Single nucleotide polymorphism of TNF alpha relationship with some serum factors in kidney injury patients.

Abdalnabi J Abid[1*], Seenaa J Allawande[2]
[1]Dept. of Biology , College Of Science for women. University of Babylon – Iraq
[2]Ministry of Education , Babil Education Directorate/Iraq
*Corresponding author email : dr_almamory59@yahoo.com
Phone no. : +9647803853959

Abstract
Acute and chronic kidney injury represent a great health problem worldwide .The current study aimed to investigate the correlation between TNF alpha levels and its single nucleotide polymorphism for kidney injury patients . Eighty blood samples were collected from kidney failure patients who admitted to hemodialysis unit at marjan hospital , Hilla –Iraq . serum concentrations of TNF a show highly increased in patients compared with healthy persons but there is no significant differences appear between patients before and after hemodialysis . Molecular investigations for TNF a -380 A/C single nucleotide polymorphism show increase in homozygous genotype AA than heterozygous AC and homozygous CC with a percentage of 60 ,30 and 10 % respectively .sodium and potassium concentrations also reveals a noticeable variations after hemodialysis processes.
Keywords: cytokines, kidney, polymorphism, genotype .electrolytes.

Introduction

Acute kidney injury (AKI) is a major source of morbidity and mortality in hospitalized patients. Acute kidney injury (AKI) occurs in 1% of hospital admissions and up to 7% of hospitalized patients develop AKI [1]. Twenty five percent of patients in the intensive care unit (ICU) develop AKI and 5% of patients in the ICU will need renal replacement therapy [2]. Approximately 40% of patients with renal disease present with AKI mostly as a result of development of acute tubular necrosis (ATN), that formed a final common pathway of severe renal dysfunction in patients who have diseases of non-renal origin[1].
Dialysis is the only Federal Drug Administration approved treatment for AKI [3]. Even though both intermittent hemodialysis (IHD) and continuous renal replacement therapy (CRRT) are widely used, the mortality of AKI is as high as 80% in ICU patients [4]. A better understanding of the inflammatory response in AKI is needed to allow interventions which would prevent the need for hemodialysis. An increase in plasma proinflammatory cytokine levels predicts mortality in patients with AKI [5]. Many cytokines are released by leukocytes and renal tubular cells into the injured kidney and are important components of both the initiation and extension of inflammation in AKI. Many pro-inflammatory cytokines/chemokines are increased in the kidney in ischemic AKI [6, 7]
TNF-alpha is a potent pro-inflammatory cytokine and important mediator of inflammatory tissue damage, AKI is mediated in-part by TNF-alpha and the inhibition of the release or action of TNF-alpha protects the kidney from nephrotoxicity [8]
The pro-inflammatory cytokine TNF-α is produced by monocytes, macrophages and to a lesser extent T cells and B cells. It stimulates macrophage function and increases MHC II antigen expression, which may lead to post transplant immune reactivity [9, 10] . The TNF-α gene is localized in the HLA Class III region of the major histocompatibility complex (MHC) on chromosome 6 [11]. Single nucleotide polymorphism (SNP) at position-308 in TNF- α promoter region results in two alleles [12]. Additionally, researchers focused on the association between donor TNF-α-308G/A polymorphism and recipients acute rejection, considering that net effects of donor and recipient derived factors may contribute to donor recipient consequence [13] .The kidneys play an important role in the fluids and electrolytes regulation, and when they malfunction it often leads to an electrolyte imbalance. According to "Fluids and Electrolytes Demystified," chronic renal failure involves progressive and irreversible loss of kidney function. kidney is the primary organ responsible for potassium excretion[14]. Chronic renal failure frequently is complicated by elevations in serum potassium. Sodium and potassium intake is minimized except in patients with prior deficiencies or GI losses. [15] . Daily rise in serum creatinine is diagnostic sign of AK , since the rate of production of metabolic waste exceeds the rate of renal excretion then urea and creatinine concentrations rise [16].

doi.org/10.31838/hiv22.01.09 Received: 20.08.21, Revised: 19.12.21, Accepted: 18.01.22

The current study designed to investigate the association of TNF alpha single nucleotide polymorphism in kidney failure patients in addition to determine the level alterations in some serum factors before and after hemodialysis.

Materials and methods

Patients and . Samples collection.

