Canadian Society of Nephrology/ Société canadienne de néphrologie CSN/SCN

CANADIAN JOURNAL OF KIDNEY HEALTH AND DISEASE
Journal canadien de la santé et de la maladie rénale

Original Basic Research

Canadian Journal of Kidney Health and Disease
Volume 10: 1–9

DOI: 10.1177/20543581221132748
journals.sagepub.com/home/cjk
SAGE

Assessment of the Relationship Between Inflammation and Glomerular Filtration Rate

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Abstract

Background: Chronic kidney disease (CKD) is a global health problem. As it progresses to end stages, renal replacement therapy is required but ultimately, the best treatment is transplantation. Decreased renal function has been associated with an inflammatory state associated to primary CKD and in kidney transplant recipients (KTRs).

Objective: To establish how the serum concentrations of some cytokines, such as interleukin (IL)-2, IL-8, IL-22, IL-17α, interferon-gamma, IL-4, and transforming growth factor-β, correlate with various CKD stages.

Methods: One hundred and forty-one KTRs between the ages of 18 and 75 years were included in the study. We also included 112 live kidney donors, 37 CKD PG_{CKD+3}, and 76 $GP_{healthy}$. Participants were grouped according to their glomerular filtration rate (GFR) and their circulating cytokine levels, previously quantified by ELISA.

Results: By linear regression analysis, we established the relation of each cytokine with the GFR. Transforming growth factor-β correlated positively with the GFR in the study population, except in healthy individuals. A negative correlation of IL-8 and IL-17α and GFR was found in all cases.

Conclusions: Whether these cytokines (IL-8 and IL-17α) could be used as inflammatory biomarkers indicating CKD progression, regardless of the type of population, remains to be prospectively determined.

Abrégé

Contexte: L'insuffisance rénale chronique (IRC) est un problème de santé mondial. Une thérapie de remplacement rénal est nécessaire au fur et à mesure que la maladie évolue vers les stades terminaux. Mais, en définitive, le meilleur traitement reste la transplantation. La réduction de la fonction rénale a été associée à un état inflammatoire associé à l'IRC primaire; une association observée aussi chez les receveurs d'une greffe de rein.

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Objectif: Déterminer la façon dont les concentrations sériques de certaines cytokines, notamment IL-2, IL-8, IL-22, IL-17a, IFN-γ, IL-4 et TGF-β, corrèlent avec divers stades de l'IRC.
Méthodologie: Ont été inclus dans l'étude 141 receveurs d'une greffe rénale âgés de 18 à 75 ans, 112 donneurs vivants de rein, 37 personnes atteintes d'IRC (PG_{IRC+3}) et 76 personnes en bonne santé ($PG_{en\ santé}$). Les sujets ont été regroupés en fonction de leur débit de filtration glomérulaire (DFGe) et de leur taux de cytokines en circulation, quantifiés préalablement par ELISA.
Résultats: Une analyse de régression linéaire a servi à établir la relation entre chaque cytokine et le DFGe. Dans la population étudiée, une corrélation positive a été observée entre TGF-β et le DFGe, sauf chez les individus sains. Dans tous les cas, la corrélation s'est avérée négative entre le DFGe et les taux d'IL-8 et d'IL-17a.
Conclusion: Il reste à déterminer prospectivement si ces cytokines (IL-8 et IL-17a) pourraient être utilisées comme biomarqueurs inflammatoires pour indiquer la progression de l'IRC, quelle que soit la population.

Keywords
inflammation, chronic kidney disease, kidney transplantation, cytokines

Received May 2, 2022. Accepted for publication August 22, 2022.

Introduction

Chronic kidney disease (CKD) is a worldwide public health priority, affecting 10% to 12% of the population. The most advanced manifestation of CKD is end-stage renal disease, frequently warranting replacement therapy (RT) with dialysis or kidney transplantation. The use of RT has significantly increased in the past 2 decades, as well as the number of renal transplants, the most recommended treatment modality in advanced long-term renal failure.[1]

Long-term inflammation is a recognized factor leading to increased premature cardiovascular mortality in CKD patients and in those on dialysis. According to several studies, over half of patients with CKD stage 3 or above have increased levels of C reactive protein (CRP).[2,3]

