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Serum levels of proinflammatory cytokines in children from an agricultural community with a high risk of chronic kidney disease in Mexico

Concentraciones séricas de citocinas proinflamatorias en niños de una comunidad agrícola de México con alto riesgo de enfermedad renal crónica

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Abstract

OBJECTIVE: To measure serum concentrations of IL-6, IL-1β, TNF-α and MCP-1 in a pediatric population and compare them with those of persistent albuminuria and with the degree of chronic kidney disease.

MATERIALS AND METHODS: Cross-sectional, longitudinal, population-based study conducted between 2016 and 2021 in participants enrolled in the Mexican Study of Albuminuria Prevalence in Children Living in a Rural Agricultural and Subsistence Community Fishing Area (Agua Caliente, Jalisco). Detailed description of the participants and methodology of this study was previously reported by Lozano-Kasten, et al in 2017.

RESULTS: Eighty-two children with persistent albuminuria grouped according to CKD stage based on glomerular filtration rate and KDIGO guidelines were studied. Significant differences in IL-6 concentrations were found between KDIGO G1-G3B ($p = 0.02$) and G3A-G3B ($p = 0.02$) groups, with a reduction in serum IL-6 concentrations as glomerular filtration rate decreases. In addition, urinary albumin concentration showed a trend of association with TNF-α concentrations ($p = 0.09$).

CONCLUSION: The gradual decrease in IL-6 concentrations as CKD progresses demonstrates the particular immunological characteristics of the pediatric population with this disease of unknown cause.

KEYWORDS: Chronic kidney disease; glomerular filtration rate; albuminuria; proinflammatory cytokines; boys and girls.

Resumen

OBJETIVO: Medir las concentraciones séricas de IL-6, IL-1β, TNF-α y MCP-1 en una población pediátrica y compararlas con las de albuminuria persistente y con el grado de enfermedad renal crónica.

MATERIALES Y MÉTODOS: Estudio transversal, longitudinal, de base poblacional, llevado a cabo entre 2016 y 2021 en los participantes inscritos al Estudio Mexicano de Prevalencia de Albuminuria en Niños que Viven en una Zona Rural Agrícola y de Pesca Comunitaria de Subsistencia (Agua Caliente, Jalisco). La descripción detallada de los participantes y la metodología de este estudio la reportaron previamente Lozano-Kasten, et al en 2017.

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RESULTADOS: Se estudiaron 82 niños con albuminuria persistente agrupados según el estadio de la enfermedad renal crónica basado en la tasa de filtración glomerular y las directrices KDIGO. Se encontraron diferencias significativas en las concentraciones de IL-6 entre los grupos KDIGO G1-G3B (p = 0.02) y G3A-G3B (p = 0.02), con una reducción en las concentraciones séricas de IL-6 a medida que disminuye la tasa de filtración glomerular. Además, la concentración de albúmina urinaria mostró una tendencia de asociación con las concentraciones de TNF-α (p = 0.09).

CONCLUSIÓN: La disminución gradual de las concentraciones de IL-6 conforme avanza la enfermedad renal crónica demuestra las características inmunológicas particulares de la población pediátrica con esta enfermedad de causa desconocida.

PALABRAS CLAVE: Enfermedad renal crónica; tasa de filtración glomerular; albuminuria; citocinas proinflamatorias; niños y niñas.

INTRODUCTION

Chronic kidney disease (CKD) is a condition of irreversible kidney damage that can further progress to end-stage renal disease (ESRD), and is a devastating disorder associated with excessive mortality and cardiovascular morbidity, and specific problems occur in children such as impaired growth and psychosocial adjustment, all of which severely impact the quality of life.[1] CKD is a major public health problem worldwide, and extensive epidemiological research in the adult population is available. In contrast, little is known about the epidemiology of CKD in the pediatric population and is defined as abnormalities of kidney structure or function, present for greater than 3 months with specific implications for health. According to the United States Renal Data System, it is estimated that 14.8% of the general population of the United States was affected by CKD from 2013 to 2016, with the most prevalent stage being CKD stage 3.[2]

One of the most important tools for predicting the risk of CKD progression or kidney failure, dialysis, adverse events including cardiovascular risk, and premature mortality is elevated albuminuria.[3,4,5]

This pathology is defined as a GFR less than 60 mL/min/1.73 m^2 or one or more markers of kidney dysfunction including albuminuria.[6]

Age has the highest correlation with low eGFR (eGFR <60 mL/min/ 1.73 m^2), and CKD has the highest prevalence in individuals over the age of 60 years.[2,7]

Compared with men, women have a slightly higher prevalence of both a GFR of less than 60 mL/min/1.73 m^2 (7.9% vs 6.4% per the above study) and albuminuria (10.9 *vs* 8.8%).[8]

The pathophysiology and mechanisms of worsening renal function are complex and multifactorial. In addition to the well-known risk factors for renal injuries, such as aging, diabetes mellitus, and hypertension, some environmental chemicals have also been shown to be important risk factors for renal injury.[9,10,11] With the ever-increasing use of synthetic compounds in all aspects of daily life, the health risks from environmental toxins and pollutants becomes increasingly important. In particular, as the kidneys are responsible for excreting waste products from the body, they are exposed to toxins and pollutants in the blood, and they are therefore susceptible to the adverse effects stemming from this exposure.[12,13]

There is much epidemiological evidence for the association between environmental pollution and kidney disease, including heavy metals, air pollution, and other environmental nephrotoxicants in the general population.[14]

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Even though environmental contamination, interracial mixing, chronic degenerative diseases, and an immune imbalance are risk factors for this disease,[15] in Mexico, the principal causes of kidney disease in children are unknown.[16]

