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ORIGINAL ARTICLE

Lymphocyte Subsets Evaluation in Kidney Transplant Patients in Basrah Center for Kidney Diseases and Transplantation

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ABSTRACT

Kidney transplantation is the most common solid organ transplant and the most effective treatment for end-stage kidney failure.

Methodology: Twenty-seven kidney transplant patients and sixteen healthy individuals were evaluated using flow cytometry to count T,B,NK cells at the Basrah Centre for Kidney Disease and Transplantation.

Results: Patients show higher mean CD3+/CD8+ cells count & lower mean CD4+/CD8+ cells count compared to control causing low CD4+/CD8+ratio, the difference is significant. Further, patients have lower B cell and NK counts. These changes are partly attributed to the effect of immunosuppression therapy received by Kidney transplant patients. In addition to the possible effect of alternation in cytokine synthesis profile.

Conclusion: Due to changes in cytokine generation, immunosuppressors decreased T cell activation and prevented the proliferation of activated B, T, and NK cells.

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INTRODUCTION

For chosen patients with end-stage renal illness, kidney transplantation is the primary therapy option. When compared to dialysis patients, successful transplant patients had lower morbidity and mortality rates and a higher quality of life[1]. Early understanding of kidney disease and understanding that life in patients with ESRD can be prolonged established the kidney as a pilot organ in developing transplantation.[2] Iraq was one of the first Middle Eastern countries to begin kidney transplantation[3]. Kidney transplantation is the ultimate treatment for patients with end-stage renal disease compared to dialysis, with lower costs, a better quality of life, and a much higher survival rate[4].

T cell subtypes in peripheral blood (CD3 +, CD4 +, CD8 +, and CD4 + / CD8 +) are clinical indicators of cellular defense function. CD4 + / CD8 + 1 T cell counts are normal in healthy adults, however altering this number increases morbidity and death [5]. The CD4 + / CD8 + T cell count was used to determine a person's ability to defend themselves. In general, the CD4 + / CD8 + ratio in healthy adults is more than one[6].

Identifying allogeneic non-self cells activates receptor T cells, which can alter cytotoxicity in transfected cells or other immune system cells, including B cells and macrophages. Dendritic cells can also serve as antigen-supplying cells, and

their activation can have several effects on the immune system, such as activating natural killer cells. In many cell types, the phenotype of natural killer cell entrance in human kidney transplant rejection has been verified [7].

Different subsets of natural killer cells become activated during different types of rejections. The complement system and the coagulation cascade are activated during kidney transplantation to express distinct immune responses. These essential components of natural defense (innate immunity) are intertwined. The complement system is a key mediator of the immune response, and as such, it impacts other endogenous systems. There are three ways to initiate the complement cascade [8].

