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# Proliferation Index of Peripheral Blood Mononuclear Cells in Patients on Peritoneal Dialysis

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## Abstract

**Aim:** Infections remain leading complication in patients undergoing peritoneal dialysis (PD). Peripheral blood mononuclear cells (PBMCs) provide first-line defense against invading pathogens. Though few studies have demonstrated reduced activation of PBMCs in hemodialysis patients, no study has systematically looked at their function among patients on PD. We evaluated stimulation index (SI) of PBMCs to various external stimuli in patients on peritoneal dialysis (PD group), patients having chronic kidney disease stage 5 not on dialysis (CKD group) and compared it with those of healthy controls (20 patients in each group).

**Methods:** PBMCs were isolated as per standard procedure and were seeded in tissue culture plates in a concentration of $1 \times 10^6$ cells/well. They were stimulated with phytohaemagglutinin (PHA, a potent T- and B-cell mitogen), peritoneal dialysate effluent (DE) and fresh peritoneal dialysis fluid (PDF; Baxter, India). The SIs for different stimuli was estimated by counting uptake of $^3$H-thymidine using scintillation counter analyzer.

**Results:** Mean SIs values of PBMCs proliferation with PHA, DE and PDF were 2.14±0.89, 1.8±0.89 and 0.29±0.21 for PD patients, 4.06±1.19, 3.26±1.04, and 0.84±0.42 for CKD patients and 6.39±2.37, 3.41±1.67 and 1.15±0.28 respectively in controls. SIs values were significantly depressed in patients with CKD as compared controls, with PD patients doing worst. SIs values were significantly more with PHA followed by DE and PDF respectively in each group.

**Conclusions:** PBMCs from CKD particularly PD patients are less immuno-responsive to T- and B-cells mitogen suggestive of attenuated immune response in PD patients.

**Keywords**: Immune status; End stage renal disease; PBMCs; PD effluent; Lymphocyte stimulation test

## Introduction

The numbers of patients with end stage renal disease (ESRD) are increasing world-wide, and the number of patients on renal replacement therapy viz. hemodialysis (HD) and continuous ambulatory peritoneal dialysis (PD) are also increasing correspondingly [1,2]. Despite significant technical improvements in both HD and PD, the mortality rate in patients undergoing renal replacement therapy is still as high as 20% per year and infection contributes significantly to this increased mortality [3,4]. It has been suggested that immune cellular dysfunction leads to this higher incidence of infection [5,6]. Innate immunity is defensively the most crucial as the first line in inhibiting infections [7]. The peripheral blood mononuclear cells (PBMCs) are important components of innate immunity as they initiate and orchestrate the innate immune response and the optimal immune response by PBMCs is likely to determine the clinical outcome in such situations. Peritonitis in PD patients is a major infectious complication and leads to technique failure with increased treatment cost and risk of mortality and morbidity [8,9].

During the course of peritoneal dialysis, dialysate is introduced into the peritoneum through an implanted catheter and uremic substances are eliminated from the body through the dialysate effluent (DE). The DE is likely to be contaminated with microbes besides various diffusible toxins present in DE can be cytotoxic or can stimulate immune system [10]**.** Even in patients not having peritonitis, the microbial products such as endotoxin (ET), peptidoglycan (PG), β-D glucan, etc. and glucose degradation products can enter into circulation through back diffusion and stimulate the production of cytokines by PBMCs [11,12].Few studies have demonstrated reduced reactivity of PBMCs to ET or PG in patients undergoing HD [13, 14]. In a small study Ando et al.[15] found that innate immune system in PD patients was more significantly depressed as compared to HD patients, indicating some mechanism which can make innate immunity even more suppressed in PD patients. Lymphocyte stimulation test follows the principle of measuring proliferation of PBMCs subsequent to exposure to an antigen and is indicative of broad non-specific immune activation [16,17]. Also no study has compared innate immunity in patients with advanced azotemia not on dialysis with that of patients on dialysis, in effect looking at contribution of dialysis to innate immune dysfunction over and above azotemia if any.

In the current study, we aim to investigate the proliferation index of PBMCs from PD patients to various external stimuli including a potent T- and B-cell mitogen and dialysate effluent and compare it with that of patients having CKD stage 5 not on dialysis and healthy controls.

## Materials and Methods

### Study Population

40 patients of chronic kidney disease (20 on PD and 20 with CKD stage 5 not on dialysis) along with 20 healthy age and sex matched controls were enrolled. All subjects enrolled had no infectious complications in past 2 months and were on no immunosuppressive or immune modulatory treatment. Only patients on PD for >3 months were enrolled. Details of cause of renal disease and any other co-morbidities were also recorded. Informed consent was obtained from each subject and the current study protocol was approved by the Institutional Review Board committee.