Recent studies in the state of Jalisco have specifically reported in the Lake Chapala region a bioaccumulation of several metals such as arsenic, lead, cadmium, copper, zinc, nickel, and chromium,[17] which severely affects the quality and availability of water that reaches the communities around the lake such as Agua Caliente. Chronic exposure to this contamination could be a determining factor in the development of renal impairment through the activation of the immune system and stimulation of inflammation, mechanisms principally as causative agents in the development of acute and CKD.[18]

The search for nontraditional risk factors that may be involved in the pathogenesis of CVD in patients with CKD has been an area of intense study. During the last few years, chronic inflammation has become recognized as a major culprit in a whole range of pathologic states, such as CVD, obesity, diabetes, malnutrition, and even aging.[19] In this regard, it is becoming increasingly appreciated that ESRD is characterized by a state of chronic inflammation that seems to be linked with oxidative stress, endothelial dysfunction, vascular calcification, and wasting.[20,21] Indeed, a wide array of inflammatory biomarkers, such as C-reactive protein (CRP), interleukin (IL)-6, and white blood cell count are robust predictors of outcome in ESRD patients.[22]

In the immune system, a complex orchestration of cytokines and other molecules acts in a paracrine, autocrine, or endocrine fashion to control the differentiation, proliferation, and activity of immune cells. As the kidney is the major site for the elimination of many of these cytokines, the delicate equilibrium of pro-inflammatory cytokines and their inhibitors is dysregulated in CKD patients.[23,24]

Some investigations[25,26] have found relationships between renal function and various inflammatory biomarkers, such as CRP, IL-6, and TNF-α suggesting that the kidney plays a role in the clearance of pro-inflammatory cytokines. Indeed, studies in rats have shown that the kidney is the main site of metabolic degradation of IL-1β.[27] Moreover, a clinical study in ESRD patients has shown that serum creatinine is a major determinant of plasma IL-6 levels.[28]

There is a vast amount of literature on cytokine alterations in ESRD patients (with or without renal replacement therapy) all reporting elevated, but markedly divergent, concentrations of both IL-6 and TNF-α. Most of the published work concentrates on a few cytokines that were measured in plasma, culture supernatants, or association with circulating cells. These studies provided a sound body of information on the chronic inflammation of the renal disease.

Proinflammatory cytokines are counterbalanced at several levels. First, the secretion of IL-1β is linked to the secretion of the IL-1 receptor antagonist (IL-1RA), which binds the cytokine and prevents its actions.[29] The same mechanism also applies to TNF-α, which is counterbalanced by soluble TNF receptors.[30] One might hypothesize that the production of active cytokines and specific inhibitors is a mechanism that allows the mediators to act locally while preventing them from acting systemically.

Although several pro-and anti-inflammatory cytokines orchestrate the inflammatory response, IL-6 is a particularly interesting molecule since it has both pro and anti-inflammatory effects. The IL-6 system promotes inflammatory events through the activation and proliferation of lymphocytes, differentiation of B cells, leukocyte

recruitment, and the induction of an acute phase protein response in the liver usually in response to physiologic stimuli, such as TNF-α, IL-1β bacterial endotoxins, physical exercise, and oxidative stress.[31] Whereas most other cytokines function via paracrine/autocrine mechanisms, the major effects of IL-6 are a consequence of its presence in the circulation and can take place at sites distinct and distant from its origin. IL-6 can be detected in the 1 pg/mile range from healthy individuals and is elevated in most, but not all, ESRD patients.[32] Lastly, Monocyte Chemoattractant Protein 1 (MCP-1) is a chemotactic factor for monocytes that induces differentiation in macrophages. The positive regulation of MCP-1 is implicated in progressive diabetes, as well as in inflammatory kidney disease according to some studies. MCP-1 is closely related to urinary protein excretion in experimental models, as well as in the pathogenesis of renal fibrosis.[33]

MATERIALS AND METHODS

This was a cross-sectional, population-based study of participants enrolled in the Mexican Study of Prevalence of Albuminuria in Children Living in a Rural Agricultural and Community-based Subsistence Fishing area (Agua Caliente, Jalisco) a longitudinal study developed in Agua Caliente, Jalisco between 2016 and 2021. A detailed description of the participants and the methodology of this study have been reported elsewhere by Lozano-Kasten, *et al.* 2017.[34]

Firstly, a representative sample was randomly captured after a census was taken of the population to identify the total number of inhabitants under 17 years of age who were invited to participate in the first stage of the project, which began in September 2016. Afterwards, sociodemographic and anthropometric data was gathered. Two first morning urine samples with 12-week intervals were requested for participants. Then the samples were analyzed for the detection of human albumin. For those presenting two positive tests for albuminuria, blood tests were carried out for creatinine, urea, and cystatin. Finally, with the data obtained, the glomerular filtration rate is calculated.

394 (69.7%) children participated with two albuminuria tests, and some of them had two or more positive test results. There were children who were not included in the study because their parents did not provide their consent.

Seven children under one year of age with negative results were excluded. A total of 180 non-hospitalized children from the general population were positive (with two or more positive albuminuria tests).

Of the 180 individuals, only 82 children without infectious or chronic diseases other than CKD and their parents agreed to continue with the study. The average age ranged from 3 to 13, and all were ultimately included in the present study.

