

Study of CD4+ T-lymphocytes in chronic kidney disease patients with COVID-19 infection

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Abstract

Chronic kidney disease (CKD) is a functional and/or structural kidney damage that lasts more than three months duration. This study aimed to analyze CD4+ T-lymphocytes levels in chronic CKD patients specifically, during the coronavirus disease 2019 (COVID-19) pandemic to assess the adaptive cell-mediated immunity. The study measured absolute CD4+ T-lymphocytes counts by flowcytometry among participating individuals. The study included 146 subjects, 40 CKD patients and tested positive for COVID-19, 44 CKD patients and tested negative for COVID-19 and 62 normal individuals as controls. There was a significant impact of COVID-19 infection in CKD patients showing lower absolute CD4+ T-lymphocytes values to more than six folds compared to the control individuals (Odds Ratio: 72.63, $p= 0.0001$). Also, there was a significant correlation between the decrease in absolute CD4+ T-lymphocytes counts and the advanced stages of CKD. Therefore, the study indicated that CKD causes an obvious alteration in the body immune system as decreased CD4+ T-lymphocytes levels alongside with the advanced CKD stages. While COVID-19 infection exposes CKD patients to be 50% more likely to express lower values of CD4+ T-lymphocytes levels compared to the negative tested CKD patients. In conclusion, poor immune response and increased morbidity and mortality could be correlated with CKD patients especially when associated with COVID-19 infection as comorbidity

Keywords: T-lymphocytes, Kidney Disease, COVID-19, Flowcytometry.

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Introduction

Chronic kidney disease (CKD) is defined as a functional and/or structural kidney damage that lasts more than three months duration. It can be assessed by estimating glomerular filtration rate (eGFR), in which it is lower than 60

mL/min/1.73 m² for three months duration or more, with or without renal structural damage.¹ CKD is a worldwide health problem with increased morbidity and mortality rates as diminishing the quality of patients' life.²

Body immune homeostasis is orchestrated and affected by the renal system in different

ways through immune cells that can mediate acute renal injury with increased progression of CKD.³ While renal function regression contributes to body immune disturbances especially modification of B and T lymphocytes.⁴ The adaptive immune disruption increases the susceptibility of CKD patients to infections, poor vaccination response with increased infection complications three to five times more in CKD patients and increased mortality by 20% due to infections.⁵

The ongoing pandemic, coronavirus disease 2019 (COVID-19) infection affects multi-organ systems, mainly the respiratory system then renal and immune systems besides liver, cardiac and cerebral systems.⁶ Kidneys are specific targets for COVID-19 infection, as there is an invasive effect on the renal glomeruli, tubules, and vascular cells by the COVID-19 causative organism, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).⁷ The clinical sequelae of the disease is very variable and not-predictable, extending from flu-like symptoms, multi-organ failure and up to death.⁸ The aim of the present study was to investigate CD4+ T-lymphocytes levels among CKD patients and the effect of COVID-19 infection on their levels as a comorbidity factor.

Subjects and Methods

This case-control study was performed in the laboratory of the Department of Clinical Pathology, Sohag University hospital during the period from April 2020 to December 2020. The study included 146 subjects. Of these, 84 patients with different stages of CKD and 62 normal volunteers as controls. Patients included in the study aged from 18 to 70 years with CKD according to Kidney Disease Outcomes Quality Initiative (KDOQI).⁹

CKD patients were classified according to their CKD severity and/or staging according to KDOQI [9]. They were CKD patients of stage IIIa with eGFR (45-59 ml/min/1.73m²), CKD patients of stage IIIb with eGFR (30-44 ml/min/1.73m²), CKD patients of stage IV with eGFR (15-29 ml/min/1.73m²) and CKD patients of stage V with eGFR (<15 ml/min/1.73 m²). Simultaneously, all patients were classified

according to COVID-19 infection as determined by real time polymerase chain reaction (RT-PCR) analysis into positive or negative tested COVID-19 CKD patients.

The exclusion criteria included seropositivity for human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV), receiving corticosteroids or immunosuppressant treatment and patients known to have any immunosuppression disorders.

