International Journal of Clinical Medicine, 2015, 6, 423-430
Published Online June 2015 in SciRes. http://www.scirp.org/journal/ijcm
http://dx.doi.org/10.4236/ijcm.2015.66055

Serum Resistin Level and Polymorphonuclear Leukocytes Dysfunctions in Children on Regular Hemodialysis

Maha Yousef Zein[1], Manal Abdel-Salam[1*], Iman Abdel-Aziz[2], Naglaa Fathy Mohamed[1]

[1]Department of Pediatrics, Faculty of Medicine (Girls), Al-Azhar University, Cairo, Egypt
[2]Department of Clinical and Chemical Pathology, Theodor Bilharz Research Institute, Cairo, Egypt
Email: [*]manal24969@gmail.com

Received 4 May 2015; accepted 21 June 2015; published 26 June 2015

Abstract

Resistin is a secretory adipocytoine, which is expressed mainly in humans by inflammatory cells especially macrophages. Resistin serum levels are elevated in end-stage renal diseases of people having an increased risk of infections as a result of impaired polymorphonuclear leukocytes (PMNLs) functions. Objectives: To evaluate neutrophil functions (phagocytosis and oxidative burst) in children with end-stage renal disease (ESRD) on regular hemodialysis and to shed light on the contribution of resistin on neutrophil functions. Patients and Methods: The study included 40 children with ESRD on regular hemodialysis. Their ages ranged from 6 to 12 years, and they were selected from children attending the pediatric hemodialysis unit of AL-Zahraa Hospital, Al-Azher University during the period from October 2012 to December 2013. Another group of 40 apparently healthy children with matched age and sex with the patient group served as a control. Serum resistin, phagocytic index and nitro blue tetrazolium test (NBT%) were assessed in both groups. Results: There was a statistically more significant increase in resistin serum levels in cases than in controls; it was (3.25 ± 0.86 ng/ml) and (0.25 ± 0.16 ng/ml) respectively ($P < 0.01$). On the other hand there was a statistically more significant decrease in neutrophil phagocytic index in cases than in controls; it was (2.57 ± 1.34) and (3.55 ± 0.74) respectively ($P < 0.01$). Also it showed a statistically more significant decrease in NBT% in cases than in controls; it was (47.98 ± 16.38%) and (61.45 ± 13.17%) respectively ($P < 0.01$). We found negative correlation between resistin serum level with phagocytic index and NBT%, while we found positive correlation between resistin serum level and hemodialysis duration. Conclusion: High resistin serum level in children with ESRD decreases phagocyte function and oxidative burst of PMNLs, and this is enhanced by the longer duration of hemodialysis.

[*]Corresponding author.

How to cite this paper: Zein, M.Y., Abdel-Salam, M., Abdel-Aziz, I. and Mohamed, N.F. (2015) Serum Resistin Level and Polymorphonuclear Leukocytes Dysfunctions in Children on Regular Hemodialysis. *International Journal of Clinical Medicine*, **6**, 423-430. http://dx.doi.org/10.4236/ijcm.2015.66055

Keywords

Chronic Kidney Disease, Resistin, Dialysis, Polymorphonuclear Leukocytes

1. Introduction

Chronic kidney disease (CKD) is common, and its prevalence is increasing worldwide. Infection is a major cause of mortality in end-stage renal disease (ESRD) and hospitalization at all stages of CKD [1].

The second commonest cause of death among patients with ESRD is septicemia and patients with ESRD are at an increased risk of death from infection compared to the general population [2].

There is a wealth of evidence that disorders of both innate and adaptive immune systems contribute to an increased rate of infections in the course of ESRD. Functional abnormalities of monocytes, neutrophils, and dendritic cells are directly linked with infection risk in this patient population [3].

Polymorphonuclear leukocytes (PMNLs) are professional phagocytes which engulf antibody-coated microbes [4]. They have many intra-cellular granules which contain bactericidal proteins. PMNs are the first line of defense against invading microbes and important players in inflammation [1].

The adipose tissue has pleiotropic functions far beyond the storage of energy, and it secretes a number of hormones and cytokines, called adipokines, which have biological effects that impact health and disease. Adipokines are markedly elevated in the plasma of uremic patients, mainly due to decreased renal excretion. They have pluripotent signaling effects on inflammation/oxidative stress (e.g. leptin, adiponectin, resistin) [5].