Analysis of albumin levels[34]

The urine samples were taken in the morning on an empty stomach and analyzed with the Micral-Test® human albumin test strips (Roche Diagnostics GmbH, Mannheim, Germany). The assay was performed according to the supplier's instructions. First, the test strip was dipped into the urine for 5 seconds, then the strip was placed on a non-absorbent surface or across the top of the collection cup to allow the excess urine to drain. After waiting 1 minute, the color of the detection pad on the strip was compared with the color scale on the test strip vial. The scale was 0 mg/L, 20 mg/L, 50 mg/L, and 100 mg/L respectively.

Glomerular filtration rate calculation[34]

The obtained results showed blood pressure, weight, height, and body mass index (BMI) measures of individuals who had two positive tests for

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albumin. In addition, they were tested for creatinine, urea, and cystatin C, with the Calibrator Set® for immunoturbidimetric tests (Diazyme Laboratories, Inc., San Diego, CA, USA). Subsequently, from the data obtained, the GFR[35] calculation was adjusted to the body surface.[36]

KDIGO classification[34]

The research subjects were classified by the type of CKD according to their GFR based on Kidney Disease: Improving Global Outcomes (KDIGO), and clinical practice guidelines.[37]

Serum sample for cytokine analysis

The blood samples were obtained by a direct puncture to a vein in the antecubital area of the arm by highly trained personnel. The material used included a 25/9 mm Luer-type puncture needle, connected to a 10 mL syringe. The samples were placed in dry tubes to obtain serum (5 mL). After coagulation of the samples, the tubes were centrifuged for 5 minutes at 2,000 rpm and the serum was separated and placed in microtubes that were stored at -80 °C.

Luminex cytokine assays and data analysis

Proinflammatory cytokines were identified by multiplexed immune assays in serum from study subjects. We used a 4-plex kit (IL-6, TNF-α, IL-1β, MCP-1) from R&D Systems, Minneapolis, Minnesota, and the United States, and multicytokine analysis was performed using a MAGPIX system powered by xMAP Luminex Technology with the xPONENT software of EMD (Merck Millipore, Darmstadt, Germany) as described previously for Corral-Jara *et al*, 2016.[38]

Statistical analysis

The data are presented as median ± interquartile range 25-75 in the figures and means and interquartile range 25-75 in the text and description of the figures. Statistical comparisons were performed using SPSS V24.0 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.), Excel 21 (Excel 2021 (18.0) Microsoft 365, Redmond, Washington, United States), GraphPad Prism software version 5.01 (GraphPad Software, Inc., San Diego, CA). Soluble level comparisons were evaluated by the Mann-Whitney U test representing the median ± interquartile range 25-75. A p-value of less than 0.05 was considered statistically significant. To rule out age and gender bias in the measurement of IL-6, TNF-α, and IL-1β, soluble levels of MCP-1 were measured using a linear regression.

RESULTS

Of the 180 children sampled with persistent albuminuria from the community of Agua Caliente, Poncitlan, Jalisco, Mexico, reported by Lozano *et al*., in 2017, 82 children without infectious or chronic diseases other than CKD and their parents agreed to continue with the study. Children not included in the study were not given permission by their parents to participate.

Clinical and demographic characteristics are shown in **Table 1**. The children included in this study ranged from 3 to 13 years of age, with the average age being 8.74 ± 2.76 years. In terms of gender, 43.9% were girls and 56.1% boys.

Among the clinical tests, mainly urea (mean 23.49 ± 7.62), creatinine (mean 0.69 ± 0.21), and cystatin C (mean ±) were measured.

The concentration of albumin in urine with the highest persistence was 20 mg/L, and was observed in 51.22% of the children.

The children in the study are grouped into 5 of the 6 groups as defined by the KDIGO guidelines according to the stage of CKD, which was determined based on the GFR in G1 (18.29%),

Table 1. Clinical and demographic characteristics of children studied in the community of Agua Caliente, Jalisco

Characteristics	n (%)	mean	SD
Demographics			
Age (years)		8.74	2.76
Female/Male	36 (43.90) / 46 (56.10)		
Clinical			
Weight (kg)		26.75	9.53
Height (cm)		127.82	16.42
BMI		16.37	2.23
Systolic BP (mmHg)		99.50	19.43
Diastolic BP (mmHg)		61.81	26.33
Urea (mg/dL)		23.4899	7.62058
Creatinine (mg/dL)		0.6934	0.21396
Cystatin C (mg/L)		0.8153	0.9740
GFR (mL/min/1.73 m^2)		50.6406	15.45641
Persistent albuminuria	82 (100)		
20 (mg/L)	42 (51.22)		
50 (mg/L)	32 (39.02)		
100 (mg/L)	8 (9.76)		
Immunological			
IL-6 (pg/mL)		0.0963	0.04471
TNF- (pg/mL)		0.5403	0.46458
IL-1 (pg/mL)		0.0810	0.05181
MCP-1 (pg/mL)		83.25	84.74354
KDIGO/GFR	82 (100)	50.64	15.46
G1	15 (18.29)	109	1.35
G2	15 (18.29)	71.58	10.60
G3a	25 (30.49)	51.36	4.46
G3b	23 (28.05)	39.26	3.21
G4	4 (4.88)	28.66	0.58

G2 (18.29%), G3a (30.49%), G3b (28.05%), and G4 (4.88%) groups. None of the individuals were classified as grade 5.

Soluble levels of proinflammatory cytokines according to the degree of CKD [KDIGO]

Subsequently, to identify the influence of levels of proinflammatory cytokines with the degree of CKD, according to the degrees defined by KDIGO, a comparative analysis of serum levels of IL-6, TNF-α, IL-1β, and MCP-1 was carried out between the groups according to the degree of CKD.