### Separation of PBMCs

Peripheral venous blood (5 ml) was drawn from each subject and collected in heparinized tubes (BD Biosciences, Singapore). Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by Ficoll-Hypaque (research grade, Pharmacia, Uppsala, Sweden) density gradient centrifugation following standard method [18]. Briefly, in a 15 ml centrifuge tube 3 ml of Ficoll-Hypaque was taken and 10 ml (5 ml blood is diluted with 5 ml of 1M phosphate buffered saline (PBS) in 1:1 ratio saline) of diluted blood was loaded without disturbing the interface and centrifuged at 1800 rpm for 30 minutes. The cells were washed with PBS thrice. Cell viability was determined by trypan blue staining (Gibco BRL, Auckland, NZ) and cells were counted on haemocytometer as per the standard procedure. Cells were finally suspended in 'T cell medium' consisting RPMI-1640 medium (Gibco BRL, Auckland, NZ) supplemented with 10% fetal calf serum, 2mM L-glutamine (Gibco, Auckland, NZ) and 1% antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin).

### Cell Proliferation Assay

Freshly isolated lymphocyte were cultured in triplicate 96-wells flat bottom tissue culture plates at a concentration of 1 × $10^6$ cells/well containing 200 µl of RPMI-1640 (supplemented with 10% FCS and 1% antibiotics) with or without stimulant as described previously [18]. Cells were cultured in presence of 5.0 µg/ml phyto haemagglutinin (PHA) (a potent T- and B-cell mitogen), 20 µl/ml of fresh peritoneal dialysis fluid (PDF) having 2.5% glucose (Baxter, India) and DE per well. We used PDF as a control stimulus. Eighteen hours before harvesting the cells, 1µCi/ml of 3H-thymidine (Bhabha Atomic Research Centre, Mumbai, India) was added to each well. The cells from PHA, DE and PDF plates were harvested at day 6, on glass fiber (Advance Micro device Pvt Ltd., Ambala, India) by semi-automatic multi-well cell harvester (Skatron, Lier, Norway); filter disks were dried by incubating at 37°C for overnight.

Once dried, filter disks were suspended in scintillation fluid for at least 6 h before counts were made. The radioactivity was measured by β liquid scintillation counter (LS-1801, Beckman, Fullerton, OA, USA) and results were reported as

stimulation index (SI) i.e. mean count per minute (cpm) of stimulant containing wells/ mean cpm of control wells.

### Statistical Analysis

Data were analyzed with SPSS statistical software, version 16.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics was performed to calculate the mean SI values for different groups. Independent t-test was performed to see the statistical difference for SIs between different stimuli across the groups. A *p*-value $\leq 0.05$ was considered statistical significant.

## Results

### Demography of Study Population

The patient's demographic details are included in Table 1. The mean age (± SD) of PD patients, CKD patients and control subjects were 52.3±7.3years, 50.4±9.4 years and 49.8±7.8 years respectively. In all three groups the male-to-female ratio was 14:6. There were more diabetics in PD as compared to CKD group (70 vs 35%, p=0.02).

**Table 1:** Demographic details of study population

| Characteristics | PD patients (N = 20) | CKDpatients (N = 20) | Healthy control (N = 20) | P value |
|---|---|---|---|---|
| Age (years) | 52.3 ± 7.3 | 50.4 ± 9.4 | 49.8 ± 7.8 | NS |
| Gender (female /male) | 6/14 | 6/14 | 6/14 | |
| PD duration (months) | 5 ± 2.2 | NA | NA | |
| Diabetes mellitus | 14 (70%) | 7 (35%) | 0 | 0.02 |
| Coronary artery disease | 0 | 2 (10 %) | 0 | __ |

PD= Chronic peritoneal dialysis; CKD= Chronic kidney disease stage 5 not on dialysis

### Cell Proliferation Assay

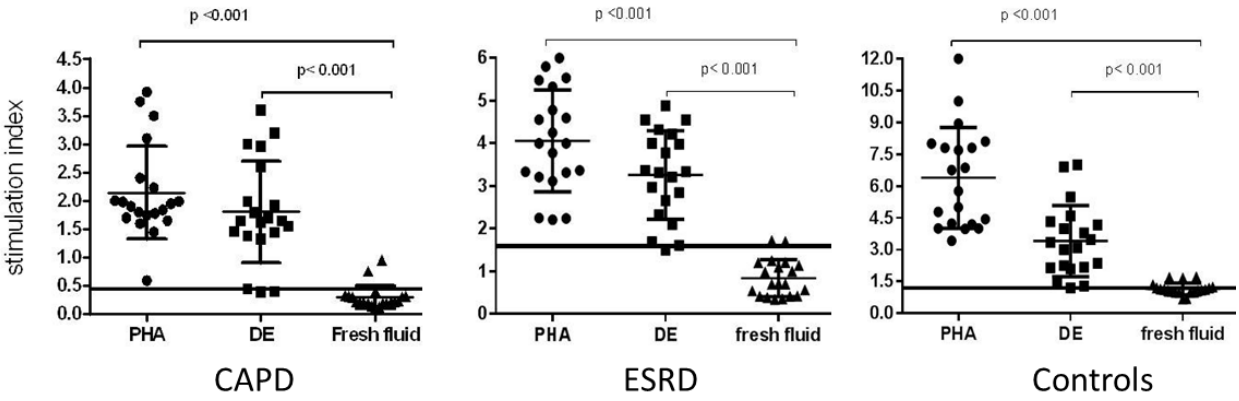
The mean SI values of the PBMCs proliferation with PHA, DE and PDF are shown in Table 2. One-way ANOVA showed a significant difference with different stimuli in all three-study groups ($p < 0.001$). PHA had significantly higher SI value in all study groups when compared to PDF. On comparing DE with PDF, DE showed significantly higher SIs values in all groups. The SIs values in PD patient to all stimuli (PHA, DE, and PDF) were significantly lowers ($p < 0.001$) than both CKD patients and healthy controls (Figure 1). The CKD patients showed significantly lower SIs values for all stimuli when compared to healthy controls ($p < 0.001$).

