Imbalance of Regulatory, Helper Th1 and Th2 cells with Expression of Permeable Glycoprotein Plays Role in Steroid Response in Nephrotic Syndrome

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Introduction

- Idiopathic Nephrotic syndrome(NS) is one of the most common glomerular disease in children.
- About 80-90% NS patients show response to steroids and others develop resistance during the course of disease.
- Almost 50-60% of steroid responsive NS have frequent relapses or steroid dependent course and 10-20% patient develops steroid resistance.
- In 1974, Shalhoub hypothesized that idiopathic minimal change disease (MCD) is a disorder of T-cell dysfunction.
- AlthoughTh1 and Th2 lymphocytes are anointed for coordinating immune system in NS, however, discrepancies have been pointed out in this hypothesis.
- Another subset of T lymphocytes non-helper regulatory T cells (Tregs) that actively suppress the immune system, and effecter T cells (Teff) may play a role
- Steroid non-responsiveness may also be because of disturbance in T-cell subset population and/or factors that modulate the disease response to pharmacological interventions, such as the expression of Permeable glycoprotein (P-gp), a product of MDR-1 gene.
- The expression of P-gp on the membrane of different types of immune cells suggest that P-gp may influence cell-mediated immune response as well.
- Immunological changes and expression of P-gp in NS patients during the natural course and after steroid therapy has not yet been studied.

Aim

➢ To demonstrate the distribution of $CD4^+IFN\text{-}\gamma^+$ Th1, $CD4^+IL\text{-}4^+$ Th2 and $CD4^+CD25^+FoxP3^+$ Treg cells in NS patients with sustained remission, during relapse and those who developed steroid resistant and its correlation to each other.

➢ To study the P-gp expression on different lymphocytes and correlate the changes with steroid response in NS patients.

Materials and Methods

Study population (N=81)

- SSNS Remission (n=22,Male-18, MeanAge-8.52±5.8)
- SSNS Relapse (n= 24,Male-20, MeanAge-8.5±4.2)
- SRNS (n= 21,Male-20, MeanAge-11.7±3.8)
- Control (n= 14,Male-11, MeanAge10.6±4.3)

All categories were defined as per criteria of ISKDC

Materials and Methods

Collected Heparinised Peripheral blood sample

Frequency analysis of T cell subpopulation and P-gp analysis

Th1 $CD4^+IFN\text{-}\gamma^+$ | Th2 $CD4^+IL\text{-}4^+$ | T regs $CD4^+CD25^+FoxP3^+$

Stimulated with PMA, ionomycin and Monensin for 5h

Surface staining for CD4, P-gp and CD25

RBCs were lysed with BD FACS lysing solution

Fixation and Permiabilize with Cytofix/Cytoperm kit (Th1 and TH2) and with BD Human FoxP3 Buffer Set (Tregs)

Intracellular staining for IFN-γ, IL-4 and FoxP3

Acquired on Flow Cytometry (BD FACS Calibur)

Statistical analysis: The means values in different groups were compared with analysis of variance (ANOVA) for parametric values. Pearson's correlation test was used to analyze the correlation between variables. $p<0.05$ is considered significant, Data analysis was done using SPSS 15.0 software.

Results

Characteristics	NS in Remission	NS in Relapse	SRNS	Control
Pgp % positive cells	4.84±2.7	10.7±5.74	12.42±5.82	4.06±2.1
RFI	6.52±1.82	8.43±2.64	8.36±1.62	8.22±1.82
RFI ×% positive cells	33.16±23.97	83.51±37.22	101.72±44.91	33.38±17.05
D-value	0.39±0.14	0.4±0.18	0.48±0.19	0.15±0.07

Table 1 | P glycoprotein expression, Relative fluorescence intensity (RFI) and D-value on lymphocytes in different group of patients.

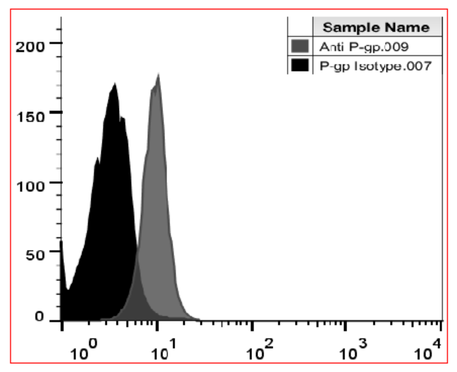

Figure 4 I Relative fluorescence intensity (RFI) in one of the representative sample from a patient with resistant NS.