The analysis of the results showed significant differences in IL-6 (**Figure 1**) between G1 and G3b (0.1275 vs 0.0792 pg/mL p = 0.02), G1 and G4

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(0.1275 vs 0.0735 pg/mL p = 0.09), G2 and G3b (0.1002 vs 0.0792 pg/mL p= 0.05), G3a and G3b (0.1021 vs 0.0792 pg/mL p = 0.02), and G3a and G4 (0.1021 vs 0.0735 pg/mL p = 0.07) groups, showing a clear decrease in serum levels of IL-6 as the degree of CKD increases.

Soluble levels of proinflammatory cytokines and persistent albumin concentration

To investigate the relationship between the concentration of proinflammatory cytokines and the level of albuminuria in urine, IL-6, TNF-α,

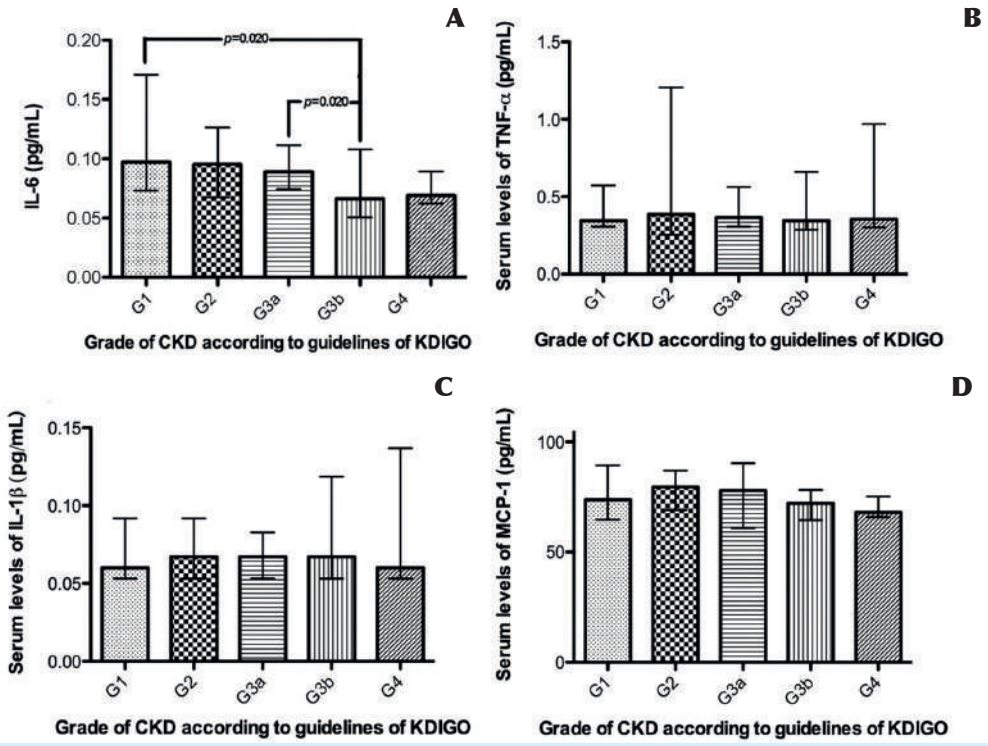

Figure 1. Comparison of IL-6, TNF-a, IL-1b, and MCP-1 serum levels with the CKD grade. **A)** Comparison of IL-6 serum levels with KDIGO groups: G1 (0.1275; IQR_{25-75}: 0.07302-0.1713), G2 (0.1002; IQR_{25-75}: 0.06751-0.1267), G3a (0.1021; IQR_{25-75}: 0.07443-0.1114), G3b (0.07916; IQR_{25-75}: 0.05091-0.1081), G4 (0.07347; IQR_{25-75}: 0.06219-0.08934). **B)** Comparison of TNF-a serum levels with KDIGO groups: G1 (0.5014; IQR_{25-75}: 0.3063-0.5735), G2 (0.7730; IQR_{25-75}: 0.2538-1.205), G3a (0.4798; IQR_{25-75}: 0.3063-0.5627), G3b (0.5288; IQR_{25-75}: 0.2870-0.6606), G4 (0.5425; IQR_{25-75}: 0.3016-0.9706). **C)** Comparison of IL-1b serum levels with KDIGO groups: G1 (0.1569; IQR_{25-75}: 0.05307-0.09183), G2 (0.07107; IQR_{25-75}: 0.05307-0.09183), G3a (0.07142; IQR_{25-75}: 0.05307-0.08284), G3b (0.1082; IQR_{25-75}: 0.05307-0.1187), G4 (0.08337; IQR_{25-75}: 0.05307-0.1369). **D)** Comparison of MCP-1 serum levels with KDIGO groups: G1 (76.74; IQR_{25-75}: 64.73-89.39), G2 (78.90; IQR_{25-75}: 69.11-87.06), G3a (75.07; IQR_{25-75}: 60.88-90.32), G3b (70.66; IQR_{25-75}: 64.53-78.25), G4 (69.71; IQR_{25-75}: 65.93-75.18).
Data are expressed as means and percentiles. Abbreviations: Interleukin-6 (IL-6); Tumor necrosis factor-alpha (TNF-a); Interleukin-1 beta (IL-1b); Monocyte chemoattractant protein 1 (MCP-1); Chronic kidney disease (CKD); Guidelines of Kidney Disease Improving Global Outcomes (KDIGO).

IL-1β, and MCP-1 serum levels were analyzed in the study groups according to the persistence of albuminuria.