**Table 2:** Mean stimulation index (SI) in peritoneal dialysis (PD), chronic kidney disease(CKD) and healthy control groups

| Population groups | Mean stimulation index (SI) | | |
|---|---|---|---|
| | PHA | DE | PDF |
| PD (20) | 2.14 ± 0.89 | 1.80 ± 0.89 | 0.29 ± 0.21 |
| CKD (20) | 4.06 ± 1.19 | 3.26 ± 1.04 | 0.84± 0.43 |
| Controls (20) | 6.39 ± 2.37 | 3.41 ± 1.67 | 1.15 ± 0.28 |

SI = (cpm experimental/ cpm background unstimulated)
Mean count per minute (cpm) = (cpm experimental - cpm background unstimulated)
PHA= Phytohaemagglutinin
DE= Peritoneal dialysate effluent
PDF= Fresh peritoneal dialysis fluid

**Figure 1**: Graph shows stimulation index of peripheral mononuclear cells (PBMCs) with PHA, DE and PDF in peritoneal dialysis (CAPD), chronic kidney disease Stage 5 (ESRD) and controls subjects.




PHA= Phytohaemagglutinin
DE= Peritoneal dialysate effluent
PDF= Fresh peritoneal dialysis fluid

## Discussion

It has been shown that patients on peritoneal dialysis exhibit compromised immune functions with increased susceptibility to infection, which is considered as the second leading cause of death among dialysis patients [19]. PBMCs, which consist of T-cells, B-cells (immunocompetent cells) and monocytes, provide first line of defense against any microbial invasions.In the current study, we have measured the proliferation or stimulation index (SI) of PBMCs, isolated form PD patients, CKD stage 5 patients and controls, after induction with PHA, DE, and PDF stimuli (PDF used as control stimulus). We observed that PBMCs derived from CKD patients were hypo reactive to *in vitro* stimulation compared with control subjects, while the patients on PD did even worse than CKD patients. This finding suggests that the innate and adaptive immune responses of CKD patients are blunted with the patients on PD being the worst. Other studies have also shown defect in innate immunity in patients on dialysis. Kuroki et al. have shown that expression of Toll-like receptor (TLR) TLR-4 and TLR-2 on PBMCs following bacterial lypo-polysaccharide (LPS) stimulation was lower in HD and peritoneal dialysis patients than healthy controls[20]. Studies have also shown reduced T cell function and compromised humoral immune response in patients on dialysis [21]. Although the exact mechanism for reduced immune response is unknown, in the present study we also for the first time demonstrate that SI of PBMCs is also impaired in CKD stage 5 patients not on dialysis suggesting that some uremia associated factors are likely to be responsible for this decreased PBMCs response.

Another interesting finding in our study is that dialysate effluent was observed to significantly stimulate the PBMCs. Importantly Ando et al.[15] showed that multiple abnormalities existed in innate immune response in dialysis patients which was worse in patients on PD as compared to those on HD. Our data may imply that the PBMCs of PD patients are 'exhausted', possibly due to chronic activation by the back-diffusion of immunogenic substances from the dialysate effluent making them refractory to pathologic stimulation.

## Conclusion

In conclusion, present study demonstrates that PBMCs from CKD stage 5 and PD patients are less immuno-responsive to T- and B cell mitogen as compared to healthy controls suggesting impaired innate immunity in these patients, which may result in increased rate of infections in these patients. In patients on PD, dialysis effluent by chronically activating the PBMCs may contribute further to this hypo responsiveness, but the pathogenic mechanism and its long-term effects on immune and peritoneal membrane cells remain unknown and calls for further studies.

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## Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

## Compliance with Ethical Standards

The study was approved by the institutional ethics committee, and written informed consent was obtained from all the study subjects.

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