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Original Article

Cardiovascular Complications and Indoxyl Sulfate Are Related to Longer Duration of End Stage Renal Disease in Children

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Abstract:
Background: Indoxyl sulfate (IS) is a non-dialyzable gut-derived uremic toxin that is reported to be cardiotoxic in patients with advanced chronic kidney disease stages.
Aim of the Work: The aim of this study was to investigate the role of IS as a risk factor for cardiovascular complications in children with end stage kidney disease (ESKD) on regular HD.
Patients and Methods: This is a cross-sectional analytical study that included children with ESKD on regular hemodialysis (HD) for at least 6 months following at Nephrology Unit of Cairo University Pediatric Hospitals. Serum IS level was measured for all patients by the enzyme-linked immunosorbent assay (ELISA). Cardiac complications was assessed using the M mode and 2D transthoracic echocardiography.
Results: The study comprised 88 children with ESKD on regular HD for a mean ± SD of 31.94 ± 26.05 months, with a mean age ± SD of 9 ± 3.2 years (range 3.3- 14 years). Of them 52 (59.1%) were males. Obstructive uropathy (28.4%), and focal segmental glomerulosclerosis (20.5%), were the main causes of ESKD in the study group. Cardiovascular complications were identified in 48 (54.5%) patients in the form of dilated cardiomyopathy in 44 (50%) children with decreased fractional shortening <30% and moderate to severe left ventricular hypertrophy above 95th for age and gender in 10 (11.4%). Cardiovascular affection correlated with duration of HD, hypertension, and IS serum level (p=<0.001 for each). Hypertension was reported in 55 (62.5%) of patients, and vascular access related complications were evident in 40 (45.4%) patients with thrombosis being the commonest complication in 16 (18.1%). The mean IS was 29.14 ± 17.43 µg/ml in ESKD patients with normal cardiac function, and 77 ± 15.18 µg/ml among those with cardiac compromise (p < 0.001). The IS level correlated with longer duration of HD (p= 0.002), and older age (p= 0.043). IS level and duration of HD did not predict cardiomyopathy, (p=0.192), and (p=0.760) respectively.
Conclusion: Cardiac complications are common among children on HD. Both cardiovascular complications and IS accumulation correlated positively with longer duration of HD, and age of children with ESKD. IS is non-dialysable and there is a need to control its production from the gut.

Level of Evidence of Study: IV (*1*)**.**
Keywords: end stage kidney disease; ESKD; hemodialysis; cardiovascular complications; uremic toxins; indoxyl sulfate.
Abbreviations: CVD: cardiovascular disease; EF: ejection fraction; ESKD: end-stage kidney disease; FS: fraction of shortening; HD: hemodialysis; HDL: high density lipoproteins; IS: indoxyl sulfate; LVH: left ventricular hypertrophy; RRT: renal replacement therapy.

Introduction

Children with end stage kidney disease (ESKD) on renal replacement therapy (RRT) are prone to complications. The cardiovascular (CVD) complications in ESKD range from heart

failure, arrhythmias, infective endocarditis, coronary artery disease and myocardial infarction. CVD complications may be a cause of death among children with ESKD (*2*). In ESKD the risk factors for CVD complications, are multiple as the chronic anemia, volume overload, oxidative stress, inflammation, accumulation of uremic toxins, mineral bone disease, hyperphosphatemia, hypoalbuminemia, hyperparathyroidism, hypertension, dyslipidemia, diabetes mellitus, obesity, and the modality of RRT (*3*). Prompt identification and management of the risk factors may help reduce the CVD in ESKD complications (*4*). Patients with ESKD accumulate toxic solutes as indoxyl sulfate (IS) which has a low-molecular-weight, and more than 90% of it is bound to plasma proteins. It is one of the gut-derived uremic toxins from the breakdown of tryptophan by colon microbes. It is cleared effectively by tubular secretion by the kidneys, but not hemodialysis (HD). Clearance by HD is limited, as HD clears only the free, unbound solute which can diffuse across the membrane (*5*), thus the dialytic clearance is limited to the free non-protein bound amount of IS (*6*). IS has been reported to augment CVD and renal disease progression (*4*). The aim of this study was to investigate the role of IS as a risk factor for cardiovascular complications in children with ESRD on regular HD.

Subjects and Methods

This is a cross-sectional analytical study at Pediatric Nephrology Unit, Cairo University Children Hospital over a period of 6 months. The research was approved by the Faculty of Medicine, Cairo University Health Ethics Review Board (IRB Approval Number: MS 283-2020). The study conforms with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans (*7*).