Kidney transplant recipients (KTRs) have an additional factor promoting inflammation resulting from allograft implantation and other clinical events that induce an inflammatory response.[4,5] According to current scientific literature, one of the major causes of death following a successful renal transplant is a cardiovascular event,[6-8] and the leading cause of long-term graft loss involves a wide variety of immunological abnormalities and associated clinical factors such as obesity,[9] dyslipidemia,[10] and other underlying diseases.[11,12]

Previous studies have documented that interleukin (IL)-6 is the most significant inflammatory biomarker playing a role in the outcomes of CKD.[13] Likewise, high sensitivity CRP and IL-6 have been independently associated with graft loss.[14] Serum tumor necrosis factor-α (TNF-α) and IL-6 have also been independently associated with the loss of a previously functional graft.[15]

Although there are many publications on the relationship between cytokine level variations in CKD,[16-19] the correlation between inflammation markers and glomerular filtration rate (GFR) has not been well studied. Another feature warranting further study is whether the association between GFR and inflammatory markers is different, when comparing KTRs, live kidney donors (LKDs), and participants in the community, with or without CKD, and apparently $GP_{healthy}$. Therefore, this study evaluated the proinflammatory and anti-inflammatory cytokines IL-2, IL-8, IL-22, IL-17α, interferon-gamma (IFN-γ), IL-4, and transforming growth factor-β (TGF-β), and their correlation with CKD stages in KTRs, LKDs, and general population ($GP_{healthy}$ and PG_{CKD+3})

Materials and Methods

Individuals participating in this study included: (1) patients who had received a kidney transplant at the *Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán* (INCMNSZ) or at the *Hospital General de México* (HGM) *Eduardo Liceaga*, between January 2014 and November 2016; (2) LKDs recruited between October 2014 and November 2016 in the same institutions (INCMNSZ and HGM); and (3) finally, a group of individuals who had previously participated in another population-based study from the state of Michoacán, Mexico. Our study was approved by the Committee of Medical Ethics and Research of the *Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán* and was conducted in accordance with the principles established in the Declaration of Helsinki, Good Clinical Practice Guidelines, and the Declaration of Istanbul. Written informed consent was obtained from all participants.

Patients in the KTR and the LKD groups were enrolled in the outpatient clinics of both participating hospitals during a follow-up consultation. Serum samples were obtained if they accepted to participate in the study. Inclusion criteria established that they had had to be between the ages of 18 and 75 years, and their transplant had been performed at least 1 year prior to inclusion. Exclusion criteria were evidence of infection, long-term liver disease, cancer, human immunodeficiency virus, human cytomegalovirus, autoimmunity, body mass index $\geq 30\ kg/m^2$, pregnancy or puerperium, hospitalization or a surgical procedure within the previous month,

being a multiple organ transplant recipient, and having had an acute graft rejection event documented by biopsy within the previous 3 months. Participants in the LKD group were enrolled using the same criteria, and serum samples were also obtained. They were drawn from a larger population (n = 4297) that had participated in a cross-sectional study on CKD prevalence in a semirural community; they were selected by balance cluster based on CKD stage, age, and sex. Selected individuals were further subdivided into 2 subgroups: one that included 76 individuals with an GFR of 60 mL/min/1.73 m^2 or above, and the second subgroup included 37 individuals with an GFR below 60 mL/min/1.73 m^2. We will refer to the first subgroup as $GP_{healthy}$ and to the second subgroup as the PG_{CKD+3}. These 2 subgroups were selected with the caliper matching method, stratifying by age, sex, and Kidney Disease—Improving Global Outcomes (KDIGO) grading. Serum samples were obtained from a bank created for the cross-sectional study during the period of July 2012 to November 2012; all serum aliquots had been cryopreserved at −70°C.

Glomerular filtration rate was estimated using the CKD-EPI formula (Chronic Kidney Disease Epidemiology Collaboration)[20] in every participant participating in the study. The assessment of CKD was based on the criteria established by the KDIGO board members in 2012.[21]

Samples were obtained after a 12-hour fast by standard venipuncture. Blood was immediately preserved, refrigerated for transportation, and centrifuged at 4°C for 20 minutes at 2000 revolutions/min to separate the serum. The serum was cryopreserved in 1 mL aliquots at −70°C until processing. Sera were assayed following the ELISA technique to determine the serum cytokine concentrations.

The following cytokines were analyzed: IL-2, IL-4, IL-8, IL-22, IL-17α, IFN-γ, and TGF-β. The samples were processed following the manufacturer's specifications (Biolegend, LEGEND MAX TM HUMAN, IL-2, IL-4, IL-8, IL-22, IL-17α, IFN-γ, TGF-β).