Resistin is a protein mediator secreted by adipocytes and macrophages within the adipose tissue [6]. In humans, resistin is essentially expressed in macrophages from white adipose tissue, rather than in human adipocytes *per se* [7].

Conventional HD does not significantly decrease plasma resistin levels. Resistin was shown to interfere with the chemotactic movement and the oxidative burst of neutrophils at concentrations seen in uremia. As neutrophils are cells of the first-line nonspecific immune defense, resistin may contribute to the disturbed polymorphonuclear leukocyte (PMNLs) functions [8] and as a consequence to the increased risk of infections in uremic patients [5].

We aimed to shed light on the contribution of resistin on neutrophil functions including phagocytosis and oxidative burst in children with end-stage renal disease (ESRD) on regular hemodialysis.

2. Patients and Methods

The study is a cross sectional comparative study. It was carried out on 80 children, their ages ranged from 4 - 16 years during the period from October 2012 to December 2013. They were divided into two groups: *Group I*: (case group): included 40 with ESRD (GFR 15 ml/min/1.73) on regular hemodialysis, at least more than 3 months at the time of the study for 4 hours/setting, 3 times weekly with low flux polysulphone membrane by Fresenius machine. They were selected from the hemodialysis unit of Al-Zahraa hospital, Al-Azhar University, they were 21 female and 19 male. The most common cause of ESRD in patients group was FSGS in 20% followed by unknown (17.5%) and polycystic kidney (17.5%).

Group II: (control group) included 40 apparently healthy children matched age and sex with group I, they were selected from patients relatives. Children with; primary immune deficiency, acute and chronic infections children receiving medications (antibiotics, cytotoxic drugs, immunosuppressive) were excluded from the study. Patients were subjected to full history taking; etiology, onset of CKD and the duration of hemodialysis, history of medications and recurrent infection, including (cellulites, gingivitis, pneumonia, UTI, otitis media) and laboratory investigations for both groups.

Informed consent was obtained from the participating parents in adherence with the guidelines of the ethical committee of AL-Zahraa hospital, AL-Azhar University, Cairo, Egypt.

2.1. Blood Sample Collection

5 ml venous blood samples were withdrawn.

- 2 ml on EDTA blood for CBC.
- 3 ml of the samples were put in a plain tube and left to clot and sera were separated for BUN, serum creatinine and CRP.
- Another portion of the serum samples were stored frozen at-20 c after careful labeling till the time of resistin assay.

4 ml of fresh venous blood samples on heparin for assessment of neutrophil functions (phagocytosis and oxidative burst).

2.2. Assessment of Serum Resistin

Serum resistin level was measured by ELISA technique (using commercially available kit) supplied by Biovender research and diagnostic products.

Principal of the Procedure

This assay employs a quantitative sandwich enzyme immunoassay technique, which measures resistin in less than 5 hours.

2.3. Assessment of Neutrophil Functions

2.3.1. Procedure

- The fresh venous blood samples were left to sediment and the buffy coat was taken and washed with PBS (Phosphate Buffer Saline) centrifuged at 800 - 1000 rpm for 10 minutes.
- Equal volumes from sediment + PBS + pooled serum was added to two tubes.
- Candida albicans was added in the first tube and incubated at 37˚C for 30 minutes and 100 micron of NBT reagent in the second tube was added and incubated at room temperature for 15 minutes.
- After incubation both samples were centrifuged for 5 minutes at 800 - 1000 rpm and slides was made and stained with Leisheman stain for 2 min, dilute with Gimesa stain for 5 minutes [9].

2.3.2. Estimation of Phagocytic Index and NBT%

Quantitate phagocytosis under oil-immersion microscopy, examining at least 200 cells and counting the number of internalized bacteria in each one. Calculate amount of phagocytosis according to the following formula:

1) Phagocytic index (PI) = (percentage of macrophages containing at least one bacterium) × (mean number of bacteria per positive cell).

The normal reference range for phagocytic index is (2.4 - 4.5) [10].

2) NBT% = (number of macrophages containing engulfed NBT/total number of counted macrophages) × 100.

The normal reference range (47% - 85%).

