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RESEARCH ARTICLE

BETA – 2 MICROGLOBULIN LEVEL IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Abstract

Background: Chronic kidney disease (CKD) is characterized by the retention of toxins that contribute to the associated morbidity and mortality. The heterogeneity of uremic toxins with respect to their molecular weight and protein binding may affect their removal by hemodialysis (HD).

Objective: The aim of our work is to determine the serum level of ß2-microglobulin (ß2-m) in patients with CKD on regular hemodialysis and its relation to dialysis-related amyloidosis (DRA) manifestatins and the factors affecting it.

Methods: This is cross-sectional cross-over, hospital based study was conducted on thirty cases, twenty cases from the pediatric nephrology department in Benha University Hospital & ten cases from Menouf Hospital. All patients with end-stage renal disease (ESRD) who were on maintenance HD for more than three months were included in the study. Demographic data were collected and details of dialysis (type of dialyzers, dialysate bath, membrane used) & frequency for attendance of dialysis session were recorded. Blood samples were drawn from all patients for hematological (hemoglobin, hematocrit), biochemical (urea, creatinine, albumin) and ß2-m level measurement. Then the cases were be further subdivided into two groups, (group IA 16 patients were switched to high flux HD for 6 months & group IB 14 patients continue on low flux HD). Also twenty patients on HD with ultrapure water & ten patients on pure water HD. 15 age & sex matched apparently healthy children as control.

Results: The mean serum level of ß2-m levels was significantly elevated in the study patients compared to the control groug with a mean (52.9± 24. mg/L) & (1.2±2.9mg/L) respectively. Post-high flux ß2-m level was significantly lower than pre-high flux levels with P value<0.01compared to low flux dialysis. Also, we found increase in level of ß2-m in cases of acidosis, infection, positive correlation between its level & duration of dialysis while there is an inverse correlation between ß2-m levels and serum albumin of the study patients.

Conclusion: use of high flux hemodialysis & ultrapure water results in the reduction of serum β2-m level.

Introduction:-

Chronic kidney disease (CKD) is defined by structural or functional kidney abnormalities for more than three months ***(KDIGO, 2013).***

β2-microglobulin (β2-m) is a polypeptide. Its molecular weight is 11.8 kDa. It forms the beta chain of the human leukocyte antigen (HLA) class I molecule. It is present on the surface of most nucleated cells and in most biological fluids, including serum, urine, and synovial fluid. Most of β2-m is normally eliminated by the kidney via glomerular filtration and subsequent tubular catabolism. Extrarenal breakdown and elimination appears to be negligible ***(Drueke and Massy, 2009)***.

During metabolism and degradation it is dissociated and released to all biological fluids. Production of β2-m is constant and low in healthy people: 0.13mg/h/kg b.w. β2-m is filtered through the glomerulal membrane due to its small size, but then it is nearly completely reabsorbed in the proximal tubules. β2-m is a marker for an activation of

the cellular immune system, as well as a tumor marker in certain hematologic malignancies (multiplemyeloma, non-Hodgkin lymphoma, Hodgkin's disease, and chronic lymphoblastic leukemia). Serum β2-m increases in patients with kidney diseases, because 99% is excreted by the kidneys ***(Svatonova et al., 2014).***

Long-term dialysis treatment for end-stage kidney disease often induces the β2-m amyloid deposition in mainly osteoarticular tissues that induces various disorders, such as carpal tunnel syndrome (CTS), destructive spondyloarthropathy (DSA), and cystic bone lesions as well as in systemic organs such as heart and gastrointestinal tract when disease advances. Recent progress of dialysis therapy has improved survival of dialysis patients, however, older age and long-term dialysis treatment may increase the onset and acceleration of DRA ***(Sipe et al., 2010).***

