VIA MEDICA

ORIGINAL ARTICLE

Tülay Kiliçaslan Ayna[1, 2], Burcu Akman[3], Aslı Özkizilcik Koçyiğit[1, 2], Derya Güleç[1, 2], Cem Tugmen[4], Mustafa Soyöz[1, 2]

[1]Department of Medical Biology and Genetics, Izmir Katip Celebi University, Izmir, Turkey
[2]Tissue Typing Laboratory, University of Health Science Tepecik Training and Research Hospital, Izmir, Turkey
[3]Izmir International Biomedicine and Genome Institute (iBG-izmir), Dokuz Eylul University, Izmir, Turkey
[4]General Surgery, University of Health Science, Tepecik Training and Research Hospital, Izmir, Turkey

Flow cytometric evaluation of T and B lymphocyte percentage in chronic kidney disease

Corresponding author:

Mustafa Soyöz, Assist. Prof.
Gaziler Cad. 1140/1 Sok.
No:468, 35180,
Yenişehir, Konak, Izmir-Turkey
Tel: 0090532 352 0362
Fax: 0090232 386 0888
E-mail: msoyoz@gmail.com

Medical Research Journal 2017;
Volume 2, Number 1, 29–33
10.5603/MRJ.2017.0005

ISSN 2451–2591

ABSTRACT

Introduction. T and B lymphocytes play crucial roles in adaptive immunity. These cells are negatively affected in multiple disorders, including chronic kidney disease. The purpose of this study was to compare T and B lymphocyte ratios between patients with chronic kidney disease and healthy controls.

Methods. In this study, we evaluated the percentages of patient and donor (healthy control) lymphocytes referred to our laboratory between 2012 and 2014. In total 103 patient-donor couples were tested by the FCXM method. CD3-PerCP and CD19-PE monoclonal antibodies were used in order to differentiate T and B cells, respectively. T and B cell percentages of the participants were statistically compared.

Results. The mean age of the investigated patients and donors was 36.3 ± 13.7 and 46.2 ± 12.4 years, respectively. Of the studied patients, 45.6% and 54.3% were female and male, whereas 54.3% and 45.6% of donors were female and male, respectively. In the investigated group, 42 patients were preemptive, 45 subjects were treated with haemodialysis, and 16 individuals were on peritoneal dialysis. T and B lymphocyte percentages in the healthy group were higher than in patients with chronic kidney disease. However, the difference reached statistical significance only for T lymphocytes ($p < 0.05$). The percentages of total lymphocytes, and T and B lymphocytes in patients treated with haemodialysis were numerically lower than in those on peritoneal dialysis. In addition, we found that patients with chronic kidney disease had lower concentrations of haemoglobin and albumin than healthy controls.

Conclusion. This study suggests that patients with advanced chronic kidney disease have lower rates of lymphocytes that healthy controls. This fact may at least partially explain impaired immunity in this setting. However, our findings require confirmation and detailed investigation of underlying mechanisms in further studies.

Key words: chronic kidney disease, dialysis, lymphocyte, flow cytometry, haemoglobin

Med Res J 2017; 2 (1): 29–33

Introduction

Chronic kidney disease (CKD) has a high worldwide prevalence and incidence. More than two million CKD patients worldwide survive due to dialysis treatment or renal transplant [1]. In Turkey, more than 62,000 people suffer from CKD, requiring long-term dialysis. Of these patients, approximately 8% are transplanted from deceased donors or living, related donors [2]. Although the death rates due to bacterial infection have decreased in the general population, it is the second most prevalent cause of death in the CKD population. This may be caused by CKD-related impaired immune response. Observed immunological abnormalities in CKD patients include: diminished antibody synthesis in B lymphocytes, defective T lymphocyte-mediated immunity, failure in antigen presentation by monocytes/macrophages, and decreased phagocytic functions of granulocytes and monocytes/macrophages. The mechanisms responsible for these abnormalities have not been completely understood [3].

In this study, we aimed to compare lymphocyte percentages between CKD patients and healthy individuals. Our additional goals were to investigate the relationship between dialysis type, duration, and the lymphocyte percentage and to assess concentrations of haemoglobin and albumin, and the percentages of lymphocyte, in the compared groups.

