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RESEARCH ARTICLE

Assessment of phagocytic activity of polymorphonuclear leukocytes in hospitalized patients with stages 4 and 5 chronic kidney disease

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ABSTRACT

Background: Infections are the main reason for hospitalization and the second common cause of death in chronic kidney disease (CKD) patients. Infections develop primarily as a consequence of deranged innate immunity. In this study, we assessed the efficiency of phagocytic function of polymorphonuclear (PMN) leukocytes in severe CKD patients. **Aims and Objectives:** The aims of the study were (i) to assess the phagocytic index and lytic index of PMN leukocytes (PMNLs) in stages 4 and 5 CKD patients. (ii) To compare the above indices with healthy age and sex-matched controls. **Materials and Methods:** The study was carried out in 60 adults in the age group of 18–60 years of which 30 were CKD patients in stages 4 and 5 taking conservative treatment (not on dialysis) and other 30 were age and sex-matched controls. After screening for inclusion and exclusion criteria, 5 ml of venous blood was collected and taken immediately for evaluation. Phagocytic index which is the number of neutrophils positive for ingested microbes per 100 neutrophils and lytic index which is the total number of microbes per 100 cells were calculated. These are indices of neutrophil functioning. **Results:** Statistically significant decrease in both phagocytic index and lytic index were found in stages 4 and 5 CKD patients as compared to controls. **Conclusion:** In the present study, we were able to establish that phagocytic capacity of PMNLs is adversely affected in severe CKD patients. This gains importance in the light of immune dysfunction being considered as a major cause of premature deaths resulting from infections in severe CKD.

KEY WORDS: Chronic Kidney Disease; Phagocytic Index; Lytic Index

INTRODUCTION

Chronic kidney disease (CKD) encompasses a spectrum of different pathophysiologic processes associated with abnormal kidney function and a progressive decline in glomerular filtration rate (GFR).[1]

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End-stage renal disease (ESRD) is a stage of CKD where there is accumulation of toxins, fluid, and electrolytes normally excreted by kidneys leading to uremic syndrome. Uremia (which develops in later stages of CKD) related immune dysfunction is a complex interaction between alterations in both the innate and adaptive immune systems. In uremic patients, there is co-existence of immune activation and immunosuppression. Immune activation is characterized by elevated cytokine levels and acute phase response and immunosuppression is characterized by impaired responses to infections. Infections are the main reason for hospitalization and the second common cause of death in ESRD patients. An increased risk of bloodstream infection is also observed in

older adults with nondialysis-dependent CKD stages 4 and 5. Infections develop primarily as a consequence of deranged functions of polymorphonuclear (PMN) leukocytes.[2]

There appears to be a definite link between the pathologic processes of cardiovascular diseases and infectious diseases which are the main causes of death in patients with CKD and immune function. Furthermore, CKD patients respond poorly to challenges of bacterial infections.[3]

PMN leukocytes (PMNLs) are phagocytes which engulf antibody-coated or complement-coated microbes, damaged cells, and cellular debris. PMNLs are an important part of innate immune system.

Although the phagocytic function of PMNs in uremic patients has been subjected to repeated evaluation, results have been inconsistent.[4-9] Furthermore, the effects of progressive uremia on PMN function have been scarcely evaluated.[10]

This study proposes to assess the efficiency of phagocytic function of PMNLs in severe CKD patients.

Aim

The aim of the study was to assess the phagocytic function of PMNLs in stages 4 and 5 CKD patients who are not on dialysis and to compare it with healthy age and sex-matched controls.

MATERIALS AND METHODS

This pilot study was carried out in a representative sample of 30 stages 4 and 5 CKD patients who were not on dialysis, hospitalized for evaluation in the Department of Nephrology in a tertiary care hospital in Tirupati and the results were compared with 30 age- and sex-matched healthy volunteers after taking Institutional Ethical Committee approval.

Hospitalized, nondiabetic CKD patients in the age group of 18–60 years were classified into different stages using GFR calculated by Cockcroft-Gault equation.[1]

$$\text{GFR } (\text{ml/min}) = \frac{140 - \text{age} \times \text{Body weight in kg}}{72 \times \text{Serum creatinine in mg/dl}}$$

In the case of females, the above formula was multiplied by 0.85.[1]

As per the Kidney Disease Outcomes Quality Initiative guidelines,[11] GFR between 29 and 15 ml/min/1.73 m^2 was classified as Stage 4 CKD and GFR <15 ml/min/1.73 m^2 was classified as Stage 5 CKD. Patients with hepatitis B and C and human immunodeficiency virus (HIV) seropositive patients and pregnant women were excluded. Phagocytic index and lytic index of PMNLs of stages 4 and 5 CKD patients were compared with that of 30 age and sex-matched healthy volunteers. Written informed consent was taken from all the participants.

Screening Procedure

The patients of severe CKD were screened for hepatitis B and C using Qualisa hepatitis B virus surface antigen and Qualisa HCV kits as part of patient management. The method used was enzyme-linked immunosorbent assay. The patients were asked to undergo HIV testing from integrated counseling and testing center strictly adhering to the National AIDS Control Organization guidelines of counseling, informed consent, and confidentiality. The patients who tested positive for hepatitis B, C, and HIV were excluded.

Collection of the Blood Sample

Under strict aseptic conditions, a 5 ml of peripheral venous blood was drawn from the antecubital veins of the subjects, collected in heparinized sterile bottles and transferred to sterile test tubes immediately for evaluation. The phagocytic index and lytic index of the neutrophils were measured on each of the samples drawn.