After long-term dialysis therapy, extra-articular symptoms of DRA manifestations such as ischemic colitis, amyloid tumor, heart failure with pulmonary hypertension, gastrointestinal tract bleeding, can also occur ***(Vanholder et al., 2010)***.
Use of steroid shows beneficial effect for the pain induced by DRA while surgical treatment will be needed for advanced carpal tunnel syndrome (CTS) and destructive spondyloarthropathy (DSA) ***(Chikuda et al., 2012).***

Subjects and Methods:-

This is across sectional cross-over, hospital based study was conducted between November 2014 to April 2015on thirty cases, twenty cases from the pediatric nephrology department in Benha University Hospital & ten cases from Menouf Hospital. Before starting the study, all patients had been on chronic regular low flux HD. All patients with ESRD who were on maintenance HD for more than three months were included in the study & subdivided on two groups 16 patients were switched to high flux HD for six months (group IA) & 14 patients still on low flux HD (group IB).

Patients who are on dialysis for less than three months were excluded. Demographic data including age, sex, cause of ESRD, HBsAg and Anti-HCV status as well as details of dialysis (dialysate bath, porosity of dialyzers and dialyzer membrane) were recorded.

Pre-dialysis blood sample of the patients was drawn to measure hematological (hemoglobin and hematocrit) and biochemical parameters (urea, creatinine, albumin) and ß2-m levels by ELISA (ORGENTEC Diagnostika Gmbh. Germany). 15 age and sex matched apparently normal individuals were included as controls.

Statistical Analysis:-

Results will be organized, tabulated and statistically analyzed using SPSS software statistical computer package version 16. For quantitative data, the mean and standard deviation will be calculated. The difference between two means will be statistically analyzed using the student (t) test and Mann-Whitney test. For qualitative data the number and percent distribution will be calculated. Chi square will be used as a test of significance which will be adopted at $p < 0.05$ for interpretation of results of tests of significance**.**

Results:-

Demographic & laboratory data of the studied groups are depicted in Table 1. The major cause of ESRD was unknown 27(90%) and the mean duration on dialysis was 5.85±3.4 years. All the study patients were being dialyzed using low flux synthetic polysulphone membrane dialyzers and bicarbonate dialysate bath. Then sixteen patients were switched to high flux HD for 6 months & fourteen patients still on low flux HD. Also twenty patients on HD with ultrapure water & ten patients on pure water HD.

Table(1):-Demographic & laboratory data of the studied groups:-

	cases	Control
Age (years)	8.9±3.6 years (5 -17years)	8.1±2.5 years (5-13years)
Gender Male Female	 15(50%) 15(%0%)	 7(46.7%) 8(53.3%)
Anthropometric measures Weight Normal Underweight Height Normal Short stature	 5(16.7%) 25(83.3%) 5(16.7%) 25(83.3%)	 15(100%) 15(100%)
Hb (gm/dl)	10.4±2.11	12±0.67
Urea (mg/dl)	106.5±29.3	23.6±4.5
Creatinine (mg/dl)	7.8±1.4	0.49±0.01
Albumin (gm/dl)	2.7±0.55	4.1±0.2
Na (mEq/L)	135.9±2.3	137.4±2.4
K (mEq/L)	5.3±0.7	3.6±0.39
Ca (mg/dl)	7.2±0.78	8.5±0.42
PH (mg/dl)	7±1.2	4.5±1.5
PTH (pg/ml)	80±10	50±11
pH	7.34±0.06	7.36±0.02
HCO3 (mEq/L)	20.8±3.8	22.8±0.8
Beta-2microglobulin (mg/L)	52.9±24.6	1.92±0.62

Results are expressed as mean± SD (minimum–maximum) and asnumber (%).

Significantly decreased levels of serum ß2-m was observed at the end of the high flux dialysis period (post-high flux) compared to pre-high flux levels (p value <0.01) & low flux levels in Table 2.