Table 1. The comparison of investigated parameters between the study and control groups

Group	Age (years)	Total lymphocytes (%)	T lymphocytes (%)	B lymphocytes (%)	Haemoglobin [g/dL]	Albumin [g/dL]	Dialysis duration (months)
Patients (n = 103)	36.3 ± 13.7	48.9 ± 15.6	61.3 ± 22.0	1.6 ± 1.3	11.6 ± 1.7	4.1 ± 0.5	15.4 ± 31.6
Controls (n = 103)	46.2 ± 12.4	54.7 ± 13.8	72.0 ± 14.1	2.5 ± 1.7	13.6 ± 1.8	4.4 ± 0.5	n/a
p-value	ns	ns	< 0.05	ns	ns	ns	n/a

n/a — not applicable; ns — non-significant

Methods

In this study, a total of 103 CKD patients and their 103 donors (healthy controls) referred to our laboratory between 2012 and 2014 were included. In our laboratory, flow cytometry cross match (FCXM) is a routine test for pre-transplant assessment. T and B lymphocytes of this cohort were analysed by flow cytometry. The overall study cohort was further divided into three subgroups according to their dialysis status.

Donor and patient lymphocytes were used in order to evaluate crossmatch and autologous results, respectively. We assessed total, T, and B lymphocyte percentages during FCXM. Anti-human CD-3 PerCP (Becton Dickenson, Sen Jose, USA) monoclonal antibody was used for the identification of T lymphocytes, while anti-human CD-19 PE (Becton Dickenson, San Jose, USA) monoclonal antibody was used for B lymphocytes. Lymphocytes were selected on the basis of their forward and side scatter characteristics by flow cytometry. Fluorescence intensity was measured with the FACS Calibur (Becton Dickenson, USA) flow cytometer using Cell Quest Pro software.

This study was performed in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. Written, informed consent was obtained from all study participants.

Descriptive and frequency analyses were performed using IBM SPSS version 21.0 for Windows (SPSS, Chicago, Ill, United States). Chi-square and Fisher's exact tests were used to compare median and percentage ratios between groups. Haemoglobin and albumin levels of the patient's ratios between the groups were compared by Student's t test. In a one-way Anova, multiple mean values were compared by Tukey's multiple comparison tests. $p < 0.05$ was accepted as statistically significant.

Results

The mean age of the 103 CKD patients and donors was 36.3 ± 13.7 and 46.2 ± 12.4 years, respectively. Importantly, none of the patients or controls presented with symptoms of an active infection during the study. Of the studied patients, 45.6% and 54.3% were female and male, whereas 54.3% and 45.6% of donors were female and male, respectively. Dialysis duration, demographic characteristics, and flow cytometry results of the study participants (mean ± SD) are shown in Table 1.

We found that all assessed parameters were numerically higher in the control group than in the overall study group (Tab. 1 and Fig. 1). However, the difference reached statistical significance only for T lymphocytes ($p < 0.05$).

In the investigated group, 42 patients were preemptive, 45 subjects were treated with haemodialysis, and 16 individuals were on peritoneal dialysis. Values of investigated parameters (percentage of lymphocytes, concentrations of haemoglobin and albumin) in the three study subgroups and in the control group are displayed in Table 2.

The percentages of total lymphocytes, and T and B lymphocytes in patients treated with haemodialysis were numerically lower than in those on peritoneal dialysis. However, the differences were not statistically significant ($p > 0.05$). In addition, total T and B lymphocyte percentages in haemodialysis-treated patients were lower than in those on peritoneal dialysis.

T and B lymphocyte percentages observed in CKD patients and in the control group are shown in Figure 1, and haemoglobin and albumin concentrations observed in all three subgroups are displayed in Figure 2. Mean concentrations of haemoglobin and albumin were numerically lowered in all three subgroups of patients with CKD when compared to healthy controls. Figure 3 shows T and B lymphocyte percentages according to the dialysis duration.