Statistical Analysis

Descriptive statistics were used according to the type of variable. Continuous variables were analyzed by means and standard deviation, and the median and interquartile range were estimated; categorical variables were tabulated and frequencies were estimated. The means and medians of the continuous variables were contrasted in the 4 groups by analysis of variance and the Kruskal-Wallis test. The distributions of the categorical variables were compared using the χ^2 and the Fisher's exact tests. In addition, mathematical transformations were performed to obtain normality and thus improve the goodness of fit of the models and to capture the possible nonlinear relationships between the variables of interest: the GFR and each of the inflammatory markers (IL-2, IL-8, IL-17α, and IFN-γ) and negative regulators (IL-4 and TGF-β). Multiple linear regression analysis was used to model the association between the GFR (dependent variable) and the inflammation markers, adjusting for the population of interest (LKD, KTR, CKD-3-5, $GP_{healthy}$, age, and sex). Furthermore, to study possible modification effects, we included the interactions between each study group and the inflammatory markers. Inflammation markers were log-transformed to better capture a potential nonlinear association and to decrease the bias introduced by outliers of the marker's distribution. Log2 transformations were used to facilitate interpretation (ie, the regression coefficient associated with IL-2 is interpreted as the expected change in GFR each time IL-2 is doubled).

Data analysis was performed with the statistical package Stata, Version 15.0. The regression model was evaluated using the F statistical method, the R^2 coefficient of determination, and the P-values for each of the variables in the model. Values of $P < .05$ were considered statistically significant.

Results

Our study population included a total of 366 individuals: 141 in the KTR, 112 in the LKD, 37 in the CKD PG_{CKD+3}, and 76 in the $GP_{healthy}$ categories. The general characteristics of each population are presented in Table 1. Most of the compared variables were similar in each of the 4 populations. However, the mean time elapsed since transplantation or donation to measurement of the GFR and inflammation markers was longer in the KTR group than in kidney donors (51 ± 65 vs 38 ± 33 months; $P \leq .01$).

In the KTR group, the most frequent cause of primary renal failure was unknown (48%, n = 67), and 79% (n = 112) received grafts from a relative. Individuals in this group were more frequently males (53%, n = 74), while individuals in the healthy group were older than the KTR, LKD, and PG_{CKD+3} individuals. Tacrolimus was the most frequent immunosuppressive maintenance treatment in the KTR group (87%, n = 123). There were no differences in terms of the presence of type 2 diabetes mellitus between LKD, but 17% of patients in the KTR group were diabetic.

The most prevalent KDIGO stage in the KTR population was stage 2, as well as in the LKD group. In the PG_{CKD+3} group, the most prevalent stage was 3. Finally, in the healthy individual category, the most prevalent stage was 2. Notably, the proportion of individuals in the first 3 stages is higher than the proportion of participants in latter stages.

In comparison with LKD, participants from the general population ($GP_{healthy}$ and PG_{CKD+3}), the KTR group had higher serum levels of IL-8, IFN-γ, and IL-4, as shown in Table 2. However, the concentrations of IL-17α and IL-2 were greater in the PG_{CKD+3} group in comparison with the KTR, LKD, and $GP_{healthy}$ categories. TGF-β1 serum levels were higher in the $GP_{healthy}$ group than in KTR, LKD, and PG_{CKD+3} groups. Statistical significance was reached in all cases.

Table 1. General Features of KTR, Kidney Donors, and Participants From the General Population ($GP_{healthy}$ and PG_{CKD+3}).