The current study include 50 renal failure patients who submitted to hemodialysis unit in Marjan Hospital – Hilla Province –Iraq from January 2016 to June 2916. Informed consent was taken from all patients

Five ml of venous blood were obtained from all renal failure patients 20 minutes in addition to 25 apparently healthy persons before and after doing the hemodialysis process. The blood samples divided in to two, one part added to EDTA tube for genetic and DNA extraction, while the other added to plain tubes and centrifuged at 3000 g for 10 minutes for serum separation ,all serum samples were saved at -20C to detect the cytokine and electrolytes concentration . [17].

Immunological assay

Detection of cytokines concentration were done by Elisa assay ,This test was conducted in accordance with the manufacturer's instructions (Boster) Serum concentrations of TNF alpha for renal failure patient before and after hemodialysis were calculated according to the optical densities of all samples and standards were read in a micro-titer plate with not more than 30 min after adding the stop solution by Elisa reader at 450nm

DNA Extraction:-

DNA was extracted from blood samples of patients and health control by using a PureLink DNA purification kit and in accordance with the manufacturer's protocols, and then the concentration and purity of extracted DNA were calculated by using Nano drop spectrophotometer at wave length 260/280 nm. The lyophilized oligonucleotide upstream and downstream primers were prepared according to the manufacturing company (Bioneer) and kept at -20°C.

Single nucleotide polymorphism of TNF- α-308G/A was investigated by Sequence specific primer PCR in duplicate reaction set ,one for TNF-α-308 G allele and the second for A allele with a final reaction volume of 20 ul. Primers used in this study depends on [18]. The mixture amplified by thermo cycler with a cycling condition that demonstrated in table 1.The reaction was performed in a PCR thermal cycler apparatus, and after several trials, and according to the manufacture's troubleshooting guide the program was adopted .

Table.1. Primers and cycling conditions of primers TNF- α-308G/A used in the study:

Markers	Sequences5'- 3'	Size of amplicon	Thermal cycler conditions
TNF- α -308 Primer G	5'TCTCGGTTTCTTCTCCATCG3'	184bp	95 C°for1min 95 C°for15sec 65 C°for50sec 72 C°for40sec 95 C°for20sec T(59 C°)for50sec 72 C°for50sec
	5'ATAGGTTTTGAGGGGCATGG3'		
Primer A	5'AATAGGTTTTGAGGGGCATGA3'		

The amplified PCR products were detected by agarose gel electrophoresis was visualized by staining with ethidium bromide. The electrophoresis results was detected by using gel documentation system. The positive results were distinguished when the DNA band base pairs of sample equal to the target product size [19].

3.2.9. Statistical analysis

All values were expressed as means SD . The data were analyzed by using of SPSS (T test) version 17 and Microsoft Excel computerized programs and taking P value less than 0.05 ($p < 0.05$) as the lowest limit of significance in addition OR and CI were calculated for cytokine genotype polymorphisim [20].

Results

Concentration of Tumor necrosis factor alpha (TNFα):

The level of TNF alpha was significantly raised in all patients suffering from chronic renal failure in comparison to the control group, renal failure patients show highly increased in Levels of TNF after hemodialysis compared with before hemodialysis and healthy control group (38.27, 30 and ,21.20), ($P<0.05$) table (1).

Table 1. Concentration of TNF alpha (pg/ml) in patients with renal failure

subject	M+SD	Range	P- value
Pre-hemodialysis	30.0 ± 1.80	18-36	0.0001
Post-hemodialysis	38.27 ± 2.89	20-42	0.0001
Control	21.20 ± 2.47	12-28	

Urea and electrolytes levels

The results showed obvious changes in biochemical parameters related with renal failure for patients with chronic renal failure undergoing hemodialysis, this changes has been observed a significant increase in the concentration of urea

levels .For the concentration of urea level a highly significantly p<0.05 has been observed in all patients before than that after hemodialysis process.

In addition serum electrolytes show noticeable changes in their levels in patients before and after hemodialysis. Sodium and potassium concentration revealed higher in renal failure patients before hemodialysis (137.63 , 7.31)mmol/L than that in post hemodialysis (118.43 , 5.64) with a significantly differences (P≤0.05) (table 3).

Table 3. levels of Urea and electrolytes in pre and post hemodialysis patients.