Participants

The study included 88 children less than 14 years with ESKD on regular HD for at least 6 months. Children with congenital heart disease, congenital thrombophilia, and autoimmune diseases affecting blood vessels as vasculitis were excluded from the study.

Methods

Demographic data were collected as well as the data related to clinical examination, lab, imaging, management and HD. Cardiovascular assessment was done using the M mode and 2 dimensional transthoracic echocardiography (LOGIQ P7, GE healthcare, echocardiography machine, South Korea), to measure the systolic and diastolic cardiac functions in the form of fraction of shortening (FS), ejection fraction (EF), and left ventricular wall diameter. Serum level of Indoxyl sulfate was sampled post dialysis for the second session in the week, done using Enzyme Linked Immunosorbent Assay (ELISA) measured in µg/ml (Catalogue number: EA0136Hu; BioAssay Technology, China). The accepted mean ± SD level among healthy subjects is known to be 0.6 ± 0.4 µg/ml (*8*).

Statistical Analysis

Analysis was performed using SPSS version 24 (IBM Inc., Chicago, USA). Results were summarized in tables and graphs. Categorical data was expressed as frequencies (numbers and percentage) and numerical data was expressed as mean ± standard deviation for normally distributed data. Chi-square test was used for comparison of frequencies of categorical variables. The Fisher exact test was used for comparison when cell frequency of five or less. Student t test and analysis of variance (ANOVA) were used to compare between two or more than two means respectively in data with normal distribution and Mann Whitney U. The correlation was calculated using Pearson and Spearman correlation coefficient when appropriate. P value < 0.05 was considered statistically significant. Multiple linear logistic regression analysis were used to suggest causality between study variables.

Results

Demographics and clinical characteristics of the studied group:

The included 88 children with ESKD received hemodialysis (HD) on regular basis (3 sessions per week, 3-4 hours each session) at Pediatric Nephrology Unit, Cairo University Children Hospital. The ages of the studied patients ranged from 3.30 to 14 years and the mean age ± standard deviation (SD) was 9 ± 3.22 years. Of them 52 (59.1%) were boys, and 36 (40.9%) were girls. Thirteen (14.8%) had consanguineous parents. The main causes of ESKD were obstructive uropathy in 25 (28.4%), focal segmental glomerulosclerosis in 18 (20.5%), primary hyperoxaluria

in 7 (8%), atypical hemolytic uremic syndrome in 4 (4.5%), and steroid resistant nephrotic syndrome in 4 (4.5%). There were 44 (50%) patients on HD using high-flux filters and 44 (50%) on HD using low-flux filters. HD duration lasted a mean of 31.94 ± 26.05 (3-129) months in our studied cohort. The mean ± SD of IS among the studied cohort was 37.66 ± 17.94µg/ml. The level of IS increased significantly as HD duration prolonged (r = 0.322, p = 0.002) and as the patient's age advanced (r = 0.216, P = 0.043). (Table 1). In this study, we found no difference in serum levels of IS among patients utilizing different dialysis filters (p = 0.66). (Table 2 and 3).

Table 1. Demographic data of the studied children with ESKD.

		Number (total=88)	%
Gender	male	52	59.1
	female	36	40.9

	Mean	Standard Deviation	Median	Minimum	Maximum
Age(years)	9.05	3.22	9.45	3.30	14.00
Weight Z score	-2.16	1.29	-2.00	-4.00	2.00
BMI (kg/m²)	14.04	2.50	13.80	9.20	23.70
Duration of hemodialysis (month)	31.94	26.05	26.50	3.00	129.00
Human indoxyl sulphate (µg/ml)	37.66	17.94	36.55	1.90	116.10

		Frequency	%
Primary disease	Obstructive uropathy	25	28.4
	Focal segmental glomerulonephritis	18	20.5
	Hyperoxaluria	7	8.0
	Cystinosis	3	3.4
	Atypical hemolytic-uremic syndrome	4	4.5
	Steroid resistance nephrotic syndrome	4	4.5
Unknown		27	30.7
Consanguinity	Positive	13	14.8
	Negative	75	85.2
Family History of cardiovascular disease	Positive	7	8.0
	Negative	81	92.0
Hypertension	Yes	55	62.5
	No	33	37.5

BMI: body mass index; CVD: cardiovascular disease; ESKD: End stage kidney disease. HD: hemodialysis; IS: indoxyl sulfate.

