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A Comparative Study of Neutrophil Phagocytic Activity with Different Types of Haemodialyser Membranes

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SUMMARY

Neutrophil phagocytic activity in Chronic Renal Failure (CRF) patients on haemodialysis with Cellulose Acetate Membrane (CAM) and Cuprophane Membrane (CuM) was investigated. Thirty diagnosed cases of CRF and 15 healthy controls were included in the study. Neutrophil phagocytic activity was assessed by determination of percentage Phagocytosis (%P), Phagocytic Index (PI) and Nitroblue Tetrazolium (NBT) reduction test. In the control group values of %P, PI and NBT reduction (%) were 69.2± 9.23, 1.45±0.39 and 73.73±10 respectively. With CAM the pre-dialysis values of %P and PI were 67.4±9.2 and 1.32±0.26 respectively, which were not significantly different from the post-dialysis values, 65.33±8.18 and 1.25±0.43 respectively. Similarly there was no significant difference in NBT reduction (%) after dialysis with CAM, the pre and post-dialysis values were 68.8±10.35 and 66.8±9.98 respectively. On comparison these values were not different from the controls. With CuM values of %P and PI before dialysis were 64.86±11.5 and 1.2±0.31 respectively. After dialysis these values were 53.66±9.86 and 0.95±0.27 respectively. Intercomparison of pre and post-dialysis values of %P and PI showed a significant decrease after haemodialysis with CuM ($p<0.01$ and $p<0.05$ respectively). When compared with the control group there was again a significant decrease in both %P and PI after dialysis with CuM ($p<0.001$). The pre- and post-dialysis values of NBT reduction (%) with CuM were 63.64±12.6 and 50.86±11.9 respectively. Comparison showed a significant decrease after dialysis ($p<0.01$). Both the values were significantly lower than the values in the control group ($p<0.05$ and $P<0.001$ respectively). Comparison of post-dialysis values of %P and PI with CAM and CuM also showed a marked reduction with CuM ($p<0.001$ and $p<0.05$ respectively). Similarly comparison of post-haemodialysis NBT reduction (%) values between CAM and CuM showed a statistically significant decrease (66.8±9.98 and 50.86±11.9 respectively with $p<0.001$). It is concluded that neutrophil function is impaired with use of CuM for haemodialysis.

INTRODUCTION

In Chronic Renal Failure (CRF) uraemia is eventually fatal unless replacement therapy, dialysis or renal transplantation is introduced[1]. Different types of dialyser membranes used for haemodialysis show significant differences in their ability to activate complement and have variable effects on neutrophil function[2,3].

Frequent bacterial infections in uraemic patients maintained on haemodialysis[4] have been attributed to various dysfunctions of Polymorphonuclear Leukocytes (PMNL) such as altered adhesiveness[5], defective chemotactic responsiveness[6], and impaired phagocytic and bactericidal capacities of neutrophils[7,8]. Depressed activities of PMNL cells from chronic haemodialysis patients was recently reported by

Tullio et al[4]. Some reports however suggest improved phagocytic activity and increased neutrophil oxidative metabolism after haemodialysis[9].

Contribution of neutrophil apoptosis to neutrophil dysfunction was recently investigated. It was shown that uraemic plasma accelerated apoptosis of normal neutrophils resulting in their dysfunction[10]. Furthermore composition of the dialysis membrane may be a determinant of accelerated apoptosis of neutrophils found in haemodialysis patients[11,12].

The main function of PMNL is to provide the first line of defense against invading organisms[13,14]. This study was undertaken to assess the neutrophil phagocytic activity in patients of CRF on haemodialysis with different types of haemodialyser membranes, namely Cellulose Acetate Membrane (CAM) and Cuprophane Membrane (CuM). Neutrophil function was assessed by determination of Percent Phagocytosis (%P), Phagocytic Index (PI) and the Nitroblue Tetrazolium (NBT) reduction test[15,16]. Phagocytic mixture was prepared according to the method of Metcalf et al[15], after separation of PMNL using Mono-polyresolving medium.

PATIENTS AND METHODS

A total of 45 subjects, 15-70 years of age, both males and females were included in the study. The patient population consisted of 30 previously diagnosed cases of CRF, selected from different hospitals of Lahore. Further inclusion criteria were absence of diseases, like diabetes mellitus, malignancy, collagen disorders, haematologic or liver diseases, active or recent infection and any drug therapy which alters neutrophil function. Fifteen healthy control subjects were age, sex and socioeconomically matched with the patient group.

