Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Apr 14; 10(B):1042-1046.
https://doi.org/10.3889/oamjms.2022.8465
eISSN: 1857-9655
Category: *B - Clinical Sciences*
Section: *Nephrology*

OPEN ACCESS

Assessment of Gut leakage Induced Systemic Inflammation in Children on Chronic Hemodialysis

Ragia Said[1], Maged A. El Wakeel[2], Hasanin Mohamed[3]*, Ghada El Kassas[2], Ahmed F. Amer[2], May M. Abdel Hamid[2], Ayman Armaneos[2], Salwa Refat El-Zayat[4], Nevein N. Fadl[4]

[1]Department of Pediatrics, Faculty of Medicine, Ain Shams University, Cairo, Egypt; [2]Department of Child Health, National Research Centre, Giza, Egypt; [3]Department of Pediatric, National Research Centre, Giza, Egypt; [4]Department of Medical Physiology, National Research Centre, Giza, Egypt

Edited by: Ksenija Bogoeva-Kostovska
Citation: Said R, El Wakeel MA, Mohamed H, El Kassas G, Amer AF, Abdel Hamid MM, Armaneos A, El-Zayat SR, Fadl NN. Assessment of Gut leakage Induced Systemic Inflammation in Children on Chronic Hemodialysis. Open Access Maced J Med Sci. 2022 Apr 14; 10(B):1042-1046. https://doi.org/10.3889/oamjms.2022.8465
Keywords: Hemodialysis-intestinal permeability; Systemic inflammation; Zonulin-children
***Correspondence:** Hasanin Mohamed, National Research Centre, Bohouth Street, Dokki, Giza, Egypt. E-mail: drhasanin1980@yahoo.com
Received: 01-Jan-2022
Revised: 15-Mar-2022
Accepted: 04-Apr-2022

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Children with chronic kidney disease (CKD) are at high risk to develop GIT complications such as intestinal wall edema and increased permeability which contributes to chronic systemic inflammation that increases morbidity and mortality burden.

AIM: This study aim was to show the relationship between inflammation and increased intestinal permeability in children on hemodialysis (HD).

METHODS: The study included 50 children with CKD on regular HD of variable duration; their age range was (5−16) years and 40 controls. They were followed up at Nephrology clinic- Eldemerdash hospital. Complete history taking, physical examination were done. Laboratory measurement in the form of zonulin, tumor necrosis factor-alpha (TNF-alpha), high sensitive C-reactive protein (hs-CRP), and alpha1-antitrypsin (AAT) were quantified in serum by ELISA method.

RESULTS: There was a highly significant decrease in anthropometric measures (weight, height and BMI) and their corresponding z score in cases compared to controls with $p \leq 0.001$. Furthermore, there was significant increase of levels of zonulin, TNF-alpha, AAT, and hs-CRP in cases compared to controls with p value of 0.009, 0.001, 0.002, and 0.003, respectively. There was significant positive correlation between zonulin and (TNF-alpha, AAT, hs-CRP, and creatinine) with P values (0.003, 0.001, 0.001, and 0.001), respectively. Zonulin is negatively correlated with weight for age Z score (WAZ) and height with p value (0.01 and 0.018), respectively. TNF-alpha and hs-CRP were negatively correlated with WAZ with P-values of 0.02 and 0.01, respectively.

CONCLUSION: Children with CKD on chronic hemodialysis had elevated levels of zonulin, TNF-alpha, hs-CRP, and AAT which reflects gut permeability induced systemic inflammatory state.

Introduction

Children with chronic kidney disease (CKD) have a state of underlying systemic chronic inflammation. It is a robust predicting factor for cardiovascular disease. Many complications are associated, such as anemia, malnutrition, cachexia, and early mortality [1], [2]. Despite in CKD patients, chronic inflammation being multifactorial, its pathophysiology is unknown. It may be due to underlying pro-inflammatory conditions, increased oxidative stress [3], [4] and complications related to the vascular access [5].

