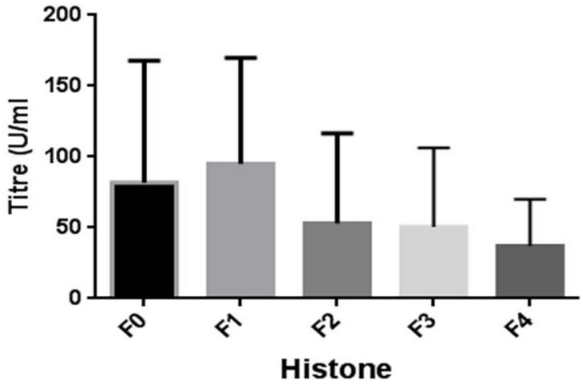
Fig.3: The frequencies of auto-antibodies of SLE patients from Assam

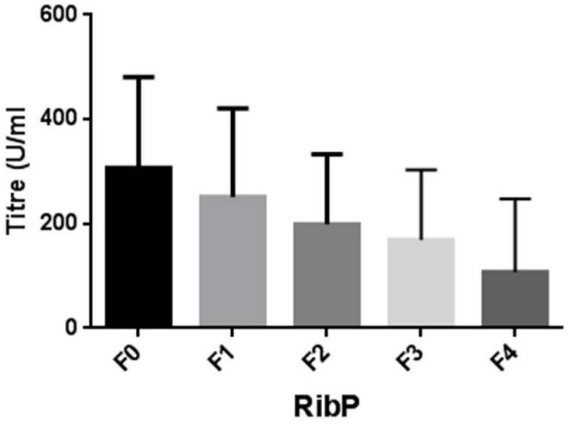
Auto-antibody titres: Presence of SLE specific auto-antibodies; anti-dsDNA, anti-nucleosome, anti-histone, anti-smith (SmD1), anti-RibP, anti-SSA and anti-SSB in patient's serum were identified by ANA –Blot. The titre of these antibodies were determined by using respective ELISA kits (Imtec-ELISA kits, Human Diagnostics Worldwide, Germany for anti-dsDNA, anti-nucleosome, anti-histone, anti-smith (SmD1), anti-SSA and anti-SSB and Askeulisa kits,

Askeu.Diagnostics, Germany for anti-RibP antibodies). These antibody titres were determined in the enrolment as well as in all the four follow-ups.









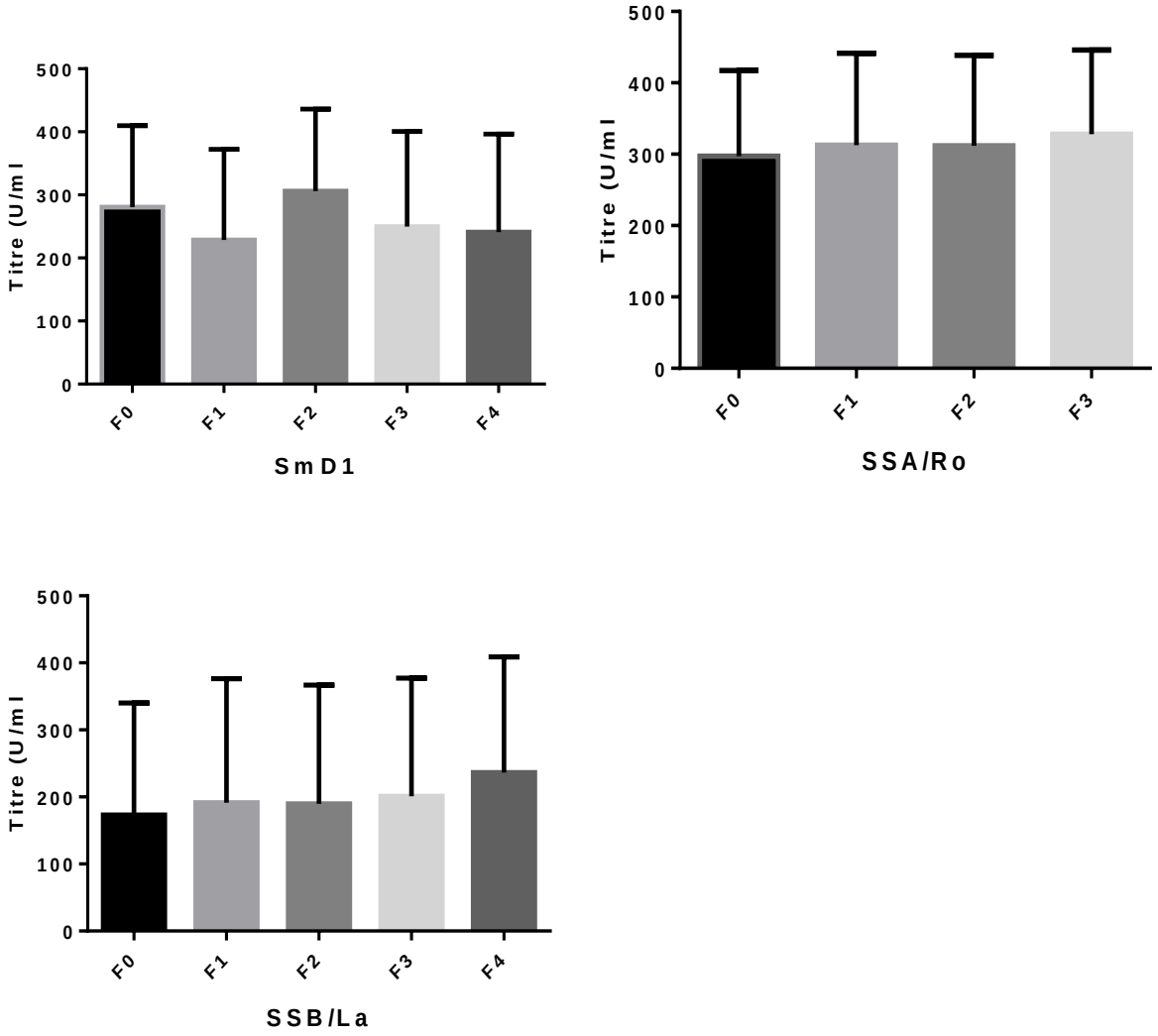


Fig.4: Titres (mean ± SD) of auto-antibodies in serum during enrolment and follow up

Correlation analysis of ELISA titers of the auto-antibodies in enrolment and the four follow-ups with SLEDAI showed weak positive correlation of dsDNA (**r= 0.324, p= <0.0001**), nucleosome (**r= 0.298, p= <0.0001**), and histone (**r= 0.244, p= <0.0001**) titers with SLEDAI.

Anti-dsDNA antibody titer showed weak positive correlation with oral/nasal ulcer(**r=0.242, p=<0.0001)** and creatinine levels (**r=0.243, p=<0.0001)** of the patients. Anti-nucleosome antibody titer correlated with oral/nasal ulcer (**r=0.270, p=<0.0001)** creatinine (**r=0.217, p=<0.0001)**, pleuritis (**r=0.239, p=<0.0001)** and thrombocytopenia (**r=0.211, p=<0.0001)**. Weak positive correlation of pleuritis was also observed with anti-histone antibody titer (**r=0.204, p=<0.0001)** . Skin rash was found to be associated with anti-RibP antibody titer (**r=0.206, p=<0.0001)**.