	Pgp expression Pearson Correlation	P-value
%Treg	-0.445	0.001*
Th1/Treg ratio	0.406	0.001*
Th2/Treg ratio	0.431	0.001*
Th1/Th2 ratio	-0.189	0.102
%Th1	0.149	0.199
%Th2	0.318	0.005*

Table 2 I Correlation of P-gp expression with different T cell profiles

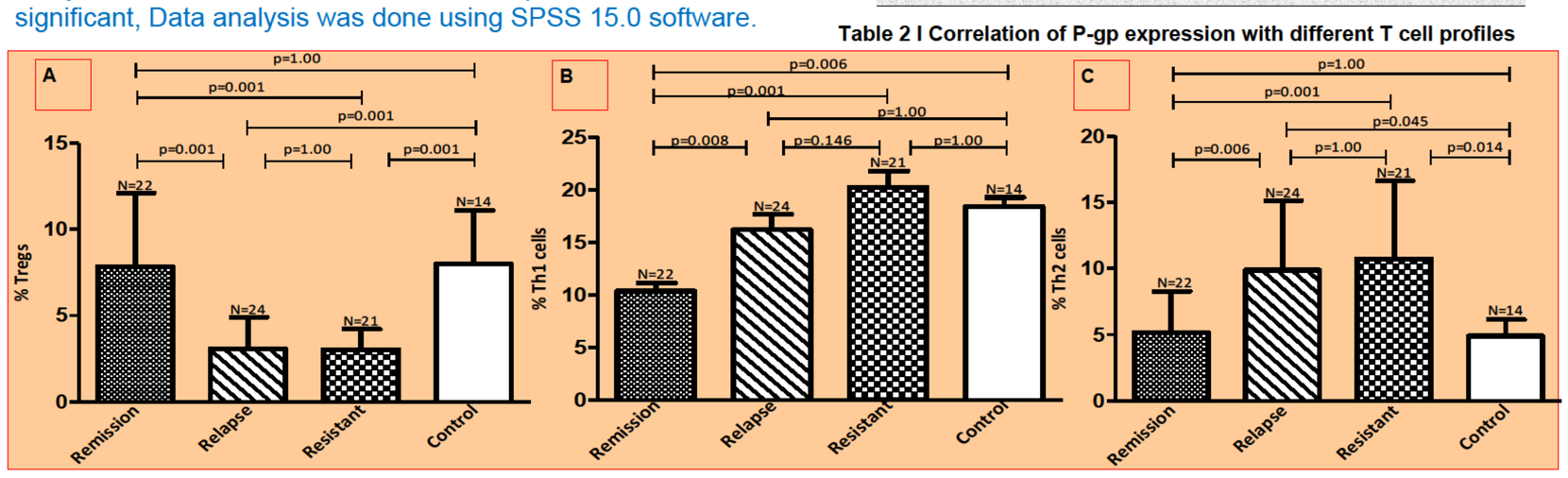

Figure1| Results are expressed as the percentage of $CD4^+CD25^+FoxP3^+$ Treg, $CD4^+IFN\text{-}\gamma^+$ Th1 and $CD4^+IL\text{-}4^+$ Th2 cells in $CD4^+$ lymphocyte in blood. Significant increase In Treg cells in sustained remission and control (A), Th1 cells were significantly decrease in remission compare to all the groups (B) and (C) shows significant increase in the population of Th2 cells in relapse and steroid resistant nephrotic syndrome patients.