The analysis showed differences without statistical significance, in TNF- α levels between persistent albuminuria in urine of 50 mg/L and 100 mg/L (50 & 100 (0.4886 pg/mL and 0.9567 pg/mL p=0.100)). Although these data do not represent a higher significance, they do demonstrate a tendency for increased TNF-α production, according to the persistence of increased albumin in the urine (**Figure 2**). In the rest of the cytokines, no relationship was shown (data not shown). To complete the analysis of the relationship between the levels of proinflammatory cytokines and the clinical markers of CKD, we proceeded to analyze the relationship between the serum concentrations of TNF-α, IL-6, IL-1β, and MCP-1 with the levels of urea, creatinine, and cystatin C. No associations were found (data not shown).

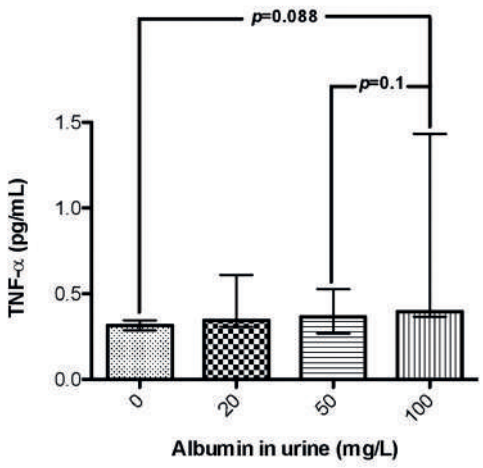

Figure 2. Comparison of the serum TNF-a levels with the persistence of levels of albumin in urine. 20 mg/L (IQR25-75: 2729-4141), 50 mg/L (IQR25-75: 2729-4141), 100 mg/L (IQR25-75: 2729-4141).
Abbreviations: Tumor necrosis factor-alpha (TNF-a).

DISCUSSION

In 2012 a study was reported on the epidemiology of CKD was carried out in some Latin American countries (Argentina, Brazil, Chile, Colombia, Mexico, Uruguay, and Venezuela) which made it clear that there is a wide variation in the incidence of between 2.8 and 15.8 new cases per 1 million inhabitants,[39] however, this result could be influenced by the lack of documentary information regarding the cases in these countries.

In addition to the problems of early detection and follow-up in order to have comparable incidence rates, the high mortality in countries that lack health resources results in a low prevalence of CKD in those places due to the lack of early diagnosis and timely treatment.[40]

These data make it clear that there are differences in the development and treatment process of children with CKD due to geographical, environmental, racial, genetic, and cultural conditions.[40]

CKD in children is a public health problem with scarce studies in Mexico. The Mexican states with the highest number of reports are Aguascalientes, Jalisco, and San Luis Potosí.[41] Furthermore, CKD affects approximately 10% of the western population and is a serious social and economic burden, especially for those who progress to renal failure and thus, require dialysis or transplantation. In this regard, the tissue injury associated with CKD is usually directly or indirectly caused by the immune system.[42]

It is very important to delve further into the disease initiation processes in order to be able to make an early diagnosis in the pediatric population, since in the adult population multiple registries show the incidence, prevalence, and comorbidities associated with CKD, but the data of pediatric CKD remain scarce and unclear [40].

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That is why we consider it important to analyze some variables that have been slightly studied in pediatric patients, such as the participation of the immune system in the process of renal deterioration, thus allowing us to identify the markers that allow us to diagnose the condition on time.

The kidneys are a frequent target of systemic immune and autoimmune disorders, which include systemic autoimmunity and vasculitis, serum sickness related to the immune complex, and complement disorders. The effects produced in the kidneys are related to the filtration process and are dependent on the load of the glomeruli that promotes the deposition of the glomerular immune complex. In addition, immune responses against autoantigens derived from the kidneys can cause autoimmune kidney diseases.[42]

This study shows that the serum levels of IL-6 undergo a decrease in the deterioration in the GFR, unlike TNF-α, IL-1β, and MCP-1. In the same sense, only TNF-α showed no significant positive relationship with urine persistence and a concentration of albumin.

Cytokines and glomerular filtration rate

There are multiple studies in which plasma and serum concentrations of cytokines are reported in patients with different levels of renal damage, with contrasting results found.[43,44] Similarly, the potential association between proinflammatory cytokines and glomerular lesions has been reported.[44] The interest of the present study was to identify the relationship between the serum levels of IL-6, TNF-α, IL-1β, and MCP-1 with GFR in children. We found that serum levels of IL-6 decreased GFR resulted in a deterioration of (p=0.002) in this population specifically.

B. Spoto, D. 2011 *et al.,* reported that plasma levels of IL-6 are elevated in patients with CKD compared to healthy individuals. They also mentioned that this increase is present early in the development of CKD, and that the increase in IL-6 levels does not vary depending on the severity of CKD.[45] This finding is inconsistent with our results since we identified that as the deterioration in the GFR progresses, IL-6 levels are also diminished, which indicates that this cytokine is not stable in the development of the disease until it reaches a chronic state. We consider that this difference with other research is based on the fact that most of the study of the report shows adult patients with a confirmatory diagnosis of CKD, which is not the case in our study because, in addition to being a pediatric population, they are in the process of developing renal impairment, and not from a solid diagnosis of CKD of unknown causes.

