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Study of IL-4 and INF –Gamma in Haemodialysis and Kidney transplant recipient patients in Basrah centre for Kidney Diseases and Transplantation

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Abstract

ESRD as the latest stage of CKD, in which the kidneys can no longer function adequately for an individual's daily life, only approximately 10% of kidney function remains.

Keywords: IL-4, INF_g, Haemodialysis, Kidney transplant recipient, Basrah center for Kidney Diseases and Transplantation

Introduction

End-stage renal disease (ESRD) as the last stage of chronic kidney disease (CKD), in which kidney function gradually decreases over time. People with ESRD have significant and permanent loss of kidney function and require regular dialysis (a procedure that removes deadly waste from a single bleeding area) or surviving kidney transplants (Scott *et al*., 2018). ESRD as the latest stage of CKD, in which the kidneys can no longer function adequately for an individual's daily life, only approximately 10% of kidney function remains (Himmelfarb and Sayegh, 2010).

Improving survival after kidney transplantation in ESRD patients (Kaballo et al., 2018) . Kidney recipients success may be due to immunosuppressive drugs, which have increased up to 90% of the survivors after 5 years (Hart *et al*., 2019).

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Interferon gamma-γ is a protein encoded by the IFNG gene (Zaidi and Merline ., 2011) . In human blood, IFN-γ is present in three fractions with different molecular mass. IFN-γ is a pleiotropic cytokine with antiviral, antitumor, and immunomodulatory functions. Hence, it plays an important role in coordinating both innate and adaptive immune response (Mendoza *et al* ., 2019). In an inflammatory environment, IFN-γ triggers the activation of the immune response and stimulates the elimination of pathogens; it also prevents over-activation of the immune system and tissue damage. This balance is maintained by complex mechanisms which are not yet fully understood (Ivashkiv ., 2018) . IFN-γ acts as a cytotoxic cytokine together with granzyme B and perforin to initiate apoptosis in tumor cells(Maimela *et al* ., 2018), but also enables then synthesis of immune checkpoint inhibitory molecules (Mojic *et al* ., 2018)The production of IFN-γ is mainly regulated by natural killer (NK) and natural killer T (NKT) cells in innate immunity while CD8+ and CD4+ T-cells are major paracrine sources of IFN-γ during adaptive immune response (Burke and Young ., 2019).These cells are stimulated by interleukins produced in situ, such as IL-12 (Kannan *et al* .,2011) , IL-15, IL-18, and IL-21(Strengell *et al* ., 2003) , tumor- or pathogen- secreted antigens (Hosking *et al* ., 2014) , Naïve CD4+ T-cells differentiate into helper T-cells, Th1 and Th2, in response to certain cytokines secreted during inflammation (Takeuchi & Saito , 2017). In such an environment, CD4+ Th1 cells are the main source of IFN-γ and are defined by the secretion of signature cytokines, namely IL-12, IL-2, and IFN-γ, as well as T-bet expression (Xu ., 2014)

The process of T-cell activation is a complex cascade consisting of three signals. First, allo antigens from the allograft are taken up by antigen-presenting cells (APCs; dendritic cells, macrophages, and B cells), which then home to the draining lymph nodes. In the lymph nodes, the alloantigens are presented on the surface of APCs by human leucocyte antigen (HLA) molecules(Mueller *et al* ., 1989)

Aims of the study

The research subjects The goal of the study was to calculate absolute values of lymphocyte subpopulations CD3+, CD3+CD4+, IL-4 (TH2) and INF -gamma (TH1) based on a few studies in Iraq, particularly Basrah, that showed light cellular immune derangement in these patients.

Material and methods

From January 2019 to January 2021, 16 healthy persons (11 men and 5 women) 18 Haemodialysis patients and 27 kidney transplant patients (3 women and 24 men) (aged 15–55 years) participated in the study. 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.

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 fluorescent markers manufacturer's protocol(Cyto staining) and flow cytometry with three lasers and eight filters using BD FACSuite TM software V 1.2.15657. An automated haematology analyser was used to calculate the absolute and differential blood cell count for all samples (Beckman soulter Cytomics FC 500, Florida, USA).

Flow Cytometry

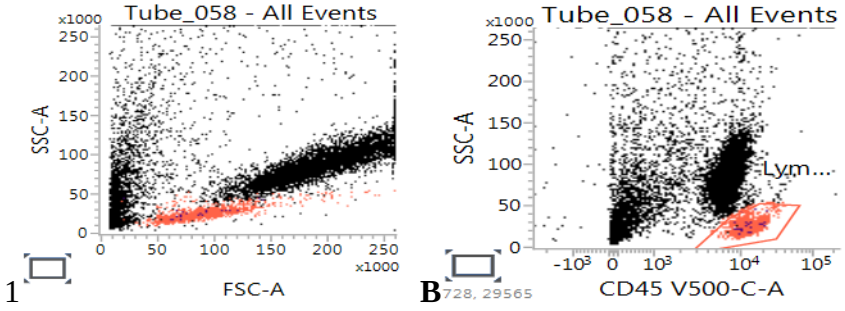
The lymphocyte subsets in lysed peripheral whole blood were identified using the BD FACS TM Permealize Solution. Blood was administered in the amount of ten ounces. The samples were made following the manufacturer's instructions. Monoclonal antibodies against T, IL-4, and INF-Gamma were used to describe lymphocyte subpopulations: T cells (CD45+, CD3+); CD4+(CD3+CD4+), IL-4 (TH2) and INF-Gamma (TH1) . 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 females 3(11.11%) less than males 24(88.88%) in KT patients and these patients from the middle age group (20-39 y). Total lymphocyte CD3+T cells, CD3+CD4+T cells and INF –gamma (Th1) are significantly lower in patients compared to control, while IL-4(TH2) cells are slightly higher in KT patients but the difference is not significant as shown in Table below.



