Oxidative Stress in Hemodialysis Pediatric Patients

Mohamed G. Shouman¹, Samar Sabry², Rasha E. E. Galal³, Emad Salama¹, Aliaa Ahmed Wahby³, Eman Awadallah³, Abeer Selim¹

¹Pediatrics Department, Medical Research Division and Medical Research Centre of Excellence (MRCE), National Research Centre, Cairo, Egypt; ²Pediatrics Department, Pediatric Nephrology Unit, Cairo University, Cairo, Egypt; ³Clinical Pathology Department, Medical Research Division and Medical Research Centre of Excellence (MRCE), National Research Centre, Cairo, Egypt

Abstract

BACKGROUND: Oxidative stress may play a role in complications of hemodialysis patients as atherosclerosis, thrombosis, and inflammation.

AIM: The aim of the study was to evaluate the oxidative stress in hemodialysis pediatric patients through measurement of oxidative stress enzymes as paraoxanase activity (PON), arylesterase activity (ASA), superoxide dismutase (SOD) and also non-enzymatic antioxidant vitamins as vitamins A, C and E levels.

METHODS: The study included 50 hemodialysis pediatric patients with mean age 11.4 ± 5.4 years and 30 normal children of matched sex and age as a control group. Assessment of oxidative stresses was done using ELIZA technique.

RESULTS: SOD, ASA, and vitamin C were significantly lower among hemodialysis patients in comparison to control group (p < 0.001).

CONCLUSION: The study concluded that oxidative stress was common finding in hemodialysis pediatric patients which may play a role in complications encountered among these patients.

Introduction

The global prevalence of chronic renal failure is on the rise in pediatric age group. It constitutes one of the major causes of death. It is associated with oxidative stress which is a significant factor in children suffering from renal failure and it may be partly responsible for complications of the disease as hypertension, anemia, atherosclerosis and related cardiovascular disturbances, neurological disorders, impaired immunity, and hemostatic abnormalities [1], [2], [3], [4], [5].

Oxidative stress is defined as tissue damage resulting from an imbalance between excessive production of free oxygen radical and oxygen scavenger which are responsible for antioxidant activity [6]. The excess generation of reactive oxygen species may be partially due to activation of peripheral polymorphonuclear leucocytes interacting with dialyzer artificial membrane [7]. The ability of cells to scavenge excess reactive species is largely dependent on the efficiency of the overall antioxidant defense system. The antioxidant defense network consists of endogenous and exogenous antioxidants. The endogenous antioxidants comprise the enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and non-enzymatic antioxidants including glutathione (GSH), vitamins A, C and E as well as small molecules. The exogenous antioxidants comprise the micronutrients and other exogenously administered...
Malonyldialdehyde (MDA) is one of the end products of polyunsaturated fatty acid peroxidation in cells which was repetitively measured in many studies, while vitamin E is a major antioxidant in biological systems and acts as a powerful chain-breaking agent because of its ability to scavenge peroxyl radicals. An increase in free radicals causes an overproduction of MDA, which is a marker of oxidative stress, and a reduction in plasma vitamin E levels, which may contribute to the development of oxidative stress conditions. Vitamin E is mainly transported by lipoproteins in the bloodstream. The vitamin E/cholesterol ratio indicates how much vitamin E can be delivered to cell membranes via the LDL receptor [10]. SOD catalyzes dismutation of superoxide to hydrogen peroxide. Hydrogen peroxide, in turn, is converted to water and molecular oxygen by catalase or glutathione peroxidase (GPX) which uses glutathione as a substrate [11].

The oxidation of low-density lipoproteins (LDL) due to oxidative stress conditions is one of the first steps in the atherosclerotic process. Oxidized LDL initiates an inflammatory reaction that ultimately leads to the formation of atherosclerotic plaques. High-density lipoproteins (HDL) have long been known to be antiatherogenic, but their exact mechanism of action has yet to be determined. Paraoxonase 1 (PON1), an enzyme associated with HDL, is thought to play a crucial role in the antioxidative properties of HDL [12].

Unfortunately, studies of oxidative stress in children had limited sample sizes and not enough convincing evidence to prove the causal relation between oxidative stress and disease conditions.

The aim of the study was to evaluate the oxidative stress in hemodialysis pediatric patients through measurement of oxidative stress enzymes as Paraoxonase activity (PON), Arylesterase activity (ASA), superoxide dismutase (SOD) and also non-enzymatic antioxidant vitamins as vitamins A, C and E levels.