A questionnaire was conducted to collect participants' data and their medical history. Blood samples (7ml) were collected from each participant by venipuncture. Of these, two aliquots of 2 ml were collected on two K3-EDTA tubes and 3 ml on a plain tube for separation of serum. One of the EDTA blood tubes was used for performing a complete blood picture analysis on an automated hematology analyzer (Cell-Dyn Ruby, automated cell counter (Abbott diagnostics, USA), according to the manufacturer's instructions. The second EDTA tube was used for determination of lymphocytes subsets count by flowcytometry.

Flowcytometric analysis of peripheral blood lymphocytes

For immunophenotyping, 50 µl EDTA blood was labeled with 5µl of CD4/8/3 isotopes. Then red blood cells were lysed using 2 ml FACS Lysing solution, then washed using 2 ml FACS sheath solution. Data acquisition was performed on a flow cytometer (FACS-Calibur flow cytometer, BD Bioscience, USA), according to the manufacturer's instructions. Events were analyzed by relevant software (CellQuest Pro Software V5.2, BD Bioscience, USA). Absolute values of each gated subset, CD4 (T helper lymphocytes), CD8 (T cytotoxic lymphocytes) and CD3 (pan T lymphocytes) were calculated using the specific subset percentage of each one that obtained by flowcytometry.

Plain serum tubes were used to perform serum creatinine, alanine transferase enzyme (ALT) and serum albumin by a blood chemistry analyzer (Cobas c311 auto-analyzer, Roche/Hitachi Diagnostics Cobas system, Switzerland), according to the manufacturer's

instructions. Assessment of serum HBV, HCV and HIV was performed by fully automated analyzer (Cobas e601 module, Roche/Hitachi Diagnostics Cobas system, Switzerland), according to the manufacturer's instructions.

Detection of COVID-19 by Real-Time PCR

We collected naso-pharyngeal swabs from all participants. Swabs were transported through Virus Transport Media (VTM) tube for diagnosis of COVID-19 by RT-PCR analysis. The RT-PCR technique was performed by two consecutive reactions, conversion of RNA into complementary DNA (cDNA) through reverse transcription enzyme, then amplification of the cDNA sample by polymerase chain reaction using gene-specific primers and fluorescently labeled probes. The RNA extraction was performed by commercial kits (catalogue number: Z-Path-COVID-19-CE, Genesig, UK) and performed on an automated extraction tool (QIAcube, Qiagen Biotechnolog, Germany), according to the manufacturer's instructions. The PCR was performed using commercial kits (CE-IVD Kits, Complied European in vitro diagnostics Kits, Genesig, UK) on a Real-Time PCR machine (StepOnePlus system, Thermo Fisher Scientifics, Applied Biosystems, USA), according to the manufacturer's instructions.

Statistical Analysis

The data were analyzed using the statistical package for the social sciences (SPSS) version 17, (SPSS Inc., Chicago, USA) software. Quantitative data are represented as mean, standard deviation (\pm SD) and compared using t-test and one-way analysis of variance (ANOVA) test. Categorical variables are presented as numbers (%) and compared using the Chi-square test. Pearson's Correlation Coefficient,

Relative Risk and Odds ratio were calculated as outcome comparison. A p value < 0.05 was considered statistically significant.

Results

The study included 146 participants. They included 84 CKD patients with the mean age \pm SD of (45.86 ± 12.85) years and 62 normal individuals as controls. The patients were 54 (64.3%) males and 30 (35.7%) females. They were further subclassified according to CKD staging and COVID-19 infection. Of the CKD patients, 35 (41.7%) received renal conservative therapy, while 49 (58.3%) CKD patients were on hemodialysis. Of the CKD patients, 40 (47.6%) tested positive for COVID-19 (Table 1).

Table 1. Characteristics of the 84 chronic kidney disease (CKD) patients.