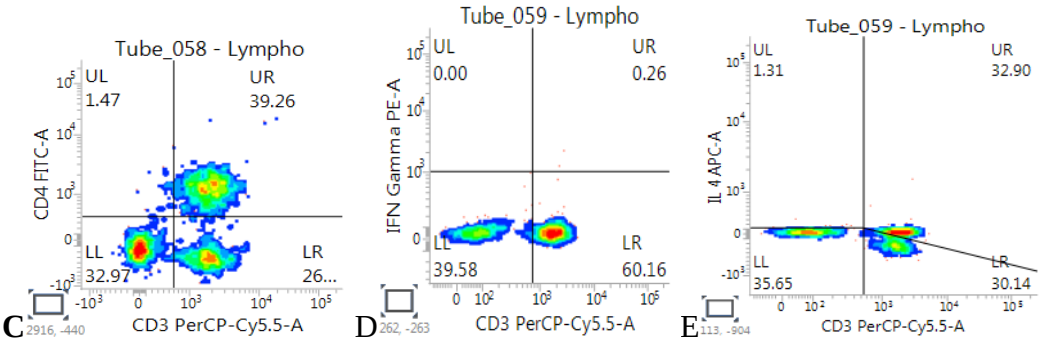


Fig (1) distinct population for healthy control

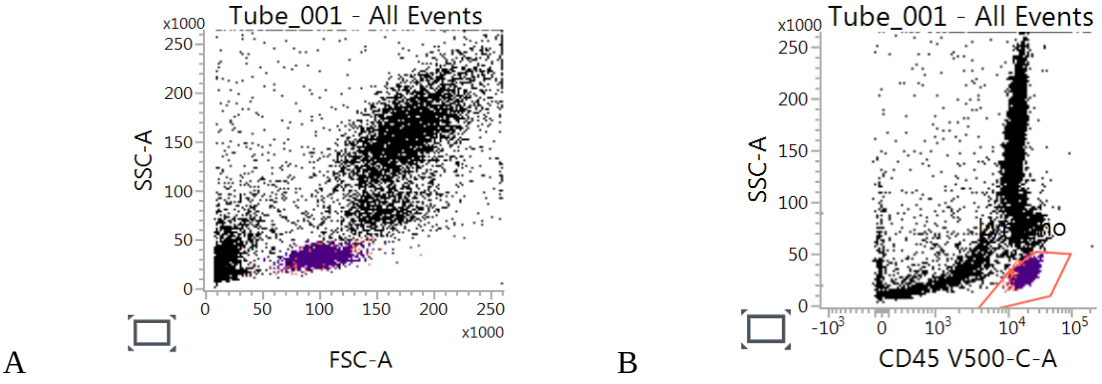
A- Lymphocyte subset population gate determination.

B- T cell quadrant determination

C- CD4+T cell quadrant determination.