In another study, a correlation analysis between mortality, GFR, and molecular marker concentrations, such as s-VCAM, D-dimer, and IL-6 in older adults, was performed. An association was reported between GFR with s-VCAM, dimer D, and IL-6 markers, in addition to mentioning that the levels of these markers modify mortality, which was higher in individuals with a significant reduction of the GFR.[46] Although the age factor significantly influences variation, other studies have reported that there are several reasons for the increase in mortality in people with reduced GFR, including malnutrition, chronic inflammation, increased oxidative stress, vascular and endothelial dysfunction related to uremia, and phosphorus and calcium deregulation.[47]

Soluble levels and persistence of albumin

Inflammation is a part of the complex biological response to vascular tissue lesions, infections, ischemia, and autoimmunity.[48] The exacerbated increase in inflammation in patients with kidney damage is associated with an increase in mortality.[49] The association between renal function and inflammation has been reported in previous investigations,[50] but one of the factors that draw attention is the association between albumin and

some inflammatory markers. It has been reported that cytokines IL-1β, TNF-α, and IL-6 activate the acute phase response in the liver,[51] thus, triggering the production of C-reactive protein, amyloid-A protein, and fibrinogen, which leads to a reduction in the synthesis and degradation of albumin where alterations, such as hypoalbuminemia can arise.[52]

In the present study, a no significant positive association was found between TNF-α levels and urinary albumin levels.

Jayanta Gupta *et al.,* conducted a study of the antagonist receptor levels of IL-1β, IL-6, TNF-α, TGF-β, high sensitivity PCR, plasma fibrinogen, and albumin in 3,939 patients with chronic renal insufficiency. A positive correlation was found between levels of the studied cytokines, mainly IL-6 and TNF-α, and albumin levels, with p= 0.001 obtained,[53] which is consistent with our results for TNF-α, where although a highly significant p was not obtained, an ascending tendency of this cytokine can be observed, as the concentration of albumin increases.[16]

In a study conducted by Sabrina Milan *et al.,* the relationship of IL-1β, IL-6, and high sensitivity CRP with serum albumin levels in patients with peritoneal dialysis due to kidney damage was analyzed. They found that there is no association between the levels of IL-1β, IL-6, and serum albumin levels, however, they did find a positive correlation between the levels of high sensitivity PCR and IL-6 ($p < 0.001$) and a negative correlation value with serum albumin levels ($p = 0.01$).[54] This finding is consistent with our results since the only cytokine that had an association with the studied population was TNF-α.

The results obtained in this study are different from those reported in the literature. This may be because most reports are in the non-open population of adults or older adults, which present multiple comorbidities, unlike children. In the case of pediatric articles related to the topic, it can be observed that most of them are children who already have a diagnosis of CRP, which can assume a divergence in the crossing of data. However, the correlation of these markers with other factors, such as exposure to environmental contamination [metals and pesticides] is associated with the proinflammatory cytokines during the development of renal damage in children,[55] genetic variants,[56] or other pathologies not diagnosed in the population, such as hepatitis E or C, which could modify the immune response.[57]

CONCLUSIONS

The gradual decrease in serum IL-6 levels as the degree of CKD increases demonstrates the particular immunological characteristics that the pediatric population presents with CKD of unknown causes. The association of the increase of TNF-α serum levels with an increase in albumin urinary levels could be an indicator of the role of the proinflammatory immune response in the development of renal impairment. These results make it clear that it is necessary to thoroughly investigate the immunological factors involved in the development of pediatric CKD of unknown cause as well as the correlation of these markers with other factors, such as exposure to environmental pollution [metals and pesticides], genetic variants or other pathologies in the population, which will modify the immune response and the development of the pathology.

This study was conducted after authorization from the Ethics Committee of the Department of Public Health of the University Center for Health Science (in Spanish: Departamento de Salud Pública, Centro Universitario de Ciencias de la Salud) (DCSP/CEI/2016/10/176) and the Investigation Committee of the Centro Universitario de Ciencias Biológicas y Agropecuarias (CINV/162/2018) of the University of Guadalajara. Informed consent was obtained from all individual participants included in the study (parents and children).

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Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper (parents and children).

Data Availability Statement

The path analysis and laboratory data of all patients used to support the findings of this study are available from the corresponding author upon request

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El título deberá ser conciso y de ser posible establecer el objetivo o resultado principal de la investigación.

Es importante mencionar que es posible ser creativo en con el título del trabajo, sin embargo, recomendamos evitar títulos que puedan mal interpretarse como ofensivos o alarmistas.

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SDE: redacción del manuscrito, análisis estadístico, conceptualización y diseño de estudio. ABC: revisión de la literatura y análisis estadístico. CBR: obtención de las muestras y bases de datos. OLP: muestreo, experimentos en laboratorio y análisis de resultados. FHG: validación de resultados, revisión y edición del manuscrito. CRA: administración del proyecto.

Este apartado deberá aparecer antes de las referencias en todos los manuscritos enviados excepto en los casos clínicos o cartas al editor.

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En caso de que exista algún tipo de conflicto de interés (participación de empresas con fines comerciales o financieros) los autores deberán especificar los detalles del mismo. En caso contrario se deberá colocar la frase siguiente:

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5. Tipos de manuscrito

5.1 Artículos originales

Este tipo de manuscritos deberán ser ideas originales y libres de plagio. En caso de que los revisores detecten algún tipo de plagio o contenido no citado o copia textual, serán rechazados para su publicación en el Boletín del Colegio Mexicano de Urología. *Los manuscritos originales no deberán superar las 2500 palabras*, excluyendo tablas, resumen y referencias. El formato deberá ser el siguiente: resumen, introducción, material y métodos, resultados, discusión y conclusiones.

- **Resumen:** el resumen del manuscrito *deberá ser de máximo 250 palabras* divididas en: objetivo, material y métodos, resultados y conclusión. El resumen deberá estar en español e inglés y no debe llevar referencias. Se recomienda no utilizar abreviaturas. Se deberá incluir 3 a 5 palabras clave en español e inglés.