The selected subjects were divided into three groups: Group A: consisted of 15 control subjects; Group B: consisted of 15 CRF patients undergoing haemodialysis with CAM for at least 3 months; Group C: included 15 CRF patients undergoing haemodialysis with CuM for at least 3 months. Detailed history and results of investigations of each patient were recorded on a proforma especially designed for the study.

5 ml. of hepranized blood from each patient was obtained aseptically for the determination of percentage phagocytosis, phagocytic index and Nitroblue Tetrazolium (NBT) reduction test. One blood sample was obtained from the control subjects, while two samples, pre- and post haemodialysis were drawn from patients of CRF on haemodialysis.

Percent Phagocytosis (%P) and Phagocytic Index (PI):

Preparation of neutrophil suspension

Neutrophils were separated using Mono-Polyresolving Medium (M-PRM) (Flow Lab. UK). Separated cells were washed three times in Hank's Buffered Saline Solution (HBSS) by centrifugation at 250g for 10 min. at RT. The supernatant was removed and the neutrophil suspension adjusted to a concentration of $5x10^6$ cells/ml by making a 1:20 dilution with acetic acid.

Preparation of yeast cell suspension

Granules of baker's yeast were dissolved in 200ml of 0.9% NaCl solution. The mixture was incubated at 80°C for 30 min. to kill the yeast cells. A standard suspension of killed yeast cells was prepared by adjusting the count at $30\text{-}40x10^6$ cells/ml by making a 1:100 dilution with HBSS. Sodium bicarbonate was added to make the suspension alkaline and was preserved in aliquots at 4°C.

Fresh 20 ml. of normal pooled serum from healthy individuals was obtained and stored frozen in aliquots at -20°C.

Preparation of phagocytic mixture

Phagocytic mixture was prepared according to method of Metcalf et al[15]. 0.3 ml of HBSS was added to 0.5 ml. of neutrophil suspension. 0.1 ml. each of normal pooled serum and yeast cell suspension were added and the mixture incubated at 37°C for 30 min. with constant agitation. After incubation the mixture was immersed in ice to stop further phagocytosis and was centrifuged at 500 rpm for 10 min. The supernatant was removed and smears made with the sediment were stained with Giemsa stain.

The slide was examined under oil immersion objective. One hundred neutrophils were examined and %P and PI were calculated as follows:

%P = number of neutrophils containing yeast cells / 100 neutrophils.

PI (Al-Tuwaijri)[16] = Total number of yeast cells within the neutrophils / 100 (number of neutrophils counted).

Nitroblue tetrazolium (NBT) reduction test

The test was performed using the NBT kit provided by Sigma chemical company, according to the principle of Metcalf et al[15].

0.1ml. of NBT solution was transferred to a siliconized glass vial. With a plastic pipette 0.05 ml of hepranized blood and 5 ul of stimulant solution were added and mixed gently. The mixture was incubated at 17°C for 10 min. and then at RT for 10 min. After mixing, it was transferred onto a clean glass slide and a moderately thick smear was prepared, air-dried and stained with Accustain Wright stain. The smear was examined under oil immersion x 100 objective and 100 neutrophils were counted. Neutrophils showing formazan deposits were counted as positive and the percentage of neutrophils that reduced NBT were recorded.

Statistical analysis

Comparison of means of different observations was done using student's `t' test.

RESULTS

Age and sex distribution

The mean age of CRF patients on haemodialysis was 42.8±13.7 years with a range of 15-70 years. The normal controls had mean age of 42.7±13.3 years with a range of 16-70 years.

Among the 30 patients of CRF on haemodialysis, 17(57%) were males and 13(43%) females. Out of 15 controls 10(67%) were males and 5(33%) females.

Percent phagocytosis (%P) and phagocytic index (PI)

Percentage phagocytosis and phagocytic index values in different study groups are given in Tables 1 and 2. In the control group A, percent phagocytosis was 69.2±9.23 with a range of 55-83, while the PI was 1.45±0.39 with a range of 0.8-1.8. In group B (CRF patients on haemodialysis with CAM) the pre-dialysis % P-value was 67.4±9.2 with a range of 52-80. The pre-dialysis PI value in this group was 1.32±0.26 with a range of 0.9-1.7. When compared with the control group, these values were not significantly different. After dialysis %P was 65.33±8.18 with a range of 54-77 and PI was 1.25±0.43 with a range of 0.8-1.7. These values were lower than those of the control group, but not significantly different. Similarly the pre- and post dialysis values in this group were not statistically different.