D- INF-γ expressed cells (TH1) quadrant determination

E-IL-4 expressed cells (TH2) quadrant determination



Fig(2) distinct population for hemodialysis patients

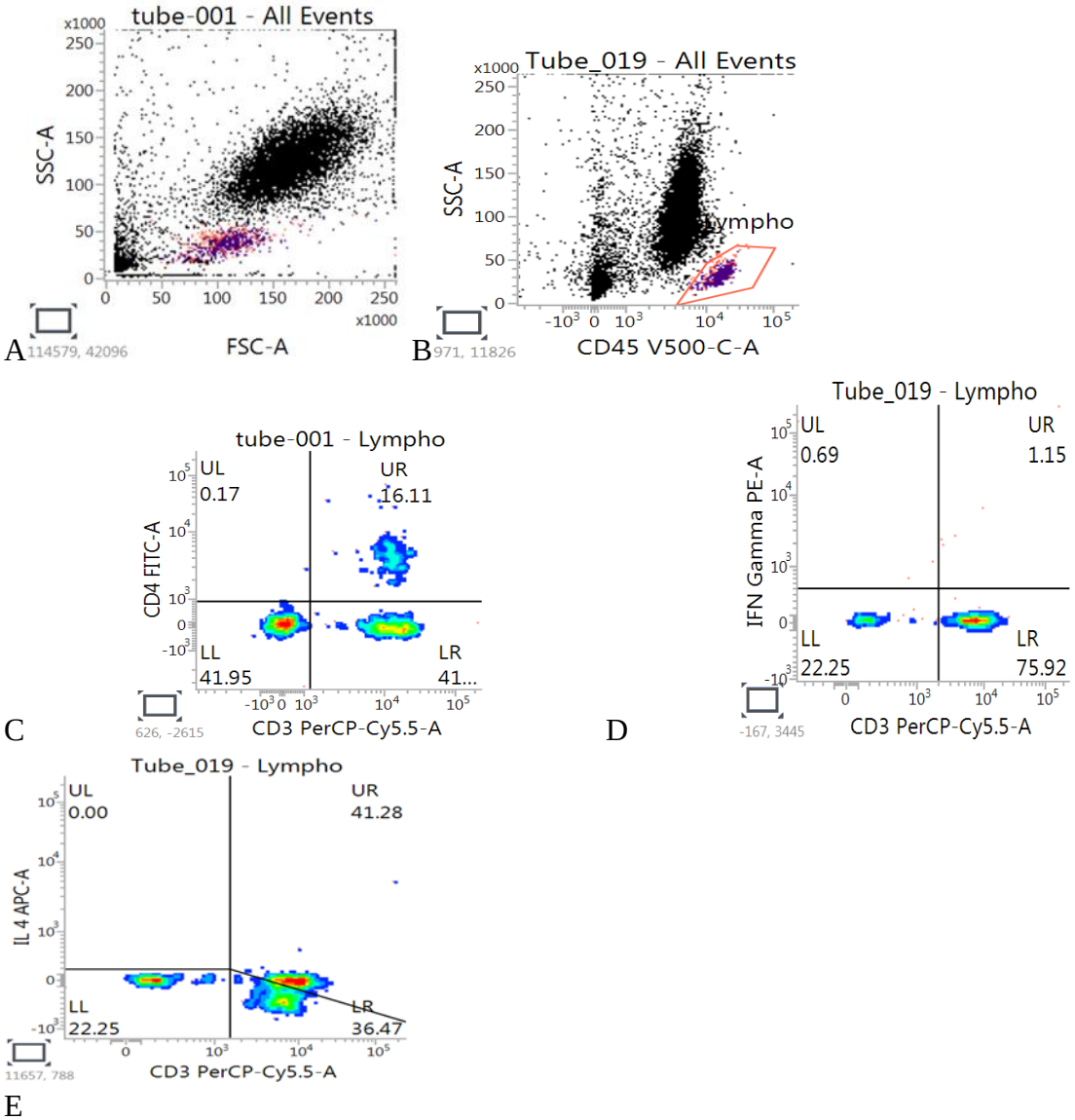
A- Lymphocyte subset population gate determination.

B- T cell quadrant determination

C- CD4+T cell quadrant determination.

D- INF-γ expressed cells (TH1) quadrant determination

E-IL-4 expressed cells (TH2) quadrant determination



Figure(3) Lymphocyte gate and subset determination for the Kidney transplant patient

A- Lymphocyte subset population gate determination.

B- T cell quadrant determination

C- CD4+T cell quadrant determination.

D- INF-γ expressed cells (TH1) quadrant determination

E-IL-4 expressed cells (TH2) quadrant determination

Table (1) differences between immunological parameters in the studied groups

Studied group Parameters	KT N=27	Control N=16	HD N=18	P-value
Total lymphocyte count	**1.270±0.6837**	**1.848±0.564**	**1.1861±0.38800**	**0.002**
$CD3^+$ absolute count	**0.7903±0.4958**	**1.1058±0.44828**	**0.7338±0.349975**	**0.037**
$CD4^+$ absolute count	**0.2691±0.2188**	**0.572±0.23114**	**0.3059±0.1727**	**0.000**
IL-4(TH2)	**0.0767±0.1473**	**0.2241±0.1527**	**0.1661±0.1187**	**0.006**
INF-γ(TH1)	**0.1635±0.3724**	**0.05006±0.0567**	**0.06082±0.09305**	**0.269**

Discussion

The use of flow Cytometry in clinical laboratories has grown substantially in the past decade. This method has emerged as a useful screening approach to evaluate whether specific cell populations and subpopulations are present or absent, which has been useful to clarify or assist the diagnosis of leukemias , lymphomas, and immune deficiencies(Oliveira and Fleisher , 2010). Nevertheless, in renal transplantation, the effect of treatment on GFR remains unclear. Changes in the T-helper and T-cytotoxic subpopulations have been more widely investigated. The majority of the research studies did not report differences in the number of T subpopulations. Instead, they reported an increase in the proportion of the T-cytotoxic subpopulation compared with that of T-helper cells (Moreso *et al*., 2014).The regulation of T-helper cells may play a key role in the prevention of negatives outcomes in patients undergoing renal transplantation. Cytokines are crucial inflammatory mediators and the generation of Th1 cytokines has been associated with both antiviral responses and allograft rejection. Our study show slightly decreases in TH2 in KT patients and slightly increased with TH1 in the same studied group .this didn't agree with (Sadeghi *et al* .,2003) may be related to small study size of patients, immune response to bacterial infections or long term of Immunosuppression drugs effects.

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