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Comprehensive Evaluation of Cystatin-C, KIM-1, and Uromodulin as Biomarkers for Early Detection of Pediatric Chronic Kidney Disease

Wijdan Rajh Hamza Al-Kraity

University of Kufa, Faculty of Medicine, Iraq.

Corresponding author Email: wijdanr.alkraity@uokufa.edu.iq

ABSTRACT

Background: Chronic kidney disease can present at any age, and the condition varies in severity. New diagnostic approaches for many diseases have been made possible by the research of biochemical markers and associated signaling pathways, which have improved the identification of high-risk patients. **Objectives:** Evaluation of cystatin-C in chronic kidney disease among children in Karbala province. **Methods:** A case-control study was conducted on 50 patients with newly diagnosed chronic kidney disease and 25 healthy controls. All participants had a measurement of Cystatin C and some biochemical markers, including lipid profile (Total cholesterol, Triglyceride, Very-low-density lipoprotein, High-density lipoprotein, and Low-density lipoprotein), bilirubin, urea, creatinine, and uric acid. **Results:** The results revealed significant differences ($P \leq 0.05$) in the levels of cystatin-C in children patients when compared with healthy groups. The results of the present study showed significant differences between CKD patients and the healthy group. Serum KIM-1 levels demonstrated a marked elevation among children with CKD, while Uromodulin levels showed a highly significant reduction compared with healthy controls. There is an increment ($p<0.05$) with high significance in serum bilirubin, urea, uric acid, and creatinine in children with CKD group compared with the healthy group**.** Significant increment ($p<0.05$) in serum levels of "TC, TG, VLDL-C, and LDL-C", while a significant decrease ($P<0.05$) in serum level of "HDL-C" in CKD patients in comparison with the healthy group. **Conclusion:** This research suggests that the Cystatin C, KIM-1, and Uromodulin tests provide more value than other indicators for assessing kidney function, and that high levels of Cystatin C and KIM-1, combined with low levels of Uromodulin, may be associated with renal failure in children.

Keywords: Cystatin-C, KIM-1, and Uromodulin, Kidney failure, CKD, Pediatrics.

Article Information

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INTRODUCTION

"Chronic kidney disease" (CKD) is a chronic disorder that causes irreversible damage to the kidneys. Reduced kidney function deteriorates with time. CKD is often referred to as chronic renal failure. People with CKD might be of any age or race, and their condition varies in severity. [1-3]. The most prevalent causes of CKD in infants and children are birth defects, congenital abnormalities, and inherited disorders such as polycystic kidney disease. Children with recurrent UTIs should receive quick medical attention and further testing because urinary tract abnormalities may eventually result in chronic kidney disease (CKD) [4]. The term CKD describes the progressive decrease of kidney function brought on by illnesses that impair kidney function over time [5].

When kidneys are damaged and unable to filter blood as well as they should, it is known as chronic kidney disease (CKD). The body stores excess fluid and blood waste as a consequence, which can cause several health issues like heart disease and stroke [6]. Kidneys and pediatric chronic renal disease are closely related to each other and to a child's

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general health. They produce urine and remove waste and water from the blood. In addition, kidneys produce red blood cells and hormones that regulate blood pressure in addition to balancing the salts and minerals in the blood. Kidney failure and other health problems can result from improper kidney function [7,9]. Immunologic responses are caused via immune cells or immune complexes, ischemia, or tissue hypoxia. The main causes of kidney injury include endogenous substances like glucose and paraproteins, external chemicals such as medications, and genetic disorders. Regardless of the underlying cause, tubulointerstitial fibrosis and glomerulosclerosis are common in CKD, In a discussion of the pathophysiology of CKD, mechanisms of glomerular, tubular, and vascular damage should get special consideration [8]. A previous Kidney function research was unclear or needs to be confirmed. In this case, the cystatin C test may be administered by your healthcare professional to determine the presence of renal disease. You are aged, overweight, or extremely muscular (like a bodybuilder). Estimates of GFR based on cystatin C are thought to be less affected by food and muscle mass than estimates based on creatinine.