In this study, cardiovascular affection was identified in 48 (54.5%) patients in the form of dilated cardiomyopathy with fractional shortening (FS) below 30% in 10 (11.4%) patients or moderate to severe left ventricular hypertrophy with LVM/height greater than 95th percentile for sex and age in 45 (51.1%) patients. The patients had an average fraction shortening of 37.32 ± 5.47% and ejection fraction of 67.32± 5.63%. The mean serum concentration of IS was significantly higher in ESKD patients with heart problems measuring 77 ± 15.18 µg/ml (range 1.90 to 88.20), while the mean value was 29.14 ± 17.43 (range 6.80 to 116.10) µg/ml in patients with normal cardiac function (p < 0.001). (Table 2). Hypertension was documented in 55 (62.5%) patients (28 males and 27 females), their ages ranged from 3.6 to 14 years with a mean of 9.46±3.28 years. The serum concentration of IS was significantly higher in hypertensive ESKD patients measuring 41.84 ± 15.72 µg/ml, while its mean was 30.71 ± 19.46 µg/ml in normotensive patients (p< 0.001).

Central venous catheters were the most prevalent type of vascular access (53.4%) among our studied cases, followed by the arteriovenous fistula in 40 patients (45.5%), and only one patient with arteriovenous graft. Vascular access related complications were evident in 40 (45.45%) patients (21 males and 19 females), thrombosis was the most prevalent complication, found in 16 (18.2%) patients, followed by vascular access malfunction in 14 (15.9%) patients, and catheter related blood stream infection in 9 (10.2%) cases. (Table 4). Complications related to vascular access correlated positively with longer duration on HD (p=004), and type of dialysis filter (p=0.047), Patients on low flux dialysis filters showed much more complications of vascular access than those on high flux filters (p=0.032). (Table 4).

Table 2. Characteristics of ESKD patients with cardiac complications versus those without.

		Cardiac affection (n= 88)				P value
		Yes (n =48)		No (n =40)		
		Count	%	Count	%	
Gender	Male	28	58.3%	24	60.0%	0.874
	Female	20	41.7%	16	40.0%	
Age (years)		9.69 ± 3.18		8.28 ± 3.15		0.053
Family History of cardiovascular disease		4	8.3%	3	7.5%	1
Duration of hemodialysis (months)		40.73 ± 27.35		21.40 ± 20		< 0.001
Echocardiographic findings	FS (%)	36.44 ± 5.57		38.37 ±5.21		0.112
	EF (%)	65.35 ± 4.67		69.68 ±5.83		0.001
	LVH	44	91.7%	1	2.5%	<0.001
	Cardiomyopathy	10	20.8%	0	0.0%	0.002
Type of dialysis filter	Low-flux	22	45.8%	22	55.0%	0.584
	High-flux	26	54.2%	18	45.0%	
Hypertension	Yes	43	89.6%	12	30.0%	< 0.001
	No	5	10.4%	28	70.0%	
Laboratory investigations						
Hemoglobin (g/dl)		10.3 ± 1.46		10.11 ± 1.54		0.83
Glomerular Filtration Rate (ml/min/1.73m²)		8.9 ± 2.2		9.1 ± 2		0.66
Serum calcium (mg/dl)		9.5 ± 0.8		9.45 ±1		0.85
Serum albumin (g/dl)		4.07±0.51		4.07±0.39		0.933
Serum phosphorus (mg/dl)		6.58 ± 1.9		5.9 ± 1.36		0.15
parathyroid hormone (pg/ml)		421.5 ± 175.9		411.48 ± 152.5		0.79
Human indoxyl sulfate (µg/ml)		44.77 ± 15.2		29.14 ± 17.4		< 0.001
Lipid Profile						
Cholesterol (mg/dl)		177.88 ± 39.59		188.8 ± 36.9		0.19
Triglyceride (mg/dl)		160.48 ± 163.7		135.2 ± 58.7		0.53
High-density lipoproteins (mg/dl)		39.54 ± 11.9		45.2 ± 11.35		0.008
Low-density lipoproteins (mg/dl)		105.1 ± 30.1		114.07 ± 33.9		0.29

ESKD: end stage kidney disease; FS: fraction of shortening; EF: ejection fraction; LVH: left ventricular hypertrophy.