<u>Comparison of disease profile of SLE patients from Assam with other Indian and world populations</u>: The disease profile of SLE patients form Assam was compared with other regions of India and rest of the world using the statistical tool Agglomerative Hierarchical Clustering (AHC). Patient cohorts from different regions were clustered based on their clinical profiles and anti-dsDNA antibody frequencies. The clinical parameters considered for this analysis were skin rash, oral/nasal ulcer, photosensitivity, arthritis, serositis, CNS and renal complications.

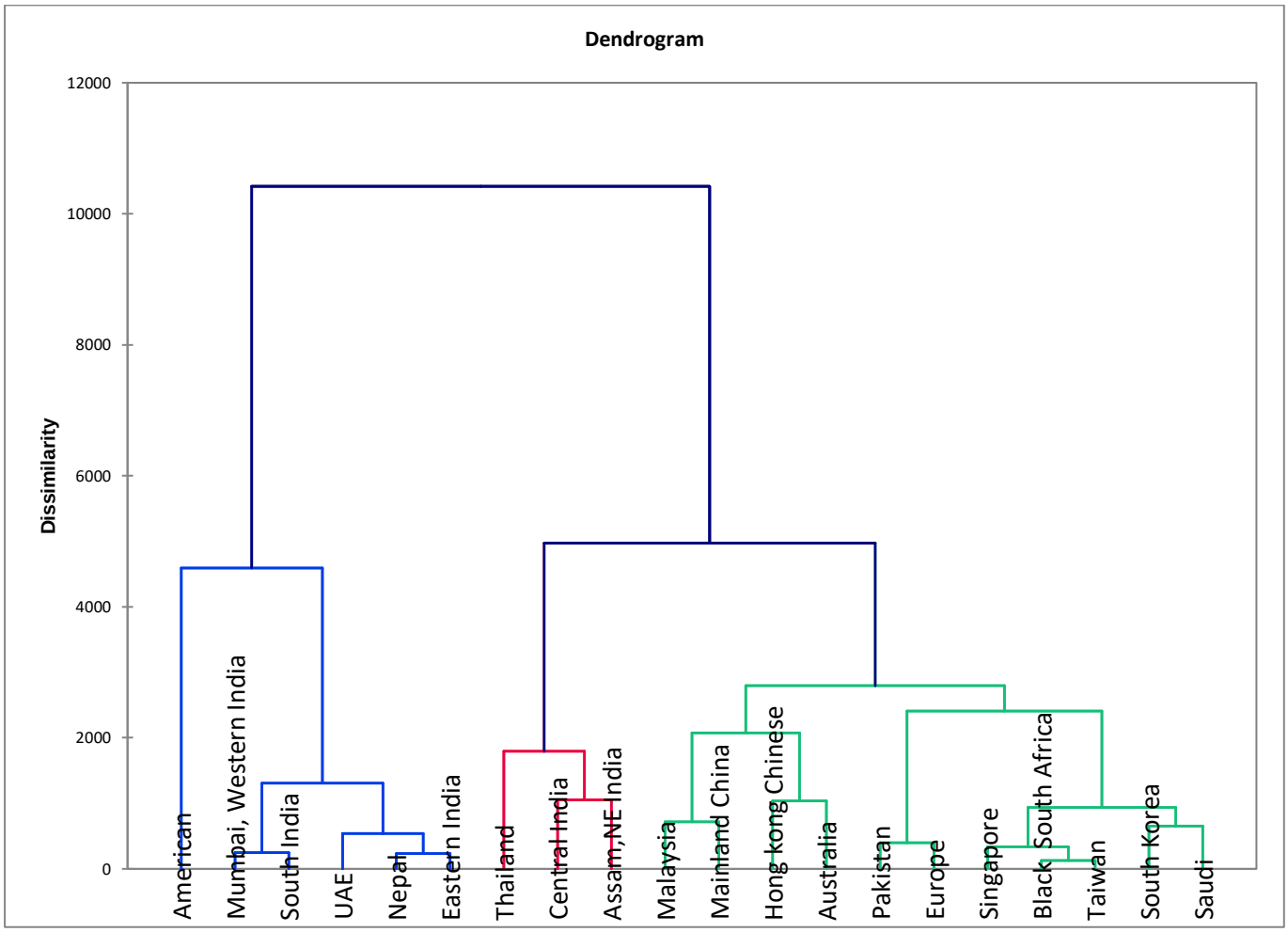


Fig.5: Dendrogram of Agglomerative Hierarchical Clustering of SLE patient cohorts from different regions of the world.

Agglomerative Hierarchical Clustering of SLE patients from Assam with other countries revealed two major groups. The patients from Assam were clustered along with Central India in the group with the South- East Asian countries. However, the patients from other parts clustered in a completely distinct group.

<u>C3 and C4 levels:</u> Serum C3 and C4 levels were determined by using nephelometer. C3 and C4 levels were low in 38.57% and 47.10% patients respectively. Serum C3 (**p=<0.0001**) and C4

(**p=0.0001**) levels were markedly low in patients with active disease in comparison to patients with inactive disease.

Clustering of patients from Assam using autoantibody titres revealed four-cluster system to have the maximum resolution among the clusters. Cluster I patients could be characterized by high titers of autoantibodies against ENAs (RibP, SmD1 and SSA). In Cluster II, the titer of autoantibody against only SSA, an ENA was high. Cluster III patients had comparatively low titers of all the antibodies in comparison to other clusters. On the other hand, cluster IV patients had high titers of antibodies against chromatin antigens (dsDNA and NUC) as well as the ENA, RibP.

No significant difference of clinical features could be observed among the first three clusters. Cluster IV with high titres of anti-dsDNA, anti-Nuc and anti-RibP autoantibodies had relatively complex disease presentation in comparison to other clusters. The frequencies of CNS manifestations (vs cluster III, **p= 0.05**, musculoskeletal complications (vs cluster II, **p= 0.036**), serositis (vs cluster II, **p= 0.05** and cluster III**, p= 0.024**) and oral/nasal ulcer (vs cluster I, **p= 0.048**; cluster II, **p= 0.022** and cluster III, **p= 0.015**) of this cluster were markedly higher.

Table 5: Clusters of patients using autoantibody titres: The titers of autoantibodies and distribution of clinical manifestations between clusters were compared by Mann-Whitney test and Fisher's exact test respectively. P-value <0.05 was considered as significant (Bold).