2.4. Statistical Analysis

The data of collected questionnaire was entered and analyzed using SPSS 16.0 (Statistical Package for Social Sciences). Mean and standard deviation was given for normally distributed quantitative variables. Frequencies and percentages were given for qualitative variables. Two-independent sample t test was applied to observe group mean differences. Pearson correlation was applied to observe correlation between quantitative variables, P value of <0.05 was considered statistically significant. Receiver operating characteristic curve (ROC) was used to assess the best cut off point with sensitivity, specificity, positive predictive value (+PV) and negative predictive value (−PV).

3. Results

Table 1: showed that there is no statistically significant difference between patients group and the controls regarding total leukocyte counts (TLCs) and absolute neutrophil counts (ANCs), while there is a high statistically significant decrease in RBCs, Hb, Hct% and platelets counts in patients group than the controls. There was a statistical significant increase in serum creatinine and BUN in patients group compared to the controls. The same table showed that there was significant differences between patients group and the controls regarding CRP, we found that 24 (60.00%) out of 40 patients have CRP +ve, while all the controls have CRP −ve.

Table 1. Comparison between patients group and the controls regarding age and routine laboratory data and CRP.

Variables	Control (No. 40)		Patients (No. 40)		Independent t-test	
	Mean	SD	Mean	SD	t	p-value
Age years	10.95	±2.86	10.01	±3.27	1.287*	0.202
TLC × 10^3 mm^3	7.64	1.47	8.25	3.82	−0.941	0.350
ANC × 10^3 mm^3	4.8	0.99	4.52	2.72	0.602	0.549
RBCs × 10^6 mm^3	4.69	0.43	3.47	0.76	8.827	0.000
Hb gm/dl	12.04	0.94	9.1	1.87	8.921	0.000
Hct%	36.37	2.55	28.19	4.79	9.537	0.000
Platelet × 10^3 mm^3	290.25	51.25	247.18	87.61	2.684	0.009
ESR mm/first hour	12.78	4.25	51.68	31.32	−7.784	0.000
Creatinine mg/dl	0.35	0.11	7.86	2.08	22.796	0.000
Urea mg/dl	20.72	6.80	143.00	84.08	−7.248	0.000
CRP	**no**	%	**no**	**%**	**X^2**	**p-value**
+ve	0	100.00%	24	60.00%	25.563	0.000
−ve	0	0.00%	16	40.00%		

p > 0.05: Non-significant; p < 0.05: Significant; p < 0.01: Highly significant.

Table 2: showed that the most common type of recurrent infection in dialysis children was pneumonia 19 (47.5%) followed by UTI 13 (32.5%) while the least common infection type was otitis media (2.5%).

Table 3: showed significant increase in resistin serum level in patient group compared to the controls, the same table showed significant decrease in phgocytic index and NTB% in patients group than in controls.

Table 4: showed significant increase in resistin serum level in patients with CRP positive, while there was a statistically significant decrease in neutrophil phagocytic index and NBT% in patients with CRP positive.

Table 5: showed significant positive correlation between resistin serum level with hemodialysis duration, while negative correlation between resistin with both neutrophil phagocytic index and NBT%.

Figure 1: showed negative correlation between the duration of hemodialysis and phagocytic index.

Table 6, **Figure 2**: showed sensitivity and specificity of resistin serum level in predicting PMNLs dysfunctions in children with ESRD on regular hemodialysis; it revealed that resistin is 100% sensitive and specific in predicting PMNLs dysfunctions in children on regular hemodialysis.

4. Discussion

The major concern of the present study is to assess resistin serum level in children with ESRD on regular hemodialysis and detect its reflection on PMNLs functions including phagocytosis and oxidative burst. We found highly significant increase in resistin level in patients group than the controls ($p < 0.01$). Resistin levels have been found to be increased in chronic inflammatory conditions such as inflammatory bowel disease, rheumatoid arthritis, and uremia [11].

Our results are in agreement with [8] who found that resistin level is higher in dialysis group than the controls ,also a study by [12] reported that increased serum resistin level in patients with ESRD. The study of [13] found that, the median serum resistin level was higher in children of uremia than controls in particular with a glomerular etiology compared with those with a non glomerular etiology (21.3 ng/mL vs 17.9 ng/mL; $p = 0.03$).