Table(2): Comparison between group IA and IB regarding serum beta2-microglobulin at the beginning& at the end of the study:

		Group I-A	Group I-B	P-value	Sign.
B2-microglobulin in mg/L At beginning	Mean	51.6	58.4	>0.05	NS
	SD	10	17.2		
B2-microglobulin in mg/L After 6 months	Mean	23.7	56.1	<0.01	HS
	SD	8.1	13.4		
Difference in level in mg/L	Mean	16.2	11.2	<0.01	HS
	SD	9.4	18.3		

There were statistically highly significant negative correlation between serum B2-microglobulin level & serum albumin level, blood PH & hemoglobin level with P-value<0.01. While there was statistically significant positive correlation between serum B2-microglobulin level& dialysis duration with P-value<0.05.

There was statistically significant decrease in serum beta2-microglobulin level in patients on HD with ultrapure water compared to its level in patients on HD with pure water in table 3.

Table(3):Relation between serum B2-microglobulin level and dialysate water purity:

Water purity	Number	B2-microglobulin in mg/L		P-value	Significance
		Mean	SD		
Ultrapure	20	45.3	18.6	<0.05	S
Pure	10	67.9	29		

There was statistically significant increase in serum B2-microglobulin level in patients with cardiovascular complications compared to patients without cardiovascular complications & in patients with osteodystrophy compared to patients without osteodystrophy in table 4.

Table (4):Relation between serum B2-microglobulin level, presence of cardiovascular complications and osteodystrophy:

	Number	B2-microglobulin in mg/L		P-value	Significance
		Mean	SD		
With CVS complications	17	58.2	25.8	<0.05	S
Without CVS complications	13	45.9	21.9		
With osteodystrophy	22	58.3	37.8	<0.05	S
Without osteodystrophy	8	37.8	10.6		

There was statistically highly significant relation between duration of dialysis& appearance of manifestations of dialysis-related amyloidosis as shown in table 5.

Table (5): Relation between duration of dialysis and appearance of manifestations of dialysis-related amyloidosis:

	Number	Dialysis duration in years			P-value	Significance
		Mean	SD	Range		
With DRA	20 (66.6%)	7.73	2.5	2-11	<0.01	HS
Without DRA	10 (33.3%)	2.1	0.94	1-4		

Discussion:-

Dialysis is the process of separation of soluble substances from colloids and their removal through a semi-permeable membrane, down a concentration gradient ***(USRDS , 2004).***

Dialysis provides incomplete replacement of the lost renal excretory function ***(Cambi et al., 2005).***

Uremic toxins are classified as small molecular weight and middle molecules (substances with a molecular weight between 0.5 and 2 kDa) as well as large peptides, which are normally excreted or metabolized by the healthy kidney. These middle molecules will accumulate in chronic HD patients if they are being dialyzed with low quality membrane ***(Erkan et al., 2008).***

Some of the morbidity associated with chronic hemodialysis such as hypertension and cardiovascular disease is thought to result from retention of middle molecule uremic toxins that are poorly removed by hemodialysis using low-flux membranes with small pores. Recent clinical studies suggest that enhancing the removal of these compounds has a beneficial effect on survival and the quality of life of patients with end stage renal disease. This can be accomplished by using high-flux dialysis membranes with large pore sizes, increasing dialysis frequency or prolonging the dialysis session ***(Vanholder et al., 2010).***

In our study we found that most patients 83.3% had both weight and height below 5th percentile for age & sex and this was expected, comparable with the control which is statistically highly significant difference.This is agrees with the finding of ***(Mehls et al., 2010)***.

In our study we found that 90% of our cases had unknown etiology, and 10% of cases due to known causes as congenital renal anomalies& obstructive uropathy. In contrast to North American Pediatric Renal Trials and Collaborative Studies ***(NAPRTCS, 2005)*** which reported that 56.2% of cases with ESRD due to congenital anomalies and 4.3% of cases due to unknown causes.

