J Bras Patol Med Lab, v. 51, n. 3, p. 153-157, June 2015

10.5935/1676-2444.20150026

Influence of hemodialysis on the plasma concentration of adenosine deaminase in patients with chronic kidney disease

Influência da hemodiálise na concentração plasmática da adenosina deaminase em pacientes com doença renal crônica

Eduardo O. Chielle[1]; Kaue A. Rigon[1]; Idania A. Arcari[1]; Vanessa Stein[1]; Gelson Antônio dos Santos[2]

1. Universidade do Oeste de Santa Catarina (Unoesc), São Miguel do Oeste-SC. 2. Clínica Renal do Extremo Oeste, São Miguel do Oeste-SC.

ABSTRACT

Introduction: Over the past years there has been a significant increase in hospitalizations and treatments due to kidney complications that eventually resulted in the increased number of patients on dialysis. The adenosine deaminase (ADA) enzyme mediates the formation of some defense cells of the organism and is therefore a marker of inflammation. **Objective:** The objective of this study was to evaluate biomarkers of renal function and serum ADA of hemodialysis patients. **Materials and methods:** Blood samples were collected from 80 patients – 40 women and 40 men – between 19 and 60 years old, before and after the completion of hemodialysis. **Results:** There was a significant difference in levels of creatinine, urea and ADA in pre- and post-hemodialysis periods ($p < 0.0001$). There was a significant increase in post-dialysis ADA regardless of sex; however there was a significantly greater increase in men. **Conclusion:** The results showed a reduction in urea and creatinine parameters, evidencing the main purpose of hemodialysis. This study suggests that the determination of ADA activity could be used to monitor inflammation in hemodialysis patients, however wider and more specific studies are needed to show the effectiveness of serum ADA activity as an inflammatory marker in patients with chronic kidney disease.

Key words: adenosine deaminase; hemodialysis; urea; creatinine; renal.

INTRODUCTION

Over the past years there has been a significant rise in hospitalizations and treatments resulting from renal complications, which increased the number of patients undergoing dialysis as the only way to keep life. The factor common to those on dialysis is renal failure, both chronic and acute, from different causes and encompassing all age groups[1].

Renal disease generally causes reduction of the glomerular filtration rate (GFR), which indicates progression of adjacent disorders with the decreased number of functioning nephrons. A patient is classified as having renal disease in case he presents decreased renal perfusion or obstruction – preventing urinary waste elimination by the kidneys – in the renal pelvis, ureters, urinary bladder or urethra. The disease comprises two phases, classified according to time and progression: acute kidney injury (AKI) and chronic kidney disease (CKD)[2].

CKD is characterized by a lesion in the renal parenchyma and/or decreased GFR for a period equal to or greater than three months, detected by markers of renal injury, including hematological and urinary alterations. On the other hand, AKI is identified by a recently (latest 30 days) raised (in at least 0.5 mg/dl) plasma concentration of creatinine[2].

A patient with CKD presents a persistent inflammatory condition that causes several complications, among them, changes in release and function of several enzymes through the action of cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α)[3], which contribute to the exacerbation of the inflammatory process. Another factor that contributes to increase the inflammatory state of CKD patients is hemodialysis, during

First submission on 27/02/14; last submission on 27/03/15; accepted for publication on 09/04/15; published on 20/06/15

which the persistent activation of monocytes and other defense cells is stimulated[4].

Adenosine deaminase (ADA) is a polymorphic enzyme of the purine metabolism pathway that irreversibly catalyzes the hydrolytic cleavage of adenosine into inosine[5]. This enzyme acts as a mediator in the formation of some defense cells, hence, as a marker of inflammation, being generally associated with processes of infectious origin. Its presence is necessary for the maintenance of cellular immunity: it is essential for the proliferation and differentiation of lymphoid cells, particularly T cells, and the maturation of monocytes[6].

Currently, the gold standard for renal function evaluation is the creatinine clearance test, which reflects GFR in a more reproducible way, along with the measurement of urea, which is almost totally excreted by the kidneys. The determination of urea alone, however, is not enough to evaluate renal function[2].