Discussion

Immunological response mediated by lymphocytes is known to be impaired in CKD patients. The mechanisms causing immunological deficiency in CKD have not been completely elucidated yet. The influence of dialysis on immune response has been intensively in-

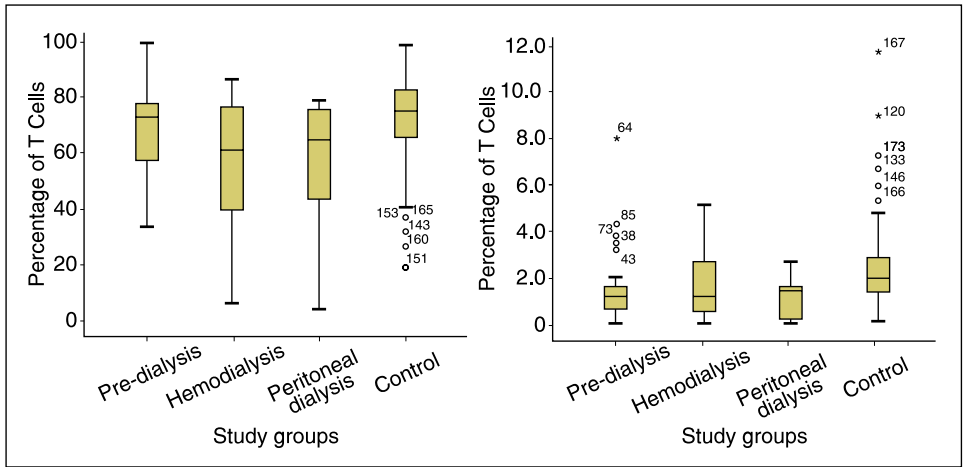

Figure 1. Comparison of T and B lymphocyte percentages

Table 2. Investigated parameters in three study subgroups and in the control group

Group	Age (years)	Total lymphocytes (%)	T lymphocytes (%)	B lymphocytes (%)	Haemoglobin [g/dL]	Albumin [g/dL]	Dialysis duration (months)
Preemptive (n = 42)	37 ± 14.7	53.3 ± 15.6	61.3 ± 22.0	1.8 ± 1.3	11.6 ± 1.6	3.9 ± 0.5	n/a
Haemodialysis (n = 45)	35.5 ± 11.3	42.1 ± 16.0	55.6 ± 24.3	1.3 ± 0.8	12.2 ± 1.8	4.2 ± 0.5	27.2 ± 41.4
Peritoneal dialysis (n = 16)	31.5 ± 11.6	52.8 ± 12.9	57.7 ± 24.2	1.6 ± 1.5	11.0 ± 1.9	3.2 ± 0.5	11.9 ± 22.5
Controls (n = 103)	46.2 ± 12.4	54.7 ± 13.8	72.0 ± 14.1	2.5 ± 1.7	13.6 ± 1.8	4.4 ± 0.5	n/a