Table 3. Indoxyl Sulfate among the studied group of children with ESKD

				IS (µg/ml)	P
		Number	%		
Gender	Male	52	59	35.05±16.4	0.1
	Female	36	40.9	41.43±19.47	
Cardiomyopathy	Yes	10	11.4	45.57±11.48	0.049
	No	78	88.6	36.65±18.41	
Left ventricle hyperterophy	Yes	45	51.1	46.18±14.01	<0.001
	No	43	48.9	28.75±17.38	
Hypertension	Yes	55	62.5	41.84±15.72	<0.001
	No	33	37.5	30.71±19.46	
Low flux dialysis filter		44	50	36.33±11.9	0.657
High flux dialysis filter		44	50	39.48±21.88	

A positive correlation was found between serum IS levels and age of patient, duration of HD, and HDL levels (Figure1), while no correlation was found between serum IS level and serum albumin (r=-0.037, p=0.73), nor with creatinine levels (r =0.14, p= 0.19).

Table 4. Characteristics of ESKD patients with complicated vascular access.

Complicated Vascular Access	Number (n=88)		P value
	Yes (n =40)	No (n = 48)	
Age (years)	9.6 ±3.28	8.59 ± 3.14	0.14
Duration of hemodialysis (months)	39.7 ± 24.9	25.5 ± 25.5	0.004
Type of dialysis filter			
High-flux	15 (37.5%)	29 (60.4%)	0.032
Low-flux	25 (62.5%)	19 (39.6%)	
Efficacy of dialysis (Kt/V)	1.4 ± 0.11	1.4±0.12	0.78

Kt/V: urea clearance(K)*dialysis time(t)/volume of water in body (V)in ml/min

Serum IS level of our patients was predictive of LVH (AUC 0.87, sensitivity 93.3%, specificity 72.1%) and cardiomyopathy (AUC 0.701, sensitivity 70%, specificity 59%); while there was no correlation found between serum creatinine level and LVH (AUC 0.563, Sensitivity 82.2%, specificity 39.5%), nor between serum creatinine level and cardiomyopathy (AUC is 0.588, sensitivity 70%, specificity 46.2%). Serum IS positively correlated with age of patients (p= 0.043), duration of disease on HD (p= 0.002), hypertension (p<0.001), and HDL levels (p=0.014), while no correlation was found between serum IS level and BMI (r=0.139, p=0.98), serum albumin (r=-0.037, p=0.73), nor with creatinine levels (r =0.14, p= 0.19). (Table 4).

Also, serum IS level of our patients could predict positively LVH (AUC 0.87, sensitivity 93.3%, specificity 72.1%), and cardiomyopathy (AUC 0.701, sensitivity 70%, specificity 59%); while there was no correlation found between serum creatinine level and LVH (AUC 0.563, sensitivity 82.2%, specificity 39.5%), or between serum creatinine level and cardiomyopathy (AUC is 0.588, sensitivity 70%, specificity 46.2%). IS level and duration of HD did not predict cardiomyopathy, (R2= 0.020, ß= -0.011(df= 0.869), p value=0.760) and (R2= 0.020, ß= -0.031 (df= 0.869), p value=0.192) respectively.

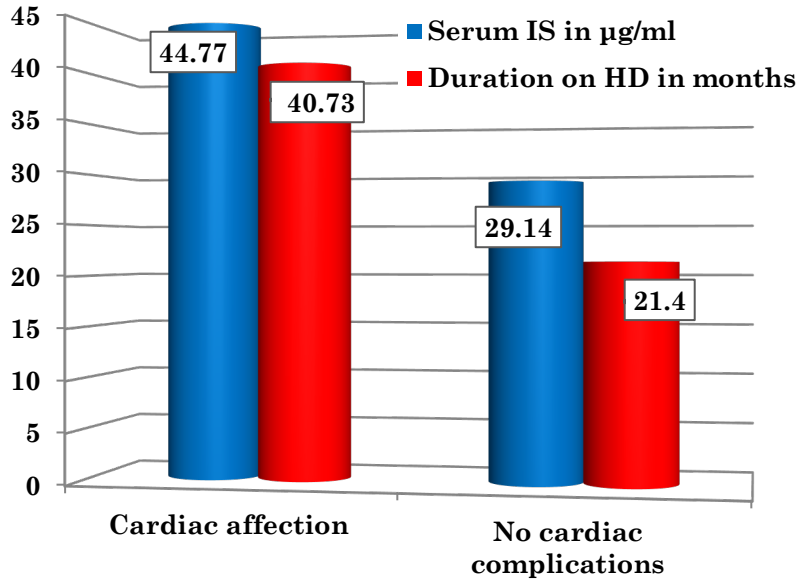

Figure 1. Comparison between the mean serum indoxyl sulfate (IS) level and duration on hemodialysis (HD) with cardiac affection in the study group.