Patients and Methods

The study included 50 pediatric patients who had been treated by conventional regular bicarbonate hemodialysis three times weekly using polysulfone filter and Fresenius 4008 dialysis system (Fresenius Medical Care, Hesse, Germany). Thirty normal children of matched age and sex served as a control group were collected from outpatient's clinic. The hemodialysis patients were recruited from the hemodialysis unit of the Centre of Pediatric Nephrology and Transplantation, in Cairo University Children Hospital. The studied patients consisted of 24 (48%) females and 26 (52%) males. The mean age of the study population was 11.4 ± 5.4 (4 -20) years for hemodialysis patients. Patients with evidence of infections, inflammation or malignancy were excluded.

This study protocol and the consents were approved and deemed sufficient by the Ethical Committee of National research Centre and informed written consent was obtained in every case from their legal guardians.

All patients were subjected to full history taking, thorough clinical examination, and laboratory investigations including routine investigations as urine analysis, complete blood picture, blood urea nitrogen, creatinine, serum sodium, potassium, calcium, phosphorus, alkaline phosphatase, SGOT, and SGPT.

Special tests for assessment of oxidative stresses were done including: enzymatic antioxidants as Paraoxonase activity (PON), Arylesterase activity (ASA), superoxide dismutase (SOD) and non-enzymatic antioxidant as serum vitamins A, C and E levels for hemodialysis and control groups. All of them were measured using ELIZA double antibody sandwich technique with the commercial available kits from Glory Science Co., Ltd (Add: 2400 Veterans Blvd. Suite 16-101, Del Rio, TX78840, USA). With catalog #: A16846 (ASA), #: 11086 (SOD), #: 90081 (vitamin C), #: 11345 (vitamin A), #: 11344 (vitamin E), #: 95462 (PON1).

Statistical Analysis

Standard computer program SPSS for Windows, release 13.0 (SPSS Inc., USA) was used for data entry and analysis. All numeric variables were expressed as mean ± standard deviation (SD). Comparison of different variables in various groups was done using Student t test. Pearson’s correlation tests (r = correlation coefficient) were used for correlating numerical variables. For all tests, a probability (P) less than 0.05 are considered significant.

Results

At the time of the study, the mean dialytic time was 55.5 ± 42.64 months (6-180). Descriptive data of studied hemodialysis pediatric patients were represented in (Table 1).

The comparison between hemodialysis and control group as regard oxidative enzymes and antioxidant vitamins revealed decreased all oxidative enzymes in hemodialysis group than healthy subjects where SOD and ASA were highly significantly reduced while PON was not significantly reduced (p = 0.004,
As regard antioxidant vitamins, vitamin C was significantly reduced in hemodialysis group while vitamins A and E were not significantly affected (p > 0.001, 0.163, 0.817) respectively (Table 2).

Table 2: Comparison between hemodialysis and control as regard oxidative stress

| Parameter                  | Hemodialysis group (50 patients) | Control group (30 patients) | t-value | p-value |
|----------------------------|----------------------------------|-----------------------------|---------|---------|
| Enzymatic Oxidative stress |                                  |                             |         |         |
| Arylesterase Activity      | 125.4 ± 18.98                    | 26.45 ± 22.43               | -2.962  | 0.004   |
| Superoxide Dismutase (SOD) | 41.5 ± 42.99                     | 159.6 ± 205.36              | -3.114  | 0.004   |
| Paraoxonase Activity (PON) | 126.83 ± 250.09                  | 232.68 ± 296.52             | -1.708  | 0.092   |
| Non Enzymatic Oxidative Stress |                                  |                             |         |         |
| Vitamin A (nmol/L)         | 427.52 ± 798.58                  | 205.30 ± 603.27             | 1.408   | 0.163   |
| Vitamin C (ng/L)           | 1342.23 ± 1380.12                | 2085.83 ± 465.52            | -3.905  | <0.001  |
| Vitamin E (nmol/L)         | 373.39 ± 107.44                  | 478.55 ± 232.3              | 0.232   | 0.817   |

In hemodialysis group, the correlation between oxidative enzymes, antioxidant vitamins and different parameters revealed positive correlation between vitamin A and age (r = 0.374, P = 0.007).