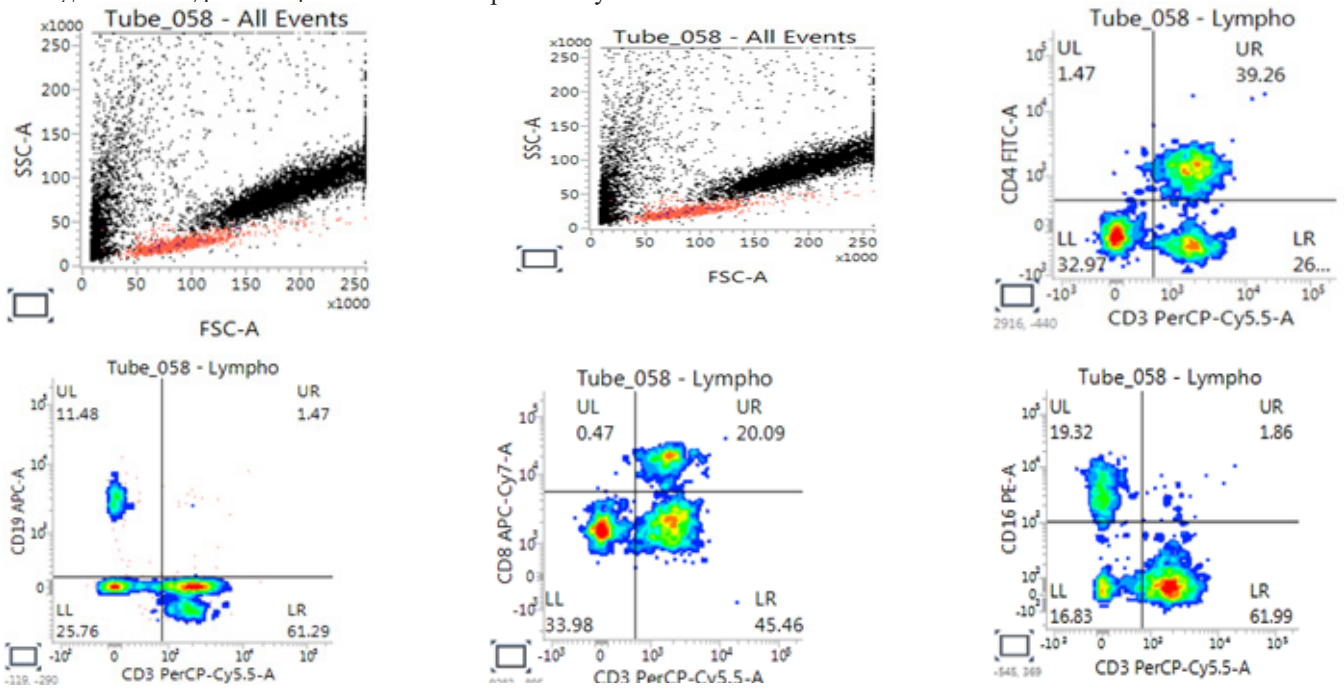

Fig 1: Lymphocyte subsets dot blots (control)
A- Lymphocyte subset population B- gate determination. C- CD4+T cell quadrant determination. D- CD8+T cell quadrant determination. E-CD3-CD19+(B cell) quadrant. F-CD3-CD16+(NK cell) quadrant determination

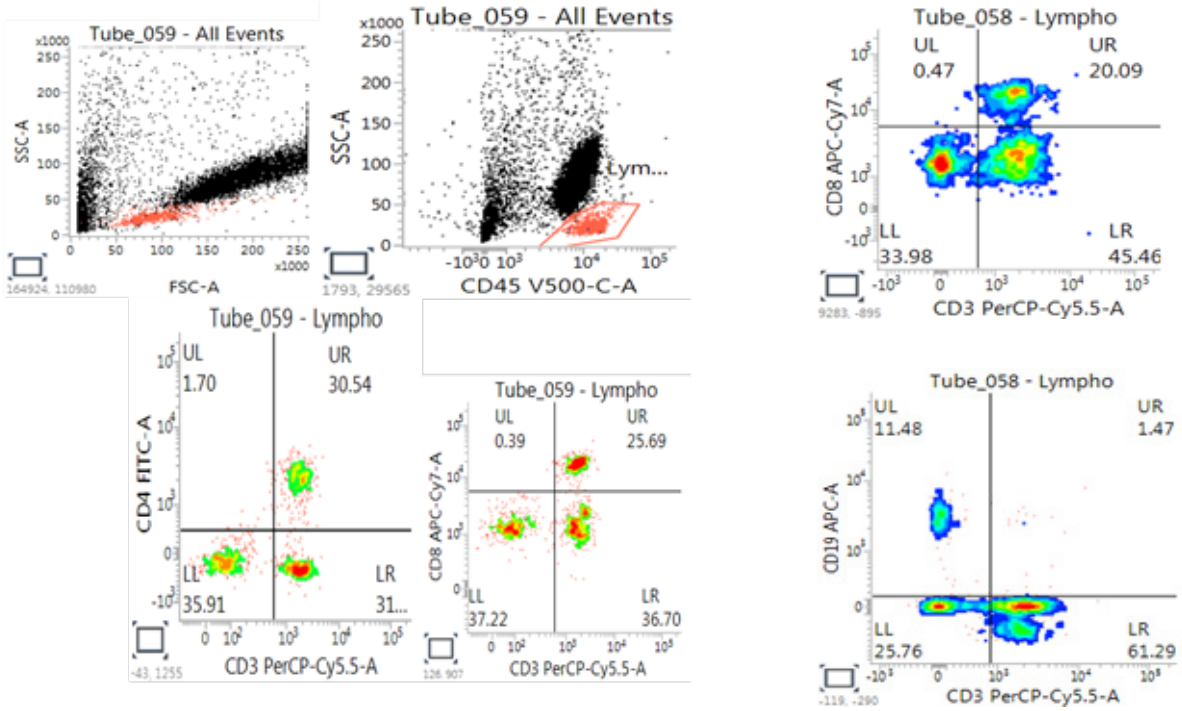

Fig 2: Lymphocyte subsets dot blots (KT patient)
A- Lymphocyte subset population B- gate determination.,C- CD4+T cell quadrant determination., D- CD8+T cell quadrant determination, E-CD3-CD19+(B cell) quadrant, F-CD3-CD16+(NK cell) quadrant determination

The complement system can cause graft inflammation as well as B and T cell responses to donor antigens showing complement inhibitory medications' function in reducing anti-allograft defense responses, as well as their prospective utility in kidney transplantation as a supplement to existing anti-rejection therapies. We recently found that kidney transplant recipients with a total NK cell count greater than 1.5 years post-transplant had a higher total NK cell count than those with a count less than 1.5 years [9].