Subjects	Pre-hemodialysis		Post-hemodialysis		P-value
	M+SD	Range	M+SD	Range	
Urea (mmol/L	30.5+2.22	18-46	11.10+1.88	8-18	0.0001
Sodium(mmol/L	137.63+2.7	118-154	118.43+3.29	122-132	0.0001
Potassium(mmol/L	7.31+0.72	4.8-8.2	5.64+0.85	4.2-6.4	0.0001

Detection of TNFα -308 polymorphism gene

Tumor necrosis factor alpha–308 allelic polymorphism gene (TNF- α) was investigated through specific primer for 50 sample,30 of them include non-hemodialysis patients and 20 hemodialysis patients A-A,homozygous genotypes appears with high percentage in both types of patients 56.66% and 70 % for non-hemodialysis and hemodialysis groups respectively while, G-G homozygous show low appearance table (4) Figure (1).

TABLE (4).Genotypic distribution of TNF-a-308 in kidney injury patients.

Subjects	Sample size	Genotype	Patients(No and %)	OR	CI 95%	P-value
Non hemodialysis patient	30	A-A	17—56.66%	2.77	0.9i7	0.0706
		A-G	9 --30-%	0.46	0.15	0.174
		G-G	4---13.33%	0.61	0.146	0.508
Hemodialysis patients	20	A-A	14---70%	4.95	1.388	0.0137
		A-G	4 ---20%	0 .16	0.044	0.007
		G-G	2 ---10%	0.28	0.05	0.157
Healthy	25	A-A	8 – 32%			
		A-G	12 –48%			
		G-G	5 ---20%			

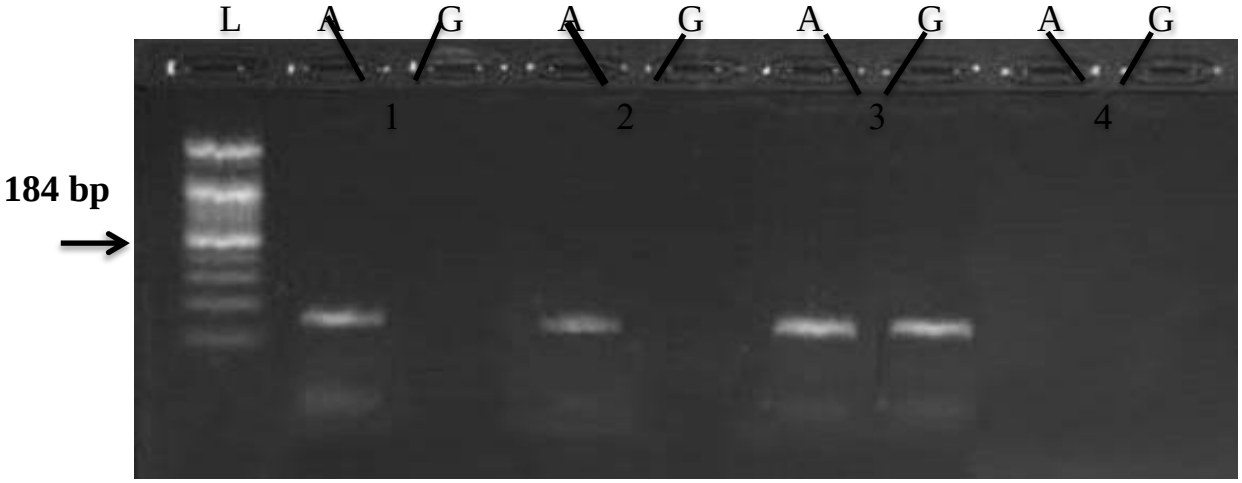

Figure (1) Agarose gel electrophoresis of PCR productes for deteciont of TNFα -308 allelic polymorphism gene amplicon product in human.Lane1-4 refer to sample number. L lader .

Ethical clearance

The study was approved by the Research Ethics Review Boards of the University of Babylon .All participants provided written informed consent.

Source of funding ; This work was done by self funding ,the practical works was done at my college university with the agreement of university

Conflict of Interest :This work was done by the author only , all the experiments were done at immunological laboratory of my college and the writing of paper was done by the author himself .

Discussion

The kidneys play an important role in the regulation of fluids and electrolytes,
acute kidney injury (AKI) remains to be an independent risk factor for mortality and morbidity. Inflammation is now believed to play a major role in the pathophysiology of AKI.[21]

Elevation of some proinflamatory and antiinflamatory cytokine Moreover, the levels of certain cytokines and gene polymorphisms for certain cytokines may have predictive value in clinical acute renal failure . [5, 22].