Cystatin test measures the level of cystatin C protein in blood. The body makes cystatin C regularly, and that protein is also found in different fluids, involve, breast milk and spinal fluid, the healthy kidneys can filter cystatin C out of the blood and excrete it in the urine [10,11, 12]. The results of the test might change based on your age, gender, medical history, and other factors. An excessively high level of cystatin C in your blood might indicate that you have chronic renal disease or another kidney illness [13]. Additionally, diabetes, cancer, HIV, hyperthyroidism, or hypothyroidism might result in higher levels of cystatin C [14].

KIM 1 acts as a biomarker of kidney injury and to a certain extent, especially during early acute kidney injury, can act as a protective factor promoting the regeneration and repair of cells. However, the continuous presence of high levels of KIM -1 maintains a pro-inflammatory environment, thus enhancing the progression of renal fibrosis as well as tubular apoptosis [15]. With regards to early onset of nephrotoxicity caused by a particular treatment agent, a vast amount of data supports the utilization of KIM perceptions and contends it is a better predictor of the cisplatin-induced acute kidney injury, and it does increase substantially before the creatinine levels start to change [15,16].

the previous studies of [16], the expression of tissue KIM -1 was stated to be superior, compared to the conventional histological analysis, in the detection of early tubular damage and has a correlation with the extent of renal dysfunction. High tissue KIM1 levels are also linked to better clinical results and can serve as an early biomarker that shows recovery in patients that receive kidney transplants [17].

One of the potential biomarkers, that is known Uromodlin (UMOD) is a glycoprotein that is produced in the tubular lining of the thick ascending limb of the loop of Henle and this turns out to be one of the possible biomarkers that can be used to assess the renal functioning in cases of chronic kidney disease. [18]. Most of the Umod produced is released in the urine which in normal persons would conceive the major protein. The effects of Uromodlin are anti-inflammatory, and it is involved in preventing renal calculi and the immunomodulatory role [19]. Some percentage of the uromodlin leaks into the blood as serum uromodulin . As serum Umod is suggested to be indicative of tubular integrity and by proxy nephron mass and reservation, it is becoming an attractive biomarker of renal functioning [19,20].

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METHODOLOGY

Study design and setting:

This is a case-control study in which, for the "chronic kidney disease" study, and the cystatin-C level study, "Peripheral blood samples" were obtained from 50 patients with newly diagnosed "chronic kidney disease" and 25 controls as healthy.

Sample size and sample procedure:

In this current study, fifty of the Children's patients (females and males) suffer from CKD, and twenty-five of the control group. From December 2023 to March 2024, samples were gathered from "Imam Hussain Medical City's Specialized Center for Diseases and Kidney Transplant", "Karbala Teaching Hospital for Children", and "Al-Kafeel Super Specialty Hospital" in Karbala, Iraq.

Patients and controls varied in age from 1 to 12 years old. Subgroups based on gender, age, and BMI subjects were created for each patient group. Patients without a full medical record or those with other health problems were not included.

Inclusion criteria

The patients were diagnosed by a physician based on history, typical kidney pain clinical presentation, angiography, and kidney disease investigation. All research that involved participants had informed consent acquired, and people who were enrolled were informed about the nature of the clinical trial.

Blood sample collection

The Medical staff or Trained phlebotomists can draw blood samples from blood vessels " veins, capillaries, and occasionally arteries". A needle is used to pierce the sample, which is then suctioned via the needle and placed into a designated collecting tube. After a 12-hour fast, 5 ml of venous blood was drawn via antecubital venipuncture using needles drained from CKD and also control participants between 8:30 and 10 AM. In a standard test-8 tube, the blood was allowed to coagulate at room temperature. After centrifugation for 10 minutes at 3000 rpm, the serum was suctioned, split into aliquots in epindroff tubes, and kept at -20°c.