The major source of chronic inflammation is increasingly being recognized in dialysis patients due to the involvement of gastrointestinal tract [6], [7]. Alteration in the intestinal microbiome is also known to affect and disturbs the function of gut barrier, which occurs as a result of deterioration of renal function [8]. Electrolytes, essential dietary nutrients and water were selectively absorbed from the lumen of the intestine. This is mediated by the intestinal barrier, which acts as a semipermeable membrane and also prevents microbial translocation into the systemic circulation [9], [10]. The breakdown of intestinal barrier function in children with CKD is strongly recognized in many observational and *in vitro* studies done on animals and human [4]. Systemic inflammation is mainly caused by breakdown, damage of intestinal barrier function which facilitates translocation of components of bacteria across the leaky gut wall into the systemic circulation [11], [12].

The changes in intestinal permeability which is mediated by hemodialysis have not been assessed in children. Hence, to demonstrate hemodialysis induced gut permeability, interventions could be targeted at the hemodialysis procedure [13].

Zonulin is a useful, valuable, non-invasive new marker used for measurement of intestinal permeability [14], [15]. Increased zonulin concentration was observed in patients with obesity [16], sepsis [17], type 2 diabetes, and autoimmune diseases.

We aim in this study to assess the association between inflammatory state and intestinal permeability

in children on hemodialysis-so reduce the impact of chronic disease-induced inflammation on CKD children and improve the survival rate.

Methods

In the present cross-sectional, case–control study, fifty children with chronic kidney disease on regular hemodialysis of variable durations were included, their age ranging from 5−16 years in addition to forty healthy age- and sex-matched controls. These children were followed up in Pediatric Nephrology unit, El-Demerdash Hospital, Ain-shams University. Patients with the previous history of GIT disorders or receiving immunosuppressant medications, those on hemodialysis for <6 months, patients with generalized inflammation and those with end-stage malignant disease were excluded from our research.

All patients in the study were subjected to full history taking from parents including (original renal disease, previous intervention for renal problems, and Concomitant medications), clinical examination, and anthropometric indices (weight, height, and body mass index BMI and their corresponding Z score were be evaluated).

An informed consent has been taken from parents before participation in the study and the study was approved by the Medical Ethical Committee of the National Research Centre.

Measurements

About 3 ml of fasting 12 h venous samples were withdrawn from cases while performing routine tests before midweek HD session. Furthermore, in control group blood samples were obtained in the morning after overnight fasting.

Serum Zonulin, hs-CRP, AAT, and TNF-alpha were quantified in serum by an ELISA method using kits purchased from (SunLong Biotch Co., LTD). Zonulin kit detection range was (30–1500 pg/ml) catalog number: SL2712Hu. hs-CRP detection range (0.3−10 ng/ml) catalog number: SL0881Hu the detection range of the 1-antitrypsin kit was (0.5−40 ng/ml) catalog number: SL1847Hu. The TNF kit detection range was (20−400 ng/L) catalog number: SL1761Hu.

Statistical analysis

Data were collected, verified, coded, and analyzed using the Statistical Package for the Social Science (SPSS) version 22 (SSPS Inc., Pennsylvania, USA). Descriptive statistics were used to summarize baseline characteristics of the study population. The mean ± standard deviation (SD) was reported for continuous variables. Independent t-test was used to compare between two groups regarding quantitative data. Pearson correlation analysis was used to assess the relationship between two quantitative parameters in the same group. Multiple linear regressions were done to determine factors mostly affecting inflammatory mediators among cases. The p-values were 2-tailed, it was considered statistically significant at $p \leq 0.05$, and highly significant at $p \leq 0.001$.

Results

This case–control cross-sectional study included 50 children as cases in addition to 40 healthy controls (age and sex matched). Anthropometric measures (weight, height, and body mass index) were significantly decreased in cases group ($p < 0.001$) and their corresponding Z score when compared to their controls as shown in Table 1.

Table 1: Comparison between cases and control groups as regard anthropometric measurements

Variable	Cases N = 50 Mean ± SD	Controls N = 40 Mean ± SD	p value
Weight (kg)	29.30 ± 8.09	64.53 ± 5.48	0.001**
Weight for age z score (WAZ)	−2.99 ± 1.72	0.53 ± 1.11	0.001**
Height (cm)	131.18 ± 13.27	160.53 ± 8.33	0.001**
Height for age z score (HAZ)	−2.86 ± 1.60	1.38 ± 0.420	0.001**
BMI	16.24 ± 3.98	25.09 ± 1.94	0.001**
BMI z score	−1.40 ± 1.24	1.38 ± 0.420	0.001**

**p ≤ 0.001 (highly significant).

Serum zonulin level was significantly higher in cases compared to control group with ($p = 0.009$). In addition, there was significant increase of systemic inflammatory markers (TNF-alpha and hs-CRP) and alpha1 AT (biomarker of intestinal inflammation) in cases in relation to controls with p value (0.001, 0.003, and 0.002), respectively, as shown in Table 2.