The current study showed enhancement in the levels of serum TNF_a in patients than healthy persons in addition this enhancement appear clearly in patients after hemodialysis than that before with no significant differences between them.

Many studies have reported an increased TNF- α levels during acute rejection in kidney allografts [23], Tumor necrosis factor alpha (TNF- α), produced by macrophage , may play a major part in various chronic inflammatory diseases, and it has been implicated in the pathogenesis of both acute and chronic transplant [11].

Unchanged in amount of TNF probably depend on cytokine kinetics. The half-lives TNF-a (5-10 min) are known to be rather short. Since plasma cytokines are rapidly bound to cell surface receptors, this implies that stable plasma concentrations are achieved by a continuously high production rate. The entire amount of cytokines potentially eliminated via hemodialysis is probably considerably lower when compared with the endogenous production[24].

However that adsorption of cytokines occurs mainly in the first minutes of HD and may not reflect a substantial amount of cytokine removal, and HD membranes may increase cytokine production by activating mononuclear cells. [25].

TNFα -308 gene

Tumor necrosis factor α -308 allelic polymorphism gene (TNF- α) was investigated through specific primer for 50 sample , homozygous alleles show enhanced in number than heterozygous in patients , 37 samples show homozygous genotype while heterozygous genotype appears with 13 samples of all patients.. This study suggested the association between TNFα-308 and patients with Kidney failure and low association with hemodialysis , this study agreed with other results [26] that pointed high association of TNFα-308 producer with acute rejection of renal transplant, and association with dialysis was trace.

A-A genotype of *TNFA* gene −308 polymorphism, is more prevalent among patients with contrast-induced acute kidney injury., [27].showed that the GG genotype of *TNFA* −308 polymorphism and AA genotype of *IL-10*–1082 polymorphism, when combined, are associated with kidney injury .[28] found that *TNFA* gene −308 G/A A-allele was associated with higher peak serum creatinine and urinary kidney injury molecule-1 (KIM-1), as well as higher multi organ failure (MOF) score in hospitalized patients with AKI[28, 29].

Polymorphism rs1800629 in the *TNFA* gene is suspected to alter the inflammatory response and to be associated with AKI susceptibility [30], where as some other studies reveals no evidence supported an association between *TNFA* and susceptibility or outcome of AKI[31]

The current study also reveals the high production of AA and AG genotype in compared with homozygous GG genotype .The high-producer genotype AA + GA of *tumor necrosis factor alpha (TNFA)* gene −308 G/A polymorphism (rs1800629) was found to be associated with higher levels of TNF-α *ex vivo* and higher mortality in patients receiving renal replacement therapy by [22].

The presence of these alleles as homozygous (AA ,GG) or heterozygous (AG) is associated with increased transcriptional activity [32]. A lot of efforts have been made to investigate whether TNF- α-308G/A polymorphism in recipients has an impact on the outcome of renal transplantation, including acute rejection, chronic rejection and long-term survival. Have implicated tumor necrosis factor alpha (TNF-α) cytokine in modulating the progression of ESRD [10].

Electrolytes

The kidneys play an important role in the regulation of fluids and electrolytes, and when they malfunction it often leads to an electrolyte imbalance and when they malfunction it often leads to an electrolyte imbalance [2].

Blood urea show decreased in concentrations for patients undergo hemodialysis than that before this process. Hemodialysis led to diffusion and ultrafiltration of patients' blood and then back into the patient's circulation , this process led to remove or decreased of toxins and excesses of electrolytes and enzymes levels [33] The movement of fluid and particles across a semipermeable membrane can help restore fluid and electrolyte balance, control acid-base balance, and remove waste and toxic material from the body. It can sustain life successfully in both acute and chronic situation where substitution for or augmentation of normal renal function is needed [34]

The present study reveals also decrease in sodium and potassium levels in patients after hemodialysis than that before. Potassium is the major intracellular ion and Sodium is the major extracellular ion and they each play a significant role in maintaining homeostasis within each of their compartments [3]. Each electrolyte serves a unique physiologic function and concentrations, dialysis can also be utilized to remove K+ from the body , the physiological regulation of serum sodium level is maintained by balancing water intake and water excretion; the former through control of thirst sensation and the latter through control of antidiuretic hormone (ADH) secretion [9].