Investigations by specific kits

Cystatin C Determination

The Sandwich-ELISA concept is employed by ELISA kits. To the micro ELISA plate wells, samples (or standards) and a biotinylated detection antibody specific for human Cys-C are applied.

KIM-1 Determination

KIM-1: Measured using a quantitative sandwich ELISA kit validated for human serum, following the manufacturer's instructions.

Uromodulin Determination

Uromodulin: Quantified using a commercially available ELISA kit sensitive to low circulating concentrations in human serum.

Creatinine Determination

Jaffé described a technique for measuring creatinine in biological fluids in 1886. Even though several techniques have since been developed, Jaffé's method remains the most commonly utilized technique today.

Uric-Acid Determination

Hydrogen peroxide and allantoin are produced from uric acid by uricase. A red quinoneimine dye complex is produced when hydrogen peroxide further reacts with a phenolic component and four aminoantipyrines via the catalytic activity of peroxidase. The color that develops and its intensity are closely correlated with the quantity of uric acid in the sample.

Lipid Profile Determination

Total cholesterol (TC): A specific kit measuring the level of human serum TC.

High-density lipoprotein (HDL): A specific kit measuring the level of human serum HDL

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Triglycerides (TG): A specific kit measuring the level of human serum TG.

Very low-density lipoprotein cholesterol (VLDL): The Very low-density lipoprotein was evaluated by the formula: **"VLDL-C = TG (mmol/l) /5"**

Low-density lipoprotein (LDL)

The "Low-density lipoprotein" was evaluated by the formula: "**LDL((mmol/l))=TC(mmol/l)-VLDL(mmol/l)-HDL(mmol/l)"**

Statistical analysis

The present study data are presented in the form of mean ± standard error and descriptive statistics and P-values have been calculated using graphpad prism. A t -test was used in conducting intergroup comparisons between two groups and one-way ANOVA was used in conducting comparisons among several subgroups. Statistical significance was taken as P 0.05.

RESULTS

Serum Cystatin-C levels

The findings of our research exposed a highly significant difference in levels of Cystatin-C in children's patients when we compared them with the healthy subjects (1.720± 1.71, and 0.778 ±0.03) respectively, Figure (1).

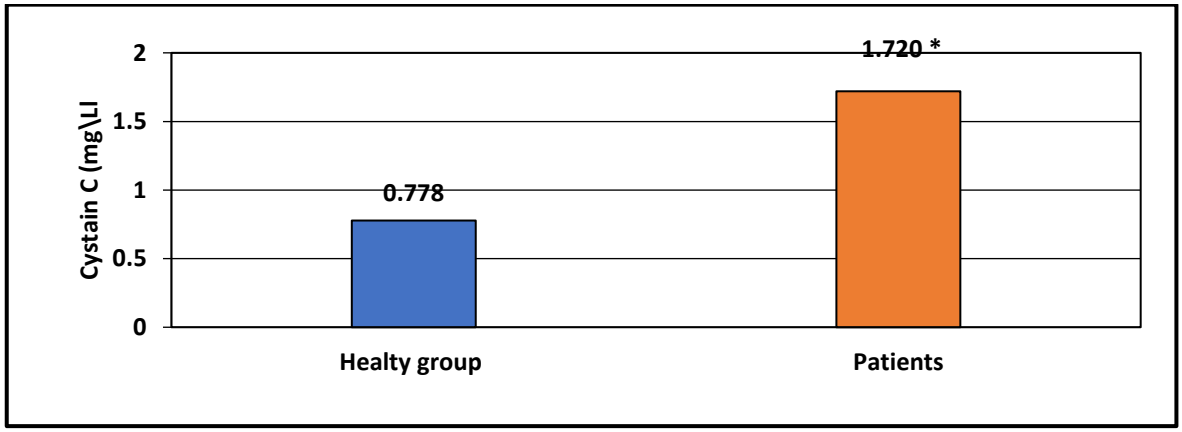


Figure (1): The serum Cystatin C levels in Children with CKD compared with the HT group.