					P-value between individual clusters						
	C -I	C-II	C-III	C-IV	C-I vs C-II	C-I vs C-III	C-I vs C-IV	C- II vs C-III	C- II vs C-IV	C- III vs C-IV	Overall p-value
	1 (n = 32)	2(n = 44)	3 (n = 55)	4 (n = 14)							
Autoantibody	Mean titer (U/ml)										
Anti-dsDNA	54.72	54.93	45.75	185.52	1	0.358	**<0.0001**	0.369	**<0.0001**	**<0.0001**	**<0.0001**
Anti-Nuc	61.93	12.84	22.49	391.66	**0.028**	0.36	**<0.0001**	0.155	**<0.0001**	**<0.0001**	**<0.0001**
Anti-SmD1	365.49	0.29	18.86	29.06	**<0.0001**	**<0.0001**	**<0.0001**	**0.017**	**0.012**	0.678	**<0.0001**
Anti- RibP	118.42	86.74	50.10	225.78	0.247	**0.009**	0.124	0.127	**0.026**	**0.002**	**0.005**
Anti-SSA	191.88	341.23	11.55	98.94	**0.0003**	**<0.0001**	0.095	**<0.0001**	**<0.0001**	0.056	**<0.0001**
Anti-SSB	26.25	47.70	5.81	14.85	0.072	0.077	0.165	**<0.0001**	0.945	**0.005**	**0.001**

Clinical manifestations	Frequencies (%)										
CNS Score	18.75	25.00	12.73	35.71	0.586	0.537	0.269	0.29	0.499	0.057	0.195
Musculsk Score	46.88	45.45	49.09	78.57	1	1	0.059	0.839	**0.036**	0.071	0.166
Renal Score	51.85	55.56	50.00	70.00	0.803	1	0.461	0.658	0.488	0.309	0.706
NEW Rash	46.88	45.45	43.64	57.14	1	0.825	0.749	1	0.545	0.387	0.841
Ulcer	25.00	22.73	20.00	57.14	1	0.6	**0.048**	0.807	**0.022**	**0.015**	**0.038**
Serositis	18.75	11.36	9.09	35.71	0.511	0.203	0.269	0.747	**0.05**	**0.024**	0.066
Hematological	50.00	60.53	54.35	75.00	0.464	0.815	0.18	0.659	0.497	0.324	0.473

ESR and CRP levels: The Erythrocyte Sedimentation Rate (ESR) of the patients with active disease was observed to be higher (**p=0.006**) than that of patients with inactive disease. ESR levels showed negative correlation with both C3 (**r= -0.307, p= 0.001**) and C4 **(r= -0.319, p= 0.001**), suggesting role of complement component in inflammation. The mean levels of ESR in patients with active and inactive disease were 66.88mm/hr and 49.6mm/hr respectively. The mean levels of C3 were 125.87mg/dL and 80.33 mg/dL while C4 were 22.47 mg/dL and15.359 mg/dL in the patients with active and inactive disease. No difference of C-reactive Protein (CRP) levels in between patients with active and inactive SLE was observed.

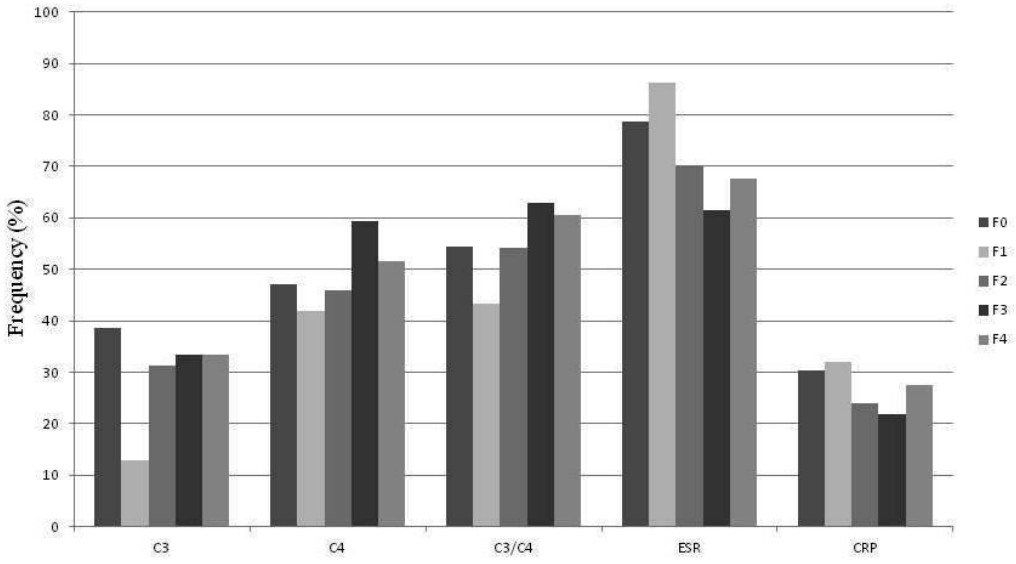


Fig.6: Frequencies of individuals with low levels of complement and high CRP and ESR during enrolment and up to 4 follow-ups

Titre of circulating immune complex: The titre of circulating immune complex (CIC) in the patients was determined by using C1q-CIC ELISA. 66.7% patients were positive, 14.8% were equivocal and 18.5% were negative for CIC. CIC showed weak positive correlation with musculoskeletal manifestations (**r=0.388, p=0.004**) and skin rash (**r=0.270, p=0.048**). Negative correlation of CIC with C3(**r=-0.259, p=0.058**) was observed which could be associated with the consumption of complement component in presence of CIC.

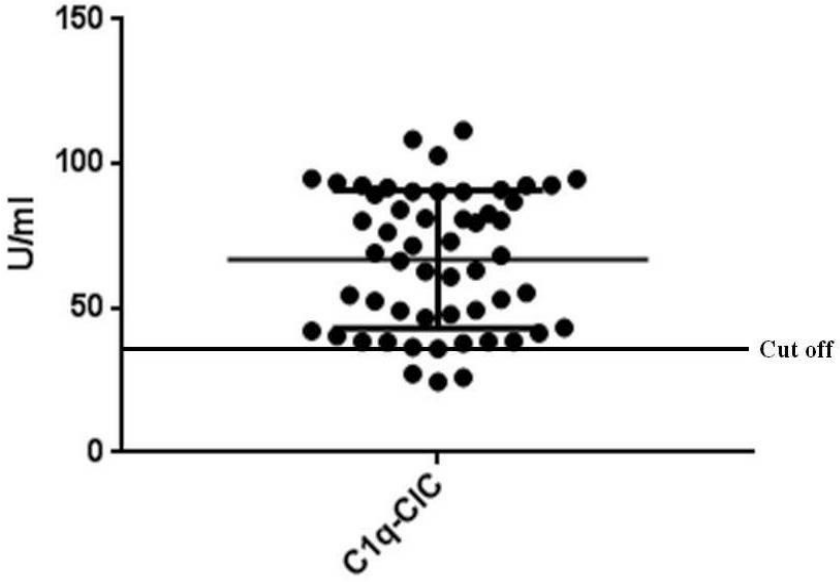


Fig.7: Titre of CIC (mean± SD) in patient serum

Summary of objective I: Mucocutaneous manifestations were the frequently observed clinical complications of the patients followed by renal, hematological and musculoskeletal manifestations. In case of haematological manifestations, lymphopenia was most frequently observed among the patients. Anti- dsDNA antibody was the most frequently found auto-antobody followed by anti-SSA antibody. Correlation of disease activity with presence of anti-dsDNA, anti-nucleosome and anti-histone antibody was observed. High titers of antibodies against chromatin antigens (dsDNA and NUC) together with RibP were found to be associated with disease complexcity. Low levels of the two complements; C3 and C4 and high ESR levels had been identified as markers to differentiate between SLE patients with active and inactive disease. Circulating Immune Complex (CIC) levels was positively correlated with musculoskeletal manifestations and skin rash.

Gene expression assay using real time PCR :

RNA extraction and cDNA preparation for real time PCR:

mRNA was extracted from blood of 35 SLE samples stored in RNA later using Ribo pure -Blood Kit (Ambion, Life Technologies, California)).RNA quantification was done using Nano Drop (GE Healthcare Corp., New Jersey).