Table 2: Comparison between cases and controls regarding to inflammatory biomarkers

Variable	Cases N = 50 Mean ± SD	Control N = 40 Mean ± SD	p value
Zonulin (ng/ml)	633.6 ± 273.8	488.8 ± 123.3	0.009**
TNF-alpha (ng/L)	71.52 ± 37.24	49 ± 16.36	0.001**
AAT(mg/dl)	13.25 ± 3.96	10.13 ± 4.14	0.002**
hs-CRP (mg/L)	5.86 ± 2.13	2.47 ± 1.18	0.003**

**p ≤ 0.001 (highly significant).

A significant positive correlation was found between serum zonulin and TNF-alpha, AAT, hs-CRP, and creatinine ($p = 0.003$, 0.001, 0.001, and 0.001), respectively, and negatively correlated with WAZ and height with ($p = 0.01$ and 0.018), respectively. TNF-alpha has significant positive correlation with zonulin, AAT, hs-CRP ($p = 0.003$, 0.001, and 0.001), respectively, and negative correlation with WAZ ($p = 0.02$). AAT (marker of nutrient wasting) has significant positive correlation

with zonulin, TNF-alpha, hs-CRP, and creatinine with p value (0.001, 0.018, 0.04, and 0.007), respectively. Hs-CRP (systemic acute phase reactant) has significant positive correlation with zonulin, TNF-alpha, AAT with (p = 0.001, 0.01, and 0.04), respectively, and negatively correlated with WAZ (p = 0.01) as shown in Table 3.

Table 3: Correlation between laboratory markers and anthropometric parameters

Variable	Zonulin (ng/ml)	TNF-alpha (ng/L)	AAT (mg/dl)	hs CRP (mg/L)	Creatinine (mg/dl)	Urea (mg/dl)
Zonulin						
r	-	0.457	0.527	0.485	0.542	0.741
p		0.003*	0.001*	0.001*	0.001**	0.054*
TNF-alpha						
r	0.457	1	0.374	0.380	0.198	-0.132
p	0.003*		0.01**	0.016*	0.22	0.41
AAT						
r	0.527	0.374	-	0.315	0.420	-0.821
p	0.001*	0.018*		0.048*	0.007*	0.03*
hs CRP						
r	0.485	0.380	0.315	-	0.253	-2.68
p	0.001*	0.016*	0.048*		0.116	0.09
Creatinine						
r	0.542	0.198	0.420	0.253	-	0.39
p	0.001*	0.22	0.007*	0.116		0.01*
Urea						
r	0.741	-0.132	0.821	-0.268	0.395	-
p	0.05*	0.416	0.037*	0.09	0.012*	
Weight						
r	-0.16	0.570-0.09*	0.979	0.531	0.599	0.795
p	0.317		0.004*	0.102	0.086	0.042*
WAZ						
r	-0.50	-0.364	0.135	0.377	0.153	0.888
p	0.001*	0.021*	0.405	0.017*	0.346	0.023*
Height						
r	-0.39	0.864	0.211	0.562	0.894	0.727
p	0.012*	0.028*	0.192	0.095	0.022*	0.057*
HAZ						
r	-0.18	0.724	0.832	0.801	0.563	0.709
p	0.259	0.058*	0.035*	0.041*	0.094	0.061
BMI						
r	0.91	0.713	0.732	0.243	0.296	0.944
p	0.019*	0.08	0.058*	0.131	0.169	0.011*
BMI z						
r	0.22	0.826	0.228	0.220	0.206	0.134
p	0.168	0.036*	0.156	0.172	0.202	0.410

Pearson's coefficient correlation test. **p ≤ 0.001 (highly significant), *p ≤ 0.05 (significant).