Discussion

Indoxyl sulfate is a putrefaction product of microbial metabolism in the colon. It is mainly cleared by tubular secretion, a function not replicated by hemodialysis. Although, IS is a small molecule (molecular weight of 213 g/mol), it is 90% bound to protein so it is not cleared effectively by HD (*9*). IS level is reported to be prognostic of cardiovascular complications in ESKD pediatric patients. In the current study, 54 % of our patients had cardiac problems in the form of dilated cardiomyopathy or left ventricular hypertrophy. The reported cardiac complications are not limited to those with ESKD, but are also notorious to complicate the course in chronic kidney

disease (CKD). The cardiovascular complications are related to the hypertension, uremia, sepsis, infective endocarditis and accumulation of uremic toxins (*10*).

Our study, being cross-sectional and of relatively small sample size could not address if elevated IS causes cardiotoxicity, yet the IS level sensitivity and specificity of LVH or cardiomyopathy was modest. Our study deems the IS level as a predictor of accumulation of non-dialysable toxins. Non-dialyzable list of protein bound uremic toxins include IS, hippuric acid, p-cresol, methyl glyoxal and many others (*11*). We have not studied other non-dialyzable uremic toxins among our studied population, but there is a need to study the validity of IS estimation as a predictor of uremic toxins accumulation that may also contribute to the CVD encountered in ESKD patients.

Continuous ambulatory peritoneal dialysis was shown to have superior efficiency in clearing IS than HD (*12*). The selection of modality of RRT depends on many factors as of patient, motivation, distance from RRT center, availability and other factors. The type of filter was not associated with elevation of IS. A hybrid RRT might be effective in clearing IS and other toxins once accumulated through individualized cycles of HD alternating with peritoneal dialysis dictated by the amount of accumulated non dialyzable uremic toxins as IS. Hybrid RRT effect on reducing cardiovascular complications remains to be studied.

The cardiovascular compromise in our studied cohort was related to hypertension, the duration on HD and the serum IS level, but not to the gender nor age of the patients. These factors are amenable to control. Early detection and proper control of hypertension in chronic kidney disease pediatric patients is a mandatory issue that will eventually affect their morbidity and mortality.

The use of high flux membrane filters are reported to have better dialysis adequacy (*12*), also in our patients it was associated with less vascular access related complications, so high flux filters seem to provide more advantages in hemodialysis units.

Obstructive uropathy march to ESKD is a grave event. Timely management of obstructive uropathy through screening, prompt diagnosis and management to prevent ESKD would decrease the frequency of ESKD among children with obstructive uropathy. Also, early screening for primary hyperoxaluria, cystinosis, and atypical hemolytic uremic syndrome in genetically predisposed infants and their early management, could prevent their progression to chronic kidney disease with its hazardous complications (*13*).

Serum calcium levels were found to be higher in the hypertensive patients (p=0.048), which is considered as an independent predictors of mortality, even in the normal range and infusion of calcium to healthy volunteers, possibly due to a calcium-dependent manner through the calcium-sensing receptors (*14*).

As a toxic metabolite, IS production could be suppressed by restricting protein intake, changing the microbial metabolism in colon by increasing dietary fiber consumption, or decreasing intestinal absorption. Life style modification as consumption of vegetarian diets is important to decrease the products of tryptophan breakdown. Effect of IS lowering interventions are awaited, as infusion of a binding competitor into the extracorporeal circuit to compete with protein bound IS for their binding sites on albumin and increases its free fraction (*6*).

Conclusions

In conclusion, hypertension, dyslipidemia, IS, and prolonged duration of hemodialysis are risk factors of cardiovascular disease in pediatric patients with ESKD. IS is not efficiently removed by HD. More detailed assessment of cardiac functions like myocardial perfusion imaging and tissue Doppler assessment and correlation with nutritional status of the patients should be highlighted in further studies. Future studies that address the role of the microbiome manipulation on reduction of IS production and improvement of the morbidity and mortality of ESKD patients are awaited.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in connection with the reported study. Authors declare veracity of information.

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