n/a — not applicable

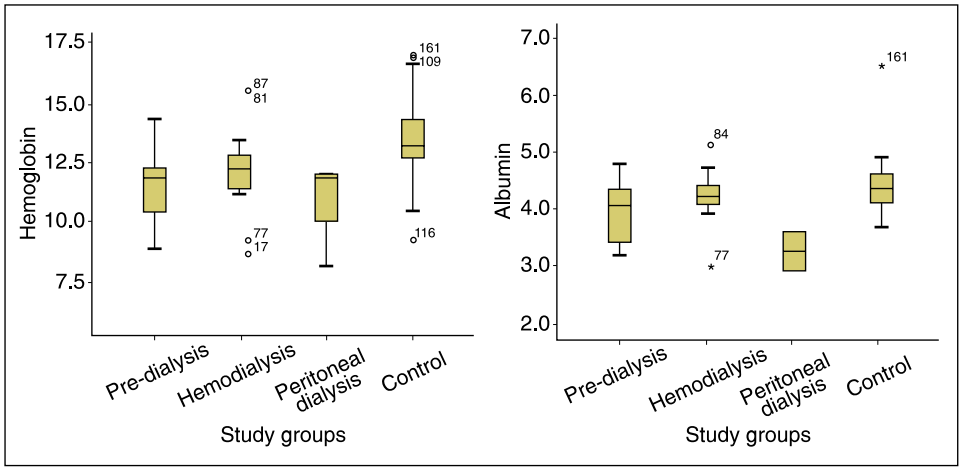

Figure 2. Comparison of haemoglobin and albumin concentrations

vestigated and discussed with inconclusive results. The majority of studies showed that numbers of $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes and B lymphocytes were decreased in haemodialysis-treated patients [4–6]. In our study, $CD3^+$ T lymphocytes and $CD19^+$ B lymphocytes were numerically lower in patients on haemodialysis than in healthy donors. During dialysis, the bioincompatibility of dialyser membrane induces various negative changes in the peripheral blood. It was reported that these decreases in T and B lymphocyte

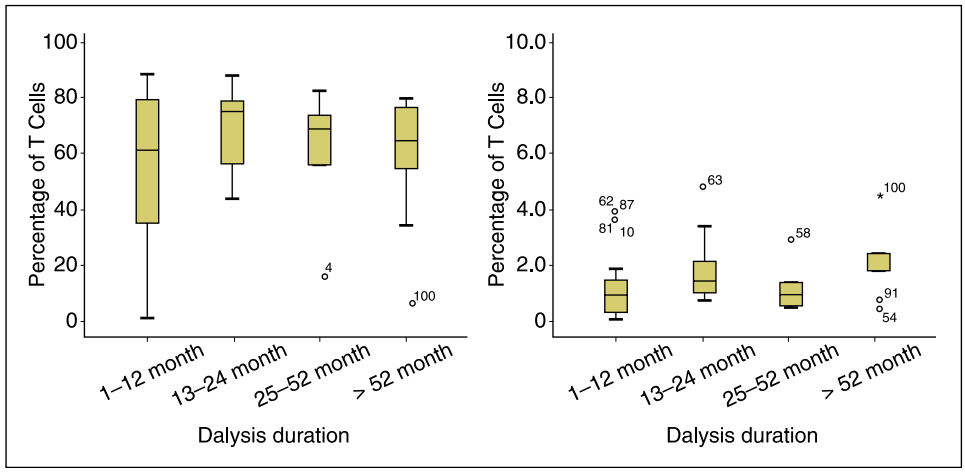

Figure 3. Changes in T and B lymphocyte percentages according to the dialysis duration

counts were consequences of the bioincompatibility of the dialyser membrane in the course of haemodialysis or due to the side effects of uremic toxins in peripheral blood [7, 8]. Also, it has been known that T-lymphocyte subpopulations depend on a number of factors, including age, gender, stress, physical activity level, hormones, protein, and trace element uptake [9]. Erythropoietin production decreases in CKD patients, and this may affect lymphocyte count because erythropoietin receptor is expressed on T and B lymphocytes and monocytes. Treatment with erythropoietin of CKD patients improves cytokine production and costimulatory molecule expression [8]. Thus, it can potentially restore the lymphocyte function. Unfortunately, we were not aware which of our study participants were treated with erythropoietin.

Anaemia, hypoalbuminaemia, and malnutrition cause significant impairment of immune response in the setting of advanced CKD [10]. In this study, concentrations of haemoglobin and albumin in CKD patients were numerically lower than in the control group.

We observed that percentages of total lymphocytes as well as T and B lymphocytes in CKD patients decreased when they started therapy with dialysis. In the second year of the therapy (13th to 24th month), an increase in T and B lymphocyte levels was seen. In the following months of dialysis therapy, T lymphocyte counts decreased. B lymphocyte counts increased to the normal range after 52 months. Similar results were obtained by Grzegorzewska et al., who found a negative correlation between B lymphocyte count and dialysis duration [11]. Several studies indicated increased T lymphocyte apoptosis in CKD patients. In addition, decreased expression of apoptotic regulatory molecule Bcl-2 was demonstrated in CKD patients [8, 12].

Limitations of this study include: small sample size, case-control design without investigation of clinical endpoints, and the lack of evaluation of underlying mechanisms.