Variable	KTR (n = 141), n (%)	LKD (n = 112), n (%)	PG_{CKD+3} (n = 37), n (%)	$GP_{healthy}$ (n = 76), n (%)
Age (years)	37 ± 13 (18-75)	45 ± 13 (18-75)	48.5 ± 18.4 (18-73)	57.5 ± 14.1 (19-75)
Male	74 (53)	58 (52)	16 (43)	37 (49)
BMI (kg/m^2):	24.8 ± 3.2	25 ± 2.8	24.9 ± 2.1	24.5 ± 2.6
<18.49	6 (4)	—	1 (3)	2 (3)
18.5-24.99	74 (53)	51 (46)	10 (28)	35 (45)
25-29.99	61 (43)	61 (54)	18 (49)	47 (62)
Complication post-surgical:				
Diabetes type 2	24 (17)	6 (5)	2 (2.6)	5 (6.6)
Hypertension	69 (49)	24 (21)	—	—
Evolution time (months)	51 ± 65	38 ± 33		
After	(12-396)	(12-240)	N/A	N/A
Previous disease:				
Unknown	67 (48)			
Diabetes mellitus	17 (12)			
Renal hypoplasia	9 (6)	N/A	N/A	N/A
Systemic lupus erythematosus in remission	15 (11)			
Others	33 (22)			
Induction:				
Thymoglobulin	22 (16)	N/A	N/A	N/A
Basiliximab	104 (74)			
No induction	15 (10)			
Kind of donor:				
Live related donor	112 (79)			
Live non-related donor	18 (13)	N/A	N/A	N/A
Dead donor	11 (8)			
Maintenance therapy:				
Tacrolimus	123 (87)			
Cyclosporine	11 (8)	N/A	N/A	N/A
Sirolimus	3 (2)			
No maintenance therapy	4 (3)			
Replacement therapy:				
Hemodialysis	62 (44)			
Peritoneal dialysis	56 (40)	N/A	N/A	N/A
Combined therapy	3 (2)			
No therapy	20 (14)			

Note. Sociodemographic and clinical features of the 3 populations. Every feature is presented with its mean ± standard deviation and frequencies. KTR = kidney transplant recipient; $GP_{healthy}$ and PG_{CKD+3}, PG: participants from the general population; LKD = live kidney donor; BMI = body mass index; CKD = chronic kidney disease.

In the results obtained by multiple linear regression analysis of the inflammatory markers and the GFR (Table 3), we observed that IL-4 (anti-inflammatory cytokine) had a positive correlation in all the studied groups, but it was statistically significant only in the KTR and PG_{CKD+3} groups. The correlation was strongest in the latter.

In terms of the proinflammatory cytokines (IL-2, IL-17α, and IFN-γ), their increase may be inversely related to the GFR, except in the case of IFN-γ in the PG_{CKD+3} group in which the correlation was positive, but of no statistical significance. Serum levels of IL-2 had a greater effect in the PG_{CKD+3} group, with a more significant associated decrease in the GFR than in the other groups. An adverse effect was observed with IL-17α and IFN-γ, particularly in the LKD group when compared with the KTR, PG_{CKD+3}, and $GP_{healthy}$ groups.

Interleukin-8 levels had a strong negative relation with the GFR across all the study groups. Thus, on the basis of our results, IL-8 seems to be the best predictor of renal function loss in terms of GFR, regardless of the study group and seems to be the cytokine that best correlates with decreased renal function.

Finally, IL-22 concentrations were found to be higher in the KTR group than in the other study groups. Correlation of

Table 2. Serum Levels of All Measured Cytokines.

Variable	KTR, (n = 141)	LKD, (n = 112)	$PG_{CKD+3,}$ (n = 37)	$GP_{healthy,}$ (n = 76)	*P* value
IL-4 (pg/mL)					
Mean (SD)	25.9 ± 43	10.5 ± 8.4	5.8 ± 7.0	11.8 ± 11.7	
Min-max	(1.2-247.3)	(1.1-52.1)	(−0.6 to 36.2)	(0.1-79.9)	.0003
Median (IQR)	11.7 (10.3)	8.3 (9.5)	3 (4)	9.9 (10.7)	
TGF-β (pg/mL)					
Mean (SD)	126.6 ± 103.6	285.3 ± 260.7	148.6 ± 144.8	326 ± 91.3	
Median (IQR)	(13.4-607.8)	(1.4-617.3)	(10.8-561.5)	(146.9-483.6)	.0003
	107.4 (72.1)	262.5 (278.2)	102.4 (222.1)	323 (97.4)	
IL-2 (pg/mL)					
Mean (SD)	69.3 ± 140.4	39.3 ± 59.9	102.3 ± 99.2	37.5 ± 31.7	
Median (IQR)	(0.1-653.5)	(0.06-459)	(3.5-528.1)	(0.33-163.4)	.0001
	2.3 (55.5)	19.6 (4.68-46.96)	100.2 (66.4)	31.8 (31)	
IL-17α (pg/mL)					
Mean (SD)	3.1 ± 6.5	6.6 ± 7	14.1 ± 8.8	11.9 ± 15.8	
Median (IQR)	(0.04-36.4)	(1.1-42.8)	(1.4-48)	(−1.9 to 73.9)	.0001
	0.6 (1.3)	4.3 (6.7)	12.4 (10.5)	5.2 (11.06)	
IFN-γ (pg/mL)					
Mean (SD)	556.44 ± 2296	23.01 ± 26.1	22.1 ± 37.2	17.9 ± 18.4	
Median (IQR)	(0.5-23 028)	(3-132)	(0.3-188.9)	(0.4-83.5)	.0001
	64.1 (410.6)	14.1 (16.2)	10.5 (9.6)	9.9 (15.3)	
IL-8 (pg/mL)					
Mean (SD)	65.3 ± 133.8	20.7 ± 20	44.8 ± 42	24.8 ± 36.4	.0006
Median (IQR)	(8.14-953.4)	(0.2-113.3)	(1.9-165.3)	(0.3-212.3)	
	24.8 (27.5)	14.1 (15)	27.7 (44.6)	13.4 (16.2)	
IL-22 (pg/mL)					
Mean (SD)	213.9 ± 195.9	58.7 ± 31.4	84.9 ± 214.5	126.4 ± 565	
Median (IQR)	(1.7-827.5)	(3.05-137.9)	(−24.6 to 1254.6)	(−30.8 to 4854.9)	.0001
	127.6 (187.7)	53.9 (29.1)	22 (92.3)	27.2 (89.8)	