1µg of isolated RNA was immediately processed for cDNA preparation using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Life Technologies, California) and cDNA was quantified using Nano Drop.

Gene expression analysis by relative quantification using Comparative Ct method:

Expression levels of the pro inflammatory cytokines; IFNɣ, IL1β, IL-6, IL18, IL21, IL22, IL23 anti-inflammatory cytokines; IL10 and TGFβ2 and transcription factors Tbet, GATA3 and FOXP3 were assessed by TaqMan based Assays on Demand using Step One Plus Real-Time PCR system (Applied Biosystems, Life Technologies, California, USA). GAPDH was used as the internal control for normalizing the expression levels. The expression levels of case samples were analyzed in terms of RQ values (2^-ΔΔCt) relative to healthy controls used as the calibrator.

Expression levels of IFNγ (RQ= 3.0±2.7) and IL6 (RQ= 2.4±2.3) were up regulated in the patients while IL1β (1.3±0.9) was observed to be up regulated in some samples. Anti-inflammatory cytokines were down regulated in majority of the samples.

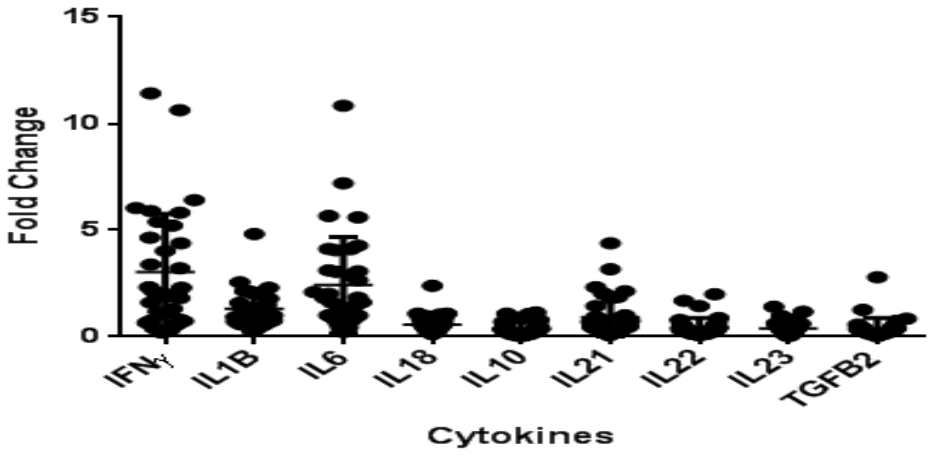


Fig.8: Fold change of pro and anti-inflammatory cytokines

Expression level of the transcription factor T bet (RQ= 3.5±2.5) and FOXP3 (RQ= 1.6±1.5) were up regulated in majority of the cases while GATA3 was up regulated in few cases.

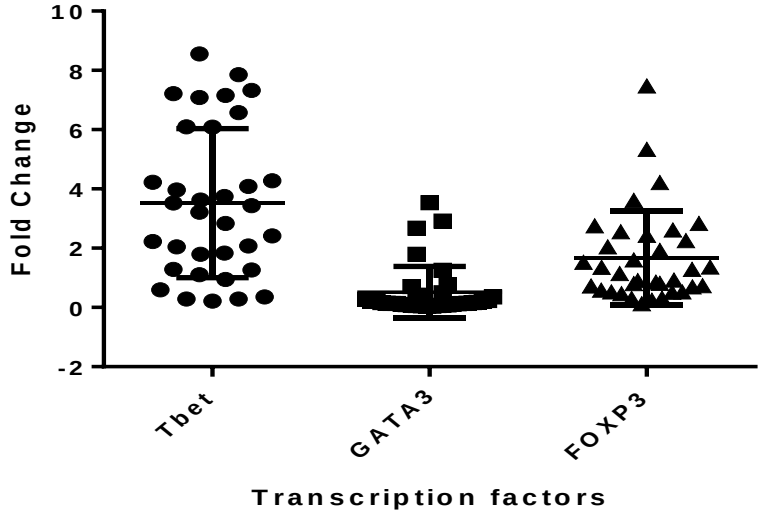


Fig.9: Fold change of transcription factors T bet, GATA3 and FOXP3

T bet expression levels were found to be negatively associated with expression levels of GATA3 and IL18 (Pearson's correlation coefficient r =-0.474, p = 0.004 and r = -0.384, p = 0.027).

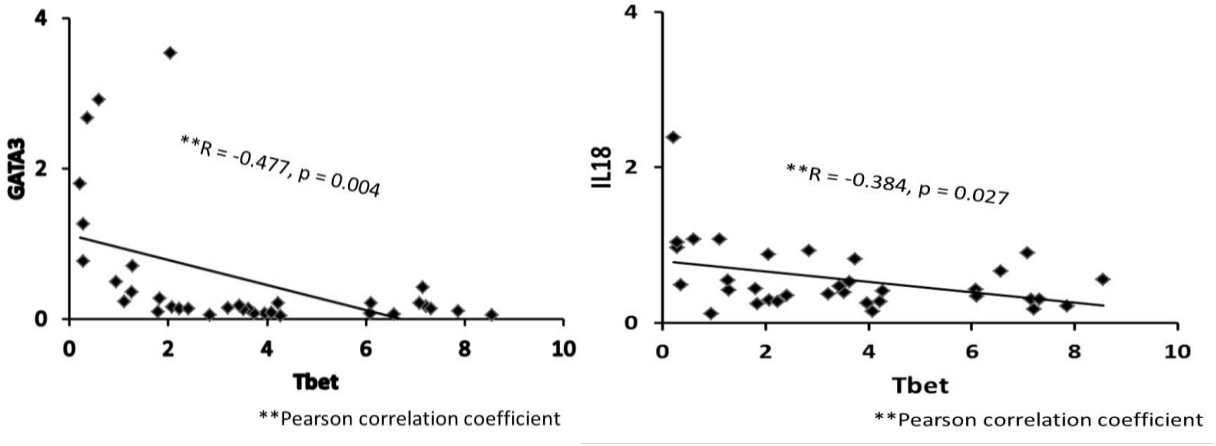


Fig.10: Correlation of T bet with GATA3 and IL18.

Expression levels of FoxP3 did not significantly correlate with IFNγ and T bet, however a simultaneous up regulation or down regulation of FoxP3 with IFNγ and T bet was observed in majority of patients. These observations suggest that in SLE patients Th1 cells were upregulated and at the same time immune system is trying to achieve homeostasis by upregulating the Th1 suppressor Treg cells, which needs to be studied.

