High circulatory level of inflammatory cells and their relation to recurrent cardiac events in Indian population

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Introduction and objective

Cardiovascular diseases (CVD) are the leading cause of global mortality . In India, CVD accounts for 28% of mortality with an alarming rise in coronary artery disease (CAD) in both rural and urban areas [1]. The role of Th17 cells in atherosclerosis has received increased attention in the past few year and Treg cells has been reported to play a crucial role in the pathogenesis of atherosclerosis[2,3]. Inflammatory and regulatory response play key role in immune regulation[4]. We earlier reported imbalance between peripheral inflammatory and regulatory T cell subsets in myocardial infarction (MI) [5]. To study the imbalance between the inflammatory and regulatory subsets in patients with myocardial infarction (MI), patients showing clinical improvement, patients with recurrent cardiac event and patients with history of CAD were analyzed that could suggest if the imbalance was cause or consequence of the disease.

Methodology

Study design: The investigation conforms to the principles outlined in the Declaration of Helsinki and the Indian council of medical research (ICMR, India) guidelines, and was approved by the Institutional ethics committees of Thrombosis research Institute and Narayana Hrudayalaya hospital. An informed consent was taken from all the participants prior to enrolment.

Follow up and re sampling: Participants were followed up starting from six months from the date of enrollment into the study. Any new events/ recurrent cardiac events were recorded. A second sample was collected from patients who showed clinical improvement as authenticated by the cardiologist, on revisit to the hospital between 6-12 months after treatment and volunteered to continue in the study. Samples were also collected from patients at the time of recurrent cardiac event who volunteered to participate in the study.

Flow Cytometry and analysis: Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll and activated for 4 hours. Surface staining was performed by using anti CD3 APC H7 and anti CD4 FITC. Intracellular staining was carried out with anti IL-17 APC. $CD3^{+}CD4^{-}$ cells were considered as CD8 cells. For regulatory T cell staining, CD25 APC and anti FOX P3 PE were used in a separate tube along with CD3 and CD4 as mentioned earlier. Fluorescence minus one and unstained control were used for setting the gates. FLOWJO Version 7.6.5 was used for data analysis and results were expressed as a percentage of $CD4^{+}$ T cells by sequential gating on lymphocytes (Fig 1).

Statistical Analysis: T cells were compared using multivariate analysis using age, gender, current smoking, BMI, WHR, diabetes and hypertension as covariates. All statistical analyses were performed using SPSS version 17.0 for Windows and p value <0.05 was considered statistically significant.

Results

- Immune imbalance observed in MI was recovered in clinically recovered MI with reduced peripheral inflammatory cells Th17 (4.45±0.36 vs 3.09±0.37, p<0.01), increased regulatory subsets $CD4^{+}CD25^{High}$ (3.69±0.45 vs 5.30±0.33, p<0.01) and Tregs (3.07±0.47 vs 4.23±0.43, p<0.05)
- There was an decrease in inflammatory to regulatory Tcell ratio (Th17/Tregs (1.71±0.16 vs 0.784±0.06, p<0.01 and $Th17/CD4^{+}CD25^{High}$ (1.43±0.13 vs 0.604±0.04, p<0.01)) compared to their first sample. (Table 1 and Fig 2)
- This suggests that there is an improvement in immune regulatory system as patients recover and Th17 and Tregs ratio is associated with disease vulnerability and recovery.
- Th17 and Treg cells in recovered patients were comparable to healthy controls (3.17±0.22 vs 3.55±0.16 for Th17 and 4.33±0.32 vs 3.9±0.1 for Tregs)
- Th17 cells and Th17/Tregs ratio were high in the patients with the history of CAD and patients who had incident of recurrent cardiac event later compared to clinically improved MI patients and control. (Fig 3)
- Th17 cells was high in the patients with the history of CAD and patients who had incident of recurrent cardiac event later (4.45±0.36 vs 4.76±0.49, p=0.67) compared to MI patients without any previous or later history of CAD.(Fig4)

Table 1 T cell Subsets at time of first event and clinical recovery

	Samples collected at the time of	
Variable	First event	After clinical improvement
Th17	4.45±0.36	3.09±0.37***
Tc17	3.6±0.29	3.52±0.23
$CD4^{+}CD25^{High}$	3.69±0.45	5.30±0.33***
Tregs	3.07±0.47	4.23±0.43*
Th17/Tregs	1.72±0.23	0.79±0.07***
$Th17/CD4^{+}CD25^{High}$	1.44±0.2	0.61±0.05***
Tc17/ Tregs	1.37±0.17	0.89±0.09**