Multiple linear regression analysis for factors affecting TNF alpha was done among children on hemodialysis and showed that Zonulin was the most effective factor as shown in Table 4.

Table 4: Multiple linear regression analysis for factors predicting TNF-alpha

Group	Unstandardized Coefficients		Standardized Coefficients	t	p value
	B	SE	Beta		
Constant	40.568	37.840		1.072	0.291
Zonulin	0.053	0.026	0.389	2.047	0.048*
AAT	1.677	1.677	0,179	1.005	0.322
Creatinine	−0.864	4.871	-0.035	0.177	0.860
Urea	−0.094	0.117	-0.0133	-0.804	0.427

[a]Dependent Variable: TNF. alpha, [b]Predictors: (Constant), urea, AAT, Zonulin, creatinine.

Multiple linear regression analysis for factors affecting hs-CRP was done among children on hemodialysis and showed that Zonulin was the most effective factor as shown in Table 5.

Discussion

Children with chronic kidney disease (CKD) have a state of underlying systemic chronic inflammation.

Table 5: Multiple regression analysis for factors predicting hs-CRP

Group	Unstandardized coefficients		Standardized coefficients	t	p value
	B	SE	Beta		
Constant	3.885	2.046		1.899	0.066
Zonulin	0.003	0.001	0.405	2.261	0.030*
AAT	0.010	0.090	0.019	0.113	0.510
Creatinine	0.232	0.263	0.165	0.880	0.385
Urea	-0.014	0.006	-0.354	−2.27	0.029

[a]Dependent Variable: hs-CRP, [b]Predictors: (Constant), urea, AAT, Zonulin, creatinine.

It is a strong predicting factor for cardiovascular disease. Many complications are associated such as anemia, malnutrition, cachexia, and early mortality [18], [19]. In this study, we evaluated zonulin level (novel biomarker of intestinal permeability), alpha1AT (marker of intestinal inflammation) and hs-CRP, and TNF-alpha (markers of systemic inflammation) in children with chronic kidney disease on regular sessions of hemodialysis of variable duration to predict early onset of complications and its prevention.

Chronic kidney disease is associated with impaired growth and malnutrition in children due to acidosis, anemia, tissue resistance to the action of human growth hormone and insulin-like growth factors, and renal osteodystrophy [20]. This is similar to our results where cases had significant decrease in anthropometric parameters (weight, height, BMI, and their corresponding z scores) with P value of 0.001 compared to their controls.

Our results were in contrast to a study done on 71 children at hemodialysis center (Baghdad Teaching Pediatric hospital), where normal weight was present in 61.9% of them, their BMI for age z score was between (–1.9 and 1.9), 8.45% of them had z score < –3, and 8.45% of them had z score from (–2 to –3).

Inflammatory markers as TNF-alpha, hs-CRP, and AAT were significantly elevated in (HD) patients compared to controls. These findings were coincident with previous works that stated higher concentrations of pro-inflammatory markers (IL-6, TNF-α, hs-CRP) in children with CKD and their relation with anemia [21]. Chronic inflammation is multifactorial and not completely understood in hemodialysis (HD) patients. The release of pro-inflammatory cytokines from activated macrophages and monocytes, contact of the dialyzer membrane with blood and increased intestinal permeability are different causes that may explain the increased inflammatory state [22].

There was also a significant increase in serum zonulin level (marker of intestinal permeability) in cases in comparison to control group with p = 0.009.

In the current study, there was a positive correlation between zonulin and TNF-alpha, hs-CRP and AAT with significant p value (0.003, 0.001, and 0.001), respectively, which is similar to the results of Ficek *et al*. (2017) who found a positive correlation between serum zonulin and some inflammatory markers (TNF and IL-6) in a group of adult patients

undergoing hemodialysis [23]. The degree of endotoxemia in CKD patients is corresponded to the level of systemic inflammation. The previous findings are similar to those present in sepsis, in which there is a link between increased intestinal permeability and systemic inflammatory response syndrome which explained elevated zonulin level [5], [24].

