Original article:

Plasma level of protein modification and inflammatory markers in snakebite induced acute kidney injury patients undergoing haemodialysis - An observational study

Debarati Mukherjee[1], Raghwendra Mishra[1,2], Priyabrata Das[3], Soma Mukhopadhyay[3], Ashis Mukhopadhyay[3], Manoj Kar[4], Roshnara Mishra[1], Pinaki Mukhopadhyay[5#].

[1] Department of Physiology, University of Calcutta, Kolkata-700009, India

[2] Department of Physiology, Ananda Mohan College, Kolkata-700009, India.

[3]Netaji Subhas Chandra Bose Cancer Research Institute, Kolkata 700016, India

[4]Ex-Biochemist, N.R.S Medical College and Hospital, Kolkata-700014, India

[5]Department of Nephrology, N.R.S Medical College and Hospital, Kolkata-700014, India

[#]Corresponding Author: Dr.PinakiMukhopadhyay

Abstract

Hemodialysis is one of the therapeutic mode of renal failure. Our study aims to evaluate its effect on plasma level of protein modification markers and inflammatory markers in snake-bite mediated acute kidney injury (SAKI) patients. The plasma level of methylglyoxal, advanced oxidation protein products (AOPP), advanced glycation end products (AGE), and other protein modification markers and inflammatory markers-soluble receptor for AGE (sRAGE) and TGFβ1 were found to be significantly elevated in SAKI patients. Methylglyoxal and AOPP shows a decreasing pattern during first few sessions ($p<0.05$) but increases gradually thereafter. Plasma AGE and advanced lipid peroxidation end products (ALE) does not shows any significant variation whereas dityrosine, pentosidine and other protein medication markers were found to be increased across the dialysis session ($p<0.05$). Among the inflammatory makers sRAGE does not show any improvement but plasma TGFβ1 level were found to be significantly reduced ($p<0.05$) after hemodialysis. These findings suggest that although hemodialysis of SAKI patients improves some markers but fails to normalize other stress and inflammatory markers and during last session all except TGFβ1 level were found to be elevated compared to first hemodialysis session.

Keywords: Snakebite induced acute kidney injury, Hemodialysis, Protein modification, Soluble receptor for advanced glycation end product, Transforming growth factor β1.

Introduction

WHO has recognized snake bite associated morbidity and mortality as one of the major neglected tropical disease (Warrell, 2010a). Mortality due to snake bite cannot be controlled by antivenom alone, without improving associated infrastructure required for supportive treatment. Acute kidney injury is one of the major clinical complication associated with snake envenomation, especially of vasculotoxic nature. 15% to as high as 39.08% (Patil and Bansod, 2012; Dharod et al., 2013) snake bite victims die due to snake bite induced acute kidney injury (SAKI). Few SAKI cases are reversible as in patients with patchy cortical necrosis but according to WHO guideline (Warrell, 2010b) patients with diffuse cortical necrosis should be given regular maintenance dialysis. The guideline mentioned that dialysis should be given in presence of - (a) Clinical uraemia (b) Fluid overload (c) blood biochemistry, one or more of the following- creatinine >4 mg/dl, urea >130 mg/dl, potassium >7 mmol/l (or hyperkalaemic ECG changes) and symptomatic acidosis. Hemodialysis has been reported to

considerably improve outcome of patients suffering from SAKI in Burma (Aye et al., 2017) and Sri Lanka (Kularatne, 2003). However, in practice the issue of dialysis in SAKI has to be critically appraised to improve the outcome from present statistics.

In developing countries with more than 40% of the population below the International Poverty Line, hemodialysis is considered an expensive treatment. Hence, despite all controversies regarding reuse of the dialysis membrane, reused membrane is a practical and cost-effective compromise considering60% of world population is dependent on reused membranes (Dhrolia et al., 2014). However, the advantages do not outnumber the associated disadvantages. Higher mortality risk from symptoms other than hemodynamic complications is increasingly being associated with dialysis. Therefore, expanding thrust on dialysis membrane to its physiological effects as inflammatory or stress response to reuse of dialyzers are being reassessed. Reuse efficiency of different types of membrane components (Rao et al, 2004), reagents for washing (Lacson et al, 2011), high flux vs low flux membranes (Palmer et al, 2012), surface structure change (Batina et al, 2013) or relative efficiency for specific molecules (Yang et al, 2009) has been studied. However, these studies have focused on stress and inflammatory changes in chronic kidney disease patients on maintenance hemodialysis, there are no such reports on AKI patients undergoing hemodialysis to best of our knowledge. Though hemodialysis reportedly decreases creatinine level in SAKI patients whether it causes some other stress and/or inflammation, was not studied so far.