Serum KIM-1 levels

The findings of our research exposed a highly significant difference in levels of KIM-1 in children's patients when we compared them with the healthy subjects (3.561± 0.189, and 1.345 ±0.09) respectively, Figure (2).

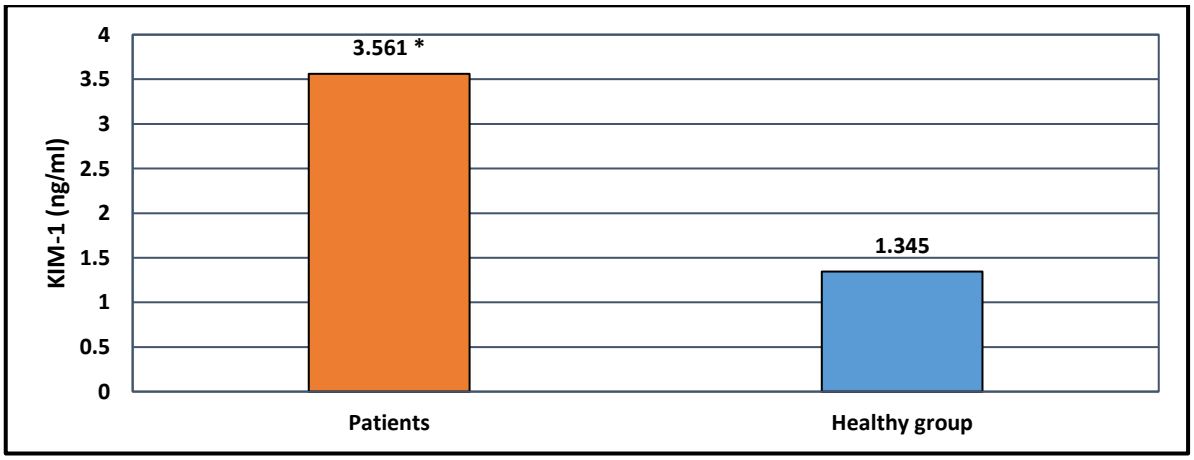


Figure (2): The serum KIM-1 levels in Children with CKD compared with the HT group.

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Serum Uromodulin levels

The findings of our research exposed a highly significant difference in levels of Uromodulin in children's patients when we compared them with the healthy subjects (17.983± 1.06, and 44.762 ±2.06) respectively, Figure (3).

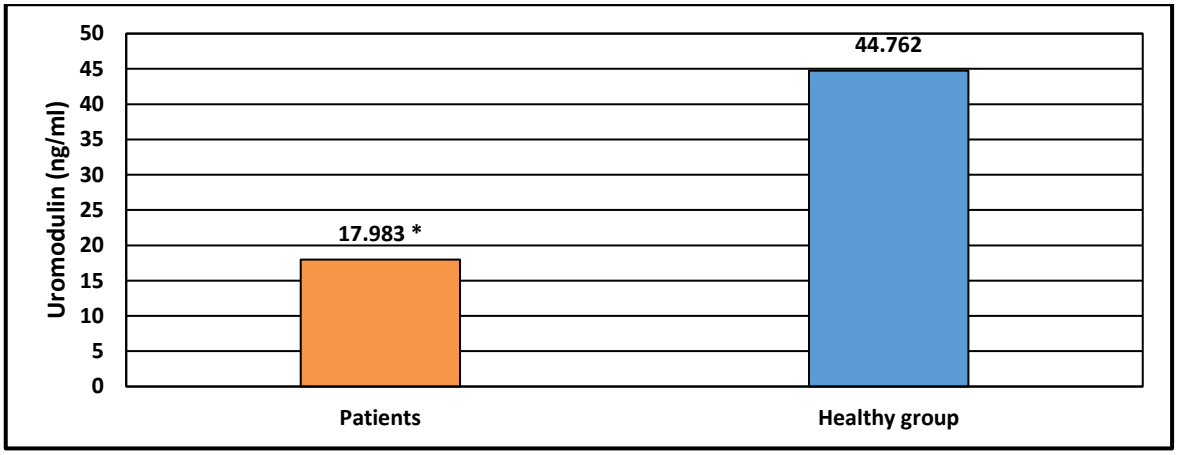


Figure (3): The serum Uromodulin levels in Children with CKD compared with the HT group.