Note. Means and medians of each inflammatory marker. The standard deviation for the mean and the interquartile range for the median are presented in parentheses. KTR = kidney transplant recipient; LKD = live kidney donor; IL = interleukin; IQR = interquartile range; TGF-β = transforming growth factor-β; IFN-γ = interferon-gamma; SD = standard deviation; CKD = chronic kidney disease; $GP_{healthy}$ and PG_{CKD+3}, PG: participants from the general population.

this cytokine with the GFR had no statistical significance in any study category (Table 3).

Discussion

According to our findings, several considerations on each of the studied cytokines can be posited as well as their effects on each population.

Interleukin-4

There is evidence suggesting that the levels of IL-4 and IL-10 have a tendency to increase in KTR.[22] Interleukin-4 has a primarily immunomodulatory function.[23] Therefore, if IL-2 and IFN-γ concentrations increase, IL-4 may dampen the inflammatory process. In addition, according to the results obtained in the regression models (Table 3), there is a significant positive correlation between IL-4 and the GFR in KTR and in the PG_{CKD+3}, reflecting a protective effect on the GFR, whereby IL-4 acts as a predominantly anti-inflammatory cytokine.

Transforming Growth Factor-β

The functions of this cytokine have been widely discussed: TGF-β is a pleiotropic molecule whose effects depend on the type of tissue upon which it acts.[24-26] Hence, under some circumstances, this cytokine seems to have proinflammatory effects,[27] and has been implicated in fibrosis development[28-30] through the inhibition of the protein Klotho.[31,32]

On the other hand, TGF-β1 is well characterized as a regulatory T cell (Treg) inducer. It has been demonstrated to increase the proliferation, differentiation, and function of Tregs by upregulating Foxp3 expression via the PP2A pathway and suppressing IL-12R. Transforming growth factor-β1 plays a protective role in CKD by suppressing inflammation and immune cell-mediated fibrosis. Notably, the abundance of peripheral Tregs is significantly reduced in

Table 3. Multiple Linear Regression Analysis of Inflammatory Markers and Glomerular Filtration Rate.

Cytokines	KTR (n = 141)	LKD (n = 112)	PG_{CKD+3} (n = 37)	$GP_{healthy}$ (n = 76)	*P* value
IL-17α					
Coef (%)	−4.6	−8.7	−6.5	−2.9	<.0001
95% CI	(−6.1 to −3)	(−11.2 to −6.2)	(−11.8 to −1.3)	(−5.5 to − 0.4)	
IFN-γ					
Coef (%)	−6.0	−7.1	0.31	−1.8	<.0001
95% CI	(−7.1 to −5.0)	(−9.8 to −4.3)	(−3.1 to 3.8)	(−4.3 to 0.8)	
IL-2					
Coef (%)	−2.2	−3.6	−9.4	−1.3	<.0001
95% CI	(−3 to −1.5)	(−5.2 to −2.0)	(−14.1 to −4.7)	(−3.8 to 1.2)	
TGF-β					
Coef (%)	10.4	309	8	−10.6	<.0001
95% CI	(5.5-15.2)	(0.9-7)	(3.5-12.5)	(−44 to 22.8)	
IL-4					
Coef (%)	3.6	2.2	5.9	2.1	.012
95% CI	(1.3-5.8)	(−0.9 to 5.3)	(0.9-11)	(−0.6 to 4.8)	
IL-8					
Coef (%)		−7.0[a]			.8499
95% CI		(−8.57 to −5.39)			
IL-22					
Coef (%)		−1.6[a]			.4093
95% CI		(−3.32 to 0.83)			