Conclusions

This study suggests that patients with advanced CKD have lower rates of lymphocytes that healthy controls. This fact may at least partially explain the impaired immunity in the CKD setting. However, our findings require confirmation and detailed investigation of underlying mechanisms in further studies.

Acknowledgements

The authors thank the staff of the Tissue Typing Laboratory, Department of Internal Medicine Nephrology, and Department of General Surgery of Izmir Tepecik TRH, Izmir Ataturk TRH, Izmir TRH, for their continuous effort in performing the study. The authors declare no financial or other conflict of interest.

References

1. Plantinga LC, Boulware LE, Coresh J, et al. Patient awareness of chronic kidney disease: trends and predictors. Arch Intern Med. 2008; 168(20): 2268–2275, doi: 10.1001/archinte.168.20.2268, indexed in Pubmed: 19001205.
2. Süleymanlar G, Altıparmak MR, Seyahi N, Trabulus S. Registry of Nephrology, Dialysis, and Transplantation in Turkey. The Turkish Society of Nephrology 2013.
3. Pahl MV, Gollapudi S, Sepassi L, et al. Effect of end-stage renal disease on B-lymphocyte subpopulations, IL-7, BAFF and BAFF receptor expression. Nephrol Dial Transplant. 2010; 25(1): 205–212, doi: 10.1093/ndt/gfp397, indexed in Pubmed: 19684120.
4. Zaher MM, Gaber A, Alrefaey AA, et al. Assessment of some trace elements: copper, zinc and magnesium and their impact on CD3

and CD4 levels in children on chronic hemodialysis. Life Sci J. 2013; 10: 222–230.
5. Lisowska KA, Dębska-Ślizień A, Jasiulewicz A, et al. Influence of hemodialysis on circulating CD4(low)CD25 (high) regulatory T cells in end-stage renal disease patients. Inflamm Res. 2014; 63(2): 99–103, doi: 10.1007/s00011-013-0679-z, indexed in Pubmed: 24189710.
6. Pahl MV, Gollapudi S, Sepassi L, et al. Effect of end-stage renal disease on B-lymphocyte subpopulations, IL-7, BAFF and BAFF receptor expression. Nephrol Dial Transplant. 2010; 25(1): 205–212, doi: 10.1093/ndt/gfp397, indexed in Pubmed: 19684120.
7. Grooteman MP, Nube MJ, van Limbeek J, et al. Lymphocyte subsets in dialyser eluates: a new parameter of bioincompatibility? Nephrol Dial Transplant. 1996; 11(6): 1073–1078, indexed in Pubmed: 8671971.
8. Lisowska KA, Dębska-Ślizień A, Jasiulewicz A, et al. Hemodialysis affects phenotype and proliferation of CD4-positive T lymphocytes. J Clin Immunol. 2012; 32(1): 189–200, doi: 10.1007/s10875-011-9603-x, indexed in Pubmed: 21993694.
9. Westermann J, Pabst R. Lymphocyte subsets in the blood: a diagnostic window on the lymphoid system? Immunol Today. 1990; 11(11): 406–410, indexed in Pubmed: 2078294.
10. Lang CL, Wang MH, Hung KY, et al. Correlation of interleukin-17-producing effector memory T cells and CD4+CD25+Foxp3 regulatory T cells with the phosphate levels in chronic hemodialysis patients. Scientific World Journal. 2014; 2014: 593170, doi: 10.1155/2014/593170, indexed in Pubmed: 24558316.
11. Grzegorzewska AE, Leander M, Grzegorzewska AE, et al. Lymphocyte subsets in the course of continuous ambulatory peritoneal dialysis. Adv Perit Dial. 2001; 17: 10–14, indexed in Pubmed: 11510253.
12. Alvarez-Lara MA, Carracedo J, Ramírez R, et al. The imbalance in the ratio of Th1 and Th2 helper lymphocytes in uraemia is mediated by an increased apoptosis of Th1 subset. Nephrol Dial Transplant. 2004; 19(12): 3084–3090, doi: 10.1093/ndt/gfh382, indexed in Pubmed: 15574999.