Table 3: Correlation between studied oxidative stress parameters

|          | VII A | VII C | PON | SOD | ASA |
|----------|-------|-------|-----|-----|-----|
| Vitamin A | r     | -0.115| -0.124| 0.111| -0.067| 0.198|
| p        | 0.248 | 0.380| 0.444| 0.644| 0.169|
| Vitamin C | r     | -0.115| 0.275| 0.554| 0.389| 0.100|
| p        | 0.428 | 0.053| 0.000| 0.005| 0.491|
| Vitamin E | r     | -0.124| 0.275| 0.295| 0.545| 0.154|
| p        | 0.390 | 0.053| 0.037| 0.000| 0.284|
| Paraoxonase | r   | -0.111| 0.554| 0.295| 0.410| 0.028|
| p        | 0.444 | 0.000| 0.037| 0.000| 0.849|
| Superoxide | r | -0.067| 0.389| 0.545| 0.297| 0.037|
| p        | 0.644 | 0.000| 0.000| 0.003| 0.997|
| Dismutase (SOD) | p | 0.198 | 0.010| 0.054| 0.284| 0.397|
| p        | 0.169 | 0.491| 0.284| 0.849| 0.004|

Discussion

Hemodialysis in addition to uremia is characterized by excessive oxidative stress which is due to loss of antioxidants as vitamins C and E, accumulation of oxidative product. During hemodialysis, activation of complement factors, platelets, and polymorphonuclear leucocytes are triggered by dialyzer membrane and dialysate and subsequently reactive oxygen species production. Also inflammatory state and lipid peroxidation, which occurred in hemodialysis, promote formation of oxidative products. Oxidative stress is also triggered by iron infusion, anemia, central venous catheter, bioincompatible dialyzers, and endotoxin challenge [13, 14].

In this study, alteration of oxidative stress and antioxidant activity has been demonstrated among hemodialysis pediatric patients in comparison to control. Antioxidant defense mechanisms have been observed including decreased all studied enzymatic antioxidant while vitamin C is the only affected among non-enzymatic antioxidant vitamins in hemodialysis group in comparison to healthy control subjects where only SOD, ASA, and vitamin C were highly reduced in significant manner.

Superoxide dismutase represents a major defense system against oxidative damage by enzymatically converting O2 to H2O2. Zwoźnirska (2006) study on 21 hemodialysis pediatric patients found that SOD was significantly decreased [15] which is in agreement with our study. Similar to our study, SOD activity was significantly lower in hemodialysis patients whatever children or adults [16, 17, 18, 19, 20, 21, 22]. In contrast to our study, activity of...
SOD has been increased in few studies which were explained by invoking adaptation to the increased rate of oxidation [23], [24], [25]. In Lim (1999) study, no significant difference of Erythrocyte-SOD (E-SOD) level was found between hemodialysis and control but the plasma level of SOD was increased in hemodialysis in comparison to control [26].

Paraoxonase (PON) is another marker of antioxidant activity. It is esterase enzyme associated with HDL and functions to protect LDL and HDL from oxidation. PON activities are decreased in subjects with renal failure with increased risk of cardiovascular disease especially the hemodialysis patients which had the lowest activity. In contrast to previous studies that revealed reduced paraoxonase levels than control [27], [28], [29], [30], [31], [32]; our results revealed insignificant reduction of PON which was used as a marker of antioxidant activity in hemodialysis patients in comparison to controls.

Human arylesterase enzyme is a member of the paraoxonase family that has a protective effect against lipoprotein oxidation in CKD through hydrolysis of organophosphate compounds. Also arylesterase displays Hcy-thiolactonase activity and poses antiatherogenic properties. Similar to our study, few studies reported that ASA decreased in CKD patients on hemodialysis when compared to healthy controls [28], [33], [34].

Vitamin E exerts its effect as antioxidant through interruption of the radical cascade to protect cell membranes from lipid peroxidation by forming a low-reactivity vitamin that attack lipid substrate. Vitamin E can protect the cell membrane against free radicals induced oxidative damage by LDL in biological membranes [35]. In this study, the vitamin E levels were insignificantly increased. Similar to our study, Nguyen-Khoa (2001) study showed that plasma vitamin E levels were insignificantly different in hemodialysis patients and control subjects [18], while in Locatelli et al., (2003) study, the intracellular levels of vitamin E were significantly reduced [3]. Also Zwolinska et al., (2006) study revealed significant reduction of plasma vitamin E levels among hemodialysis patients in comparison to control while the erythrocyte vitamin E levels of HD pediatric patients were not different [36]. The reduced level of vitamin E can be explained by reduced dietary intake, malnutrition, and loss during hemodialysis. In contrast to previous studies, Joyce et al., (2018) study revealed that hypervitaminosis E was present in 87% of pediatric patients [37].