The metabolic disturbances in CKD favor gut pathogen overgrowth, translocation of components of bacteria (e.g., lipopolysaccharide), loss of barrier function, and inflammation. All these factors activate the release of inflammatory mediators and cytokines [23] which increases the intestinal permeability and gut leakage and eventually reflects on occurrence of growth complications, for example, stunting that increases the progression of CKD. This is consistent with our results where zonulin is negatively correlated with weight for age z score (WAZ) and height with p value (0.01 and 0.018), respectively, also TNF-alpha and hs-CRP were negatively correlated with WAZ with significant P value (0.02 and 0.01), respectively.

It is not clear whether increased intestinal permeability can be accompanied by progression of kidney disease or can be due to influence of uremic toxins on the intestinal wall.

When applying multiple regression models to determine factors affecting serum hs-CRP and TNF-alpha, we found that serum zonulin was the most effective one. This supports the hypothesis that increased permeability of intestinal wall participates to the inflammation development in HD patients.

Conclusion

In our study, elevated zonulin level (marker of intestinal permeability and gut leakage) and its association with TNF-alpha and hs-CRP (markers of systemic inflammation) may indicate that increased intestinal permeability contributes to the chronic inflammation development in HD patients. Hence, early measurement of these non-invasive markers can decrease incidence of complications and improve the survival rate.

References

1. Cobo G, Lindholm B, Stenvinkel P. Chronic inflammation in end-stage renal disease and dialysis. Nephrol Dial Transplant. 2018 Oct 1;33(Suppl 3):iii35-40. https://doi.org/10.1093/ndt/gfy175
   PMid:30281126
2. Colombo I, Aiello-Battan F, Elena R, Ruiz A, Petraglia L, Musso CG. Kidney-gut crosstalk in renal disease. Irish J Med Sci. 2020;190(3):1205-12. https://doi.org/10.1007/s11845-020-02437-7
   PMid:33216316
3. Khoury T, Tzukert K, Abel R, Rmeileh AA, Levi R, Ilan Y. The gut-kidney axis in chronic renal failure: A new potential target for therapy. Hemodial Int. 2017;21(3):323-34. https://doi.org/10.1111/hdi.12486
   PMid:27634464
4. Said RM, El Wakeel MA, El-Kassas GM, Mostafa HH, Kamhawy AH, Galal EM, *et al*. Protein energy wasting in a sample of Egyptian children on regular hemodialysis: Relation to anorexigenic hormones. Open Access Maced J Med Sci. 2020;8(B):658-65. https://doi.org/10.3889/oamjms.2020.4981
5. Klaus DA, Motal MC, Burger-Klepp U, Marschalek C, Schmidt EM, Lebherz-Eichinger D, *et al*. Increased plasma zonulin in patients with sepsis. Biochem Med (Zagreb). 2013;23(1):107-1. https://doi.org/10.11613/bm.2013.013
   PMid:23457771
6. Lau WL, Vaziri ND. The leaky gut and altered microbiome in chronic kidney disease. J Ren Nutr. 2017;27(6):458-61. https://doi.org/10.1053/j.jrn.2017.02.010
   PMid:29056165
7. Lin TY, Wu PH, Lin YT, Hung SC. Gut dysbiosis and mortality in hemodialysis patients. NPJ Biofilms Microbiomes. 2021;7(1):20. https://doi.org/10.1038/s41522-021-00191-x
   PMid:33658514
8. Long J, Vela Parada X, Kalim S. Protein carbamylation in chronic kidney disease and dialysis. Adv Clin Chem. 2018;87:37-67. https://doi.org/10.1016/bs.acc.2018.07.002
   PMid:30342712
9. March DS, Graham-Brown MP, Stover CM, Bishop NC, Burton JO. Intestinal barrier disturbances in haemodialysis patients: Mechanisms, consequences, and therapeutic options. Biomed Res Int. 2017;2017:5765417. https://doi.org/10.1155/2017/5765417
   PMid:28194419
10. El Wakeel MA, El-Kassas GM, Ahmed GF, Ali WH, Elsheikh EM, El-Zayat SR, *et al*. Fecal markers of environmental enteric dysfunction and their relation to faltering growth in a sample of Egyptian children. Open Access Maced J Med Sci. 2021;9(B):1117-22. https://doi.org/10.3889/oamjms.2021.7029
11. Sánchez-Alcoholado L, Ordóñez R, Otero A, Plaza-Andrade I, Laborda-Illanes A, Medina JA, *et al*. Gut Microbiota-mediated inflammation and gut permeability in patients with obesity and colorectal cancer. Int J Mol Sci. 2020;21(18):6782. https://doi.org/10.3390/ijms21186782
    PMid:32947866
12. El-Kassas GM, El Wakeel MA, Elabd MA, Kamhawy AH, Atti MA, Abd El-Gaffar SA, *et al*. Vitamin D status in neonatal pulmonary infections: Relationship to inflammatory indicators. Open access Maced J Med Sci. 2019;7(23):3970-4. https://doi.org/10.3889/oamjms.2019.592
    PMid:32165938
13. Sriwijitalai W, Wiwanitkit V. Ralstonia infection among hemodialysis patients: A summary. Saudi J Kidney Dis Transpl. 2019;30(3):749-50. https://doi.org/10.4103/1319-2442.261367
    PMid:31249248
14. Tanigasalam V, Bhat BV, Adhisivam, B, Sridhar MG, Harichandrakumar KT. Predicting severity of acute kidney injury in term neonates with perinatal asphyxia using urinary neutrophil gelatinase associated lipocalin. Indian J Pediatr. 2016;83(12-13):1374-8. https://doi.org/10.1007/s12098-016-2178-z
    PMid:27299341
15. El Wakeel M, El-Kassas G, Hashem S, Mohamed H, Ali W,