Earlier we have reported elevated oxidative and carbonyl stress and associated protein damage in SAKI patients (Mukhopadhyay et al., 2016), in this work we have studied the variation in plasma level of carbonyl stress and associated protein modification markers and their downstream inflammation modulators among SAKI patients during successive hemodialysis session.

Aims: To evaluate the effect of hemodialysis on plasma level of protein modification markers and inflammatory markers in snake-bite mediated acute kidney injury (SAKI) patients.

Materials and Methods

Sample Collection

Blood sample were collected in heparinised vials from 36 normal healthy subjects, randomly selected with no proteinuria, diabetes or recent history of renal impairment. About 38 SAKI patients admitted in NRS Medical college were enrolled randomly who were undergoing hemodialysis (HD) using a biocompatible polysulfone hollow-fiber dialyzer .Clinical histories and related biochemical investigation of all patients were documented. The cause of acute renal failure was snake bite (serum creatinine concentration > 2.5mg/dl). In a sequence of 6 consecutive HDcarried out on patients, whose blood sample were collected during the study period, it was observed that 38, 34,24,17,13, and 8 patients received 1^{st}, 2^{nd},3^{rd},4^{th}, 5^{th} and 6^{th} dialysis respectively. Plasma was separated by centrifuging the heparinised blood at 5000 rpm for 5 minute and used for biochemical markers of carbonyl stress and associated protein damage. For immunological assay plasma samples of the patients collected prior to first haemodialysis session (n=42) and at the end of last dialysis(n=34) were used. Twelve healthy subjects were considered as a control group for the above immunoassaystudy.The study was approved by institutional ethical committee of N.R.S. Medical College & Hospital, Kolkata.

2.2 **Estimation of biochemical parameters**

Plasma concentrations of the following parameters of both normal and HD patients were estimated. MG was estimated spectrophotometrically, following Ghosh *et al.*, (2006) method. AOPP was determined according to

the method of Witko-Sarsat*et al.* (1996), the result was expressed as µM Chloramin T equivalent. AGE (Münch et al., 1997), Pentosidine, advanced lipid peroxidation end products (ALE) (Meng*et al.*, 2000), dityrosine (Giulivi& Davies, 1993), argpyrimidine (Gomes et al., 2005)and fluorescent imidazolone-like product (Wittmann et al., 1999) were measured from plasma by spectrofluorometric technique after hundred times dilution with phosphate buffer saline (20mM, pH=7.4) and expressed as arbitrary unit/mg protein. Plasma protein and creatinine estimation were done by commercial kit according to manufacturer's guidelines.Soluble receptor for AGE (sRAGE) and transforming growth factor β1 (TGFβ1) levels were detected by an enzyme-linked immunosorbent assay (ELISA) kit (R&D, USA) according to manufacturer's guideline.

2.3 **Statistical Analysis**

The results of different assays of HD patients and normal control were analyzed statistically and represented as mean±S.E using bar diagram. Oneway ANOVA followed by Tukey HSD *post hoc* analysis was performed to check any statistical difference, significance level was considered at $p< 0.05$.