Vitamin C is considered as a potent antioxidant in biological fluids and thus prevents oxidative damage to important biological macromolecules. As regard vitamin C levels, Francesco Locatelli and Zwolinska et al., [3], [36] studies showed reduced vitamin C levels which coincide with our results and can be explained by reduced dietary intake of fresh fruits and vegetables to avoid hyperkalemia, malnutrition and loss of the vitamin during hemodialysis with clearance rate 30%-53% and losses from 80 to 280 mg per session [3], [36], [38], [39].

As regard vitamin A level, hypervitaminosis A is present in hemodialysis pediatric patients as in CKD patients and is associated with hypercalcemia among hemodialysis [40]. In Joyce et al., (2018) study, hypervitaminosis A was present in 94% of pediatric patients [37]. Hypervitaminosis A is explained by homeostatic dysregulation of its plasma carrier, the retinol binding protein [41]. Only one study done by Zwolinska (2006) revealed reduced levels of plasma vitamin A [36] but in our study no significant difference was found between hemodialysis patients and control.

The correlation between different parameters of this study revealed positive correlation between different antioxidant which means that oxidative stress may act synergistically to increase cardiovascular morbidity and mortality risk in maintenance hemodialysis patients.

There are few limitations to our study findings:
1. The nutritional data is not collected from our patients to assess the amount of vitamins ingestion; 2. There is wide range of variability in enzymes and vitamins levels.

The study concluded that oxidative stress was common finding in hemodialysis pediatric patients who may play a role in complications as atherosclerosis and adequate vitamin supplementation may be recommended and have therapeutic potential.

Acknowledgements

Authors thank National Research Centre for funding this research. And also thank Pediatric Nephrology Unit, Cairo University for their collaboration during this research.

References

1. Deicher R, Horl WH. Vitamin C in chronic kidney disease and hemodialysis patients. Kidney Blood Press Res. 2003; 26:100-106. https://doi.org/10.1159/000070991 PMid:12771534
2. Vaziri ND, Dicus M, Ho ND, Boroujerdi-Rad L, Sindhu RK. Oxidative stress and dysregulation of superoxide dismutase and NADPH oxidase in renal insufficiency. Kidney Int. 2003; 63:179-185. https://doi.org/10.1046/j.1523-1755.2003.00702.x PMid:12472781
3. Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Warner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. Nephrology Dialysis
Antioxidant enzymes show adaptation to oxidative stress in athletes and increased stress in hemodialysis patients. Therapeutic Apheresis and Dialysis. 2009; 13(4):300-5. https://doi.org/10.1111/j.1744-9887.2009.00728.x PMid:19659063

21. Meerashivashake WK, Revathi R. Padmanaban. Effect of oxidative stress in pre and post hemodialysis patients with chronic renal failure patients. Int J Biol Med Res. 2012; 3(1):1335-7.

22. El-Shafei AM, El-Mashad GM, Azzam AA. Oxidative stress markers in children with end stage renal disease. Journal of Clinical Pediatric Nephrology. 2014; 2(2).

23. Toborek M, Wasik T, Drzdóć M, Klin M, Magner-Wróbel K, Kopieczenia-Grzebienia E. Effect of haemodialysis on lipid peroxidation and antioxidant system in patients with chronic renal failure. Metabolism. 1992; 41:1229-1232.

24. Trznadel K, Luciak M, Pawlicki L, Kędziora J, Błaszczyk J, Buczyński A. Superoxide anion generation and lipid peroxidation processes during hemodialysis with reused cuprophan dialyzers. Free Rad Biol Med. 1992; 8:429-432. https://doi.org/10.1016/0891-5849(90)90055-N

25. Aysun Hacîşevki. Effect of oxidative stress on muscle in patients with chronic renal failure. J Fac Pharm Ankara. 2008; 37(2):990-100. https://doi.org/10.1501/Eczfak_0000000493

26. Paik-Seong Lim, Yau-Huei Wei, York Leng Yu, Benny Kho: Enhanced oxidative stress in hemodialysis patients receiving intravenous iron therapy. Nephrol Dial Transplant. 1999; 14:2680-87. https://doi.org/10.1093/ndt/14.11.2680 PMid:10534512

27. Johnson-Davis KL, Fernelius C, Elison NB, Wilson A, Beddhu S, Roberts WL. Blood enzymes and oxidative stress in chronic kidney disease: A cross sectional study. Annals of Clinical & Laboratory Science. 2011; 41(4):331-9.