In group C (CRF patients on haemodialysis with CuM) before dialysis %P value was 64.86±11.55 with a range of 42-78 and PI value was 1.2±0.31 with a range of 0.6-1.6. These values were decreased as compared to the control group, but statistically the difference was not significant. After dialysis %P and PI values were further decreased and were 53.66±9.86 with a range of 34-64 and 0.95±0.27 with a range of 0.45-1.31 respectively. When compared with the control group, this decrease was statistically significant ($p<0.001$, Tables 1 and 2). Intercomparison of pre and post-dialysis values of %P and PI also showed a significant decrease after dialysis ($p<0.01$ and $p<0.05$ respectively, Tables 1 and 2).

When the post-dialysis %P and PI values of group B and C were compared, a marked reduction with CuM was observed which was statistically significant ($p<0.001$ and $p<0.05$ respectively, Table 4.

Nitroblue tetrazolium (NBT) reduction test

Comparison of NBT reduction (%) in patients and controls is given in Table 3. In group A, NBT reduction was 73.73±10% with a range of 55-90%.

In group B the pre-dialysis NBT reduction value was 68.8±10.35% with a range of 46-83%. It was decreased as compared to the control group but the decrease was statistically non-significant. In the same group the post-dialysis NBT value was further decreased to 66.8±9.98% with a range of 45-81%, but the difference was statistically non-significant. The pre- and post-dialysis values when compared were also not different (Table 3).

In group C the pre-dialysis NBT reduction value of 63.64±12.6% with a range of 38-80% was significantly decreased as compared to the control subjects ($p<0.05$). After dialysis NBT reduction value was 50.86±11.9% with a range of 26-66%. It was also significantly decreased when compared with the control group ($p<0.001$). Intercomparison of pre- and post-dialysis NBT values also revealed a markedly significant reduction after dialysis with CuM ($p<0.01$, Table 3).

Table 1: Comparison of Percent Phagocytosis (%P) in Controls and Patients

Subjects	Group A	Group B		Group C	
		Pre	Post	Pre	Post
Controls (n=15)	69.2±9.23 (55-83)				
Patients (n=30)		67.4±9.2 (52-80)	65.33±8.18 (54-77)	64.86±11.55 (42-78)	53.66±9.86 (34-64)
Patients vs Controls		N.S.	N.S.	N.S.	P<0.001
Pre vs Post		N.S.		P<0.01	

(The values are expressed as Mean±SD. The range values are given in parenthesis)
Key: Group A = Controls
Group B = Patients on haemodialysis with cellulose acetate membrane.
Group C = Patients on haemodialysis with cuprophane membrane.

Table 2: Comparison of Phagocytic Index (PI) in Controls and Patients

Subjects	Group A	Group B		Group C	
		Pre	Post	Pre	Post
Controls (n=15)	1.45±0.39 (0.8-1.8)				
Patients (n=30)		1.32±0.26 (0.9-1.7)	1.25 (0.8 7)	1.2±0.31 (0.6-1.6	0.95±0.27 (0.45-1.31)
Patients vs Controls		N.S.	N.S	N.S.	P<0.001
Pre vs Post		N.S.		P<0.05	

(The values are expressed as Mean±SD. The range values are given in parenthesis)
Key: Group A = Controls
Group B = Patients on haemodialysis with cellulose acetate membrane.
Group C = Patients on haemodialysis with cuprophane membrane.

Post-dialysis NBT values of group B and C were also significantly different ($p<0.001$), with a marked decrease in NBT reduction % after dialysis with CuM as compared to CAM (Table 4).

DISCUSSION

Bacterial infections and sepsis remain important causes of morbidity and mortality in CRF

Table 3: Comparison of Nitrobluetetrazolium reduction (%) in Controls and Patients

Subjects	*Group A*	*Group B*		*Group C*	
		Pre	*Post*	*Pre*	*Post*
Controls (n=15)	73.73±10 (55-90)				
Patients (n=30)		68.8±10.35 (46-83)	66.8±9.98 (45-81)	63.64±12.6 (38-80)	50.86±11.9 (26-66)
Patients vs Controls		N.S.	N.S.	P<0.05	P<0.001
Pre vs Post		N.S.		P<0.01	

The values are expressed as Mean±SD. The range values are given in parenthesis)
Key:
Group A = Controls
Group B = Patients on haemodialysis with cellulose acetate membrane.
Group C = Patients on haemodialysis with cuprophane membrane.