3. **Result**

Hemodialysis,the preferred renal replacement therapy for SAKI, does not show any significant improvement of the studied markers of carbonyl stress (MG) and related protein modification markers (AOPP, AGE. dityrosine, pentosidine, ALE, argpyrimidine and protein imidazolone) (Figure 1a-h). Plasma MG level of SAKI patients was found to be increased by about 3.8 time ($p<0.001$) compared to healthy control. MG shows a decreasing pattern over first three sessions of HDthough insignificantly and remain significantly higher than control level ($p<0.001$).It starts increasing afterward and shows highest level during last session (about 4.5 times of control; $p<0.001$) (Figure 1a). Plasma AOPP was found to be elevated throughout HD session ($p<0.001$) compared to healthy control (figure 1b). The variation across HD session was found to be random and insignificant. The highest AOPP level was found during the third HD session (about 2.3 times of control) the mean AOPP level at sixth HD session was noted to be 1.93 times higher than the control. All the other protein modification markers, except argpyrimidine and imidazolone, shows insignificant but increasing pattern, though random, across the HD sessions ($p<0.001$). AGE and ALE remain elevated (about 1.6-1.9 times of control; $p<0.001$)) across HD session (Figure 1c and 1f). Pentosidine and dityrosine level shows similar pattern across HD session (Figure 1d and 1e). Their level shows minimal variation during first three session of HD but increases gradually thereafter ($p<0.001$) compared to control and predialysis level in SAKI patients. Their highest level was noted during terminal HD session (2 and 2.8 times of control respectively). Argpyrimidine and imidazolone shows gradual increasing pattern across HD session ($p<0.05$) (Figure 1g and 1h). Their highest concentration was noticed at sixth HD session which is about 1.25 and 1.75 times higher compared to predialysis level in SAKI patients ($p<0.001$). PlasmasRAGE, both pre and post dialysis were found to be significantly elevated compared to healthy control ($p<0.001$) and shows a similar increasing tendency as indicated by elevated post-dialysis sRAGE level, though insignificantly compared to pre-dialysis level in SAKI patients (Figure 2a). Plasma TGFβ1 level was also found to be significantly higher in both pre and post dialysis plasma sample compared to healthy control level ($p<0.001$). In contrast to all other parameters, TGFβ level were found to be significantly low in post-dialysis sample compared to pre-dialysis sample ($p<0.001$) (Figure 2b).

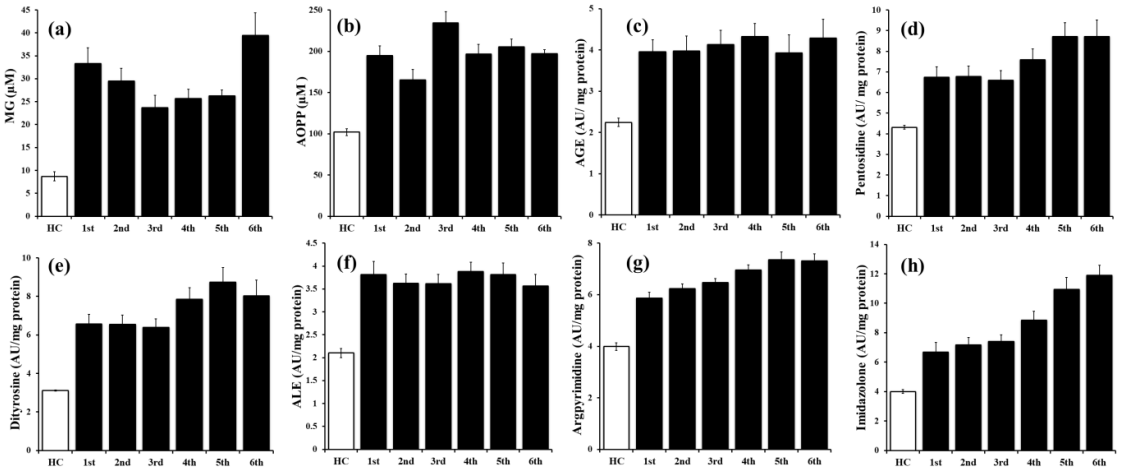

Figure 1: Bar diagram representing plasma levels of the studied stress and protein modification markers across the hemodialysis session. (a) Methylglyoxal (µM); (b) AOPP (µM Chloramine T equivalent); (c) AGE (AU/mg of protein); (d) ALE (AU/mg of protein); (e) Dityrosine (AU/mg of protein); (f) pentosidine (AU/mg of protein); (g) Argpyrimidine (AU/mg of protein) and (h) fluorescent imidazolone-like productfluorescent imidazolone-like product. Numbers 1^{st} – 6^{th} represent respective hemodialysis session, HC-Healthy control.