In our study we found significant decrease in neutrophil phagocytic function in patients group than in controls, which assessed by phagocyte index (PI) by measuring of phagocyte activity of candida albicans or bacterial cells ingested per phagocyte (neutrophil) during a limited period of incubation of a suspension of bacteria and phagocytes in serum. PMNLs are professional phagocytes which engulf antibody-coated and complement-coated microbes, damaged cells & cellular debris. They have many intra-cellular granules which contain bactericidal

Table 2. Types of recurrent infections in patients group.

Recurrent infections	Frequency	Percent
Pneumonia	19	47.5
UTI	13	32.5
Cellulites'	4	10
Tonsillitis	3	7.5
Otitis Media	1	2.5
Total	40	100

Table 3. Comparison between patients group and the controls regarding resistin level, neutrophil phagocyic index and NBT%.

Variables	Controls (No. 40)		Patients (No. 40)		Independent t-test	
	Mean	SD	Mean	SD	t	p-value
Resistin ng/ml	0.25	0.16	3.25	0.86	−21.681	0.000
Phagocytic index	3.55	0.74	2.57	1.34	4.055	0.000
NBT%	61.45	13.17	47.98	16.38	4.055	0.000

Table 4. The relation between CRP and resistin level, phagocytic index and NBT%.

Variables	Negative CRP		Positive CRP		Independent t-test	
	Mean	SD	Mean	SD	t	p-value
Resistin ng/ml	2.96	0.67	3.67	0.95	−2.8	0.008
Phagocytic index	3.05	1.37	1.84	0.94	3.087	0.004
NBT%	52.13	16.91	41.75	13.78	2.041	0.048

Table 5. Correlation between resistin level and some of clinical and laboratory parameters.

Variable	Resistin ng/ml	
	r	p-value
Age (y)	−0.036	0.828
Duration of dialysis/month	0.351	0.027
Urea mg/dl	−0.011	0.947
Creatinine mg/dl	0.092	0.574
Phagocyte index	−0.569	0.000
NBT%	−0.569	0.000

Table 6. Correlation between resistin level and some of clinical and laboratory parameters.

Best cut off point	AUC	Sensitivity	Specificity	+PV	−PV
>0.741	100.00	100.00	100.00	100.0	100.0

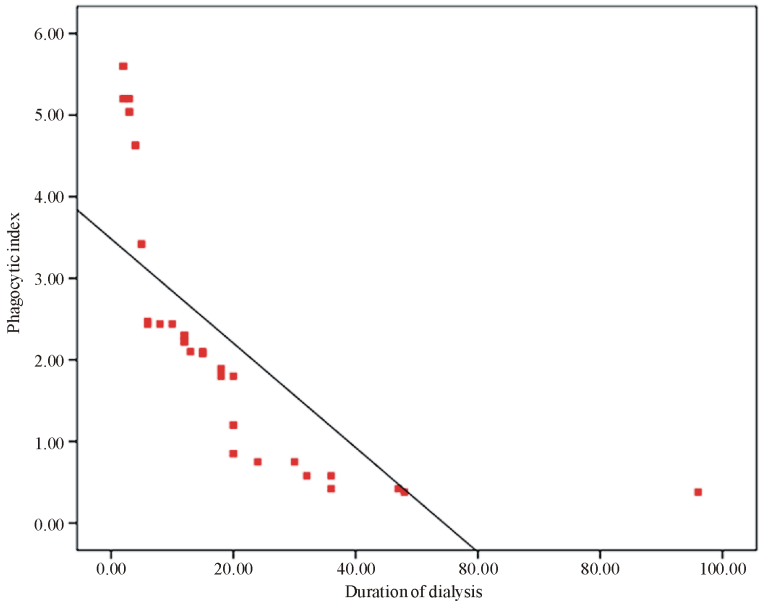

Figure 1. Correlation between hemodialysis duration and phagocyte index.