- **Introducción:** La introducción del manuscrito deberá ser concisa y con información sustentada en la bibliografía. Se recomienda que la última frase de la introducción mencione el objetivo principal de la investigación.

- **Material y métodos:** en esta sección se deberá incluir el diseño de estudio, el periodo de tiempo y el lugar donde se realizó el estudio, además del permiso de las autoridades sanitarias o Comité de Ética que autorizó la investigación.

En caso de ensayos clínicos será necesario indicar el número de registro.

Es importante mencionar las variables del estudio (dependientes e independientes) y la forma en que se determinó la muestra.

Se debe incluir el tipo de análisis estadístico utilizado y los programas de cómputo empleados. Ejemplo:

"Los datos fueron procesados con Excel® (Microsoft, Redmond, WA, USA) y EpiInfo version 7.2.4 (Centers for Disease Control and Prevention, Atlanta, GA, USA)"

En esta sección se deberá especificar si fue necesario el uso de consentimiento informado.

En caso de experimentos se podrá hacer un apartado en donde se mencionen los métodos utilizados. Ejemplos:

1. "Urine samples were collected; then, each sample was centrifuged at 2,000 rpm for 5 min. The pellet was washed three times with PBS and resuspended in PBS buffer added to Fc Receptor Blocking Solution® (BioLegend, San Diego, CA, USA). Afterward, the cells were incubated with antihuman TREM-1-PE® (phycoerythrin) (R&D Systems, Minneapolis, MN, USA) for 30 min at room temperature."

2. "Urine samples were transported to the laboratory and were processed for the determination of pesticides with the HPLC/MS/MS (high-performance liquid chromatography coupled with tandem mass spectrometry) method with Agilent Technologies® Model 1200 equipment for HPLC and Model 6430B for MS/MS spectrometry."

Resultados: Esta sección podrá tener subdivisiones. En caso de presentar tablas o figuras, deberán ser mencionadas e incluidas en el texto. Las tablas deben ser usadas como una herramienta para resumir los datos, por lo que se recomienda no describir a detalle las tablas en el texto y solo señalar lo más trascendental de la tabla. Todas las tablas y figuras deberán ser numeradas y contar con título. El número de tablas o figuras está limitado a un máximo de seis.

Discusión: En este apartado es necesario que se cubran los hallazgos clave de su investigación. Se sugiere resaltar datos novedosos y comparar los resultados obtenidos con otras investigaciones.

También sugerimos agregar un párrafo en donde se mencionen las fortalezas y debilidades del estudio. Se invita a los autores a que en este apartado de su trabajo se realice un análisis extenso de los resultados obtenidos para hacer reflexionar a los lectores de manera crítica sobre el tema. Es importante también

cuestionar sobre lo investigado y generar nuevas hipótesis para futuras investigaciones sobre el tema cuando esto sea posible.

Conclusiones: Esta sección deberá contener los comentarios propios de los autores destacando los puntos más relevantes de su trabajo. En esta sección no se deberá incluir referencias.

Referencias: El número de referencias no deberá exceder de 25. Las referencias deberán ser indicadas en superíndice en el texto y en orden numérico. El formato para las referencias es exclusivamente en AMA (American Medical Association). Deberán enlistarse de acuerdo con el orden en el texto. El formato AMA se puede copiar directamente de PubMed y en caso de que la referencia no se encuentre en esta base de datos, existen varias herramientas en línea para dar formato a las referencias. Se invita a los autores a seguir esta regla al pie de la letra ya que la mayor parte de los trabajos no aceptados o devueltos para revisión es por falta de formato adecuado y orden de las referencias.

5.2 Casos clínicos

La presentación de los casos clínicos deberá ser puntual y concisa. Para este tipo de envíos el número de autores no podrá ser mayor a cinco.

Los casos clínicos están limitados a 1000 palabras (excluyendo resumen, tablas, figuras y bibliografía). Las citas bibliográficas deben contar con información actualizada de no más de cuatro años y limitada a cinco referencias.

Resumen: El resumen se debe presentar en español e inglés. Debe presentarse en un solo párrafo sin secciones. El resumen deberá presentar los detalles de la enfermedad que se presentará en el caso clínico y evitar agregar los datos del caso en particular.

Ejemplo:

Los tumores neuroendocrinos primarios o carcinoides del testículo son una entidad rara que se presentan en menos del 1% de las neoplasias testiculares. Clínicamente se caracterizan por ser masas testiculares con o sin dolor. En esta estirpe se debe realizar inmunohistoquímica para llegar al diagnóstico definitivo, siendo principalmente positivos para cromogranina, sinaptofisina y citoqueratina. La orquiectomía radical es el tratamiento de elección.

Las terapias adyuvantes no han mostrado utilidad, aunque la quimioterapia y la radioterapia adyuvante se han utilizado en casos metastásicos.

Introducción: La introducción del manuscrito deberá ser concisa y con información sustentada en la bibliografía. Se recomienda que la última frase de la introducción mencione el objetivo principal de la investigación.

Presentación del caso: En esta sección se presentarán los datos más relevantes de la historia clínica del paciente. Es importante solo agregar los datos que son trascendentes para la presentación del caso y no una historia clínica completa. Los resultados de laboratorio se deben presentar con las unidades de medición de forma adecuada. El pronóstico y los resultados finales del caso deben estar en esta sección y evitar repetirlo en la discusión. En caso de incluir tablas o figuras, el número está limitado a un máximo de tres.