28. Gugliucci A, Kotani K, Kimura S. Paraoxonase 1 in Chronic Kidney Failure: Review Article. Journal of Lipids. 2012; Article ID 726048. https://doi.org/10.1155/2012/726048 PMid:22523692 PMCID:PMC317201

29. Kannamazhu J, Darling PB, Maguire GF, Donnelly S, McFarlane P, Chan CT, Connelly PW. Paraoxonase 1 arylesterase activity and mass are reduced and inversely related to C-reactive protein in patients on either standard or home nocturnal hemodialysis. Clinical nephrology. 2010; 73(2):131-8.

30. El-Behairy HF, Elmenshawy AA, Khalifa NM, Elsawy DH, Eid SR, Salem KA, et al: Determination of the Antiatherogenic Role of Paraoxonase-1 in Uremic Children on Hemodialysis. Journal of Applied Sciences Research. 2013; 9(4):2994-3000.

31. Prakash M, Phani NM, Kayara V, Supriya M. Paraoxonase: Its antiatherogenic role in chronic renal failure. Indian journal of nephrology. 2010; 20(1):9-14. https://doi.org/10.4103/0971-4065.62088 PMid:20535264 PMCID:PMC2878404

32. Sawant J, Nair S, Redkar N. Oxidative stress and serum paraoxonase activity in patients on maintenance hemodialysis. The Internet Journal of Nephrology. 2009; 6(1). https://doi.org/10.5580/6f

33. Sung CC, Hsu YC, Chen CC, Lin YF, Wu CC. Oxidative stress and nucleic acid oxidation in patients with chronic kidney disease. Oxidative medicine and cellular longevity. 2013; 2013.

34. Tucker PS, Dalbo VJ, Han T, Kingsley M. Clinical and research markers of oxidative stress in chronic kidney disease. Biomarkers. 2013; 18(2):103-15. https://doi.org/10.3109/1354750X.2012.749302 PMid:23339563

35. Descamps-Latscha B, Drüeke T, Wilko-Sarsat V. Dialysis-induced oxidative stress: biological aspects, clinical consequences, and therapy. Seminars in Dialysis. 2001; 14(3):193-199. https://doi.org/10.1046/j.1525-193X.2001.00952.x PMid:11422926

36. Zwalinska D, Grzeszczak W, Szczepanska M, Kiliś-Pstrusinska K, Szpyrny K. Vitamins A, E and C as non-enzymatic antioxidants and their relation to lipidperoxidation in children with
chronic renal failure. Nephron Clin Pract. 2006; 103:c12-8. 
https://doi.org/10.1159/000090506 PMid:16374033

37. Joyce T, Wallace D, Reid CJ, Sinha MD. Trace element and vitamin concentrations in paediatric dialysis patients. Pediatr Nephrol. 2018; 33:159-165. https://doi.org/10.1007/s00467-017-3773-6 PMid:28799141

38. Handelman GJ. Vitamin C deficiency in dialysis patients—are we perceiving the tip of an iceberg? Nephrol Dial Transplant. 2007; 22:328-331. https://doi.org/10.1093/ndt/gfl534 PMid:17107966

39. Kosmadakis G, Da Costa Correia E, Carceles O, Somda F, Aguilera D. Vitamins in dialysis: who, when and how much?. Renal failure. 2014; 36(4):638-50. https://doi.org/10.3109/0886022X.2014.882714 PMid:24502653

40. Manickavasagar B, McArdle AJ, Yadav P, Shaw V, Dixon M, Blomhoff R, O'Connor G, Rees L, Ledermann S, van't Hoff W, Shroff R. Hypervitaminosis A is prevalent in children with CKD and contributes to hypercalcemia. Pediatric Nephrology. 2015; 30(2):317-25. https://doi.org/10.1007/s00467-014-2916-2 PMid:25119682 PMCid:PMC4282719

41. Holden RM, Ki V, Morton AR, Clase C. Fat-soluble vitamins in advanced CKD/ESKD: a review. Semin Dial. 2012; 25:334-43. https://doi.org/10.1111/j.1525-139X.2012.01084.x PMid:22607215