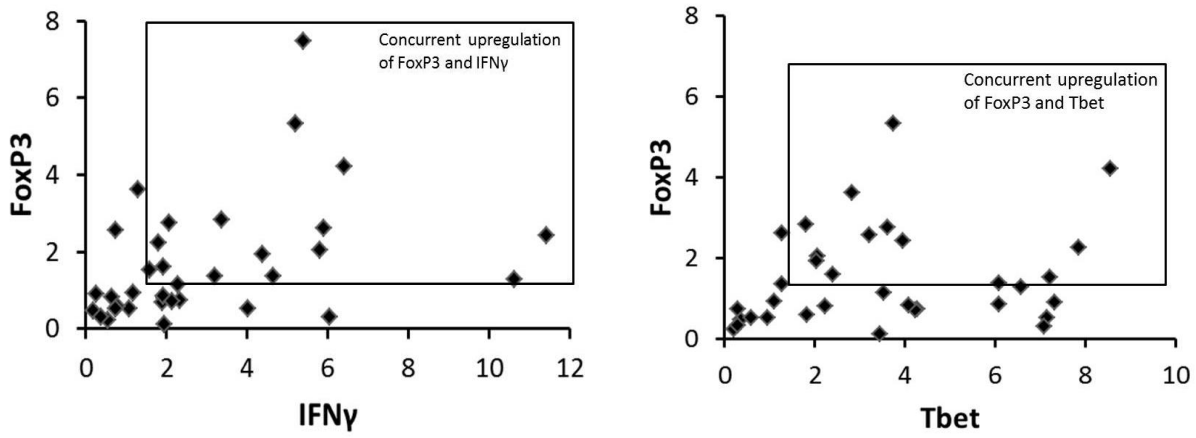
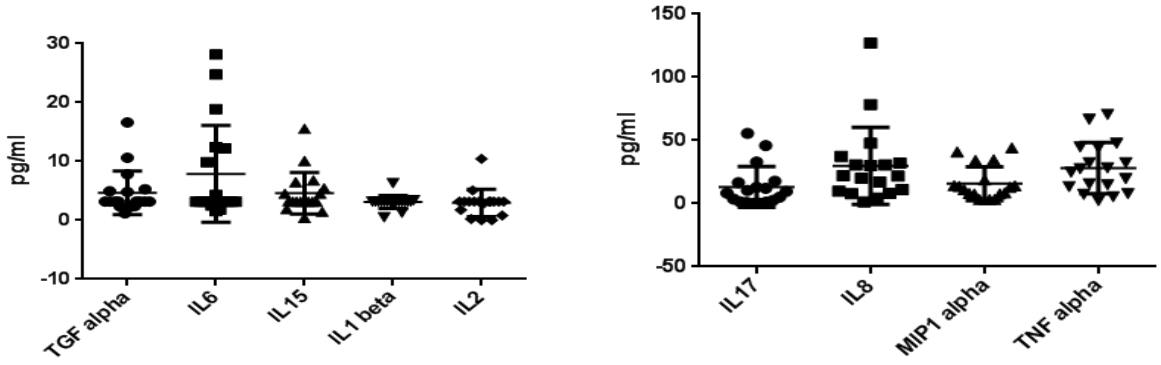


Fig.11: Relation of FoxP3 with IFNγ and T bet.

Serum levels of pro and anti-inflammatory cytokines: Pro and anti-inflammatory cytokine levels in patient serum during enrolment and follow-up were determined by using Milliplex MAP kits; 24 plex human cytokine. Serum concentrations of the cytokines



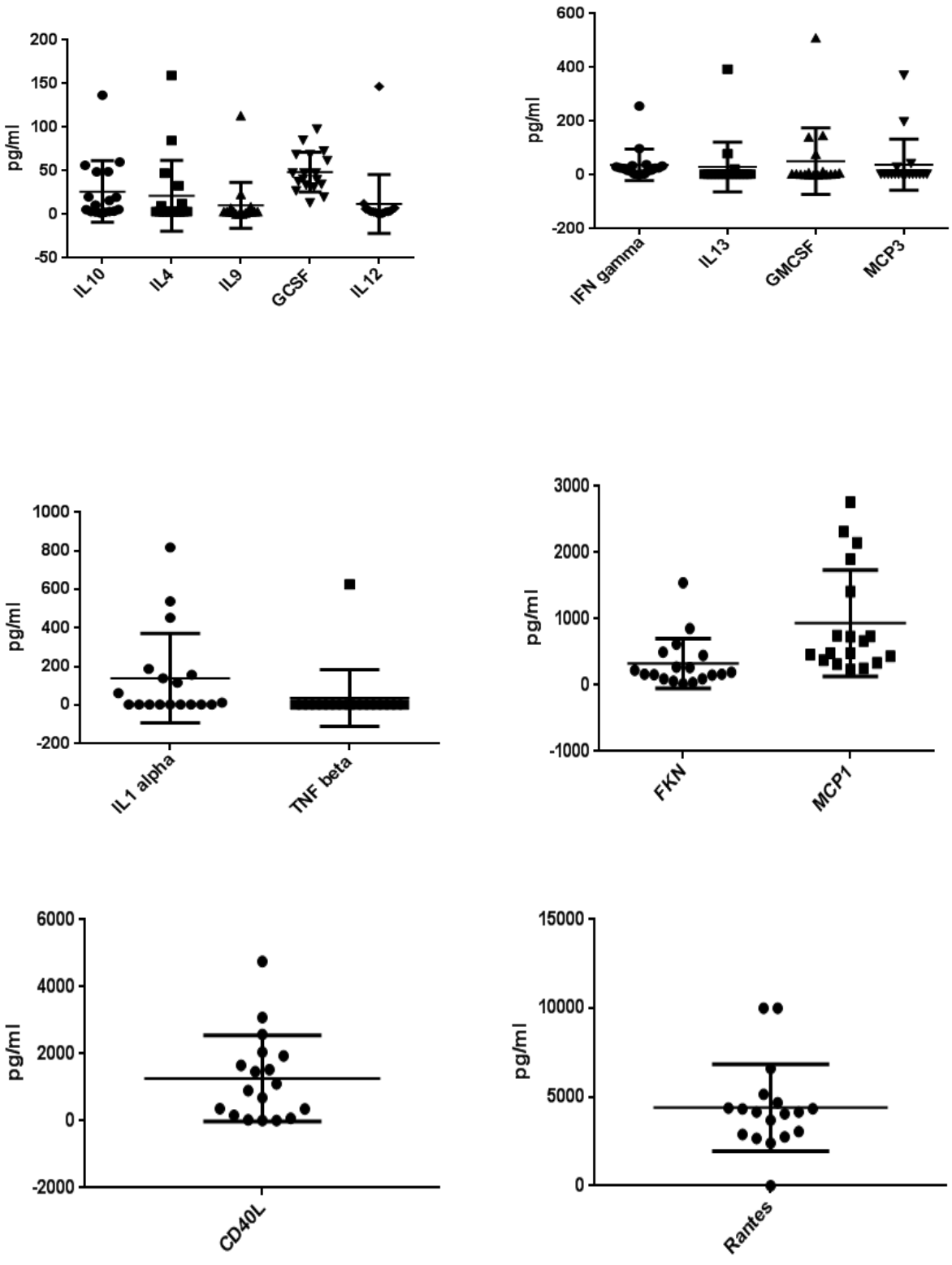


Fig.12: Serum concentrations of 24 cytokines

The Monocyte Chemoattractant Protein; MCP1 level correlated with the inflammatory marker CRP(r= 0.495, p=0.043). Serum level of the pro-inflammatory cytokine; IFNγ was correlating

with monocyte count (r=0.476, p=0.046) of the patients. The correlation of CRP and MCP1 suggests infiltration and activation of monocytes in response to inflammation. The negative correlation of complement; C3 with TNFα (r= -0.528, p=0.024) suggests consumption of C3 due to ongoing inflammation in these patients.

Correlation of the pro-inflammatory cytokines; IFNγ with IL12 (r=0.531, p=0.023) and TNFα with MCP1 (r=0.569, p=0.014) was observed. IL12 showed correlation with IL10 (r=0.531, p=0.023); IL12 has been reported as a strong inducer of IL10.