Characteristics		CKD Patients
Age		45.86 \pm 12.85
Gender	Males	54 (64.3 %)
	Females	30 (35.7 %)
CKD severity/stage	Stage (IIIa)	2 (2.4 %)
	Stage (IIIb)	5 (6.0 %)
	Stage (IV)	28 (33.3 %)
	Stage (V)	49 (58.3 %)
COVID-19 infection	Negative	44 (52.4 %)
	Positive	40 (47.6 %)

Variables are expressed as mean \pm SD and number (%).

The study showed a significant decrease of absolute CD3+, CD4+ and CD8+ T-lymphocytes counts in CKD patients compared to control individuals ($p= 0.0001$, for all) (Table 2). Also, among CKD patients with negative COVID-19, the counts of CD3+, CD4+ and CD8+ T-lymphocytes were affected by the progressive loss of renal functions definitely from CKD stage III to CKD stage V ($p= 0.0001$, for all) (Figure 1).

Table 2. Comparison of T-lymphocyte subsets between chronic kidney disease (CKD) patients and controls.

	Controls (n=62)	CKD patients (n=84)	p -value
Absolute CD3 ($\times 10^3/\mu$ l)	1.21 \pm 0.48	0.57 \pm 0.38	0.0001
95% CI*	1.088 – 1.331	0.487 – 0.652	
Absolute CD4 ($\times 10^3/\mu$ l)	0.65 \pm 0.26	0.31 \pm 0.22	0.0001
95% CI	0.584 – 0.716	0.262 – 0.357	
Absolute CD8 ($\times 10^3/\mu$ l)	0.47 \pm 0.23	0.22 \pm 0.16	0.0001
95% CI	0.411 – 0.528	0.185 – 0.254	

Table 2. Continued.

	Controls (n=62)	CKD patients (n=84)	p-value
CD4/CD8 Ratio	1.48 ± 0.51	1.54 ± 0.72	NS
95% CI	1.350 – 1.609	1.383 – 1.696	
HB level (g/dl)	12.86 ± 0.95	10.89 ± 2.84	0.0001
Range	11.1 – 15.1	4.66 – 20.1	

Variables are expressed as Mean ± SD and number (percentage), *CI: Confidence Interval. $p > 0.05$ is not significant (NS).

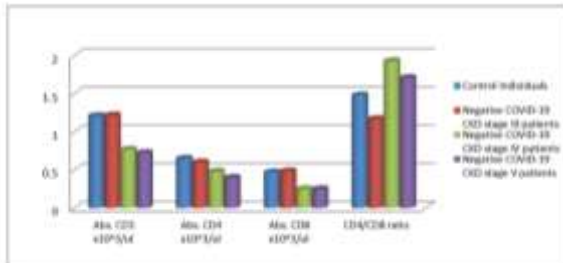


Figure 1. Comparison of CD3, CD4, CD8 lymphocytes and CD4/CD8 ratio in negative COVID-19 patients with different CKD stages.

In addition, there was a statistically significant decrease in hemoglobin levels in the CKD patients when compared to controls ($p = 0.0001$) (Table 2). However, hemoglobin levels were not different between CKD patients with positive COVID-19 and COVID-19 negatives (Table 3). Of

the whole CKD patients, ALT levels were high in 18%, and of those patients with high ALT levels, 67% tested positive for COVID-19 ($p = 0.001$). Also, albumin levels were low in 37% of CKD patients, and almost all of them tested positive for COVID-19 ($p = 0.0001$) (Table 3).

It was noted that 53% of the CKD patients with positive COVID-19 developed absolute lymphopenia versus 9% of the CKD patients with negative COVID-19 test ($p = 0.0001$) (Table 3). Also, absolute levels of CD3 T-lymphocytes, CD4 T-helper lymphocytes, and CD8 T-cytotoxic lymphocytes were markedly decreased in CKD patients with positive COVID-19 compared to CKD patients with negative COVID-19 test ($p = 0.0001$, $p = 0.0001$, and $p = 0.008$, respectively) (Figure 2).

Table 3. Comparison of T-lymphocyte subsets between negative and positive COVID-19 CKD patients.