Early post-transplant infections, infections during peak immunosuppression, and late-onset infections are the three types of infections that can occur after a kidney transplant [10]. Many factors influence infection time, including receptor-specific and determinant factors including prior or uninfected infection, antimicrobial prophylaxis use, and net immunosuppressive condition[11].

Glucocorticoids (mainly oral prednisone), azathioprine, and mycophenolate mofetil (MMF, cyclosporine) are the most common immunosuppressants encountered in combination therapy[12].

Immunosuppressants with different mechanisms of action are used in traditional maintenance regimens. This technique lowers the rates of morbidity and mortality associated with each drug class while improving overall efficacy. Treatments may differ depending on the patient, transfer center, and geographic area[13].

Table 1: Demographic characters for study groups

Variables	KT (n=27)	Healthy control (n=16)
Gender Male Female	24(88.88%) 3(11.111%)	11(68.75%) 5(31.25%)
Age	2(7.407%)	1(3.22%)
>20	15(55.55%)	11(35.48%)
20-39	10(37.037%)	4(12.903%)
40+		
Blood Urea	31.75556 ± 13.684	24 ± 7.509
Serum creatinine	1.3911 ± 0.758	0.45 ± 0.216

Aims of theSstudy

The research subjects The goal of the study was to calculate absolute values of lymphocyte subpopulations CD3+, CD3+CD4+, CD3+CD8+ T cells, CD19+ B cells, CD3-CD16+ natural killer cells, and the CD4+: CD8+ index based on a few studies in Iraq, particularly Basrah, that showed high light cellular immune derangement in these patients.

MATERIAL AND METHODS

Table: 2 Kidney transplant patients and control means

Studied groupParameters	KT N=27 Mean	Control N=16 Mean	p-value
Total lymphocyte count	1.270 ± 0.6837	1.848 ± 0.564	0.002
CD3+ absolute count	0.7903 ± 0.4958	1.1058 ± 0.44828	0.037
CD4+ absolute count	0.2691 ± 0.2188	0.572 ± 0.23114	0.000
CD8+ absolute count	0.5015 ± 0.3241	0.4744 ± 0.2045	0.585
CD4+/CD8+ ratio	0.535 ± 0.3128	1.22 ± 0.225	0.000
CD19+ absolute count(B-cell)	0.2092 ± 0.2347	0.2515 ± 0.1234	0.0631

From January 2019 to January 2021, 16 healthy persons (11 men and 5 women) and 27 kidney transplant patients (aged 15–55 years) participated in the study (3 women, 24 men). Based on written informed consent, participants were recruited from the Basra Center for Kidney Diseases and Transplantation, Al-Sader Teaching Hospital. They provided information such as age, gender, origin, Diagnosis, Date of Renal Failure, Dialysis Duration, Treatments, Family history, Date of Kidney Transplantation, Relationship to Donor, and other pertinent information. -Chronic Diseases, Urea in the Blood, and Creatinine in the Serum.

They filled out a questionnaire that asked for information. Peripheral blood samples were collected in vacutainer tubes containing the anticoagulant ethylene diamine tetra acetic acid (EDTA). The samples were immediately transferred to Bayan Investment laboratory and processed according to the flourecent markers manufacturer's protocol and flow cytometry with three lasers and eight filters using BD FACSuite TM software V 1.2.15657. An automated hematology analyzer was used to calculate the absolute and differential blood cell count for all samples (Beckman soulter Cytomics FC 500, Florida, USA).

Flow Cytometry

The lymphocyte subsets in lysed peripheral whole blood were identified using the BD FACS TM Lysis Solution. Blood was administered in the amount of ten ounces. The samples were made following the manufacturer's instructions. Monoclonal antibodies against T, B, and NK cells were used to describe lymphocyte subpopulations: T cells (CD45+, CD3+); T cells (CD45+, CD3+, CD4+); T cells (CD45+, CD3+, CD8+); T cells (CD45+, CD3+, CD4+); T cells (CD45+, CD3+, CD8+); T cells (CD45+, CD3+, CD8+ CD45+, CD19+ B cells CD45+, CD16+, CD3-NK cells BD FACSuite TM software V 1.2.15657 was used to analyze the data. The percentages acquired using a dual platform were used to compute absolute counts of lymphocyte subpopulations: Absolute count (cells per liter) = lymphocyte count (number of cells per liter of blood) x fraction of cell subpopulation of interest times 100.