Similar to ***Chavers et al. (2009)*** who stated that children and adolescents with chronic renal failure and ESRD represent a growing group of patients suffering from hypertension**,** in our study we found that 10 patients (33.3%) had blood pressure≥95th percentile and 17 patients (56.7%) had cardiovascular complications that is explained by ***Park,* (*2012*)** by several mechanisms as increased cardiovascular risk in patients with renal failure is due to overactivation of the sympathetic nervous system and other factors include oxidative stress, inflammation, decreased nitric oxide bioavailability, anemia, extracellular volume overload, fluid and electrolyte shifts, malnutrition, abnormal calcium and phosphorus metabolism, infection, uremic toxins, as well as sympathetic nervous system overactivity.

22 patients (73.3%) had osteodystrophy in our study. ***Pavlović et al., 2015*** explained that is due to impaired kidney function, there is reduced phosphate excretion that leads to phosphate retention. As a result of that phosphate retention, there is increased secretion of fibroblast growth factor 23 (FGF 23) on one hand and impaired synthesis of calcitriol on the other. Reduced synthesis of calcitriol is the result of reduced renal mass, phosphate retention and the effect of increased FGF 23 level. A low level of calcitriol together with phosphate retention leads to hypocalcaemia. The net effect is increased synthesis and secretion of PTH. In the early stages of CKD, hyperphosphatemia and hypocalcaemia are counteracted by increased PTH concentrations. Therefore, in many patients normal levels of calcium and phosphate are present, but the price is a high PTH level. As with other endocrine organs, parathyroid gland overactivity is associated with hypertrophy and hyperplasia with a significant reduction of vitamin D and calcium receptors**.**

More than half percentage of our patients (53.3%) were anemic (normocytic normochromic). In agreement with **(*K/DOQI, 2005 ; NAPRTCS, 2005)*** who stated that anemia is common in children with CKD (Increasing from 18.5% in stage 2 CKD to 68% in stage 5 predialysis patients). Normochromic normocytic anemia principally develops from decreased renal synthesis of erythropoietin, the hormone responsible for bone marrow stimulation for red blood cell (RBC) production. It starts early in the course of disease and becomes more severe as the GFR progressively decreases with the availability of less viable renal mass. No reticulocyte response occurs. RBC survival is decreased, and tendency of bleeding is increased from the uremia-induced platelet dysfunction. Other causes of anemia in chronic kidney disease patients include chronic blood loss, secondary hyperparathyroidism, inflammation, nutritional deficiency, and accumulation of inhibitors of erythropoiesis ***(Stauffer and Fan, 2014)***.

Friedman and Fadem, (2010) observed that serum albumin is a reliable index of malnutrition; because serum albumin is typically low in patients with CKD which matches with our results, most of the patients were associated with low albumin level & the mean value of albumin is (2.7±0.55g/dl).

In this study , serum beta2-microglobulin is statistically highly significant increased in group I compared to group II at the beginning of the study with P value <0.01.

Drueke and Massy, (2009) who reported that beta2-microglobulin serum level increases with the progression of chronic kidney disease, to reach very high concentrations in patients with end-stage kidney disease.

Also ***Jeloka et al. (2001)***, ***Traut et al., (2007) & Mumtaz et al., (2010)*** observed that baseline pre-dialysis serum levels of ß2-m are considerably higher in chronic HD patients than in controls matching with our results.

After usage of high flux membrans for 6 months, mean serum level of beta2-microglobulin is statistically highly significant decreased in group I at the end of the study compared to mean serum beta2-microglobulin at the beginning of the study & (23.7±8.1mg/l) & (54±13.4mg/l) with P value<0.01respectively .

These results are similar to ***Cheung et al., (2008) & Mumtaz et al., (2010)*** who found that the cumulative mean pre-dialysis serum ß2-m level was significantly lower with use of high-flux dialyzers than with low-flux dialyzers (23.7*versus* 51.6mg/L).The major reason for such a high level of ß2-m in this study was that the dialyzer used for HD in our patients was of the low-flux type. As ß2-m is a middle molecule of molecular weight of 12000 Da,

conventional, low-flux dialyzers do not clear these molecules which lead to accumulation of this silent killer in the body that decreases markedly after the use of high flux dialysers.

