IMMUNE PROFILE OF GLUCOCORTICOID RESISTANT NEPHROTIC SYNDROME PATIENTS

ABSTRACT

of

Thesis

SUBMITTED TO

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

(A CENTRAL UNIVERSITY)

LUCKNOW

BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

FOR THE DEGREE OF

Doctor of Philosophy

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2016

Abstract

Nephrotic syndrome, one of the most common kidney ailments in children, is characterized by excessive protein loss known as proteinuria, hypoalbuminemia (decrease in serum albumin), edema (swelling of tissues due to fluid accumulation), hypercoagulable state (increased ability of blood clotting) and increased susceptibility to infection. Minimal change nephrotic syndrome (MCNS) accounts for most of the cases of childhood NS. In healthy individual, interdigitated foot process of podocytes present on glomerular basement membrane prevents protein loss. In MCNS foot process of podocytes are effaced and this effacement and loss of foot process results into leakage of proteins in urine.

The immune dysregulation in MCNS always remain a matter of debate. It has been postulated that the increased frequency of effector T cell and decreased frequency of regulatory T cell may be responsible of occurrence of NS. The initial studies focused the role of Th1and Th2 lymphocytes as effector cells playing role in NS . Recent studies focused role of Th17 in pathogenesis of NS. Th17 cells secrete IL-17, which lead to damage of glomerular filtration barrier, slit diaphragm of glomerular epithelial cells (podocytes). Podocin and nephrin required for maintenance of foot processes of podocytes. Changes in podocin and nephrin lead to leaky membrane and proteinuria. Regulatory cells required to check the effector T cell response. The increased effector T cells response over regulatory T cells may result in to leaky glomerular basement membrane and NS.

Steroid is the cheapest, easily available, first line therapy used for treatment MCNS. Steroids exert anti-inflammatory effect by inducing apoptosis of lymphoid cells. Steroid passively diffuses inside the cells, and binds to the steroid receptors and gets translocated into the nucleus and competes with NFkB, AP1, STAT5, CREB molecule. Steroid inhibits various inflammatory transcription factors and reduces the production of inflammatory cytokines IL-1 beta and TNF-alpha and the 'immunomodulatory' cytokines IL-2, IL-3, IL-4, IL-5, IL-12 and IFN-gamma, IL-6, IL-8 and the growth factor like GM-CSF.

The majority of the patients respond to steroid therapy and approximately 10% of patients are primary non responder. About 60% of initial responsive patients show frequently relapsing and dependence course and some of them become secondary non

responder. These patients are treated with repeated course of steroid which leads to steroid toxicity. Steroid leads to multiple complications affecting musculoskeletal, endocrine, cardiovascular, central nervous systems and bone. The changes of immune parameters during this therapy in different phenotypes of NS are not well studied part of NS.

Therefore we plan to study the different population of T cell in circulating blood with steroid treatment in different phenotype of NS like patients in remission, during relapse and those who develops resistant. We also studied the pharmacological factors regulating effective availability of steroid in these patients. There are two important questions which has been addressed in the study; does alteration of T cell subpopulation responsible for resistant and relapse condition of MCNS patients during steroid treatment and second question is pharmacological factor like expression of P-gp drives the drug resistance and related with alteration of T cell subpopulation?

We analyzed the effector T cell and regulatory T cells in relapse and resistant patients from peripheral blood. Among effector cells, Th1 remains same in all groups of NS, while Th2 and Th17 cells are upregulated in resistant and relapsed patients as compared to those patients in remission. For the first time, we have shown pathogenic Th17 populations are up regulated in resistant and relapse cases, where T regs population was lower as compared to patients in remission.

To evaluate the role of steroids on regulating the effector function, PBMCs were stimulated with mitogen. Culture supernatant of mitogen stimulated PBMCs of resistant and relapse patients have more inflammatory cytokines i.e. IL-4, 6, 17, IFN-γ, TNFα and less anti-inflammatory cytokines i.e. TGF-β and IL-10 as compared to patients in remission and healthy controls. While it was well established fact that steroid increases the anti-inflammatory cells and cytokines production.

In order to evaluate the cause of steroid response, we also studied the expression of P-gp on lymphocytes which are target cells of steroid during treatment and its correlation with steroid therapy. It might be possible that efflux of steroids by P-gp may be contributing factor for this resistance. P-gp is one of the major factor involved in effective therapeutic concentration. During our study, we found that P-gp

expressing lymphocytes i.e. Th1, Th2 and Th17 are significantly higher on resistant and relapse patients as compared to the patients in remission and healthy control.

Our study had suggested that P-gp expression increases on pathogenic T cells in diseased condition. P-gp has been recently targeted as the molecule responsible for poor response to many other chemotherapeutic agents as well. Increased P-gp expression could be one of the possible factors responsible for resistance besides other immunological and non immunological factors. Do the monitoring of P-gp expression will be helpful to predict steroid resistance or relapsing course of the disease. Steroid is the substrate of the P-gp and calcineurin inhibitors is both substrate and inhibitor of P-gp, therefore it is possible that those patients who are resistant to steroid alone, responds to calcineurin inhibitors if added in treatment regimen. Non-responsive patients may be treated with other therapies instead of using higher dose of steroid, which have toxic effect. Rituximab and Tacrolimus with low dose steroid had shown effectiveness in resistant NS. Tacrolimus and other calcineurin inhibitors may be used as steroid sparing agents in this scenario.

Our observation has translational potential in treatment of SRNS patients. Predicting steroid resistance and adding steroid sparing medicine from beginning may help in avoiding toxic effect of steroid in this scenario.