Note. KTR = kidney transplant recipient; LKD = live kidney donor; IL = interleukin; CI = confidence interval; IFN-γ = interferon-gamma; TGF-β = transforming growth factor-β; $GP_{healthy}$ and PG_{CKD+3}, PG: participants from the general population; CKD = chronic kidney disease.
[a]The interaction has no statistical significance for neither of the 2 cytokines, suggesting that the type of population is a confusion factor. In this case, this effect is observed overall.

CKD patients compared with healthy controls. Furthermore, TGF-β1 induces membrane-bound TGF-β1 on the Treg cells to suppress naive CD4+ T cells expansion for immune suppression via activating Smad3.

The results of our study showed that the levels of TGF-β are elevated in the group of healthy individuals and decreased in the KTR and the PG_{CKD+3} study groups, which could suggest a lower number of Treg cells; this same instance was observed in the study by Kumar et al.[33] However, our regression models suggest that this cytokine has a GFR-protective effect, but only in populations with evident kidney injury: KTR, LKD, and PG_{CKD+3}.

On the other hand, in $GP_{healthy}$ in the initial stages of CKD, TGF-β has an GFR–non-protective effect. We can therefore suggest that in the initial stages, this cytokine could play a pro-fibrotic role, but in the presence of renal injury it could be contributing to tissue remodeling. However, this conclusion still requires further confirmation.

Interleukin-2

Interleukin-2 levels are higher in KTR and even higher in the PG_{CKD+3} groups than in the other 2 populations. This could be due to the proinflammatory role played by IL-2 in the immune response against cancer cells, pathogenic microorganisms, or external antigens, such as those found in the renal allograft.

It should be emphasized that KTR and PG_{CKD+3} had the highest levels of this IL. Interestingly, it would be expected that stable KTR on immunosuppressive therapy, including a calcineurin inhibitor, to have the lowest IL-2 levels among the studied groups. According to our linear regression analysis, this cytokine's impact on the GFR is regularly noted to be negative in all the populations, but particularly in the PG_{CKD+3} group. Similar findings were reported[34] when comparing the levels of this cytokine in KTR and in the general population. The administration of IL-2 can also lead to rejection in primates.[35]

Interleukin-17

Interleukin-17 is an IL family of which IL-17α is the most studied member. Although it can be produced by various cells, it is the characteristic IL produced by Th17 lymphocytes.[36] This is relevant since early differentiation of virgin lymphocytes into Th17 lymphocytes can be inhibited by certain ILs such as IFN-γ and IL-4.[37] This could explain why the serum IL-17α values observed in KTR are lower than in the other 3 groups, given that the serum values of IFN-γ are highest in the KTR group. We were unable to detect a gradient effect between our study categories, suggesting a possible inverse relationship between IL-17α serum values and the levels of IFN-γ.

In addition, it has been observed that IL-17 is related to acute rejection,[38] high levels of angiotensin II,[39] and the development of renal fibrosis.[40] It has also been studied as a marker of long-term allograft injury.[41]

This could explain the negative correlation between this cytokine and the GFR in all of the populations. Actually, an association between an IL17RA polymorphism (rs4819554) with a negative effect on the GFR has been described in a Spanish cohort.[42]