Serum Kidney Function Markers

This study revealed a highly significant increase (p<0.05) in "Bilirubin (3.778± 0.665), Urea (34.771± 1.737), Uric acid (4.328± 0.236), and Creatinine (0.974± 0.110) " levels in the children in the CKD group compared with those in the HT group "Bilirubin (0.774 ± 0.023), Urea (4.20± 0.264), Uric acid (6.740 ± 0.192) and Creatinine (5.426 ± 0.092) Figure (4).

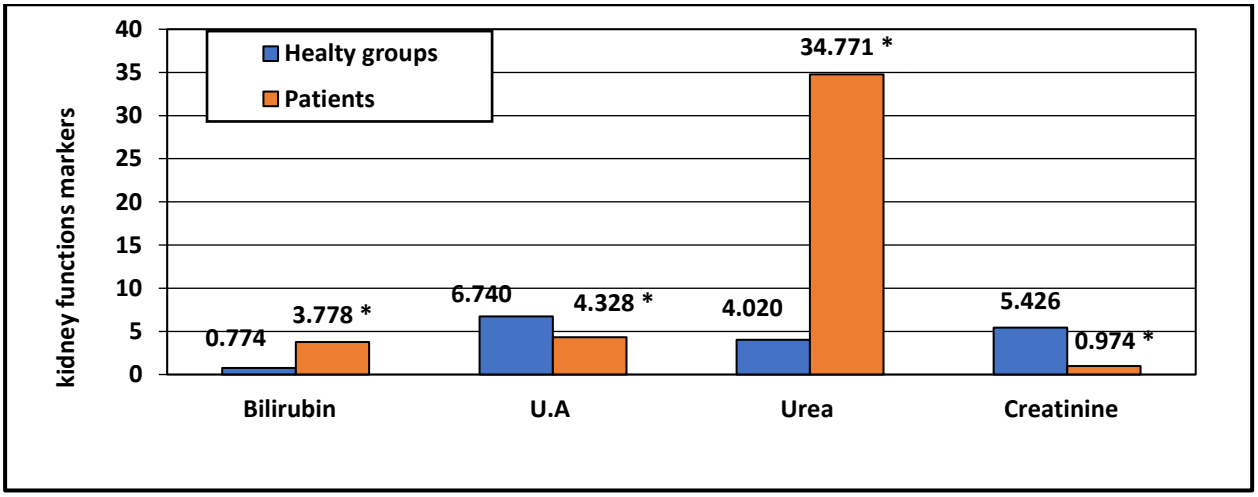


Figure (4): The levels of Kidney function markers of Children with CKD compared with the HT group.

Serum lipid profile level

The findings of this research exposed a highly significant differences in levels of lipid profile between the Children with CKD, and the healthy subjects, a highly significant increase (p<0.05) in "TC (290.403 ± 2.441), TG (181.021 ± 3.687), VLDL-C(36.204 ± 0.737), and LDL-C (223.200 ± 0.813)" levels, but a highly significant decline (P<0.05) in "HDL-C(30.999 ± 0.891)" level in CKD patients in comparison with the healthy subjects "TC (186.906 ± 0.523), TG (65.587 ± 0.271), VLDL-C(13.117 ± 0.054), and LDL-C (119.879 ±0.185)", "HDL-C (53.91 ± 0.654) ", Figure (3.778± 0.665), TG (3.778± 0.665), VLDL-C(3.778± 0.665), and LDL-C (3.778± 0.665)", Figure (5).