The early detection of renal disease and of the increased inflammatory process in these patients, as well as the therapeutical conducts appropriate to retard progression, of both the disease and the inflammatory process, may reduce patients' suffering and the financial costs associated to CKD. Patients with slight renal disorder almost always present progressive, insidious and asymptomatic evolution, what makes early diagnosis difficult. Thus, capacitation, conscientization, and surveillance by the physician and the primary health care are essential for diagnosis and early referral to nephrologists and the appropriate institutions. This makes it possible to retard CKD progression, avoid complications, modify present comorbidities, and allow adequate preparation for renal replacement therapy[7].

The present study was aimed at assessing the levels of kidney function biomarkers (urea and creatinine) and the activity of serum ADA as a marker of inflammatory response in patients undergoing hemodialysis at Clínica Renal do Extremo Oeste, in São Miguel do Oeste (SC), helping to monitor treatment and to follow-up alterations caused by dialysis.

MATERIALS AND METHODS

Study population

A cross-sectional study was conducted in 80 CKD patients undergoing hemodialysis at Clínica Renal do Extremo Oeste. The study protocol was approved by the human research ethics committee of Universidade do Oeste de Santa Catarina (Unoesc), under number 449.917. The study included 40 women and 40 men at the 19-60 age group, who had been undergoing hemodialysis for more than 12 months with an arteriovenous fistula as the vascular access. Patients with inflammatory, acute or malignant diseases were excluded. The mean dialysis session length was 3-4 hours, three to four times per week, with blood flow rate greater than 250 ml/min, dialysate flow rate of 500 ml/min and bicarbonate buffer. The main causes of CKD were hypertensive nephrosclerosis, chronic glomerulonephritis, diabetic nephrosclerosis, polycystic kidney disease, and other underlying diseases. Other patients presented CKD of unknown causes.

Laboratory analysis

Collection was performed at Clínica Renal do Extremo Oeste, after explanation of the research and signing of the informed consent. Blood samples were collected in two moments: the first, at fasting, before the hemodialysis session; the second, just after the end of the procedure. The samples were placed in gel separator tubes, which were centrifuged for 20 minutes, at 4000 rpm; the serum was separated and stored at -80ºC for later analysis. Measurements of creatinine and urea were carried out in serum samples, according to instructions provided by the manufacturer (Labtest Diagnóstica SA, MG, Brasil), and results were expressed in mg/dl. ADA measurement was performed in serum samples according to techniques supplied by the manufacturer (Ebram Produtos Laboratoriais Ltda, SP, Brasil), using the enzymatic deamination methodology. The assay was based on the enzymatic deamination of adenosine to inosine, which was converted in hypoxanthine by purine nucleoside phosphorylase. Hypoxanthine was converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase. H_2O_2 reacted with N-ethyl-N-(2-hidroxi-3-sulfopropyl)-3-methylaniline and 4-aminoantipyrine in the presence of peroxidase, generating a quinone dye that was monitored in a kinetic manner. Values were expressed in U/l.

Data analysis

Results were expressed in mean ± standard deviation (SD). Data were statistically analyzed with the aid of the Statistical Package for the Social Sciences (SPSS) 17.0 (Chicago, USA). The normality of the results was verified by the Kolmogorov-Smirnov test. Differences between measurements before and after hemodialysis were analyzed by Student's *t*-test for paired samples, when they presented parametric distribution, and by Wilcoxon test for dependent samples, when distribution was non-parametric. Results with $p < 0.05$ were considered significant.

RESULTS

Comparison between laboratory determinations is available in **Table 1**. A total of 80 samples from patients on hemodialysis (40 males and 40 females) were analyzed. The population belonged to the 19-60 age group.

Significant difference was observed in the levels of creatinine and urea when comparing pre- and post-hemodialysis measurements. Creatinine presented mean of 9.7 mg/dl ± 0.5 before hemodialysis and 4.2 mg/dl ± 0.3 after it ($p < 0.0001$); urea, mean of 123.8 mg/dl ± 4.6 before and 43.5 mg/dl ± 2.5 after hemodialysis ($p < 0.0001$).

There was significant difference between serum ADA pre- and post-hemodialysis results: post-hemodialysis ADA showed a significant increase ($p < 0.0001$). We observed averages of 17.18 U/l ± 1 in pre-hemodialysis and 28.96 U/l ± 1.5 in the post-hemodialysis moment.