Statistical Analysis

SPSS software statistics tool (SPSS for Windows ver.26.00) 2020 was used to analyze the data using the Chi square test and ANOVA test. For comparison of differences in outcomes across groups, a significant difference under probability P 0.05 was employed.

RESULTS

Our results show male 24(88.88%) in KT patients more than women 3(11.11%), and these patients from the middle age group 20-39 y.KT patients show higher serum creatinine 1.3911 and blood urea 31.7555 than control 0.45 and 24 respectively as shown in Table (1).

Total lymphocyte CD3+T cells, CD3+CD4+T cells,CD19+ B cells and NK cells are significantly lower in patients compared to control, while CD3+CD8+ T cells are slightly higher in patients but the difference is not significant as shown in Table (2).

DISCUSSION

The majority of kidney transplant recipients are given a cocktail of calcineurin inhibitors (tacrolimus), azathioprine, mycophenolic acid, and prednisone (two or more) (14). Clinical indicators of cell defense function include CD3 +, CD4 +, CD8 +, and CD4 + / CD8 + T cell subtypes in peripheral blood. CD4 + / CD8 + 1 T cell counts are normal in healthy adults, however altering this number increases morbidity and death[5]. According to our findings, CD3 + T cell deficit implies a low defense function of the human body, and CD3 + T cell proliferation was dramatically reduced in KT patients.

In the initial years after a kidney transplant, CD4 + depletion is common. The only clinical factor linked to long-term CD4 + lymphopenia was pre-transplant dialysis. Accept our KT findings. In controls from kidney transplant patients, we found a substantial number of CD4 + T cells. Some kidney disorders, such as lupus nephritis, have been linked to lower CD4 + T cell and CD4 + / CD8 + T cell numbers in the peripheral blood than healthy people[15]. T lymphocytes are the most important cell in cellular defense and are involved in practically all of the body's unique defense responses [16]. In the KT group, CD3 + CD4 + levels were lower than in the control group, resulting in a lower CD4 + / CD8 + ratio.

Reduced CD4 + / CD8 + T cell ratios in KT due to fewer helper T cells and more cytotoxic T cells in CD4 + / CD8 + T cell ratios indicate that immunological function is being assessed, and this rate balances various functions [17]. CD8 + T cells are important in generating cytokines that protect against infection, provide antitumor resistance, and decrease immune system function[18]. B-cell lymphopenia has been confirmed in patients with KT in other research. These investigations[19] and[20]support our findings that CD3-CD19 + levels are lower than in the control group. As a result of alterations in cytokine generation, several poor suppressors suppressed T cell activation and hindered the proliferation of activated B, T, and NK cells[21].

Our findings revealed that in patients with KT, there was a significant reduction in NK[22] The number of NK cells in the blood is thought to be a predictor of kidney transplant problems[23]. Immunosuppressants are linked to a lower NK cell count[24]. In short, additional research is needed, as well as a high number of kidney transplant recipients in Basra and Iraq.