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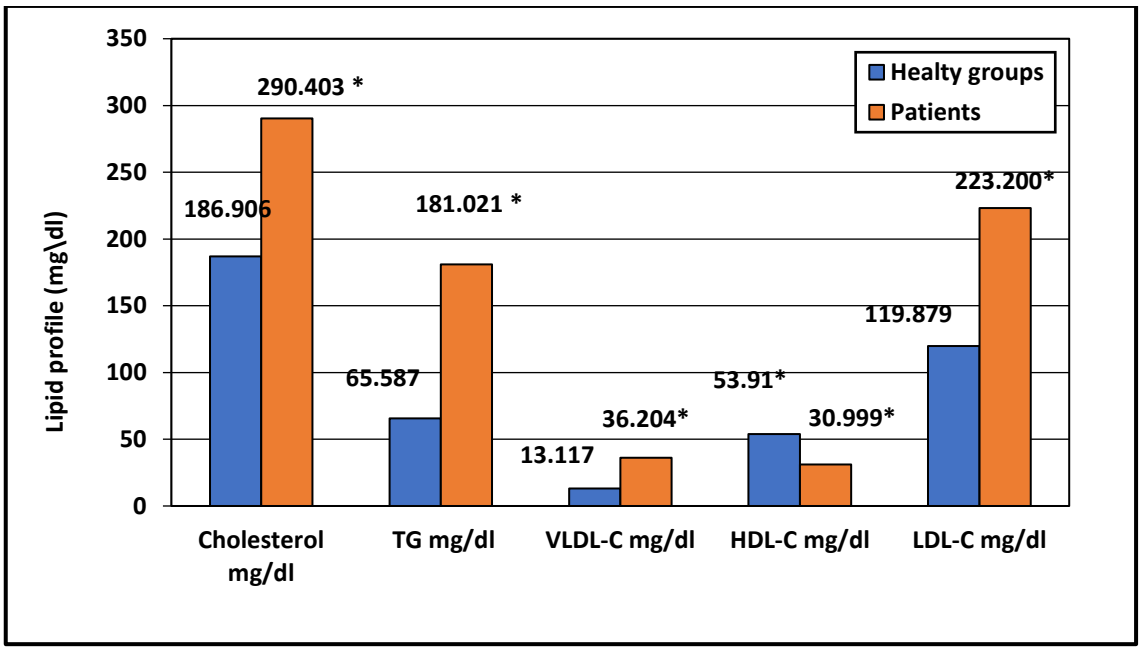


Figure (5): Levels of serum lipid profile of Children with CKD compared with the healthy group.

Table (1): Matrix correlation (R) among biochemical markers Cystatin-C, KIM-1, and Uromodulin, and with kidney function tests and lipid profile.

Results of the correlation coefficient among biomarker concentrations in pediatric chronic kidney disease patients are revealed in Table (1):

There is a positive association between Cystatin C and KIM-1, and these biomarkers revealed a positive association with Bilirubin, Urea, VLDL, LDL, CH, and TG, there is a negative association between Cystatin C and KIM-1 with Uromodulin, Uric acid, Creatinine, and HDL. While the Uromodulin revealed a negative association with Bilirubin, Urea, VLDL, LDL, CH, and TG, and a positive association with Uric acid, Creatinine, and HDL.

Table (1): The Correlation among biomarkers in pediatric chronic kidney disease.

Matrix correlation (R)	Cystatin C mg/l	KIM-1 (ng/ml)	Uromodulin (ng/ml)
Cystatin C mg/l	1.000		
KIM-1 (ng/ml)	.774	1.000	
Uromodulin (ng/ml	-.832	-.650	1.000
Bilirubin (mg/dl)	.710	.845	-.673
Uric acid (mg/dl)	-.878	-.834	.783
Urea (mg/dl)	.712	.853	-.721
Creatinine (mg/dl)	-.804	-.679	.872
VLDL (mg/dl)	.848	.824	-.878
HDL (mg/dl)	-.840	-.871	.746
LDL (mg/dl)	.872	.758	-.837
Ch (mg/dl)	.534	.501	-.588
TG (mg/dl)	.534	.540	-.502

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DISCUSSION

The associated comorbidities of CKD, such as diabetes or cardiovascular disease, maybe the cause of the rise in the prevalence of CKD among the elderly. The results of our study show a strong negative relationship between serum creatinine and chronic kidney disease (CKD), which is statistically significant ($p < 0.05$). According to Randers [21], this study indicates that creatinine and cystatin C are both reliable indicators of renal function and that they have a strong correlation with chronic kidney disease. Nevertheless, as cystatin C was elevated in all instances [22], it was determined that these levels were a more reliable diagnostic.