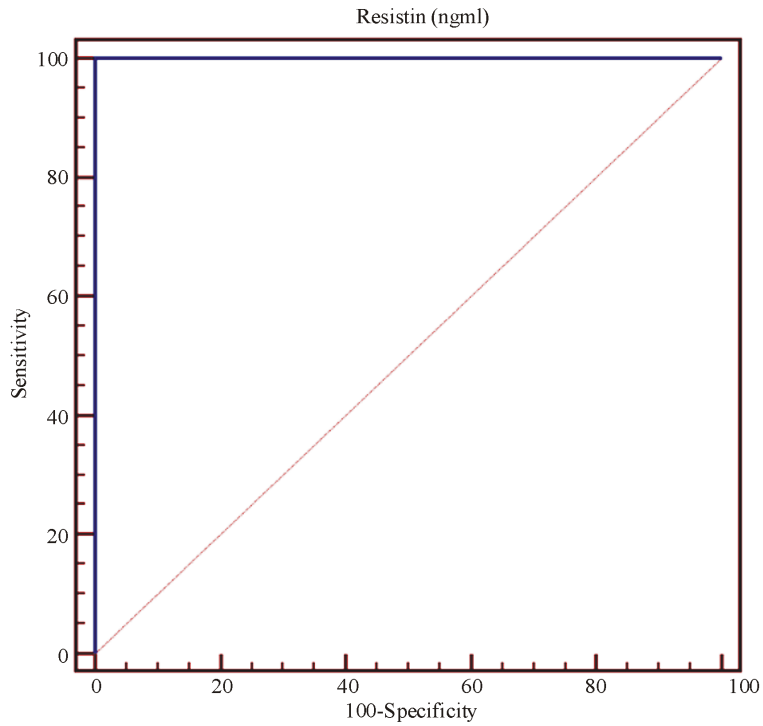

Figure 2. Receiver operating characteristic (ROC) curve for resistin serum level in predicting PMNLs dysfunctions in hemodialysis children.

proteins. PMNLS are the first line of defense against invading microbes and important players in inflammation [3]. The susceptibility of CKD patients to infections as a result of defective phagocytosis is caused by a variety of factors, including uremic toxins such as resistin, iron overload, anemia of renal disease and dialyzer bioincompatiblity [14].

Pneumonia is the commonest encountered infection type in the studied patients group, it is detected in (47%) followed by UTI (32.5%) and cellulites (10%), tonsillitis and otitis media represent (7.5%) and (2.5%) respec-

tively. The susceptibility of CKD patients to infections as a result of defective phagocytosis caused by a variety of factors, including uremic toxins such as resistin, iron overload, anemia of renal disease and dialyzer bioincompatiblity [14]. Although clinical experience suggests that pneumonia occurs frequently in dialysis patients, its clinical epidemiology in that group remains poorly defined [15].

Our results are in agreement with the results of [16] who reported that PMNLs ability to phagocyte is related to kidney function.

In our study there was a highly statistically significant decrease in oxidative burst of neutrophils in patients group than the controls which assessed by nitroblue tetrazolium test (NBT%). Nitroblue tetrazolelium test (NBT) is a test for assessment of oxidative function of neutrophils by measuring the activity of the NADPH oxidase system which generate superoxide and related oxygen intermediates which control bacterial and fungal infections [17].

Our results are in agreement with. [18] who found that oxidative system of neutrophils are severely impaired in CKD patients and worsen progressively with the degree of renal failure. Ghobrial *et al.* [19] found that CKD is a state of oxidative stress and impaired oxidative functions.

In our study positive correlation between resistin level and the duration of hemodialysis ($r = 0.351$, $p = 0.027$), as resistin is an inflammatory cytokine, that was clearly associated with general inflammation and renal disease, it is increased with the longer duration of the disease and not improved by dialysis because conventional heamodialysis does not significantly decrease plasma resistin level [8] [20] [21].

In our study there was a highly statistically significant increase in CRP level in patients group than the control group. In agreement with our results [19] who reported that children with CKD had higher percentages of positive CRP than the controls. Also in the same line [22]-[25], they reported highly significant increased CRP levels in maintenance hemodialysis patients. Accordingly, we found that significant increase in serum resistin level with CRP positive than CRP negative patients, as well as significant decrease in the neutrophil functions (phagocytic index and nitroblue trtrazolium test NBT%) with CRP positive children than CRP negative, as CRP positive conditions are associated with inflammations and chronic infections that alter neutrophil functions. CRP and other acute phase proteins which are elevated in dialysis patients represent the single largest cause of mortality and morbidity in chronic renal failure patients due to impaired immunity**.**

In our study there was significant negative correlation between serum resistin level and neutrophil functions (phagocytosis and oxidative burst) as well as it is highly specific and sensitive in predicting PMNLs functions. *Cohen et al.* [8] found that high uremic resistin levels attenuate chemotaxis and the oxidative burst of PMNLs compared with normal levels, but they did not found a statistically significant difference as regard PMNL phagocytosis of opsonized *E coli.* The difference may be attributed to that [8] Cohen *et al.* [8] using PMNLs of healthy subjects and expose them to high levels of resistin.