Another study done by ***Li et al., (2010)*** demonstrate that pre-dialysis ß2-m level was significantly lower with the use of high flux dialyzers than with low-flux dialyzers.

In this study , serum albumin level had a statistically highly significant negative correlation with ß2-m levels with P value <0.01 while there was statistically highly significant positive correlation between presence of acidosis & serum B2-microglobulin level with P value <0.01.

According to ***Cianciolo et al., (2007)*** who stated that this negative correlation supports the direct effect of hypoalbuminemia & malnutrition on the mortality of dialysis patients

According to ***Raikou and Kyriaki, (2015)*** who confirmed that metabolic acidosis, which is a common condition particularly in ESRD patients. Metabolic acidosis promotes inflammation releasing cytokines as increased accumulation of ß2-m.

Herrero-Morín et al., (2007) reported that serum beta2-microglobulin level is not influenced by age which goes in hand with our results but in contrast to ***Mumtaz et al., (2010) &Yamamoto et al., (2013)*** who stated that the progress of dialysis treatment as well as treatment for other diseases makes better survival of dialysis patients and older age of initiation of dialysis treatment.

In our study, there was statistically significant relation between presence of hepatitis infection & serum B2-microglobulin level with P value <0.05 similar to ***Drueke and Massy, (2009)*.**

When we study the manifestations of DRA, there was statistically highly significant increase in serum B2-microglobulin level in patients with manifestations of dialysis –related amyloidosis compared to its level in patients without manifestations of dialysis –related amyloidosis & this agree with ***Yamamoto et al., (2013)*** who stated that dialysis-related amyloidosis (DRA) is associated with high level of beta2-microglobulin which is the precursor protein for DRA manifestations.

In this study, there was statistically significant decrease in serum beta2-microglobulin level in patients on HD with ultrapure water compared to its level in patients on HD with pure water.

According to ***Baker, (2011)*** who confirmed that since hemodialysis patients have diminished renal function, they have less ability to excrete toxic substances in their urine. The combination of this diminished capacity and their extensive exposure, places the hemodialysis patient at much greater risk to waterborne contaminants than the normal population. Today, there is a consensus that purer water (ultra-pure) with a very low level of endotoxin and other bacterial byproducts is best for patients.

In our study, there was statistically highly significant negative correlation between serum B2-microglobulin level& hemoglobin level and this agree with ***Sipe et al., (2010)*** who stated that DRA leads to deposition of amyloid material in systemic organs as heart, gastrointestinal tract& leads to bone cysts. All these complications can lead to anemia.

During studying the manifestations of DRA, there was statistically significant increase in serum beta2-microglobulin level in patients with cardiovascular complications as heart failure & pulmonary hypertension compared to patients without cardiovascular complications with P value<0.05. This was in agreement ***Okuno et al., (2009)*** who found that a significant relation between higher level of ß2-m & the mortality rate.

Mumtaz et al., (2010) stated that after long-term dialysis therapy, extra-articular symptoms such heart failure with pulmonary hypertension can also occur. ß2-m is an important predictor of mortality in dialysis patients.

In our study we found that there is statistically significant increase in serum beta2-microglobulin level in patients with osteodystrophy compared to patients without osteodystrophy with P value<0.05.

Also ***Susantitaphong et al., (2012)*** stated that long-term complication in patients with chronic renal failure on long-term dialysis are bone and periarticular tissue B2m deposition. Typical clinical manifestations include carpal tunnel syndrome, chronic arthropathy, spondyloarthropathies, subchondral bone cysts & fractures.