References

.1 Nisula S, Kaukonen K-M, Vaara ST, et al. Incidence, risk factors and 90-day mortality of patients with acute kidney injury in Finnish intensive care units: the FINNAKI study. Intensive care medicine. 2013;39(3):420-8. https://doi.org/10.1007/s00134-012-.5-2796

.2 Bagshaw SM, George C, Dinu I, et al. A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients. Nephrology Dialysis Transplantation. 2008;23(4):1203-10. https://doi.org/10.1093/ndt/gfm744.

.3 Esson ML, Schrier RW. Diagnosis and treatment of acute tubular necrosis. Annals of internal medicine. 2002;137(9):744-52. https://doi.org/10.7326/0003-4819-137-9-200211050-00010.

.4 Liano F, Junco E, Madero R, et al. The spectrum of acute renal failure in the intensive care unit compared with that seen in other settings. Kidney International-Supplements. 1998;66:16-24.

.5 Simmons EM, Himmelfarb J, Sezer MT, et al. Plasma cytokine levels predict mortality in patients with acute renal failure. Kidney international. 2004;65(4):1357-65. https://doi.org/10.1111/j.1523-1755.2004.00512.x.

.6 Rice JC, Spence JS, Yetman DL, et al. Monocyte chemoattractant protein-1 expression correlates with monocyte infiltration in the post-ischemic kidney. Renal failure. 2002;2 .23-703:(6)4 https://doi.org/10.1081/JDI-120015673.

.7 Setty HN, Rama C, Murthy P, et al. Dissecting aortic aneurysm associated with severe aortic regurgitation in an asymptomatic young female. Journal of Natural Science, Biology and Medicine. 2020;11 .-81:(1) https://doi.org/10.4103/jnsbm.JNSBM_149_19.

.8 Ramesh G, Reeves WB. TNF-α mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. The Journal of clinical investigation. 2002;110(6):835-42. https://doi.org/10.1172/JCI200215606.

.9 Rossol M, Heine H, Meusch U, et al. LPS-induced cytokine production in human monocytes and macrophages. Critical Reviews™ in Immunology. 2011;31(5). https://doi.org/10.1615/CritRevImmunol.v31.i5.20.

.10 Sankaran D, Asderakis A, Ashraf S ,et al. Cytokine gene polymorphisms predict acute graft rejection following renal transplantation. Kidney international. 1999;56(1):281-8. https://doi.org/10.1046/j.1523-1755.1999.00536.x.

.11 Azarpira N, Aghdai MH, Raisjalali GA, et al. Influence of recipient and donor IL-10, TNFA and INFG genotypes on the incidence of acute renal allograft rejection. Molecular biology reports. 2009;36(6):1621-6. https://doi.org/10.1007/s11033-008-9361-x.

.12 Melk A, Henne T, Kollmar T, et al. Cytokine single nucleotide polymorphisms and intrarenal gene expression in chronic allograft nephropathy in children. Kidney international. 2003;64(1):314-20. https://doi.org/10.1046/j.1523-1755.2003.00050.x.

.13 Hoffmann U, Bergler T, Rihm M, et al. Upregulation of TNF receptor type 2 in human and experimental renal allograft rejection. American Journal of Transplantation. 2009;9(4):675-86. https://doi.org/10.1111/j.1600-6143.2008.02536.x.

.14 Choi HY, Ha SK. Potassium balances in maintenance hemodialysis. Electrolytes & Blood Pressure. 2013;11(1):9-16. https://doi.org/10.5049/EBP.2013.11.1.9.

.15 Lameire N, Van Biesen W, Vanholder R, editors. Electrolyte disturbances and acute kidney injury in patients with cancer2010: Elsevier. https://doi.org/10.1016/j.semnephrol.2010.09.00.2

.16 Abuelo JG. Normotensive ischemic acute renal failure. New England Journal of Medicine. 2007;357(8):797-805. https://doi.org/10.1056/NEJMra064398.

.17 Lewis PA, Donhauser ZJ, Mantooth BA, et al. Control and placement of molecules via self-assembly. Nanotechnology. 2001;12(3):231. https://doi.org/10.1088/0957-4484/12/3/306.