REFERENCES

1. Brar, A., Markell, M., Stefanov, D. G., Timpo, E., Jindal, R. M., Nee, R & Salifu, M. O. (2017). Mortality after renal allograft failure and return to dialysis. American journal of nephrology, 45(2), 180-186.
2. Leon G.Fine.(2013).A 2000-year history of nephrology:10 enduringscientific landmarks. J NEPHROL. 26(Suppl 22): S6-S17
3. Rifat U N.(2006) Kidney Transplantation in Iraq. Saudi J Kidney Dis-Transpl, 17(3): 399-40
4. Jagdale A, Cooper DKC, Iwase H, Gaston RS.(2019) Chronic dialysis in patients with end-stage renal disease: relevance to kidney xenotransplantation. Xenotransplantation. ;26(2)
5. Hadrup, S. R., Strindhall, J., Køllgaard, T., Seremet, T., Johansson, B., Pawelec, G.,... & Wikby, A. (2006). Longitudinal studies of clonally expanded CD8 T cells reveal a repertoire shrinkage predicting mortality and an increased number of dysfunctional cytomegalovirus-specific T cells in the very elderly. The Journal of Immunology, 176(4), 2645-2653.
6. Vaziri, N. D., Pahl, M. V., Crum, A., & Norris, K. (2012). Effect of uremia on structure and function of immune system. Journal of Renal Nutrition, 22(1), 149-156.
7. Kildey, K., Francis, R. S., Hultin, S., Harfield, M., Giuliani, K., Law, B. M.,... & Healy, H. (2019). Specialized roles of human natural killer cell subsets in kidney transplant rejection. Frontiers in Immunology, 10, 1877.
8. Grafals, M., & Thurman, J. M. (2019). The role of complement in organ transplantation. Frontiers in Immunology, 2380.
9. Trojan, K., Zhu, L., Aly, M., mer, R., Bulut, N., Morath, C.,... & Daniel, V. (2017). Association of peripheral NK cell counts with Helios+ IFN-γ– Tregs in patients with good long-term renal allograft function. Clinical & Experimental Immunology, 188(3), 467-479.
10. Van Delden, C., Stampf, S., Hirsch, H. H., Manuel, O., Meylan, P., Cusini, A.,... & Mueller, N. J. (2020). Burden and timeline of infectious diseases in the first year after solid organ transplantation in the Swiss transplant cohort study. Clinical infectious diseases, 71(7), e159-e169.
11. Dorschner, P., McElroy, L. M., & Ison, M. G. (2014). Nosocomial infections within the first month of solid organ transplantation. Transplant Infectious Disease, 16(2), 171-187.
12. Wong, W., Venetz, J. P., Tolkoff-Rubin, N., & Pascual, M. (2005). immunosuppressive strategies in kidney transplantation: which role for the calcineurin inhibitors?. Transplantation, 80(3), 289-296.
13. Hardinger, K., & Brennan, D. C. (2020). Kidney transplantation in adults: Maintenance immunosuppressive therapy.
14. Lim MA, Kohli J, Bloom RD.(2017). Immunosuppression for kidney transplantation: where are we now and where are we going? Transplant Rev. 31:10–7
15. Shah, D., Aggarwal, A., Bhatnagar, A., Kiran, R., & Wanchu, A. (2011). Association between T lymphocyte sub-sets apoptosis and peripheral blood mononuclear cells oxidative stress in systemic lupus erythematosus. Free radical research, 45(5), 559-567.
16. Hu, Z., Zou, Q., & Su, B. (2018). Regulation of T cell immunity by cellular metabolism. Frontiers of Medicine, 12(4), 463-472.
17. Pichler, R., Sfetsos, K., Badics, B., Gutenbrunner, S., Berg, J., & Auböck, J. (2009). Lymphocyte imbalance in vitiligo patients indicated by elevated CD4+/CD8+ T-cell ratio. Wiener Medizinische Wochenschrift, 159(13), 337-341.
18. Shaban, E., Bayliss, G., Malhotra, D. K., Shemin, D., Wang, L. J., Gohh, R.,... & Gong, R. (2018). Targeting regulatory T cells for transplant tolerance: new insights and future perspectives. Kidney Diseases, 4(4), 205-213.
19. Saad K, Elsayh KI, Zahran AM, Sobhy KM. (2014)Lymphocyte

populations and apoptosis of peripheral blood B and T lymphocytes in children with end stage renal disease. Renal failure.36(4):502-7

20. Xiang FF, Zhu JM, Cao XS, Shen B, Zou JZ, Liu ZH, et al. (2016) Lymphocyte depletion and subset alteration correlate to renal function in chronic kidney disease patients. Ren Fail. 38:7–14
21. Zaza G, Leventhal J, Signorini L, Gambaro G, Cravedi P.(2019) Effects of antirejection drugs on innate immune cells after kidney transplantation. Front Immunol. 10:2978.
22. Nagai, K. (2021). Dysfunction of natural killer cells in end-stage kidney disease on hemodialysis. Renal Replacement Therapy, 7(1), 1-7.
23. Fernández-Ruiz, M., Silva, J. T., López-Medrano, F., Allende, L. M., San Juan, R., Cambra, F.,... & Aguado, J. M. (2016). Post-transplant monitoring of NK cell counts as a simple approach to predict the occurrence of opportunistic infection in liver transplant recipients. Transplant Infectious Disease, 18(4), 552-565.
24. Hutchinson, P., Chadban, S. J., Atkins, R. C., & Holdsworth, S. R. (2003). Laboratory assessment of immune function in renal transplant patients. Nephrology dialysis transplantation, 18(5), 983-989