

Study of T CD4 Lymphocytes by Flow Cytometry in Chronic Kidney Disease Patients in Abidjan

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Abstract

Objective: We undertook this study to analyze the T CD4 subset of non-HIV CKD patients and to investigate factors that may influence their rate.

Materials and methods: It was a cross-sectional, three-month study, on the determination of T CD4, count by Flow Cytometry (FACS Calibur), in patients aged 18 to 65 years, chronic kidney disease according to KDOQI and non-HIV.

Results: Sixty-three cases were collected with an average age of 41 years and sex ratio of 1.79. The median BMI was 22.9 kg/m² and 69.9% had normal weight. 36 of patients (69.2%) were at stage 5 of chronic kidney disease. CD4 rate was low in 23 patients (36.5%), normal in 37 patients (58.7%) and high in 3 patients (4.8). There was a significant correlation between the decrease in absolute CD4 rate and the grade of chronic kidney failure (CKD) ($p=0.02$). In linear regression, a statistically significant correlation was observed between changes in absolute CD4 values and white blood cell level ($p=0.000003$), total lymphocyte rate ($p=0.00006$) and urea rate ($p=0.04$); on the other hand between changes in the absolute values of CD3 and the levels of white blood cells ($p=0.000001$) and lymphocytes rate ($p=0.000002$).

Conclusion: The decrease in GFR is accompanied by a decrease in CD4 rate, which increases the risk of infections. This situation could contribute significantly to the morbidity and mortality of chronic kidney disease patients.

Keywords: CKD; Ivorian adult; CD4; Flow cytometry

Introduction

The progressive and irreversible loss of renal function is accompanied by a great variety of disturbances among which qualitative and quantitative modifications of the immune system elements. This is the hyperactivity of monocytes resulting in the high production of proinflammatory cytokines [1], functional abnormalities of macrophages and dendritic cells, reduction of B and T lymphocytes including CD4 and CD8 subpopulations [2]. Disruption of adaptive immunity (T and B) will result in increased susceptibility to infection, poor response to vaccination, and skin test anergy of these patients [3]. Infection complications are 3 to 5 times more common in CKD patients than in general population [4]. The mortality due to infections is 20% [5]. The deleterious consequences of the disorders of the immunity in the renal insufficiency impose their evaluation within the framework of the optimal care of these patients. In a poor country where the exploration of the immune system is very limited, we conducted this study to study the CD4 T cells level, a subpopulation of T cells in chronic kidney disease patients.

Materials and Methods

Type and context of the study

It was a cross-sectional and analytical study carried out at the University Hospital Center of Yopougon in Abidjan, Côte d'Ivoire, from May 17th, 2016 to August 16th, 2016 for a period of 3 months.

Population of study

Patients aged 18 to 65 years with chronic Kidney disease according to KDOQI: *Kidney Disease Outcomes Quality Initiative* [6], untreated by hemodialysis, seronegative for HIV, not treated with corticosteroids or immunosuppressants three months before the study and having no known cause of secondary immunodepression were included in the study. A questionnaire was used to collect epidemiological data, the history of metabolic diseases, the recent infectious past, the clinical and laboratory data of each patient who freely agreed to be part of this study. All information gathered in this study was processed in accordance with the Code of Ethics. The patient has given his consent regarding this article.

Method

We took at Yopougon University Hospital, in all patients who gave his free and informed consent to participate in the study, 5 ml of blood in an EDTA tube. The blood samples were stored at room temperature containing accumulators and transported in the immunology and hematology laboratory of COCODY University Hospital (Abidjan) for the determination of T CD4 lymphocyte count by flow cytometry according to the LTCD4 enumeration method adapted to the device used. For the immunophenotyping, 50 µl blood was labeled with 4 mAbs in one tube containing CD4-phycoerythrin, CD3-fluorescein isothiocyanate and CD45- peridin chlorophyl II protein. Red cells were lysed by using FACS-lysing solution and the samples were washed with PBS. Sample data were acquired by using a FACS Calibur flow cytometer (Serial number: 3CHE97300368). CD4 T lymphocyte population was selected on the flow cytometer according these criterias: morphological (small cells weakly granulated) and triple extrinsic fluorescence (cells labeled positively by anti-CD45 antibody (pan leukocytes) by the anti-CD3 antibody (pan-T) and by the anti-CD4 antibodies coexpressing CD3. Absolute values were expressed as number of cells per mm³, were determined in a single platform using an internal standard consisting of a volume of auto fluorescent beads of known concentration equivalent to the volume of the blood sample added to the preparation tube.