Elkhatib AA, *et al*. Serum biomarkers of environmental enteric dysfunction and growth perspective in egyptian children. Open Access Maced J Med Sci. 2020;8(T2):246-53. https://doi.org/10.3889/oamjms.2020.7023

16. Vancamelbeke M, Vermeire S. The intestinal barrier: A fundamental role in health and disease. Expert Rev Gastroenterol Hepatol. 2017;11(9):821-34. https://doi.org/10.1080/17474124.2017.1343143
PMid:28650209
17. Wong J, Lenaerts K, Meesters DM, Olde Damink SW, van Eijk HM, Vilar E, *et al*. Acute haemodynamic changes during haemodialysis do not exacerbate gut hyperpermeability. Biosci Rep. 2019;39(4):BSR20181704. https://doi.org/10.1042/bsr20181704
PMid:30898976
18. Yu J, Shen Y, Zhou N. Advances in the role and mechanism of zonulin pathway in kidney diseases. Int Urol Nephrol. 2021:53(10):2081-8. https://doi.org/10.1007/s11255-020-02756-9
PMid:33428167
19. Elzaree FA, Shehata MA, El Wakeel MA, El-Alameey IR, AbuShady MM, Helal SI. Adaptive functioning and psychosocial problems in children with beta thalassemia major. Open access Maced J Med Sci. 2018;6(12):2337. https://doi.org/10.3889/oamjms.2018.367
PMid:30607187
20. Gupta A, Mantan M, Sethi M. Nutritional assessment in children with chronic kidney disease. Saudi J Kidney Dis Transpl. 2016;27(4):733-9. https://doi.org/10.4103/1319-2442.185235
PMid:27424690
21. Goyal KK, Saha A, Sahi PK, Kaur M, Dubey NK, Goyal P, *et al*. Hepcidin and proinflammatory markers in children with chronic kidney disease: A case-control study. Clin Nephrol. 2018;89(5):363-70. https://doi.org/10.5414/cn109132
PMid:29451472
22. Anders HJ, Andersen K, Stecher B. The intestinal microbiota, a leaky gut, and abnormal immunity in kidney disease. Kidney Int. 2013;83(6):1010-6. https://doi.org/10.1038/ki.2012.440
PMid:23325079
23. Ficek J, Wyskida K, Ficek R, Wajda J, Klein D, Witkowicz J, *et al*. Relationship between plasma levels of zonulin, bacterial lipopolysaccharides, D-lactate and markers of inflammation in haemodialysis patients. International Urol Nephrol. 2017;49(4):717-25. https://doi.org/10.1007/s11255-016-1495-5
PMid:28044237
24. El Wakeel MA, El-Kassas GM, Hashem SA, Abouelnaga MW, Elzaree FA, Hassan M, *et al*. Potential role of oxidative stress in childhood obesity and its relation to inflammation. Biosci Res. 2018;15(4):3791-9.