.18 Perrey C, Turner SJ, Pravica V, et al. ARMS-PCR methodologies to determine IL-10, TNF-alpha, TNF-beta and TGF-beta 1 gene polymorphisms. Transplant immunology. 1999;7(2). https://doi.org/10.1016/S0966-3274(99)80030-6.

.19 Bartlett JS, Stirling D. PCR protocols method in molecular and biology ..Human press Inc. 2nd edition. . 1998.

.20 Paulson DS. Biostatistics and microbiology: a survival manual vol 4: Springer Science & Business Media, 2008. https://doi.org/10.1007/978-0-387-77282-0.

.21 Edelstein CL, Schrier RW. Pathophysiology of ischemic acute renal injury," in Diseases of the Kidney and Urinary Tract, (2) R. W. Schrier, Ed., Lippincott Williams & Wilkins, Philadelphia, Pa, USA,8th edition.chapter 36, pp. 930–961,. 2007.

.22 Jaber BL, Rao M, Guo D, et al. Cytokine gene promoter polymorphisms and mortality in acute renal failure. Cytokine. 2004;25(5):212-9. https://doi.org/10.1016/j.cyto.2003.11.004.

.23 Manchanda PK, Kumar A, Sharma RK, et al. Association of pro/anti-inflammatory cytokine gene variants in renal transplant patients with allograft outcome and cyclosporine immunosuppressant levels. Biologics: targets & therapy. 2008;2(4):875. https://doi.org/10.214/7BTT.S2459.

.24 Sander A, Armbruster W, Sander B, et al. Hemofiltration increases IL-6 clearance in early systemic inflammatory response syndrome but does not alter IL-6 and TNFα plasma concentrations. Intensive care medicine. 1997;23(8):878-84. https//:doi.org/10.1007/s001340050425.

.25 Pertosa G, Grandaliano G, Gesualdo L, et al. Clinical relevance of cytokine production in hemodialysis. Kidney International. 2000;58:S104-S11. https://doi.org/10.1046/j.1523-1755.2000.07613.x.

.26 Ram S, Bass K, Abreo K, et al. Tumor Necrosis Factor-a-308 Gene Polymorphism Is Associated with Synthetic Hemodialysis Graft Failure. Journal of investigative medicine. 2003;51(1):19-26. https://doi.org/10.1097/00042871-200302000-00023.

.27 Dalboni MA, Quinto BMR, Grabulosa CC, et al. Tumour necrosis factor-α plus interleukin-10 low producer phenotype predicts acute kidney injury and death in intensive care unit patients. Clinical & Experimental Immunology. 2013;173(2):242-9.

.28 Susantitaphong P, Perianayagam MC, Tighiouart H, et al. Tumor necrosis factor alpha promoter polymorphism and severity of acute kidney

injury. Nephron Clinical practice. 2013;123(1-2):67-73. https://doi.org/10.1159/000351684.

.29 Thurman JM, Lenderink AM, Royer PA, et al. C3a is required for the production of CXC chemokines by tubular epithelial cells after renal ishemia/reperfusion. The Journal of Immunology. 2007;178(3):1819-28. https://doi.org/10.4049/jimmunol.178.3.1819.

.30 Chang C-F, Lu T-M, Yang W-C, et al. Gene polymorphisms of interleukin-10 and tumor necrosis factor-α are associated with contrast-induced nephropathy. American journal of nephrology. 2013;37(2):110-7. https://doi.org/10.1159/000346528.

.31 Jouan J, Golmard L, Benhamouda N, et al. Gene polymorphisms and cytokine plasma levels as predictive factors of complications after cardiopulmonary bypass. The Journal of thoracic and cardiovascular surgery. 2012;144(2):467-73. https://doi.org/10.1016/j.jtcvs.2011.12.022.

.32 Wilson AG, Symons JA, McDowell TL, et al. Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation. Proceedings of the National Academy of Sciences. 1997;94(7):3195-9. https://doi.org/10.1073/pnas.94.7.3195.

.33 Hootkins R. Lessons in dialysis, dialyzers, and dialysate. Dialysis & Transplantation. 2011;40(9):392-6. https://doi.org/10.1002/dat.20609.

.34 Coresh J, Byrd-Holt D, Astor BC, et al. Chronic kidney disease awareness, prevalence, and trends among US adults, 1999 to 2000. Journal of the American Society of Nephrology. 2005;16(1):180-8. https://doi.org/10.1681/ASN.2004070539.