Mumtaz et al., (2010) found that the duration on HD treatment plays an important role in the development of DRA. For the development of clinical effects of ß2-m, more than five to seven years are required. However, a recent large scale post mortem study showed that incipient dialysis related amyloid deposits occur in as many as 21% of cases within two years, and in 33% of cases within four years, which increased to 100% in patients treated for more than 13 years after the start of renal replacement therapy. Similar to our results we found that there is statistically highly significant relation between duration of dialysis& appearance of manifestations of dialysis-related amyloidosis with P value <0.01.

Conclusion:-

Our study suggests that ß2-m levels are significantly high in dialysis patients which is significantly reduced after usage of high flux membranes & ultrapure water HD.

Recommendations:-

- We should use high flux HD& ultrapure water HD for better removal of beta2-microglobulin.
- We should avoid infection & acidosis in patients with ESRD to avoid high level of beta2-microglobulin.

References:-

1. ***Baker JW:*** Improved Hemodialysis WaterThrough Ultra-pure Water Treatment. An AmeriWater White Paper. AmeriWater, Inc.Publication;January 2011.

2. ***Cambi V, David S ,Tagliavini D.*** Dialysis stratigies. In: Davison AM, editor. Oxford textbook of clinical nephrology 2005. New York: Oxford university press; vol 3, ch 12. 1, pp 1899-1909.

3. ***Chavers BM, Solid CA, Daniels FX, Chen S, Collins AJ, Frankenfield DL, et al.*** Hypertension in Pediatric Long-term Hemodialysis Patients in the United States Clin J Am Soc Nephrol 2009; 4(8): 1363-1369.

4. ***Cheung AK, Greene T, Leypoldt JK, Yan G, Allon M, Delmez J, et al.*** Association between serum 2-microglobulin level and infectious mortality in hemodialysis patients. Clin J Am Soc Nephrol.2008;3(1):69-77.

5. ***Chikuda H, Yasunaga H, Horiguchi H, Takeshita K, Kawaguchi H, Matsuda S, et al.*** Mortality and morbidity in dialysis-dependent patients undergoing spinal surgery: analysis of a national administrative database in Japan. J Bone Joint Surgery Am 2012;94(5):433-8.

6. ***Cianciolo G, Colí L, La Manna G.*** Is beta 2-microglobulin-related amyloidosis of hemodialysis patients a multifactorial disease? A new pathogenetic approach. Int J Artif Organs.2007;30(10):864-78.

7. ***Drueke TB and Massy ZA.*** Beta2-Microglobulin. PROGRESS IN UREMIC TOXIN RESEARCH. Inserm Unit 845, Necker Medical School, University Paris-Descartes, Paris, France, and Inserm ERI-12, University of Picardie and Amiens University Hospital, Amiens, France Seminars in Dialysis 2009;22, (4):378–380.

8. ***Erkan D, Yonca A, Sarper E, Gur A, Ahmad Y.*** Beat 2 m amyloidosis in hemodialysis and peritoneal dialysis patients. Therap Apheresis Dial 2008;12(4):306-10.

9. ***Friedman AN, Fadem SZ :*** Reassessment of Albumin as a Nutritional Marker in Kidney Disease. J Am Soc Nephrol.2010;21:223-230.

10. ***Herrero-Morín JD, Málaga S, Fernández N, Rey C, Diéguez MA, Solís G, et al.*** Cystatin C and beta2-microglobulin: markers of glomerular filtration in critically ill children. Critical Care 2007,11:R59

11. ***Jeloka T, Mathur G, Kaur R, Kohli R, Singh NP, Rizvi SN.*** β2 Microglobulin in chronic renal failure and effect of different dialyzer membrane on its clearance. Indian J Nephrol.2001;11:160-4.

12. ***K/DOQI.*** Clinical practice guidelines for cardiovascular disease in dialysis patients, Am J Kidney Dis 2005, 45(4/3):S1-S153.

13. ***Kidney Disease Improving Global Outcomes (KDIGO):*** Clinical practice guidelines for the evaluation& management of chronic kidney disease. Kidney Inter suppl., 2013;3(1):1-150.