Operational definitions

The CD4 rate was considered: low if <500 cells/ml; normal if between 500 and 1750 cells/ml; and high if >1750 cells per ml.

Chronic kidney disease was classified on the basis of estimated glomerular filtration rate based on calculated creatinine clearance using the MDRD (Modification Diet in Renal Disease) formula [7]. This classification distinguished five degrees of severity according to clearance values calculated according to KDOQI [6]. Kidney disease was stage 1 for calculated clearances greater than 90 ml/min; stage 2 for calculated clearances between 60 and 89 ml/min; stage 3 for a calculated clearance of between 30 and 59 ml/min; stage 4 for a calculated clearance ranging from 15 to 29 ml/min and stage 5 for a clearance below 15 ml/min.

Statistical Analysis

The data was analyzed on Excel 2007 and then on the Statistical Package Social Sciences software (SPSS) version 17.0 and Epi Info

version 6.0 for the calculation of the chi² test. The significant value was set at p < 0.05.

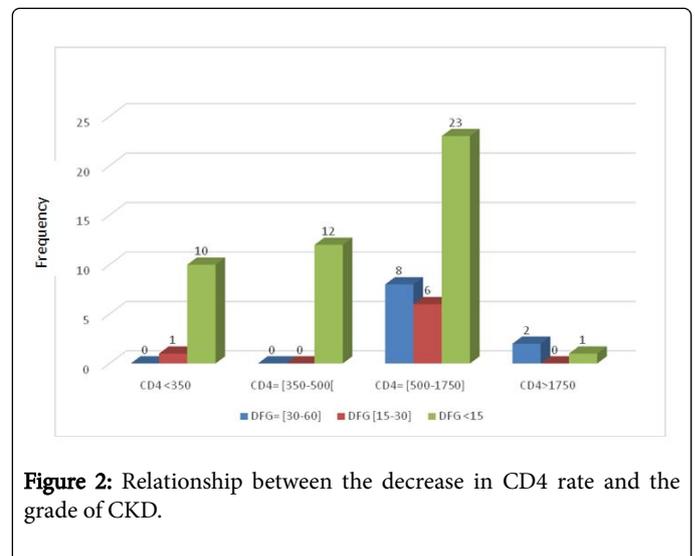
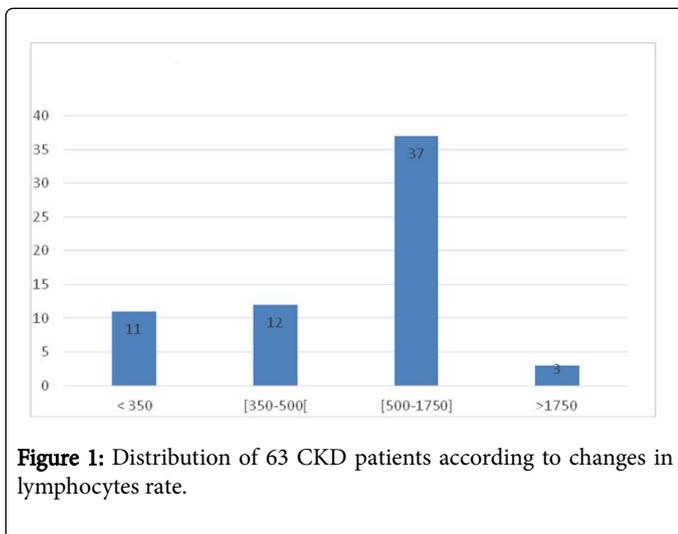
Results