Concerning pre- and post-hemodialysis periods for men and women, a significant difference was observed in the results of post-hemodialysis ADA. Both sexes had significant increase in post-hemodialysis ADA ($p < 0.0001$) (**Table 2**), but levels were significantly higher in men than in women ($p = 0.001$). Values of urea and creatinine showed significant reduction ($p < 0.0001$) in both sexes, as can be observed in Table 2.

TABLE 1 – Comparison of laboratory analyses between pre- and post-hemodialysis periods

	Pre-hemodialysis ($n = 80$)	Post-hemodialysis ($n = 80$)	p
ADA (U/l)	17.18 ± 1	28.96 ± 1.5	0.0001
Urea (mg/dl)	123.8 ± 4.6	43.5 ± 2.5	0.0001
Creatinine (mg/dl)	9.7 ± 0.5	4.2 ± 0.3	0.0001

Data are expressed in mean ± SD. They were analyzed by t-test (parametric data) and Mann-Whitney test (non-parametric data). Significant difference was considered when $p < 0.05$.
ADA: adenosine deaminase; SD: standard deviation.

DISCUSSION

In this study we observed the activity of ADA and of markers of kidney injury in 80 CKD patients of both sexes undergoing hemodialytic treatment, in two moments, before and after the hemodialysis session. The chronic inflammatory state in CKD patients undergoing hemodialysis may be attributed to the constant activation of circulating monocytes and neutrophils during blood passage through the hemodialysis circuit, endotoxin transfer from the dialysis capillary membrane to blood during the sessions, activation of inflammatory and proinflammatory cytokines and, principally, endothelial alterations. Endothelial cells are activated with expression of cellular adhesion molecules that bind to leukocytes and migrate into the inflammed tissues[4]. A significant increase of serum ADA ($p < 0.0001$) was also observed after hemodialysis, in both sexes, with results of 17.18 U/l ± 1 in pre- and 28.96 U/l ± 1.5 in post-hemodialysis periods. In blood cells, the highest ADA activity is found in lymphocytes and in the maturation of monocytes[5]. Accordingly, the increased activity of this enzyme observed in this study may be closely connected with an increased activation of both cells during the passage of blood through the hemodialysis circuit. The encountered results suggest that during hemodialysis an increase in inflammatory markers may occur, for example in ADA, which could be used as an important biomarker to assess the level of the inflammatory process caused by hemodialysis.

ADA increase stimulates deamination of adenosine to inosine, and consequently, the reduction of adenosine; unfortunately, it was not possible to quantify adenosine levels in this study. Marleswki *et al*. (2000)[8], though, observed accelerated degradation of adenine nucleotides (adenosine) in erythrocytes of CKD patients when compared with erythrocytes of healthy volunteers; adenine nucleotide catabolism was much faster in CKD patients than in cells of healthy volunteers.

TABLE 2 – Comparison of laboratory analyses in pre- and post-hemodialysis periods between male and female patients

	Males ($n = 40$)		Females ($n = 40$)		p
	Pre-hemodialysis	Post-hemodialysis	Pre-hemodialysis	Post-hemodialysis	
ADA (U/l)	18.6 ± 1.6	35.6 ± 2.4	15.8 ± 0.9	26.3 ± 1.4	0.0001
Urea (mg/dl)	118 ± 6.6	49 ± 2.9	129 ± 6.5	44 ± 3.9	0.0001
Creatinine (mg/dl)	9.9 ± 0.7	4.3 ± 0.3	9.5 ± 0.6	4.1 ± 0.3	0.0001

Data are expressed in mean ± SD. They were analyzed by t-test (parametric data) and Wilcoxon test (non-parametric data). Significant difference was considered when $p < 0.05$.
ADA: adenosine deaminase; SD: standard deviation.

Our results showed for the first time a significant increase in post-hemodialysis serum ADA in men compared with women. We did not find available data in the literature explaining the reason for that, but men have higher blood volumes than women, and, as a result, larger amounts of lymphocytes and monocytes, which are directly involved in ADA activation. This is perhaps one of the possible reasons for the difference, besides other factors that may be involved, such as hormone, genetic, and body mass differences.