Phenotyping of monocyte subpopulations of SLE patients by flow cytometry: The proportion of the three monocyte subpopulations; $CD14^{++}CD16^{-}$, $CD14^{++}CD16+$ and $CD14^{+}CD16++$ were determined in SLE patients with flare and remission as well as in healthy controls.

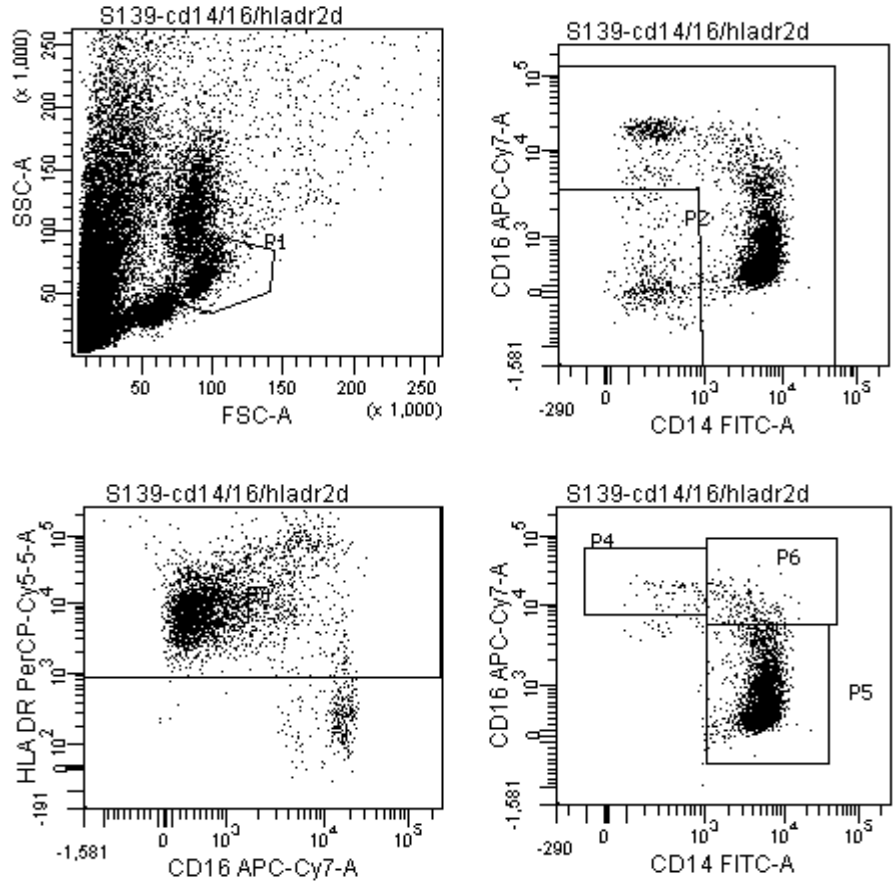


Fig.13: Phenotyping of monocyte subpopulations CD14++CD16-, CD14++CD16+ and CD14+CD16++ by using flurochrome antibodies to CD14, CD16 and HLADR.

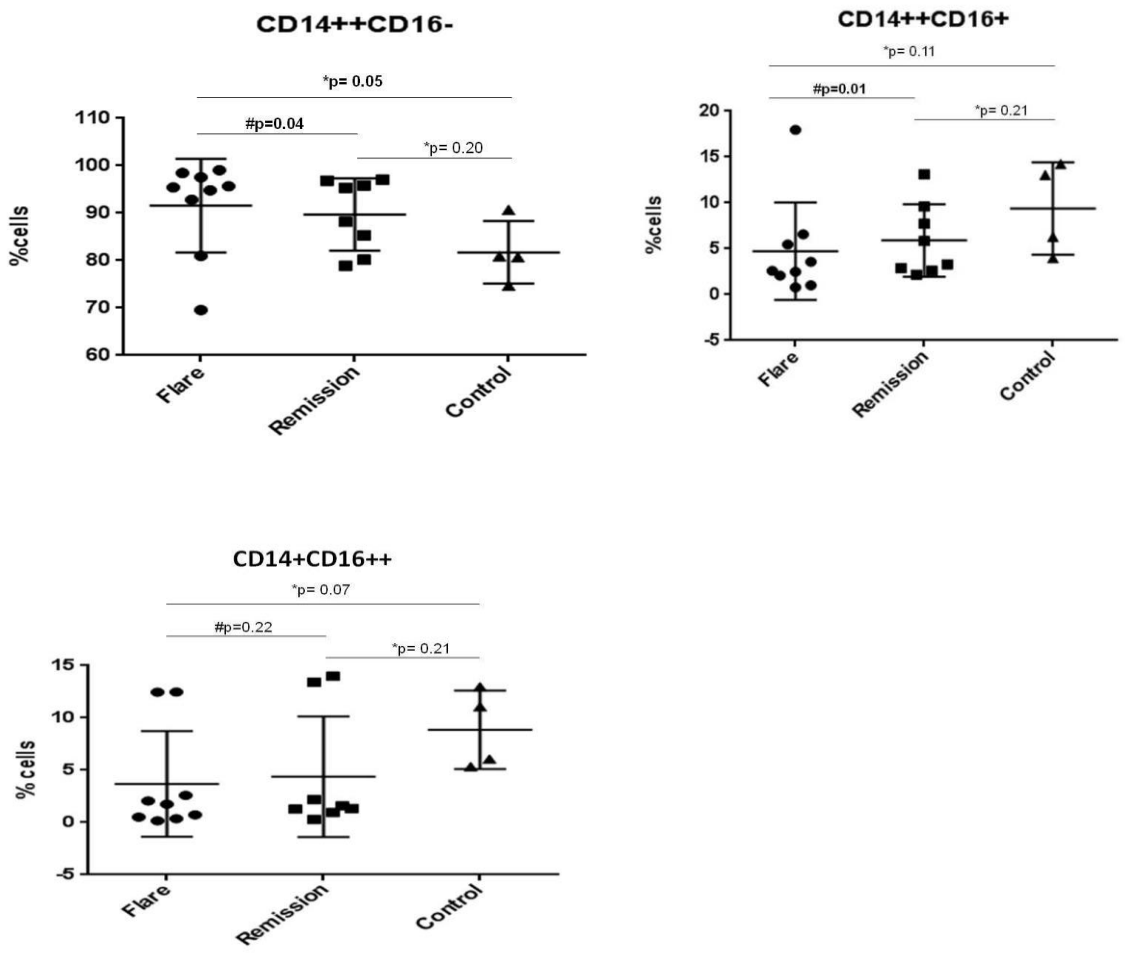


Fig. 14: Monocyte subpopulations in patient and control groups.*Mann- Whitney U test & # Wilcoxon signed ranked test

Percentage of classical/inflammatory CD14++CD16- population increased during flare as compared to disease resolution (p=.04) and healthy controls (p=.05). Percentage of intermediate CD14++CD16+ population decreased during flare compared to disease resolution (p=.01). The percentage of CD14+CD16++ subpopulation also decreased during disease but the change was not statistically significant. A marked difference of CD14++CD16-/ CD14++CD16+ ratio was observed between flare and remission (0.016). A change in monocyte proportions of SLE patients was observed during flare and remission.