Procedure for Estimating Phagocytic Index and Lytic Index

The heparinized blood sample was centrifuged at 2500 rpm for 10 min and plasma was discarded. The buffy coat was aspirated carefully and transferred to another test tube. Two hundred µl of pooled sera, 100 µl of candida suspension, and 200 µl of Hank's medium were added to the buffy coat preparation from the test subject. The tube was kept in a water bath for incubation at 37°C for 30 min. The test tube was centrifuged at 1500 rpm for 5–10 min. The clear supernatant solution was discarded and the buffy coat was aspirated, taken on glass slides and smears were prepared.

The smears were stained with the Gram's stain and examined under oil immersion for the presence of microbes inside the neutrophils (Figure 1 polymorphonuclear cell with ingested candida in CKD patient [arrow mark, × 100]). The number of neutrophils positive for ingested microbes per 100 neutrophils was recorded as the phagocytic index[9] and the total number of microbes per 100 cells was recorded as lytic index. These are indices of neutrophil functioning.[12,13]

Statistical Analysis

The data was recorded on a pre-designed pro forma and managed using MS Excel 2007 (Microsoft Corporation, Redmond, WA). The descriptive statistics such as mean and standard deviation were calculated. The unpaired student's *t*-test was performed to compare the means between cases

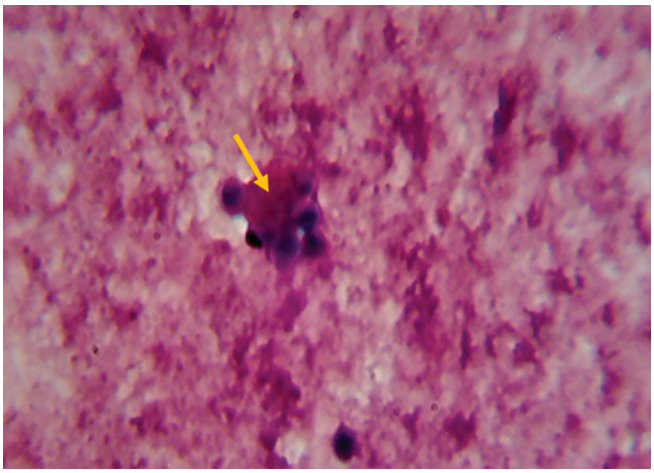

Figure 1: Polymorphonuclear cell with ingested candida in CKD patient (arrow mark, X 100)

and controls. $P \leq 0.05$ was considered significant. All statistical analyses were performed with the help of SPSS (Statistical Package for Social Sciences) version 20.0 (IBM corp., Armonk, NY).

RESULTS

This study was conducted in a total of 60 subjects. The study group consisted of 30 CKD (stages 4 and 5) patients in the age group of 18–60 years and control group consisted of 30 age and sex-matched controls.

There was a statistically significant decrease in the phagocytic index in the study group (CKD stages 4 and 5) when compared to the control group (64.1 ± 28.8 vs. 36.8 ± 16.6, $P = 0.001$). Furthermore, there was a statistically significant decrease in lytic index (446.33 ± 189.8 vs. 207.26 ± 101.2, $P = 0.001$) in stages 4 and 5 CKD patients as compared to controls [Table 1].

Table 1: The mean±standard deviation of phagocytic index and lytic index in severe CKD patients and controls

Parameters	Controls (Mean±SD)	CKD (Mean±SD)	*P* value
Phagocytic index	64.1±28.8	36.8±16.6	0.001
Lytic index	446.33±189.8	207.26±101.2	0.001

SD: Standard deviation, CKD: Chronic kidney disease

DISCUSSION

The present study was conducted to show the effect of the CKD (stages 4 and 5 who are not on dialysis) on innate immunity. In the present study, the phagocytic index and lytic index of PMNLs were found to be significantly reduced in severe CKD patients when compared to age and gender-matched controls. The main cause of increased risk of morbidity and mortality among CKD patients is deranged functioning of PMN cells leading to frequent bacterial infections. This derangement of PMNL functioning in severe CKD patients may be due to various factors such as increased circulating levels of uremic toxins, iron overload, or anemia due to uremia.[14,15] Some of the uremic toxins delay apoptosis which leads to neutrophils becoming more prone to necrosis causing low-grade inflammation. Other uremic toxins induce apoptosis which decreases necrosis induced inflammation but at the same time response to infections is compromised. Although the PMNLs are activated, their functional capacity is diminished.[16,17]

Massry and Smogorzewski in a study showed that the phagocytic capacity of PMNLs is impaired in uremia and the cause for this was attributed to excess of parathyroid hormone levels leading to increase in cytosolic calcium levels in PMNLs.[5] In a study conducted by Saeki *et al*., neutrophils of predialysis patients showed almost same phagocytic capacity as that of controls, but phagocytosis activity was significantly reduced in hemodialysis patients.[6]

In the present study, we have studied the phagocytic activity of PMNLs in stages 4 and 5 CKD patients who are not on dialysis and found it to be significantly reduced as compared to controls. Both phagocytic index and lytic index were assessed in this study which are indices of neutrophil functioning. Since it is a pilot study, the results cannot be generalized. The process of phagocytosis involves various steps such as margination, diapedesis, chemotaxis, opsonization, engulfment, degranulation, and finally killing phase. Since the high rate of infectious diseases in CKD patients due to immune failure is multifaceted; further, research has to be done to throw light on which of the above steps of phagocytosis are affected in CKD and its causes. This gains importance in the light of immune dysfunction being considered as a major cause of premature deaths resulting from infections in severe CKD.[18]

CONCLUSION

In the present study, we were able to establish that phagocytic capacity of PMNLs is adversely affected in severe CKD patients. Further study on which of the steps in phagocytosis are affected would give us targets for interventions aiming to reduce mortality in CKD patients. Furthermore, it would help to improve outcome in this patient group.

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