Table 4: Comparison of Post-Heamodialysis Percent Phagocytes (%P), Phagocytic Index(PI) and NBT Reduction (%) values between Cellulose Acetate and Cuprophane Membranes

Investigation	*Cellulose acetate Membrane (CAM)*	*Cuprophane Membranes (CuM)*	**P value*
Percent Phagocytosis	65.33±8.18	53.66±9.86	<0.001
Phagocytic Index	1.25±0.43	0.95±0.27	<0.05
NBT reduction %	66.8±9.98	50.86±11.9	<0.001

The values are expressed as Mean±SD. *CAM vs CuM

patients[17] and CRF patients on haemodialysis[4]. Although this outcome is attributed to several factors, neutrophil dysfunction may be one of the factors for increased risk of infections in these patients. A few clinical studies have investigated the potential effect of different dialysis membranes on neutrophil function. Decreased chemotactic responses and impaired phagocytic and bactericidal activities in CRF patient's haemodialysed with cuprophane dialysis membrane have been reported[3,8,18]. This study was thus designed to observe the effects of haemodialysis with two different types of membranes on neutrophil function.

In the present study values of %P and PI after dialysis with CAM were not significantly different from the pre-dialysis mean values. However haemodialysis with CuM resulted in a significant decrease in both the %P and PI. Other workers have also reported similar significant decrease in phagocytosis in 38% of cases after completion of dialysis with CuM[8,19]. This decreased phagocytic activity may be due to greater activation of the alternate complement pathway[20] with resultant granulocyte aggregation and entrapment and a subsequent release of younger cells from the bone marrow. These cells have different phagocytic activity. Alternatively impaired Fc receptor function

has been suggested[19,21].

Vanholder et al[8]. have suggested that recurrent complement activation with CuM and subsequent activation of neutrophils with release of their intracellular enzymes plays a role in decrease of their phagocytic response to further challenge. Different dialysis membranes show significant differences in their ability to activate complement. The products of complement activation have several biological actions which may contribute to adverse clinical and biochemical changes[22]. Complement activating dialysis membranes have been shown to have an adverse effect on neutrophil function[2,3]. A number of workers have reported greater complement activation with cuprophane dialyser membrane than the cellulose acetate (CA) and Polymethylmethacrylate (PMMA) membranes. Nguyen et al[2]. showed decreased leucocyte count as well as neutrophil oxidative metabolism with cuprophane membrane but no significant change was observed with Polyacrylonitrile (PAN) membrane. Naveceral et al[23]. studied the effect of membrane contact with four different dialyser membranes, Cuprophane (Cu), Polycarbonate (PC), Polysulphone (PS) and Polyacrylonitrile (PAN). They observed defective neutrophil function with CuM but not with others. More recently Thorlacius et al[24]. reported impaired leukocyte rolling, adhesion and transendothelial migration, following cuprophane haemodialysis. Decreased leukocyte rolling has also been shown experimentally with cuprophan and polysulphone membranes[25].

In the present study NBT reduction by PMN cells before and after haemodialysis with CAM was found to be within normal control range. Markert et al[3]. examined production of oxygen radicals by PMN cells using Chemiluminescence (CL) technique and reported no significant difference in oxygen radical production by pre- and post-dialysed PMN cells when CAM was used.

However in the present study a significant decrease in mean NBT reduction values was observed prior to haemodialysis with CuM and after dialysis a further significant decreased as compared to the pre-dialysis values was observed. Oxidative metabolism[26], oxygen radical production by PMN cells[3,23] and superoxide anion (O_2) production by PMNL[18] have all been reported to be significantly decreased after haemodialysis with CuM, when compared to normal controls. A transient but significant reduction in oxygen radical production in patients haemodialysed with Cu and PC membranes has also been observed[3]. PMNL contact with CuM dialysis membrane coils might cause damage to the NADPH oxidase enzyme[8,26]. Winchester et al[27]. on the contrary reported a significantly increased postdialysis mean NBT value in patients haemodialysed with CuM.

Another possibility of significantly reduced NBT reduction in patients haemodialysed with CuM might be blocking of C5a receptors on PMN cells due to complement activation and subsequent generation of C5a[16]. Alternatively haemodialysis with CuM removes functionally active cells and the remaining PMN cells become less responsive[3]. Complement activation occurs to a much lesser extent with CAM than with CuM, this could explain the difference in responses with the two membranes[20]. Neutrophil response to phagocytic challenge was assessed by Vanholder et al[8] in uraemic and haemodialysed patients using Cu, PS and PMMA membranes. They concluded that patients with CRF developed worsening metabolic response of phagocytes, which was markedly exacerbated by initiation or maintenance of dialysis with CuM. The higher incidence of infections in these patients was attributed to decreased metabolic response of neutrophils because of use of CuM, a complement activating membrane.

It is thus concluded that neutrophil function is impaired with use of CuM, so haemodialysis using CAM is more useful than CuM. Understanding the impact of different types of dialysis membranes on the neutrophil phagocytic function may help in selection of appropriate dialysis membranes for patients on haemodialysis.

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