Dussol *et al*. (2004)[9] carried out a study with 12 CKD patients – eight man and four women – that were about to start hemodialysis. The study followed 36 sessions of the procedure. Serum levels of ADA transiently increased in CKD patients between the first and the third sessions, and then decreased, with increased serum adenosine, what is in conflict with our results. It is important to highlight that different methodologies were used for measurement of the enzymatic activity; still we used a highly sensitive and specific methodology for ADA determination.

Since the decades of 1950 and 1960, ADA activity has been determined in the serum of patients with neoplasias; inflammatory, hepatic, and autoimmune diseases; and human immunodeficiency virus (HIV)[10-12]. In addition, medicines, as well as materials and procedures used in hemodialyzed patients (time on dialysis, membrane and dialysate bath, for instance) could stimulate the increase in adenosine, and as a consequence, in ADA, which occurs simultaneously due to the increased substrate of this enzyme. Studies with CKD patients undergoing hemodialysis without associated diseases, besides *in vitro* studies with the most commonly medicines used by patients with CKD must be completed for further elucidation of the exact mechanisms leading to increased or decreased activity of this enzyme. This may help to diagnosis or exclude inflammatory abnormalities in CKD patients undergoing hemodialysis.

Urea and creatinine levels are important parameters in the diagnosis and follow-up of CKD, being useful for control and treatment. Urea, one of the by-products of protein metabolism, accumulates in the blood in CKD, causes uremia, and its serum concentration increases as GFR decreases[13]. In our study all individuals presented high levels of urea before hemodialysis, as expected; levels after hemodialysis were normal. Urea presented an average of 123.8 mg/dl ± 4.6 before hemodialysis and 43.5 mg/dl ± 2.5 after it ($p < 0.0001$). This fact may also happen due to dietary neglect, medication, routine or interference with treatment. During hemodialysis, excess urea is just partially removed. In order to prevent accumulation, it is very important to balance the amount of consumed proteins, avoiding excessive production of urea. In any case, the hemodialytic process in the studied patients was perceived to be efficient, because significantly reduced levels were found after it. Draczevski and Teixeira (2011)[14], in a study that assessed pre- and post-hemodialysis urea levels, obtained results in which there was a significant reduction in serum levels, showing that this process is efficient, favoring the relationship with the present study. Similarly, this study found decreased post-hemodialysis creatinine levels: creatinine presented an average of 9.7 mg/dl ± 0.5 before and 4.2 mg/dl ± 0.3 after hemodialysis ($p < 0.0001$). These results confirm that hemodialysis is an efficient method to remove undesired substances from CKD patients, even when values are above the desired reference levels.

CONCLUSION

We concluded that hemodialysis is an efficient and indispensable process for filtration of undesired metabolites in CKD patients, increasing their life expectancy. We also observed that post-hemodialysis serum levels of ADA are increased, regardless of sex, although they are higher in men than in women, what suggests evidence of an increased inflammatory process during hemodialysis. These preliminary results corroborate serum ADA determination as a biomarker to monitor the post-hemodialysis inflammatory process in CKD patients. Nonetheless, despite significant results, wider and more specific studies are necessary to explain the exact mechanisms of this enzyme's increase during hemodialysis, to confirm the efficiency of the increased serum ADA activity as a post-hemodialysis inflammatory marker for CKD patients, and to solve the contradictions existing in the literature over the activity of this enzyme in hemodialyzed patients.

ACKNOWLEDGEMENTS

The authors thank Universidade do Oeste de Santa Catarina and Clínica Renal do Extremo Oeste, of São Miguel do Oeste (SC), Brazil, and all the volunteers that participated in this study.