14. ***Li PK, Cheng YL, Leung CB, Szeto CC, Chow KM, Kwan BC, et al.*** Effect of membrane permeability on inflammation and arterial stiffness: a randomized trial. Clin J Am Soc Nephrol.2010;5(4):652–8.

15. ***Mehls O, Lindberg A, Nissel R, Haffner D, Hokken-Koelega A, Ranke MB.*** Predicting the response to growth hormone treatment in short children with chronic kidney disease, Journal of Clinical Endocrinology and Metabolism 2010; 95(2):686–692.

16. ***Mumtaz A, Anees M, Bilal M, Ibrahim M.*** Beta-2 Microglobulin Levels in Hemodialysis Patients. Saudi Center for Organ Transplantation. Saudi J Kidney Dis Transpl.2010;21(4):701-706.

17. ***North American Pediatric Renal Transplant Cooperative Study. NAPRTCS:*** Annual report 2005. The EMMES Corporation, Rockville, MD.

18. ***Okuno S, Ishimura E, Kohno K, Fujino-Katoh Y, Maeno Y, Yamakawa T, et al.*** Serum beta2-microglobulin level is a significant predictor of mortality in maintenance haemodialysis patients. Nephrol Dial Transplant. 2009;24(2):571-7.

19. ***Park J.*** Cardiovascular Risk in Chronic Kidney Disease: Role of the Sympathetic Nervous System. Cardiology Research and Practice 2012; (319432), 1-8.

20. ***Pavlovic D, Katicic D, Gulin T, Josipovic J, Orlic L.*** Chronic Kidney disease mineral bone disorder. Periodicum Biologorum 2015;117 (1): 81-85.

21. ***Raikou VD, Kyriaki D:*** The relationship between glycemic control, beta2- microglobulin and inflammation in patients on maintenance dialysis treatment. Journal of Diabetes & Metabolic Disorders 2015 ;14-34.

22. ***Sipe JD, Benson MD, Buxbaum JN, Ikeda S, Merlini G, Saraiva MJ, et al.*** Amyloid fibril protein nomenclature: 2010 recommendations from the nomenclature committee of the International Society of Amyloidosis. Amyloid :the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis 2010;17(3-4):101-4.

23. ***Stauffer ME, Fan T.*** Prevalence of anemia in chronic kidney disease in the United States. PLoS One. 2014. 9(1):e84943.

24. ***Susantitaphong P, Dember LM, Jaber BL.*** Dialysis-Associated Amyloidosis. Chapter Amyloid and Related Disorders, part of series Current Clinical Pathology 2012, pp69-80.

25. ***Svatonova J, Borecka K, Adam P, Lanskas V.*** Beta2-Microglobulin as a Diagnostic Marker in Cerebrospinal Fluid: A Follow –Up Study. Hindawi Publishing Corporation. Disease Markers 2014: 2014:1-6.

26. ***Traut M, Haufe CC, Eismann U, Deppisch RM, Stein G, Wolf G.*** Increased binding of beta-2-microglobulin to blood cells in dialysis patients treated with high-flux dialyzers compared with low-flux

membranes contributed to reduced beta-2-microglobulin concentrations. Results of a cross-over study. Blood purification.2007;25(5-6):432-40.

27. ***United States Renal Data System, USRDS.*** Annual Data Report: Atlas of End-Stage Renal Disease in the United State 2004.NIH.National Institute of Diabetes and Digestive and Kidney Disease,Bethesda,MD.
28. ***Vanholder R, Glorieux G and Van Biesen W.*** Advantages of new hemodialysis membranes and equipment. Nephron Clin Pract 2010 ;114:c165–72.

29. ***Yamamoto S, Kazama J, Maruyama H, Narita I.*** In: Feng D editor. Dialysis-related amyloidosis: pathogenesis and clinical features in patients undergoing dialysis treatment, amyloidosis Chapter4,InTech;2013.ISBN:978-953-51-1100-9.