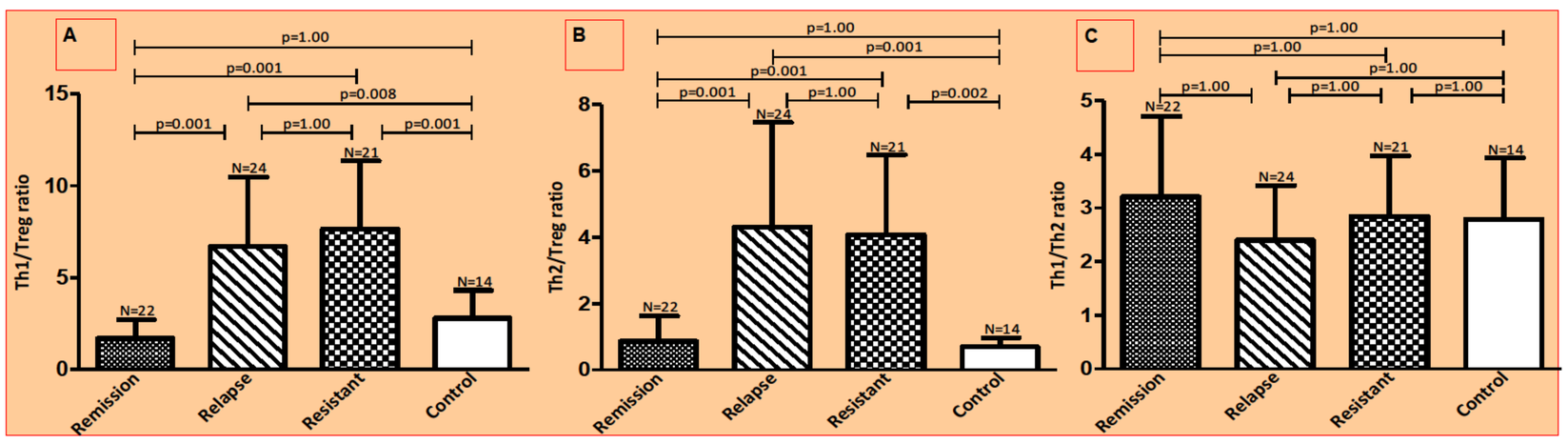

Figure 2 | Bar-diagram shows significant increase in the ratio of Th1/Treg (A) and Th2/Treg (B) cells during relapse and in resistant patients when compared to remission and control group whereas no difference in the ratio of Th1/Th2 (C)cells between the groups.

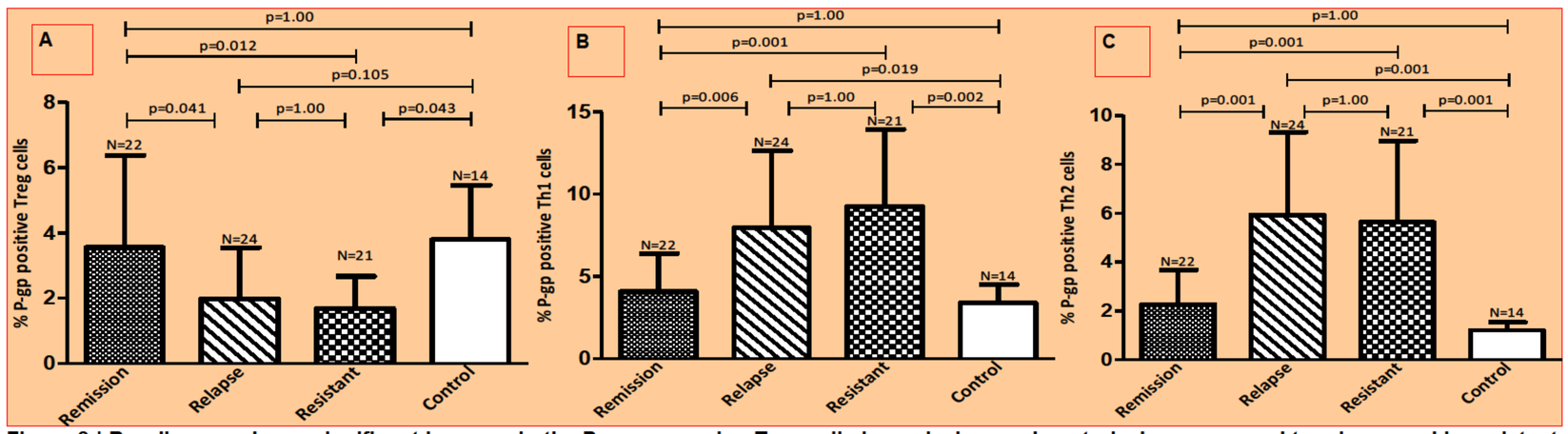

Figure 3 | Bar-diagram shows significant increase in the P-gp expressing Treg cells in remission and control when compared to relapse and in resistant patients whereas P-gp expressing Th1 and Th2 cells were significantly lower in remission and control group compare to relapse and resistant patients.

Conclusions

- ✓ The imbalance of Tregs and T eff cells may result in state of sustained remission, relapse and resistant in childhood NS patients.
- ✓ Greater Treg and lesser ratio of Th1/Treg, and Th2/Treg results into sustained remission.
- ✓ P-gp expression is greater in relapsed and resistant state and may be responsible for resistance in NS.
- ✓ P-gp is negatively correlated with Treg and positively with Th1/Treg and Th2/Treg, indicating imbalance of T cells as a possible factor for expression of P-gp on lymphocytes..

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Clinical nephrology - miscellaneous

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