RESUMO

Introdução: *No decorrer dos últimos anos, houve aumento significativo nas internações e nos tratamentos decorrentes de complicações renais que resultaram, consequentemente, no aumento de pacientes sujeitos a diálise. A adenosina deaminase (ADA) atua como enzima mediadora na formação de algumas células de defesa do organismo, sendo, portanto, marcadora de processos inflamatórios.* ***Objetivo:*** *O objetivo deste trabalho foi avaliar biomarcadores da função renal e da ADA sérica de pacientes em hemodiálise.* ***Materiais e métodos:*** *Amostras de sangue foram coletadas de 80 pacientes – 40 mulheres e 40 homens – entre 19 e 60 anos, antes e após a realização da hemodiálise.* ***Resultados:*** *Houve diferença significativa nas dosagens de creatinina, ureia e ADA no pré e pós-hemodiálise (*$p < 0,0001$*). Observou-se aumento significativo da ADA no pós-hemodiálise independentemente do sexo, no entanto houve aumento considerável nos homens.* ***Conclusão:*** *Os resultados mostraram redução nos parâmetros de ureia e creatinina, evidenciando o propósito principal da hemodiálise. Por meio deste estudo, sugere-se que a determinação da atividade da ADA pode ser utilizada para monitorar o processo inflamatório de pacientes em hemodiálise, contudo estudos mais amplos e específicos são necessários para mostrar a eficiência da dosagem de ADA sérica como marcador inflamatório para pacientes com doença renal crônica.*

Unitermos: adenosina deaminase; hemodiálise; ureia; creatinina; renal.

REFERENCES

1. Cherchiglia ML, Gomes IC, Alvares J, et al. Determinantes dos gastos com diálises no Sistema Único de Saúde, Brasil, 2000 a 2004. Cad Saúde Pública. 2010; 26(8): 1627-41.

2. Rennke HG, Dennker BM. Fisiopatologia renal: princípios básicos. 2 ed. São Paulo: Livraria Médica Paulista Editora; 2009.

3. Mak RH, Ikizler AT, Kovesdy CP, Raj DS, Stenvinkel P, Kalantar-Zadeh K. Wasting in chronic kidney disease. J Cachexia Sarcopenia Muscle. 2011; 2(1): 9-25.

4. Alves CMP, Teixeira MCB, Martino MC. Dosagem de marcadores de lesão endotelial em pacientes com doença renal crônica em hemodiálise. J Bras Patol Med Lab. 2010; 46(3): 207-14.

5. Livi AL. Adenosina deaminase no diagnóstico de rejeição aguda após o transplante renal [dissertação]. Porto Alegre: Universidade Federal do Rio Grande do Sul (USGRS); 1999.

6. Bota A, Gella FJ, Profilis C, et al. Production and certification of an enzyme reference material for adenosine deaminase 1 (BCR 647). Clin Chim Acta. 2001; 306(1-2): 79-89.

7. Romão Jr JE. Doença renal crônica: definição, epidemiologia e classificação. J Bras Nefrol. 2004; 26(3 Suppl 1).

8. Marlewski M, Smolenski RT, Szolkiewicz M, Aleksandro Wicz Z, Rutkowski B, Swierczynski J. Accelerated degradation of adenine nucleotide in erythrocytes of patients with chronic renal failure. Mol Cell Biochem. 2000; 213(1-2): 93-7.

9. Dussol B, Fenouillet E, Brunet P, et al. Kinetic study of adenosine concentrations and the expression of adenosine deaminase in mononuclear cells during hemodialysis. Kidney Int. 2004; 66(4): 1640-6.

10. Goldberg DM. Serum adenosine deaminase in the differential diagnosis of jaundice. Br Med J. 1965; 1(5431): 353-5.

11. Taylor A. Serum adenosine deaminase activity is increased in sarcoidosis. Clin Chem. 1984; 30(3): 499-500.

12. Gakis C, Calia G, Naitana AG, Pirino D, Serru G. Serum adenosine deaminase activity in HIV positive subjects. A hypothesis on the significance of ADA2. Panminerva Med. 1989; 31(3): 107-13.

13. Souza C, Oliveira S, Guedes AM, et al. Avaliação das dosagens de ureia pré e pós-hemodiálise em uma unidade de saúde. Cadernos de Graduação – Ciências Biológicas e da Saúde. 2011 Jan/Jun; 13(13): 11-17.

14. Draczevski L, Teixeira ML. Avaliação do perfil bioquímico e parâmetros hematológicos em pacientes submetidos à hemodiálise. Rev Saud Pesq. 2011; 4(1): 15-22.

MAILING ADDRESS

Eduardo Ottobelli Chielle
Universidade do Oeste de Santa Catarina; Rua Oiapoc, 211; Agostini; CEP: 89900-000; São Miguel do Oeste-SC, Brazil; e-mail: eduardochielle@yahoo.com.br.