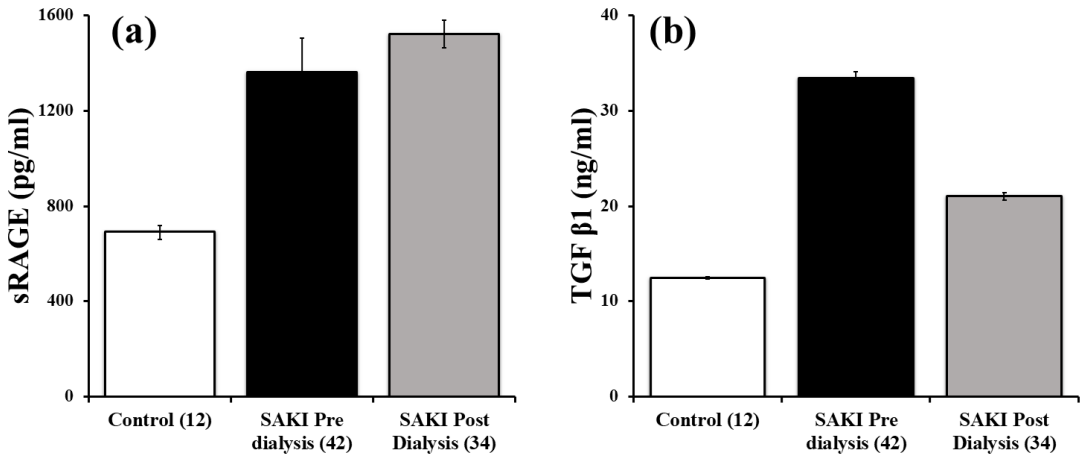

Figure 2: Bar diagram representing plasma levels of inflammatory markers- (a) sRAGE (pg/ml) and (b) TGFβ1 (ng/ml). Number in the parenthesis represents the respective sample size.

4. Discussion

Hemodialysis has been linked to secondary rise in inflammatory markers as polymorphonuclear leucocytes (Rao et al., 2004); CRP and IL-6 (Jofré et al., 2006)to stress markers as**,** serum lipid peroxidation (Velayeti et al., 2016), which results from interaction with elevated reactive oxygen species. Treatment with antioxidants as Silymarin, a herbal medicine (Firuzi et al., 2016) or unfermented grape juice (Corredor et al., 2016) in dialysis patients have been proven beneficial. Stępniewska et al. (2016) have reported that the duration of renal replacement therapy significant changes platelet antioxidative enzymes activities and concentration of GSH,

leading to thrombotic complications. Gonzalez et al. (2015) have studied cardiovascular complication resulting from dialysis and have mention the role of advanced oxidation protein products (AOPPs) in this respect.

Snakebite induced acute kidney injury has been associated with oxidative stress and carbonyl stress (Mukhopadhyay et al., 2016), which seem to be aggravated by dialysis. In the present study we have found significant increase in plasma MG level in SAKI patients. In consecutive dialysis MG level though reduces but does not return to the control value and remain higher during consecutive hemodialysis session. However, marked increase of MG in later stages of dialysis indicates that hemodialysis does not improve carbonyl stress marker level.

AOPP belong to the compounds that could not only be exquisite marker of oxidative stressbut also active mediators of the improve the inflammation associated with the uremic state. AOPP has been linked with dialysis related complications (Witko-Sarsat*et al.*, 2003), which was elevated in SAKI patients receiving dialysis. AOPP level shows only a transitory dip at second dialysis (Figure 1b) but remain elevated thereafter in SAKI patients during the course of HD. This elevation in plasma AOPP during HD session may be attributed to neutrophil activation due tto dialysis membrane (Witko-Sarsat*et al.*, 2003; Rao et al., 2004). MG along with other lipid peroxidation end products, viz. malondialdehyde, hydroxynonenoletc. also results in non-enzymatic glycation of proteinthrough Maillard reactionproducing AGE and ALEproducts (Miyata *et al.*, 2000; Ramasamy*et al*., 2012). Both AGE and ALE and other fluorescent protein modification markers were found to be elevated at all stages of hemodialysis, representing the continuous presence of carbonyl and oxidative stress and inadequate removal of modified protein which is in accordance with other studies in this field (Agalou et al., 2003) and may be attributed to adverse outcome in SAKI patients.