Discusión: Es necesario que en esta sección los autores realicen un análisis de la trascendencia del caso y utilizar la bibliografía como soporte de la información que se presenta. *Los casos clínicos no llevan sección de conclusiones* por lo que los autores podrán incluirlas en el último párrafo de la discusión.

Referencias: El número de referencias no deberá exceder de cinco. Las referencias deberán ser indicadas en superíndice en el texto y en orden numérico. El formato para las referencias es exclusivamente en AMA (American Medical Association). Deberán enlistarse de acuerdo con el orden en el texto.

6. Tablas

El formato de las tablas deberá ser en Word o Excel® (Microsoft, Redmond, WA, USA). Las tablas se deben incluir en el texto con un título y pie de tabla señalando el significado de las abreviaciones o el método estadístico por ejemplo para el cálculo de la p. Ejemplo:

Table 1. Arithmetic mean differences between study variables

Variable	Mean (SD)	*P**
Age in years Female Male	45.7 (14.15) 43.3 49.1	0.003
Height (meters) Female Male	1.63 (0.09) 1.57 (0.06) 1.70 (0.07)	> 0.05
Weight (kg) Female Male	75.5 (15.0) 70.8 (15.3) 81.5 (12.5)	>0.001
BMI Female Male	30.4 (5.1) 32.5 (6.1) 27.6 (3.6)	>0.05
Stone size (cm3) Female Male	3.1 (2.3) 3.1 (2.5) 3.0 (2.4)	>0.05
Essence (HU) Female Male	928 (366) 906 (392) 954 (337)	>0.05

SD: Standard deviation; **BMI:** Body Mass Index; **HU:** Hounsfield Units
*Mann Whitney U test

En caso de que las tablas contengan información obtenida de algún artículo, esta deberá llevar agregada la cita bibliográfica.

El número de tablas o figuras está limitada a seis en artículos originales y a tres en casos clínicos.

7. Figuras

Las figuras o imágenes serán aceptadas en formatos comunes como TIFF, JPEG, PDF y EPS. La resolución de las anteriores debe ser de al menos 1000 pixeles o resolución de 300 dpi o mayor. Cada figura debe ser mencionada en el texto e incluida en el mismo, siempre numerada y con la descripción del contenido. Ejemplo:

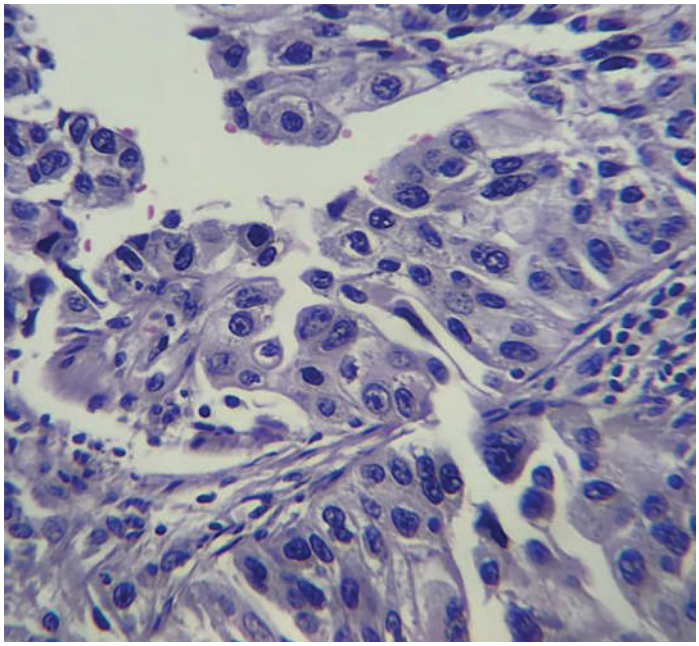

Figure 5. Histological section (40x) H&E. The neoplasm shows epithelial cells showing and areas with eosinophilic cytoplasm and ovoid vesicular nuclei.

El número de tablas o figuras está limitada a seis en artículos originales y a tres en casos clínicos.

8. Formato de las referencias:

Como ya se ha mencionado en apartados previos, las referencias deberán colocarse en el texto en superíndice y en estricto orden numérico. Es importante evitar colocarlas entre paréntesis o corchetes.

La sección de referencias es la parte final del manuscrito y es obligatorio que el orden de las referencias sea de acuerdo con su aparición en el texto. El formato es estrictamente AMA (American Medical Association).

Ejemplos:

Artículos:

1. Murillo-Garzón V, Kypta R. WNT signalling in prostate cancer. Nat Rev Urol. 2017;14(11):683-696. doi:10.1038/nrurol.2017.144

2. Schweizer L, Rizzo CA, Spires TE, et al. The androgen receptor can signal through Wnt/beta-Catenin in prostate cancer cells as an adaptation mechanism to castration levels of androgens. BMC Cell Biol. 2008;9:4. Published 2008 Jan 24. doi:10.1186/1471-2121-9-4

3. Ranasinghe W, Shapiro DD, Zhang M, et al. Optimizing the diagnosis and management of ductal prostate cancer [published online ahead of print, 2021 Apr 6]. Nat Rev Urol. 2021;10.1038/s41585-021-00447-3. doi:10.1038/s41585-021-00447-3 Para citar libros, sitios de internet u otras fuentes de información sugerimos visitar el sitio "AMA Manual of Style 11th Edition":www.amamanualofstyle.com

9. Material suplementario (opcional)

Los autores podrán agregar un apartado de material suplementario en donde se puede ofrecer a los lectores la base de datos de donde se obtuvo la información. Esto puede limitarse debido a las normas y estatutos institucionales.

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