Comparison of clinical presentation, disease severity and immunological parameters of SLE patients from Assam and Mumbai, Western India:

100 patients were enrolled in NIIH for a comparative study to identify the differences of SLE pathogenesis between the patients from Assam and Mumbai. Renal manifestations were markedly higher in patients from Assam, while arthritis and oral/nasal ulcer were remarkably higher in Mumbai patients. Within haematological manifestations the difference was contributed by higher leucopenia in patients from Mumbai. The low levels of complement were frequently observed in patients from Mumbai. Surprisingly, negligible difference of auto-antibodies between the two groups was noted (Fig. 13).

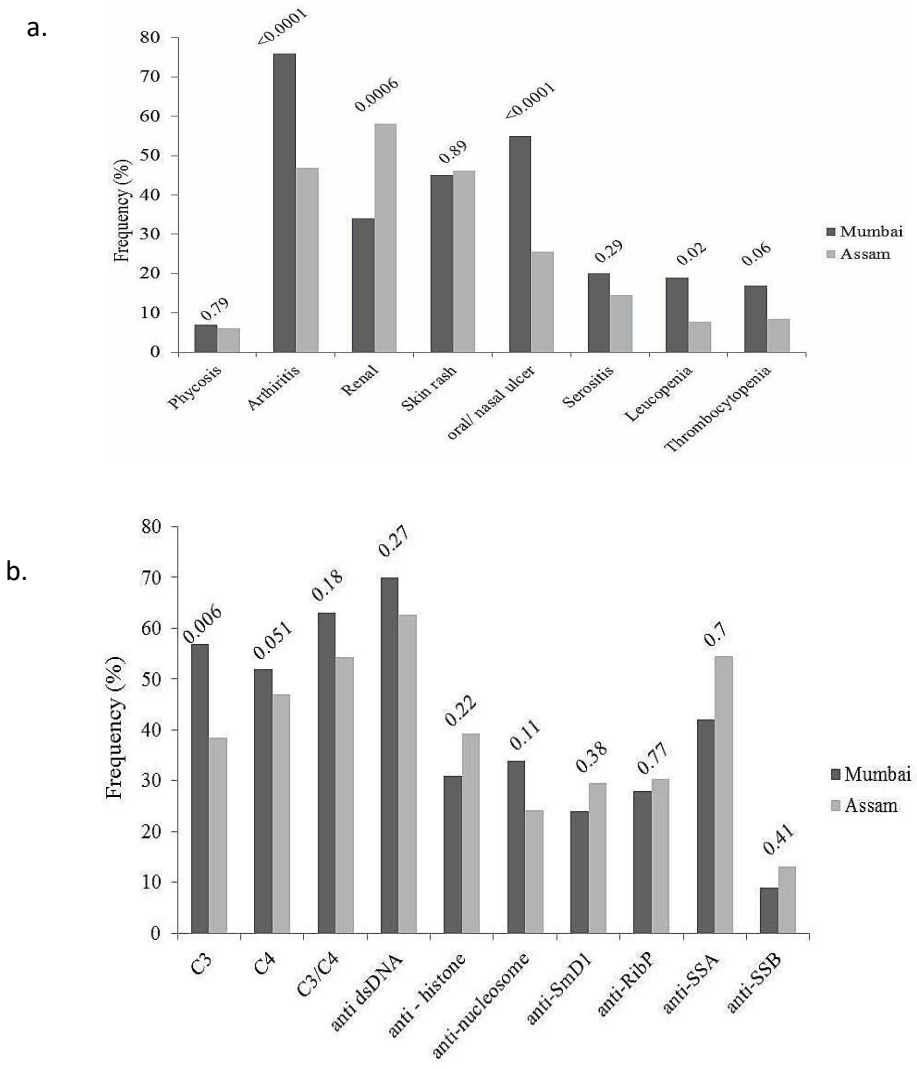


Fig. 15: Comparison of clinical manifestations and immunological parameters of SLE

patients from Assam and Mumbai. The * P values were obtained by performing Fisher's exact test. $P < 0.05$ was considered as significant.

Though mean SLEDAIs of the two patient groups were comparable. However, a markedly higher percentage (20%) of patients from Assam was occurred in severe category than the patient group from Mumbai (6%).

Disease activity of patients from Mumbai correlated with anti- dsDNA antibody (r=0.294, p=0.003) and histone (r=0.267, p=0.007) which was also observed in patients from Assam. The concentration of anti-dsDNA antibody in Mumbai was noticeably higher (p=<0.0001) than patients of Assam.

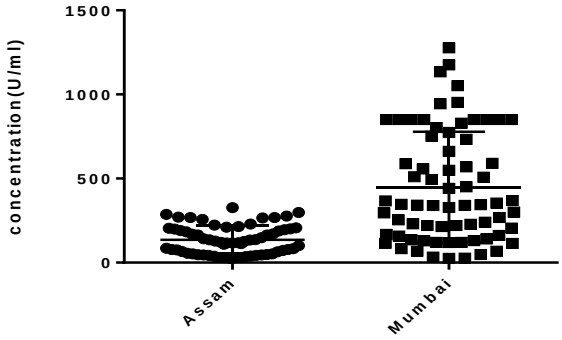


Fig.16: Concentrations of anti-dsDNA antibody of SLE patients from Assam and Mumbai

Comparison of serum cytokines: The serum cytokine levels were found to be different in the patients groups from Assam and Mumbai. The pro-inflammatory cytokine IFNγ levels were higher in SLE patients from Assam in comparison to the patients from Mumbai (p=0.0002). On the other hand, IL1β (p=<0.0001) was higher in Mumbai patients. Mean IL6 levels (p=0.01) were also higher in some patients from Mumbai. The difference in the levels of anti-inflammatory cytokine; IL4 (p=0.03) between the two groups was contributed by high levels of IL4 in few patients from Assam. No difference in the levels of TNFα between the two groups was observed.