Sixty-three (63) patients were enrolled during the study period, 23 women (36.5%) and forty (40) men (63.5%); which corresponded to a sex ratio of 1.73. The average age was 41 ± 10 years old. Forty-eight (48) patients (76.2%) had a low socio-economic level, fifteen (15) patients (23.8%) an average socioeconomic level. Twenty three (23) patients (36.5%) were workers, twenty (20) patients (31.7%) were unemployed, and ten (10) patients (15.9%) were traders. Fifty-two (52) patients (80.5%) were hypertensive, sept (7) patients (11.1%) were diabetic, and eleven (11) patients (17.5%) were smokers. Thirty six (36) patients (57.1%) had chronic glomerular nephropathy, twenty four patients (38.1%) chronic vascular nephropathy, and three (3) patients (4.8%) chronic tubulo-interstitial nephropathy. Table 1 show the socio-demographic and clinical characteristics of the study population. Simple nutritional indices were BMI and albumin level tested or 17 patients. The median were 22.9 kg/m² for the body mass index (BMI) and 32.6 g/l for albumin. Among the patients, we noticed 7.9% underweight (BMI <18.5 kg/m²), 69.9% with normal weight (BMI: 18.5-24.9 kg/m²), 15.9% overweight (BMI: 25-29.9 kg/m²) and 6.3% obese (BMI ≥ 30 kg/m²). Twenty three (23) patients (36.5%) had a low CD4 count, thirty seven (37) patients (58.7%) had a normal CD4 count, three (3) patients (4.8%) had a high CD4 count. Forty (40) patients (63.5%) had lymphocyte count below 2000/mm³. Figure 1 show the variation of absolute CD4 lymphocytes. The average hemoglobin level was 8.8 g/dl with extremes of 7 and 11 g/dl. Table 2 shows the biological characteristics of the population studied. Ten (10) patients (15.9%) were in CKD stage 3, seven (7) patients (11.1%) in stage 4 and forty six (46) patients (73%) in stage 5. A significant correlation between the decrease in absolute CD4 and the grade of CKD was observed with p=0.02 (Figure 2). Of the forty six (46) patients with CKD stage 5, twenty two (22) or 47.8% had low CD4 counts. Table 3 shows the relationship between the decrease in CD4 count and the stage of CKD. No statistically significant relationship was found between uremia and CD4 count. In linear regression, a statistically significant correlation was observed on the one hand between changes in the absolute values of CD4 and white blood cell, lymphocyte and uremia levels and on the other hand between changes in the absolute values of CD3 and levels of white blood cells and lymphocytes (Table 4).

Characteristics		Frequency (n=63)	Percentage (%)	Trusted Range at 95%
Sex	F	23	36.5	24.7-49.6
	M	40	63.5	
Classes Ages	[20;30]	12	19.1	50.4-75.3
	[30;40]	20	31.7	
	[40;50]	15	23.8	9.1-29.1
	[50;60]	16	25.4	20.6-44.7
Socioeconomic Level	Low	48	76.2	14-36.2
	Average	15	23.8	15.3-37.9

	High	0	0.0	---
Profession	Simple Framework	7	11.1	4.6-21.6
	Senior Worker	2	3.2	0.4-11
	Traders	10	15.9	7.9-27.3
	Workers	23	36.5	24.7-49.6
	Pensioner	1	1.6	0.0-8.5
	Unemployed	20	31.7	20.6-44.7
Comorbidities	Palustre Infection	14	22.2	12.7-34.5
	Traditional therapy	34	54.0	40.9-66.6
	Diabetes	7	11.1	4.6-21.6
	alcoholism	13	20.6	11.5-32.7
	Smoking	10	15.9	7.9-27.3
	HTA	52	82.5	70.9-90.9
Initial nephropathy	GNC	36	57.1	44.0-69.5
	Vascular	24	38.1	26.1-51.2
	NTIC	3	4.8	1.0-13.3

Table 1: Sociodemographic and clinical characteristics of the 63 patients evaluated.



Items		Frequency (n=63)/ (%)	IC at 95(%)	Average	Extremes Min/Max	Standard Deviations
Hb (g/dl)	<7	16/(25.4)	9.5-54.2	8.8	4.1/13.9	2.5
	[7;11]	32/(50.8)	24.5-91.7			
	>11	15/(23.8)	8.0-52.7			