In the downstream of AOPP and AGE, receptor for AGE (RAGE) and TGF β1 are well documented in the literature for their role in progression of renal impairment. Consistent with the rise of stress markers the inflammatory marker sRAGE rises after dialysis which is in accordance with other studies (Borazan et al., 2004; Jofré et al., 2006). However, TGFβ1 decreases after dialysis. TGF-β1, the anti-inflammatory cytokine and thekey driver of fibrosis, has been found to be depleted levels in hemodialysis patients as compared to normal population (Füth et al., 2004; Knerr et al., 2005). However, the constitutive production of it from monocyte is under the influence of several factors and should be studied in detain in relation to hemodialysis.

Conclusion: The stress and inflammatory markers increase significantly even after hemodialysis in SAKI patients and the levels of some markers were remain unaltered and not affected by the sessions of hemodialysis. On going inflammatory cascade or hypercatabolic state may contribute. Single use dialyzer membrane or hemodiafiltration may show improvement but need more study. The level of these intrinsic toxins/markers may be monitored to check the efficacy of hemodialysis in SAKI patients. Uses of some potent antioxidants and carbonyl quenchers, such as N-acetyl cysteine which is known for their therapeutic potentials can also be considered for better outcome.

Abbreviations

AGE- advanced glycation end products, ALE- advanced lipid peroxidation end products, AOPP-advanced oxidation protein product, AKI- acute kidney injury, HD- hemodialysis, MG-methylglyoxal, SAKI-snake-bite mediated acute kidney injury, SE- standard error of mean.

Acknowledgement:

This work is supported by West Bengal State Department of Science & Technology, Kolkata, West Bengal, India [Grant numbers: 647(Sanc.)/ST/P/S&T/9G-7/2009 Dated 29.01.2010 & 368(Sanc.)/ST/P/S&T/9G-7/2009 Dated 16.09.2011].

References

1. Agalou S, Ahmed N, Dawnay A, Thornalley PJ. Removal of advanced glycation end products in clinical renal failure by peritoneal dialysis and haemodialysis. BiochemSoc Trans. 2003 Dec;31(Pt 6):1394-6.
2. Aye KP, Thanachartwet V, Soe C, Desakorn V, Thwin KT, Chamnanchanunt S, Sahassananda D, Supaporn T, Sitprija V. Clinical and laboratory parameters associated with acute kidney injury in patients with snakebite envenomation: a prospective observational study from Myanmar. BMC Nephrol. 2017 Mar 16;18(1):92. doi: 10.1186/s12882-017-05100.
3. Batina, N.; Acosta García, M.C.; Avalos Pérez, A.; Alberto Ramírez, M.; Franco, M.; Pérez Gravas, H.; Cadena Méndez, M. Structural changes of polysulfone membrane use for hemodialysis in the consecutive regime: nanometric analysis by AFM. 4th International Conference on Smart Materials and Nanotechnology in Engineering, 2013, 8793, 87931Q-87931Q.DOI: 10.1117/12.2027844.
4. Borazan A, Ustün H, Ustundag Y, Aydemir S, Bayraktaroglu T, Sert M, Yilmaz A. The effects of peritoneal dialysis and hemodialysis on serum tumor necrosis factor-alpha, interleukin-6, interleukin-10 and C-reactive-protein levels. Mediators Inflamm. 2004 Jun;13(3):201-4.
5. Corredor Z, Rodríguez-Ribera L, Coll E, Montañés R, Diaz JM, Ballarin J, Marcos R, Pastor S. Unfermented grape juice reduce genomic damage on patients undergoing hemodialysis.FoodChemToxicol. 2016 Jun;92:1-7. doi: 10.1016/j.fct.2016.03.016.
6. Dharod MV, Patil TB, Deshpande AS, Gulhane RV, Patil MB, Bansod YV. Clinical predictors of acute kidney injury following snake bite envenomation. N Am J Med Sci. 2013 Oct;5(10):594-9. doi: 10.4103/1947-2714.120795.
7. Dhrolia MF, Nasir K, Imtiaz S, Ahmad A. Dialyzer reuse: justified cost saving for south Asian region. J Coll Physicians Surg Pak. 2014 Aug;24(8):591-6. doi: 08.2014/JCPSP.591596.
8. Firuzi O, Khajehrezaei S, Ezzatzadegan S, Nejati M, Jahanshahi KA, Roozbeh J. Effects of silymarin on biochemical and oxidative stress markers in end-stage renal disease patients undergoing peritoneal dialysis.Hemodial Int. 2016 Oct;20(4):558-563. doi: 10.1111/hdi.12413.
9. Füth R, Herder C, Förster S, Müller-Scholze S, Kruse N, Rieckmann P, Heinig A, Koenig W, Scherbaum WA, Kolb H, Martin S. Evaluation of diagnostic relevance of mRNA levels in peripheral blood: predictive value for mortality in hemodialysis patients. Cytokine. 2004 Sep 21;27(6):166-72.
10. Ghosh M, Talukdar D, Ghosh S, Bhattacharyya N, Ray M, Ray S. In vivo assessment of toxicity and pharmacokinetics of methyl glyoxal. Augmentation of the curative effect of methyl glyoxal on cancer bearing mice by ascorbic acid and creatine. ToxicolAppl Pharm. 2006; 212: 45–58.