	Negative COVID-19 CKD patients (n=44)	Positive COVID-19 CKD patients (n=40)	p-value
Absolute CD3 ($\times 10^3/\mu\text{l}$)	0.795 ± 0.379	0.330 ± 0.216	0.0001
95% CI*	0.679 – 0.910	0.264 – 0.395	
Absolute CD4 ($\times 10^3/\mu\text{l}$)	0.444 ± 0.205	0.170 ± 0.138	0.0001
95% CI	0.381 – 0.506	0.128 – 0.212	
Absolute CD8 ($\times 10^3/\mu\text{l}$)	0.300 ± 0.177	0.140 ± 0.096	0.008
95% CI	0.246 – 0.354	0.110 – 0.169	
CD4/CD8 ratio	1.70 ± 0.681	1.37 ± 0.740	0.035
95% CI	1.493 – 1.907	1.140 – 1.595	
Lymphocytes ($\times 10^3/\mu\text{l}$)	1.48 ± 0.50	0.91 ± 0.66	0.0001
Range	0.58 – 2.77	0.19 – 1.89	
HB (g/dl)	10.88 ± 3.12	10.9 ± 2.53	NS
Range	4.66 – 20.1	5.73 – 16.3	
ALT (IU/L)	19.4 ± 13.38	35.8 ± 27.31	0.001
Range	6 – 61	8 – 131	
Alb (g/dl)	4.32 ± 0.70	3.10 ± 0.47	0.0001
Range	2.3 – 5.9	2.4 – 4.2	

Variables are expressed as Mean ± SD and number (percentage), *CI: Confidence Interval. $p > 0.05$ is not significant (NS).

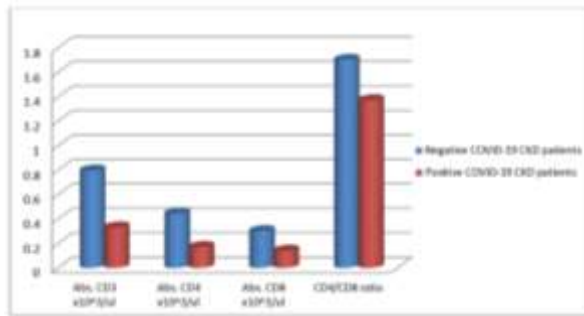


Figure 2. Comparison of CD3, CD4, CD8 and CD4/CD8 ratio in negative & positive COVID-19 CKD patients.

In addition, CD4+ T-lymphocytes were lower by more than six folds in CKD patients with positive COVID-19 when compared to normal control individuals ($p= 0.0001$). CD4+ T-lymphocytes were lower by more than four folds in CKD patients with negative COVID-19 test when compared to normal control individuals ($p= 0.0001$). Finally, CKD patients with positive COVID-19 test were 50% more likely to have lower levels of absolute CD4+ T-lymphocytes than CKD patients with negative COVID-19 test ($p= 0.002$) (Table 4).

Table 4. Relative Risk and Odds Ratio of CD4+ T-lymphocytes in chronic kidney disease (CKD) patients with positive and negative COVID-19 test.

	Relative Risk	Odds Ratio	* p -value
Positive COVID-19 CKD patients Vs Controls	6.37	72.63	0.0001
Negative COVID-19 CKD patients Vs Controls	4.54	11.39	0.0001
Positive Vs Negative COVID-19 CKD patients	1.51	7.77	0.002

* $p \leq 0.05$ is significant.

Discussion

This study analyzed levels of different T-lymphocytes subsets (CD3+ CD4+ CD8+) in CKD patients with different stages according to KDOQI guidelines⁹ and according to COVID-19 infection, the study presented precise data to assess the adaptive cell-mediated immunity in CKD patients and the impact of COVID-19 infection on the same patient groups.