5. Conclusion

High resistin levels in children with ESRD decrease phagocytic function and oxidative burst of PMNLs and this effect is enhanced by the progression of the disease and longer duration of dialysis. We recommend targets for pharmacological intervention or new hemodialysis modalities to reduce resistin serum level, consequently improving PMNLs functions in these patients. Further studies are required in a large number of patients to determine the diagnostic implications of resistin in CKD patients.

References

[1] Mayadas, T.N., Cellere, X. and Towell, C.A. (2014) The Multifaceted Functions of Neutrophils. *Annual Review of Pathology*: *Mechanism of Disease*, **9**, 181-218. http://dx.doi.org/10.1146/annurev-pathol-020712-164023

[2] McDonald, B., Urrutia, R., Jenne, C.N. and Kubes, P. (2012) Intravascular Neutrophil Extracellular Traps Capture Bacteria from the Bloodstream during Sepsis. *Cell Host Microbe*, **12**, 324-333. http://dx.doi.org/10.1016/j.chom.2012.06.011

[3] Vaziri, N.D., Pahl, M.V., Crum, A. and Noris, K. (2012) Effect of Uremia on Structure and Function of Immune System. *Journal of Renal Nutrition*, **22**, 149-156. http://dx.doi.org/10.1053/j.jrn.2011.10.020

[4] Mocsai, A. (2013) Diverse Novel Functions of Neutrophils in Immunity, Inflammation and Beyond. *Journal of Excremental Medicine*, **210**, 1283-1299. http://dx.doi.org/10.1084/jem.20122220

[5] Teta, D. (2012) Adipokines as Uremic Toxins. *Journal of Renal Nutrition*, **22**, 81-85.

http://dx.doi.org/10.1053/j.jrn.2011.10.029

[6] Korpela, L.S., Lehtimäki, L., Vuolteenaho, K., Nieminen, R., Saarelainen, H.K.S. and Moilanen, E. (2011) Adipokine Resistin Predicts Anti-Inflammatory Effect of Glucocorticoids in Asthma. *Journal of Inflammation*, **8**, 12. http://dx.doi.org/10.1186/1476-9255-8-12

[7] Patel, S.P. and Raju, P.A. (2013) Resistin in Serum and Gingival Cervical Fluid as a Marker of Periodontal Inflammation and Its Correlation with Single-Nucleotide Polymorphism in Human Resistin Gene at −420. Dental College and Research Institute, Bangalore, 192-197.

[8] Cohen, G., Ilic, D., Raupachova, J. and Ho, W.H. (2008) Resistin Inhibits Essential Functions of Polymorphonuclear Leukocyte. *Journal of Immunology*, **181**, 3761s-3768s. http://dx.doi.org/10.4049/jimmunol.181.6.3761

[9] Muniz-Junqueira, M.I,. Pecanha, L.M., de Silva-Filho, V.L., de Almeida, Cardoso, M.C. and Tosta, C.E. (2003) Novel Micotechnique for Assessment of Postnatal Maturation of the Phagocytic Function of Neutrophil and Monocytes. *Clinical and Diagnostic Laboratory Immunology*, **10**, 1096-1102.

[10] Carneir, O.V.M., Bezerra, A.C., Machado, M. do C., Guimarães, M.D. and Muniz-Junqueira, M.I. (2012) Decreased Phagocytic Function in Neutrophils and Monocytes from Peripheral Blood in Periodontal Disease. *Journal of Applied Oral Science*, **20**, 5. http://dx.doi.org/10.1590/s1678-77572012000500002

[11] Almehed, K., d'Elia, H.F., Bokarewa, M. and Carlsten, H. (2008) Role of Resistin as a Marker of Inflammation in Systemic Lupus Erythematosus. *Arthritis Research & Therapy*, **10**, R15. http://dx.doi.org/10.1186/ar2366

[12] Kader, N.A., Ali El-Din, F.A., Khatab, E.A.A. and Elabd, N.E. (2010) Does Plasma Resistin Level Have a Role in Predicting Inflammatory Bowel Disease Activity? *Indian Journal of Gastroenterology*, **29**, 126-127. http://dx.doi.org/10.1007/s12664-010-0008-1