11. Gomes R, Sousa Silva M, Quintas A, Cordeiro C, Freire A, Pereira P, Martins A, Monteiro E, Barroso E, Ponces Freire A. Argpyrimidine, a methylglyoxal-derived advanced glycation end-product in familial amyloidotic polyneuropathy. Biochem J. 2005 Jan 15;385(Pt 2):339-45.
12. Gonzalez E, Bajo MA, Carrero JJ, Lindholm B, Grande C, Sánchez-Villanueva R, Del Peso G, Díaz-Almirón M, Iglesias P, Díez JJ, Selgas R. An Increase of Plasma Advanced Oxidation Protein Products Levels Is Associated with Cardiovascular Risk in Incident Peritoneal Dialysis Patients: A Pilot Study. Oxid Med Cell Longev. 2015;2015:219569. doi: 10.1155/2015/219569.
13. Giulivi C, Davies KJA. Dityrosine and tyrosine oxidation products are endogenous markers for the selective proteolysis of oxidatively modified red blood cell hemoglobin by (the 19 S) proteasome. J Biol Chem. 1993; 268: 8752-9.
14. Jofré R, Rodriguez-Benitez P, López-Gómez JM, Pérez-Garcia R. Inflammatory syndrome in patients on hemodialysis. J Am SocNephrol. 2006 Dec;17(12 Suppl 3):S274-80.
15. Knerr K, Füth R, Hemsen P, Mohné W, Heinig A, Kleophas W, Scherbaum WA, Martin S.Chronic inflammation and hemodialysis reduce immune competence of peripheral blood leukocytes in end-stage renal failure patients.Cytokine. 2005 May 7;30(3):132-8.
16. Kularatne SA. Epidemiology and clinical picture of the Russell's viper (Daboiarusseliirusselii) bite in Anuradhapura, Sri Lanka: a prospective study of 336 patients. Southeast Asian J Trop Med Public Health. 2003 Dec;34(4):855-62.
17. Lacson E Jr, Wang W, Mooney A, Ofsthun N, Lazarus JM, Hakim RM. Abandoning peracetic acid-based dialyzer reuse is associated with improved survival.Clin J Am SocNephrol. 2011 Feb;6(2):297-302. doi: 10.2215/CJN.03160410.
18. Meng J, Sakata N, Takebayashi S. Increased glycoxidation and lipoperoxidation in the collagen of myocardium in hemodialysispatients.Cardiovasc Res. 2000; 47: 306-13.
19. Miyata T, Ueda Y, Saito A, Kurokawa K. 'Carbonyl stress' and dialysis-related amyloidosis. Nephrol Dial Transplant. 2000;15 Suppl 1:25-8.
20. Mukhopadhyay P, Mishra R, Mukherjee D, Mishra R, Kar M. Snakebite mediated acute kidney injury, prognostic predictors, oxidative and carbonyl stress: A prospective study. Indian J Nephrol. 2016 Nov-Dec;26(6):427-433.
21. Münch G, Keis R, Webels A, Riederer P, Bahne U, Heidland A, Niwa T, Lemke HD, Schinzel R. Determination of advanced glycation end products in serum by fluorescence spectroscopy and competitive ELISA. Eur J ClinChemClinBiochem. 1997; 35: 669-77.
22. Palmer SC, Rabindranath KS, Craig JC, Roderick PJ, Locatelli F, Strippoli GFM. High-flux versus low flux membranes for end-stage kidney disease. Cochrane Database Syst Rev. 2012 Sep 12;(9):CD005016. doi: 10.1002/14651858.CD005016.pub2.
23. Patil TB, Bansod YV. Snake bite-induced acute renal failure: A study of clinical profile and predictors of poor outcome. Ann Trop Med Public Health 2012;5:335-9.
24. Ramasamy R, Yan SF, Schmidt AM. Advanced glycation endproducts: from precursors to RAGE: round and round we go. Amino Acids. 2012 Apr;42(4):1151-61. doi: 10.1007/s00726-010-0773-2.