The current study highlights clear changes in biomarkers in children with CKD. Elevated serum KIM-1 indicates ongoing proximal tubular injury, reflecting active damage in the nephrons. [23], At the same time, the significant reduction in serum Uromodulin points to a loss of tubular epithelial mass, suggesting early nephron loss [24]. The opposing trends of these biomarkers provide complementary information; KIM-1 serves as a marker of acute tubular stress or injury, while Uromodulin reflects the structural integrity and functional capacity of the tubules. Measuring both biomarkers together can therefore improve early detection and monitoring of CKD progression in pediatric patients.

The results of the current investigation showed that CKD patients had significantly higher blood levels of bilirubin, creatinine, urea, and uric acid ($p<0.05$) than the healthy group. According to research by [25], individuals with chronic kidney disease (CKD) have higher blood levels of urea and creatinine, which can lead to many additional serious disorders. Creatinine and urea are good markers for kidney function, and a rise in these chemicals in the serum is indicative of renal dysfunction., The quantity of bilirubin in the blood rises. Therefore, the kidneys only filter the direct or conjugate form of bilirubin, which is then discharged into the urine (urine bilirubin). Consequently, the research conducted by [26] concludes that a malfunction in the "glomerular filtration rate GFR" is a source of the extreme increase in the serum level of cystatin C As a result, there is an imbalance in the latter stages of bilirubin excretion in urine, which causes the proportion of bilirubin in children's blood to rise.

In comparison to the healthy group, the current study found that patients with chronic kidney disease had significantly higher serum concentrations of "TC, T.G., LDL, and VLDL" ($p<0.05$) and lower serum concentrations of "HDL" ($p>0.05$). Renal dysfunction is also linked to numerous disruptions in lipoprotein metabolism that result in dyslipidemia and the build-up of atherogenic particles [27]. A previous study by [28]indicated that children with chronic kidney disease (CKD) are linked to dyslipidemia, which is defined as hypertriglyceridemia, raised "LDL cholesterol", a buildup of "lipoprotein-containing apolipoprotein B (Apo B)" particles, increased levels of "lipoprotein(a) particles, and low HDL". Because of a lecithin-cholesterol acyltransferase (LCAT) deficit, HDL metabolism is compromised in CKD, and HDL-3 does not mature into HDL-2 [29], There is strong evidence that excess LDL builds up in CKD, particularly in HD patients. Children who have severe CKD, monocytosis, and monocyte activation caused by a loss in renal function represent an additional risk factor for accelerated atherosclerosis.

Kidney functional markers and lipid profiles correlated with Cystatin-C, KIM-1 and Uromodlin with significant rates of correlation. There were strong positive and negative correlations between Cystatin-C and KIM-1 ($r = 0.774$) and between Uromodulin and Cystatin-C ($r = -0.832$) and KIM-1 ($r = -$

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0.650) [23,24]. Cystatin-C and KIM-1 also had a significant correlation with the markers of the kidney functions and lipid parameters, and Uromodulin had the opposite associations [30-33]. These results indicate that a combination of these biomarkers can be used to complement each other to determine kidney functioning in pediatric CKD.

Study limitations

Restricted capacity to follow up with patients for an extended duration might account for these discrepancies.

CONCLUSION

This research suggests that the Cystatin C, KIM-1, and Uromodulin tests provide more value than other indicators for assessing kidney function, and that high levels of Cystatin C and KIM-1, combined with low levels of Uromodulin, may be associated with renal failure in children.

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