[13] Nehus, E., Furth, S., Warady, B. and Mitsnefes, M. (2012) Correlates of Resistin in Children with Chronic Kidney Disease: The Chronic Kidney Disease in Children Cohort. *The Journal of Pediatrics*, **161**, 276-280. http://dx.doi.org/10.1016/j.jpeds.2012.01.055

[14] Zaza, G., Granata, S., Rascio, F., Pontrelli, P., Dell, M., Cox, S.H., Pertosa, G., Grandaliano, G. and Lupo, A. (2013) A Specific Immune Transcriptomic Profile Discriminates Chronic Kidney Disease Patients in Predialysis from Hemodialyzed Patients. *BMC Medical Genomics*, **6**, 17. http://dx.doi.org/10.1186/1755-8794-6-17

[15] Guo, H., Liu, J., Collins, A.J. and Foley, R.N. (2008) Pneumonia in Incident Dialysis Patients—The United States Renal Data System. *Nephrology Dialysis Transplantation*, **23**, 680-686. http://dx.doi.org/10.1093/ndt/gfm474

[16] Sardenberg, C., Suassuna, P., Andreoli, M.C., Watanabe, R., Dalboni, M.A., Manfredi, S.R., *et al.* (2006) Effects of Uraemia and Dialysis Modality on Polymorphonuclear Cell Apoptosis and Function. *Nephrology Dialysis Transplantation*, **21**, 160-165. http://dx.doi.org/10.1093/ndt/gfi095

[17] Gómez-Ochoa, P., Sabateb, D., Homedesb, J. and Ferrerc, L. (2012) Use of the Nitroblue Tetrazolium Reduction Test for the Evaluation of Domperidone Effects on the Neutrophilic Function of Healthy Dogs. *Veterinary Immunology and Immunopathology*, **146**, 97-99. http://dx.doi.org/10.1016/j.vetimm.2012.01.018

[18] Libetta, C., Sepe, V., Esposito, P. and Galli, F. (2011) Oxidative Stress and Inflammation: Implications in Uremia and Hemodialysis. *Clinical Biochemistry*, **44**, 1189-1198. http://dx.doi.org/10.1016/j.clinbiochem.2011.06.988

[19] Ghobrial, E.E., Mahfouz, N.N., Fathy, G.A., El Wakkad, A.A. and Sebaii, H.M. (2013) Oxidative Stress in Egyptian Heamodialysis Children. *Iranian Journal of Kidney Diseases*, **7**, 485-491.

[20] Nusken, K.D., Kratzsch, J., Wienholz, V., Stohr, W., Rascher, W. and Dotsch, J. (2006) Circulating Resistin Concentrations in Children Depend on Renal Function. *Nephrology Dialysis Transplantation*, **21**, 107-112. http://dx.doi.org/10.1093/ndt/gfi084

[21] Mehdi, W.A. (2011) Determination of Resistin and Several Antioxidants in Sera of Patients with Chronic Renal Failure. *Australian Journal of Basic and Applied Sciences*, **5**, 196-203.

[22] Abraham, G., Sundaram, V., Sundaram, V., Mathew, M., Leslie, N. and Sathiah, V. (2009) C-Reactive Protein, a Valuable Predictive Marker in Chronic Kidney Disease. *Saudi Journal of Kidney Diseases and Transplantation*, **20**, 811-815.

[23] Shroff, R. (2009) Monitoring Cardiovascular Risk Factors in Children on Dialysis. *Peritoneal Dialysis International*, **29**, S173-S175.

[24] Gheissari, A., Kelishadi, R., Roomizadeh, P., Abedini, A., Haghjooy-Javanmard, S., Abtahi, S.H., *et al.* (2013) Chronic Kidney Disease Stages 3-5 in Iranian Children: Need for a School-Based Screening Strategy: The CASPIAN-III Study. *International Journal of Preventive Medicine*, **4**, 95-101.

[25] Ohtake, T., Ishioka, K., Honda, K., Oka, T., Maesato, K., Mano, T., Ikee, R., Moriya, H., Hidaka, S. and Kobayashi, S. (2010) Impact of Coronary Artery Calcification in Hemodialysis Patients and Association with Prognosis. *Hemodialysis International*, **14**, 218-225. http://dx.doi.org/10.1111/j.1542-4758.2009.00423.x