25. Rao M, Guo D, Jaber BL, Sundaram S, Cendoroglo M, King AJ, Pereira BJ, Balakrishnan VS; HEMO Study Group. Dialyzer membrane type and reuse practice influence polymorphonuclear leukocyte function in hemodialysis patients. Kidney Int. 2004 Feb;65(2):682-91.
26. Stępniewska J, Dołęgowska B, Cecerska-Heryć E, Gołembiewska E, Malinowska-Jędraszczyk A, Marchelek-Myśliwiec M, Ciechanowski K. The activity of antioxidant enzymes in blood platelets in different types of renal replacement therapy: a cross-sectional study.IntUrolNephrol. 2016 Apr;48(4):593-9. doi: 10.1007/s11255-015-1204-9.
27. Velayeti J, Mansourian AR, Mojerloo M, MarjaniA.Evaluation of oxidative stress and thyroid hormone status in hemodialysis patients in Gorgan.Indian J EndocrinolMetab. 2016 May-Jun;20(3):348-53. doi: 10.4103/2230-8210.179986.
28. Warrell DA. Snake bite. Lancet. 2010a Jan 2;375(9708):77-88. doi:10.1016/S0140-6736(09)61754-2.
29. Warrell DA. WHO/SEARO Guidelines for the clinical management of snakebite in the Southeast Asian Region. SE Asian J Trop Med Pub Hlth 1999; 30:1-85.
30. Warrell DA. World Health Organization. Guidelines for the Management of Snake-bites. Geneva, Switzerland: World Health Organization, Regional Office for South-East Asia; 2010b.
31. Witko-Sarsat V, Friedlander M, Capeillere-Balndin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, Jungers P, Descamps-Latscha B. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int. 1996; 49: 1304-13.
32. Witko-Sarsat V, Gausson V, Nguyen AT, Touam M, Drüeke T, Santangelo F, Descamps-Latscha B. AOPP-induced activation of human neutrophil and monocyte oxidative metabolism: a potential target for N-acetylcysteine treatment in dialysis patients. Kidney Int. 2003 Jul;64(1):82-91.
33. Wittmann I, Wagner Z, Pótó L, Wagner L, Mazák I, Nagy J. Detection of glycation end products in the urine of diabetic patients. Orv Hetil. 1999 Sep 5;140(36):1997-2001. [Article in Hungarian]
34. Yang Y, Xu X, Wang X, Zhu G.Species and characteristics of protein adsorption on reused hemodialysis membranes.Sheng Wu Yi Xue Gong Cheng XueZaZhi. 2009 Feb;26(1):67-71.