* -p Value<0.05, ** -p Value<0.01 and *** -p Value<0.001

Fig 1: Gating strategy for Flow cytometry analysis

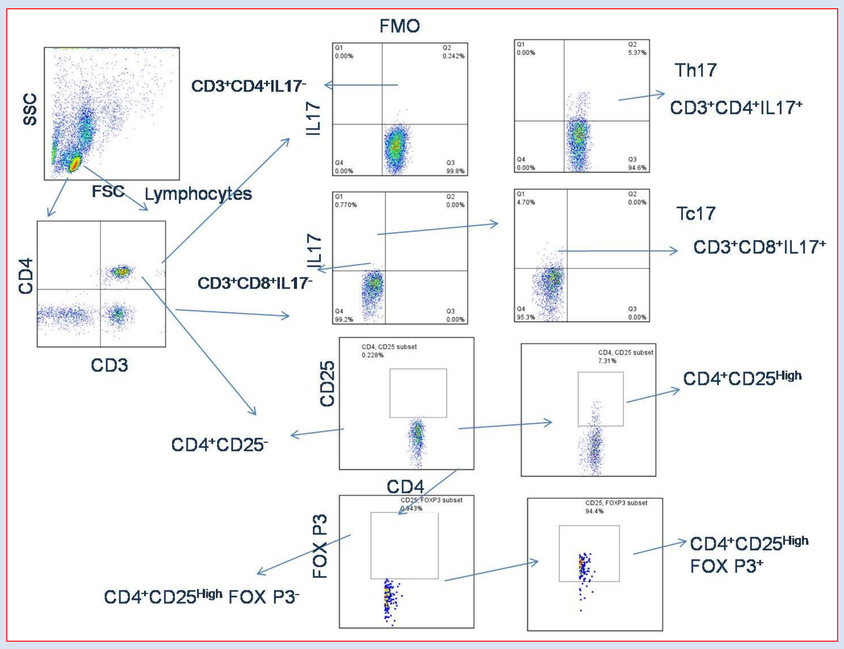


Fig 2: Tcell subsets distribution in MI and recovered MI

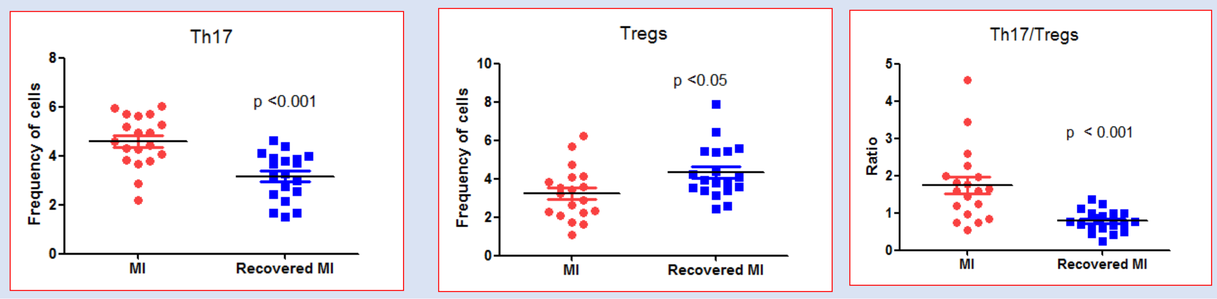


Fig 3: Tcell subsets distribution in Control, MI, recovered MI, recurrent event and patients with history of CAD

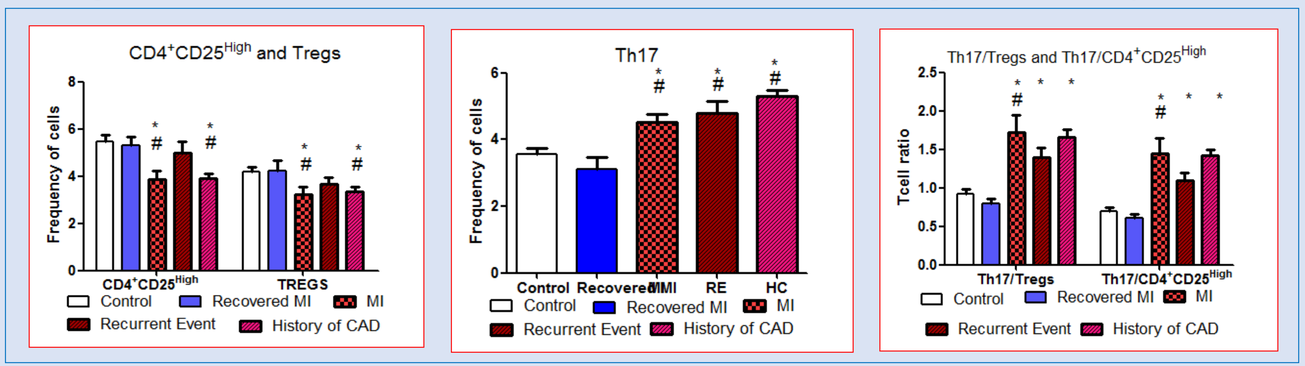
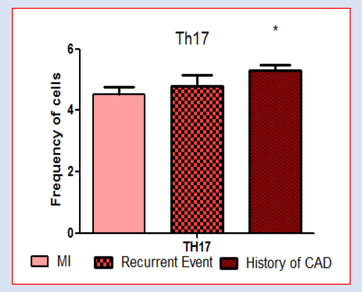


Fig 4:Th17 in MI , recurrent event and patients with history of CAD



* -p Value compared to control and # - p Value compared to recovered MI

Conclusion

- Our results suggest that immune imbalance could be one of the cause of occurrence of acute coronary syndrome
- High circulatory level of inflammatory play an important role in recurrent cardiac events

References

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