Many previous studies discussed the effect of COVID-19 infection on T-lymphocytes subsets in CKD patients. For example, Ahmadian et al., 2021, demonstrated that COVID-19 infection affects kidneys in different ways as initial impact on the renal parenchyma through a mechanism starting from activating the angiotensin converting enzyme-II (ACE-II), that acts as COVID-19 receptor to invade the renal tissue cells.¹⁰ Shi et al., 2020, claimed that COVID-19 causes immune system dysregulation at both levels; innate and acquired immune activity through early specific response of the acquired immunity system trying to get rid of the viral infection.¹¹ Also, Alshammary et al., 2022, demonstrated the dysregulated lymphocyte subset parameters in patients with COVID-19.¹² In 2020 Wan et al., explained that T lymphocytes necrosis process that was initiated

by the cytokine storm leading to reduction of T lymphocytes exhaustion, CD4+, CD8+, interleukin 10 (IL10) and tumor necrosis factor (TNF)- α levels.¹³ Cameron et al., 2008, studied severe acute respiratory syndrome (SARS) patients and expressed delayed adaptive immune response with prolonged viral clearance from the body.¹⁴ Also, Yoshikawa et al., 2009 and Zhao et al., 2009, confirmed that there is alternation in the function of antigen presenting cells and impairment of dendritic cells migration,^{15,16} although T helper cells modulate the activity of other immune cells as B cells and macrophages; besides promoting intrinsic and extrinsic apoptosis pathways for T lymphocytes side by side to the pro-inflammatory cells produced from the cytokine storm.^{17, 18, 19}

The present study showed that CD4+ T-helper lymphocytes alongside with CD3+ and CD8+ lymphocytes were markedly decreased in CKD patients with positive COVID-19 test compared to CKD patients with negative COVID-19 test and the normal control individuals, indicating that not only CKD affecting the different variants of lymphocytes, but also COVID-19 infection aggravates that effect to clearly low absolute values. Diao et al., 2020, showed an obvious dramatic drop and

functional exhaustion of CD4+T-lymphocytes²⁰ followed by increased incidence of co-infection by other pathogens.^{21,22} Consumption and exhaustion of CD4+ and CD8+ T cells might lead to aggravated inflammatory response, as well as cytokine storm production with eventually worsening of the tissue damage. Huang et al., 2019, and Qin et al., 2020, studies reported that patients with weakened immunity due to a pre-existing disease such as kidney, liver diseases, different malignancies and diabetes are more susceptible to catch COVID-19 infection with bad prognostic disease sequences.^{23,24}

In the present study, it was found that CD4+ T-lymphocytes were affected by the progressive loss of renal functions definitely from CKD stage III to CKD stage V in negative COVID-19 CKD patients, as there was a disturbance of T cell homeostasis with CKD progression. These are in accordance with findings of Applegate et al., 2020, Chiu et al., 2018, and Dounousi et al., 2012, who reported that T-cells apoptosis appeared to be increased across CKD stages.^{25,26,27} Also, Meier et al., 2009, and Hendriks et al., 2009, showed that the magnitude of T-cells dysfunction and depletion was more in hemodialysis CKD patients; the matter that resulted in decrease CD4+ T-cell proliferation with decreased naïve T-cells, anti-inflammatory capacity reduction hence increased T-cell aging and apoptosis.^{28,29}

In conclusion, CKD causes marked alterations in the immune system in which low eGFR is accompanied by the decrease in CD4+ T cells levels. Although COVID-19 infection is considered mainly as a respiratory disease, it suppresses body immune responses sharply, as CD4+ T lymphocyte levels markedly dropped among CKD patients with positive COVID-19 test with late CKD stage patients are highly susceptible risk group. Impaired T lymphocytes activation after COVID-19 infection causes abnormal immune stimulation, may contribute to severe COVID-19 outcomes in CKD patients. Therefore, CKD patients combined with COVID-19 infection; especially patients of late stage and hemodialysis, are highly susceptible and at-risk population with increased morbidity and mortality that requires special care to avoid poor outcomes. Marked reduction in CD4+T

lymphocytes and eGFR can be considered as poor prognostic markers.

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Author Contributions

SPA, TM and AS contributed to the study design. SPA, AA, SAS, TM, OM and AS contributed to material preparation, data collection and laboratory investigations. SPA, AA, SAS, TM and AS wrote the manuscript draft. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The protocol of the study was reviewed and approved by the Medical Research Ethics Committee of the Faculty of Medicine; Sohag University (dated 2019).

Informed consent

An informed consent was taken from each participant before being included in the study.

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