Interferon-Gamma

We detected increased IFN-γ values in the KTR individuals. Interferon-gamma is a cytokine with a predominant antiviral effect. However, it has multiple functions in both innate immunity and adaptive immunity; for example, it can induce the production of reactive oxygen species, the secretion of proinflammatory cytokines such as TNF-α and IL-12, and can facilitate the presentation of antigens through several mechanisms that result in greater expression of the major histocompatibility complex, both I and II.[43] The latter is essential due to its role in the presentation of foreign antigens, specifically those of the graft in KTR individuals. This could explain the high levels of IFN-γ in the KTR group. It must be noted that this cytokine may play a role in the development of atherosclerosis through monocyte chemoattractant protein-1 in patients with CKD and type 1 diabetes mellitus[44] and hypercoagulable states have also been shown to be associated with elevated serum levels of this IL.[45] For these reasons, it is not unusual that on average, the correlation between IFN-γ and GFR is negative in almost all populations, but only significant in LKD and in KTR individuals. This agrees with a study conducted in renal transplant recipients that described a positive correlation between positive ELISPLOT-IFN-γ and lower GFRs.[46] The effect is more significant in LKD than in the other 3 groups, perhaps for 2 reasons: the first is that PG_{CKD+3} and healthy groups have the advantage provided by 2 kidneys, unlike LKD and KTR individuals. The second is that KTRs were exposed to immunosuppressive drugs, which did not occur in LKD. A greater impact would have been expected in the LKD population.

Interleukin-8

Higher IL-8 concentrations were found in the KTR group. Interleukin-8 is the prototype cytokine of a subgroup of chemokines known as CXC. This chemokine is one of the main chemoattractants of neutrophils. In some long-term inflammatory diseases, IL-8 has been found to be overexpressed,[47] ergo IL-8 has a proinflammatory role. Higher levels of this chemokine may be expected in KTR individuals, given that long-term inflammation leads to CKD *per se* and the need for a kidney graft. In fact, it has been suggested that this cytokine can be used as a predictor of acute renal rejection and of dismal renal function, since high levels of this cytokine in KTR individuals have been associated with these 2 unfavorable outcomes.[48] The same finding was observed in our multiple linear regression analysis, in which this IL negatively compromised the GFR in all of the groups.

Interleukin-22

Last, we detected that the value of serum IL-22 levels was increased in the KTR group. Although there is not much information on the role of this IL in renal function, this molecule has been posited to be implicated in the regeneration of renal tissue or linked to the development of nephropathy.[49] Our results suggest that there is no concluding evidence of these suggestions, since there is no detected statistical significance (according to *P*-values and confidence intervals).

Conclusion

We conclude that the relationship between inflammatory markers and CKD requires further study; particularly the TGF-β1, which may be linked to fibrosis development and may also have an GFR protective effect in some individuals; and the role of IL-8 and IL-17A, which could potentially act as impaired renal function biomarkers in any population.

Limitations

Our study has several limitations, particularly its cross-sectional design. The ideal timing for the measurements may have been immediately prior to transplantation and 1 year later during follow-up, since this is the period in which kidney transplant patients are expected to have stable renal function and management, but we lacked the resources needed to obtain these observations.

Therefore, the GFR measured in these 4 populations helps to elucidate the cytokine participation in the different phases of the clinical evolution of CKD.

We also faced the problem of reverse causation, that is the cross-sectional design precluded our ability to determine whether renal function is the result of the direct effects of the studied cytokines or vice versa. Hence, further research will have to be conducted in longitudinal studies to characterize the changes in renal function following donation and graft reception.

List of Abbreviations

CKD, chronic kidney disease; GFR, glomerular filtration rate; ESRD, end-stage renal disease; RT, replacement therapy; HIV, human immunodeficiency virus; HCV, human cytomegalovirus; BMI, body mass index; KDIGO, Kidney Disease—Improving Global Outcomes; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; IL-2, interleukin-2; IL-4, interleukin-4; IL-8, interleukin-8; IL-22, interleukin-22; IL-17α, interleukin-17α; IFN-γ, interferon-gamma; TGF-β, transforming growth factor-β; KTR, kidney transplant recipient; LKD, live kidney donor; $GP_{healthy}$ and

PG_{CKD+3}, PG: participants from the general population; ROS, reactive oxygen species; MHC, major histocompatibility complex.

Ethics Approval and Consent to Participate

This study was approved by the INCMNSZ research ethics committe (Ref. number: 1346). AS this was study with available data patient consent was required.

Consent for Publication

All authors agreed to the publication of this manuscript.

Availability of Data and Materials

The data for this study is available and the models used for the analysis are available upon request.

Author Contributions

This study is conceived original idea, drafted the first version. All authors contributed to the research idea/conception and reviewed/revised the original draft. All authors provided final approval for the manuscript in its current form.

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.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Funds: Universidad Nacional Autónoma de México (UNAM).

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