Leuk	<4000	7/(11.4)	3.6-28.1	7021.1	2500/19060	3013,7
	[4000-10000]	47/(74.6)	26.7-138.5			
	>10000	9/(14.3)	3.0-45.6			
Lymp	<2000	40/(63.5)	45.1-85.7	1912.9	400/4200	833.9
	[2000-4000]	21/(33.3)	17.9-55.5			
	>4000	2/(3.2)	0.4-11			
CD4 Absolute	<350	11/(17.5)	3.0-50.4	828.9	112/9999	1232.5
	[350-500]	12/(19)	5.4-46.4			
	[500-1750]	37/(58.7)	39.5-88.7			
	>1750	3/(4.8)	1.0-13.3			
Uremia	≤ 0.53	10/(15.9)	4.4-41.6	1.6	0.3/4.5	0.9
	[0.53-3.0]	47/(74.5)	36.7-130.1			
	>3.0	6/(9.6)	1.8-32.5			

Hb: Hemoglobin concentration; Leuk: Leukocyte ; Lymp: Lymphocyte.

Table 2: Clinical and biological characteristics of the 63 CKD patients.

CD4 Rate (elts/ μ l)	GFR= [30-60] (n=10)	GFR [15-30] (n=7)	GFR <15 (n=46)	p
CD4<350	0	1 (14.3%)	10 (2.7%)	0.02
CD4= [350-500]	0	0	12 (26.1%)	
CD4= [500-1750]	8 (80%)	6 (85.7%)	23 (50%)	
CD4>1750	2 (20%)	0	1 (2.2%)	
Total	10	7	46	63

Table 3: Distribution of CD4 levels according to CKD stage.

Variables	Absolute CD4			Absolute CD3				
	Simple regression (r)	linear	Coefficients a and b of the regression line	value p	Simple regression	linear	Coefficients a and b of the regression line	value p
Age	0			0,8	0		4.8; 1061	0.7
Sex	0			0,6	0		16.7; 1252	0.9
BMI	0		-1.4; 1147.6	0.7	0		-0.02; 1266.8	0.9
Hb	0,01		-3.8; 1166.3	0,5	0		-0.2; 1279	0.9
Leukocyte	0,3		0.2; -754.7	0,000003	0,32		0.2; -404.8	0.000001
Lymphocyte	0,18		0.6; -356.6	0,0006	0.31		0.8; -350.5	0.000002
Uremia	0.07		3.2; 317.5	0,04	0.03		2.2; 911.5	0.174028
GFR	0,01		0.08; 717.4	0,4	0.03		0.1; 1067.6	0.2
Traditional therapy	0,05		-544; 1122.5	0,08	0.05		-576.6; 1573.7	0.06

Hb: Hemoglobin concentration.

Table 4: Factors associated with changes in the absolute values of CD4 and CD3.

Discussion

We analyzed by immunophenotyping CD4 levels in adult patients with chronic kidney disease according to KDIGO. Our study presents data to evaluate adaptive cell-mediated immunity in a resource-limited country where immunological examinations are reduced. The average rate of CD4 in our series (824 cells/ml) was lower than the average of 1196 CD4/mm³ found in a population of healthy subjects in Ivory Coast [8]. However, this rate was higher than the average rate of 442 CD4/mm³ of a cohort of 755 HIV-infected Ivorian subjects [9]. Infectious agents such as HIV and metabolic disorders such as kidney failure affect the intrinsically normal immune system of the host; this explains the variations between the CD4 levels observed in the different populations previously described. The drop in glomerular filtration rate is accompanied by the decline in the absolute number of CD4 cells. This is confirmed by other works [10,11]. Indeed, the aggravation of chronic kidney disease is accompanied by functional and phenotypic modifications of lymphocytes resulting in accelerated aging, the decrease in the number of naive T lymphocytes, an increase in the number of memory T cells, a shortening of the telomeres, a thymic involution and an increase in sensitivity to apoptosis [2]. It should also be noted that apart from the accumulation of uremic toxins that affect the immune system of the chronic kidney disease subject, there are other causes of secondary immunodeficiency in these patients. These include undernutrition, calcitriol deficiency, secondary hyperparathyroidism, iron overload secondary to multiple transfusions and the use of erythropoietin [12].

Conclusion

The decrease in GFR is accompanied by a decrease in the CD4 rate. The consequences of a low CD4 level are the increase in the frequency of infections, a factor that aggravates mortality in chronic kidney disease patients. If there is no treatment for these immunodeficiency disorders at the present time, management of superimposed immunosuppression causes would reduce its magnitude.

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