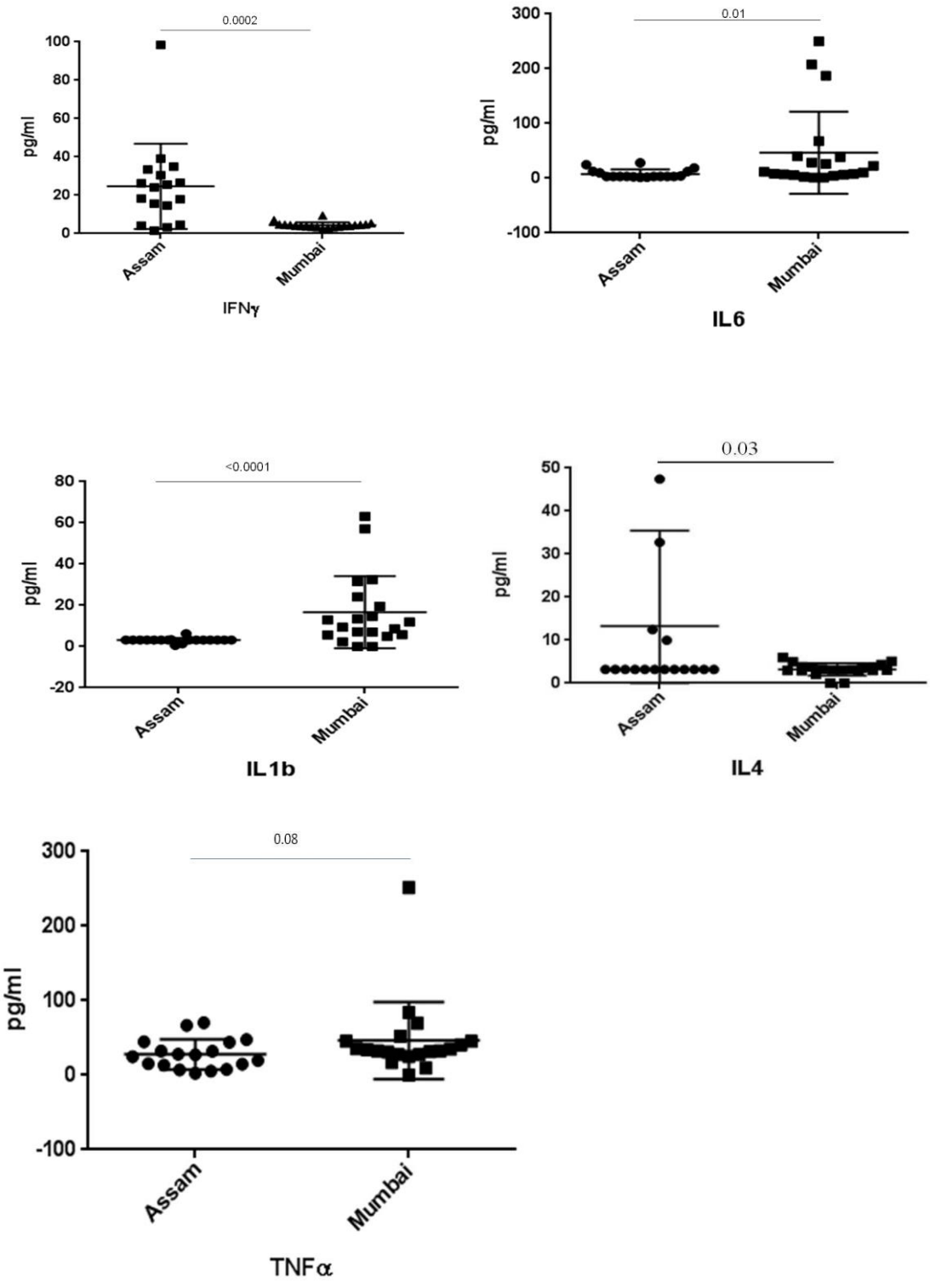


Fig.17: Difference in the levels of serum cytokines of SLE patients from Assam and Mumbai

Summary of objective II:

Differences of SLE disease presentation with respect to clinical manifestation serological (auto-antibody &complement)and immunological parameters (cytokine & monocyte subpopulations)

were observed between the patients Assam and Mumbai. Frequencies of renal complications were markedly higher in the SLE patients from Assam compared to the patients of Mumbai. However, incidences of arthiritis, oral and nasal ulcer and leucopenia were higher in the patients of Mumbai.

No difference in the frequencies of auto-antibodies of the two patient groups was observed. However, concentration of dsDNA of patients from Mumbai was markedly higher than patients from Assam. No of patients with low C3 levels were higher in Mumbai group than that of Assam, suggesting difference in disease pathogenesis.

The pro-inflammatory cytokine INFγ was identified as a mediator of SLE disease pathogesis in patients from Assam while IL1β and IL6 were observed to be associated with disease pathogenesis in patients from Mumbai. Although, significant difference of IL4 levels was observed but higher levels of these two cytokines was found in particular cases.

Objective 3: Determine markers for resolution between SLE related flares and active infections in SLE patients (AMC&H,TU)*.

Serological and haematological parameters were investigated among three groups of SLE patients, i.e. patients with inactive disease, active disease and inactive disease with active infection. It was observed that lymphocyte count, complement levels C3 and C4 were higher in patients with inactive disease with active infection as compared to patients without infection irrespective of their disease status. Although, the mean CRP level was found to increase in infection but the change was not significant. However, ESR level was higher in patients without infection (Table5).

Table 6: Serological and haematological parameters which were found to be different among different status of SLE.

	Groups			Mann-Whitney test p value		
Parameters	Active SLE without infection (Group1)	Inactive SLE without infection (Group 2)	Inactive SLE with active infection (Group 3)	Group1 Vs Group 2	Group1 Vs Group 3	Group2 Vs Group 3
Lymphocyte count	1784 ± 942	1594 ± 710	2179 ± 821	0.55	0.089	**0.034**
C3	97 ± 32	107 ± 47	134 ± 44	0.27	**0.004**	0.28
C4	14.6 ± 7.2	14.3 ± 5.9	19.9 ± 8.0	0.79	**0.013**	**0.023**
CRP	0.63 ± 0.89	0.97 ± 1.4	1.56 ± 2.35	0.59	0.18	0.45
ESR	38.8 ± 32.1	42.6 ± 22.8	26.4 ± 19.5	0.34	0.24	**0.029**

Summary of objective III:

Lymphocyte count, complement levels C3 and C4 were higher in patients with inactive disease with active infection as compared to patients without infection irrespective of their disease status. ESR level was higher in patients without infection.

Conclusion:

1. Auto-immune diagnostic laboratories were established in AMC&H and department of MBBT, Tezpur University. Manpower of both the institutes was trained in auto-immune diagnosis.
2. Formation of SLE registry and DNA bank of 145 SLE patients had been completed.
3. 52.41%, 42.75%, 37.93% and 22.76% of total enrolled patients showed for F1, F2, F3 and F4 follow-ups respectively. 11.03% of the patients expired during the study period. Infection and/or renal complications were the major causes of mortality among the patients.
4. Mucocutaneous manifestations were the major clinical complications of the patients followed by renal and musculoskeletal manifestations. In case of haematological manifestations, lymphopenia was most frequently observed among the patients.
5. Anti- dsDNA antibody was the most frequently found auto-antibody followed by anti-SSA antibody. Correlation of disease activity with presence of anti-dsDNA, anti-nucleosome and anti-histone antibody was observed.
6. C3 and C4 levels showed negative and moderate correlation with SLEDAI. Weak positive correlation of ESR was observed with SLEDAI. No correlation of CRP with SLEDAI was observed. Circulating Immune Complex (CIC) levels was positively correlated with musculoskeletal manifestations and skin rash.
7. mRNA levels of IFNγ, IL-6, T-bet and FoxP3 levels were unregulated in the SLE patients suggesting Th1, Treg and monocyte/macrophage cell activity in the patients.
8. Serum levels of IFNγ showed moderate and positive correlation with monocyte count. Moderate and positive correlation was also observed between CRP and monocyte chemoattractant protein (MCP1). These suggest activation of monocytes and their tissue specific migration in response to inflammation.
9. The proportion of pro-inflammatory classical monocyte population had increased during flare in comparison to remission

10. Frequencies of renal complications were markedly higher in the SLE patients from Assam compared to the patients of Mumbai. However, incidences of arthritis, oral and nasal ulcer and leucopenia were higher in the patients of Mumbai.
11. No difference in the frequencies of auto-antibodies of the two patient groups was observed. However, concentration of dsDNA of patients from Mumbai was markedly higher than patients from Assam. No of patients with low C3 levels were higher in Mumbai group than that of Assam.
12. Lymphocyte count, complement levels C3 and C4 were higher in patients with inactive disease with active infection as compared to patients without infection irrespective of their disease status. ESR level was higher in patients without infection.