01/17

BJ

BANTAO Journal

Official Publication of the BANTAO Association

Incorporating Proceedings of the BANTAO Association

Published by: Balkan Cities Association of Nephrology, Dialysis, Transplantation and Artificial Organs

Printing: BANTAO, 2015

Contents

I. Review articles

II. Original Articles

III. Short Communication

IV. Case Reports

V. Letter to the Editor

BANTAO Journal 2017; 15(1): 1-5

BJ

BANTAO Journal

Review

Prevention of Diabetic Kidney Disease in the Light of Current Literature

Harun Akar[1], Emin Taskiran[1], Dilek Taskiran[2] and Oytun Erbas[3]

[1]University of Health Sciences, Izmir Tepecik Health Research and Application Center, Department of Internal Medicine, Izmir, [2]Ege University, Medical School, Department of Physiology, Izmir, [3]School of Medicine, Department of Physiology, Istanbul Bilim University, Istanbul, Turkey

Abstract

Diabetes is a rapidly growing problem of the community health. The resulting morbidity and mortality are responsible for the complications of diabetes. Nephropathy caused by diabetes often causes serious morbidity and mortality. In this review, we discuss the current approaches to prevent diabetic nephropathy based on the available literature evidence.

Keywords: nephropathy, novel approaches, diabetes

Introduction

Type 2 diabetes mellitus (T2DM) is one of the rapidly spreading illnesses universally [1]. When the macro- and microvascular complications develop, problems related to diabetes are multiplied. Simply, diabetic microvascular complications contain nephropathy, retinopathy, and neuropathy. Renal and cardiovascular complications are the most problematic faces of T2DM. One of the goals of diabetes treatment is to prevent morbidity and mortality due to these complications.

Lifestyle Modifications

Treatment is based on controlling blood glucose and blood pressure levels pharmacologically in an ailment that is firmly related to lifestyle components such as bad diet habits, stationary and sedentary life [2]. One of the most important element of diabetes care should compose dietary interventions [1]. Nutrition information helps keep blood sugar levels optimal in patients with T2DM [3]. Increasing nutritional knowledge and experience provides a balanced approach to the care of T2DM [3]. Decreasing hemoglobin A1c (HbA1c) can be presented along with the oral anti-diabetic drugs with patient education programs such as medical nutrition therapy (MNT). Individualized MNT was found effective in decreasing HbA1c level in patients diagnosed with pre-diabetes [4]. Regular movement prevents disease progression in subjects with impaired glucose tolerance [5]. The majority of people with T2DM are individuals with weight management problems, and weight loss is an approach which is preferable. Most of the weight-loss initiatives in overweight or obese adults with T2DM result in less than 5 percent weight loss and it would not lead to useful results metabolically. When more than five percent weight loss it can provide positive effects on glycemic control and blood pressure. Achieving this level of weight loss needs concentrated attempts such as energy limitation and tidy physical activity. Losing weight in many diabetics with weight problems is not a realistic approach to primary treatment of meticulous glycemic control. Nutrition therapy for individuals with T2DM should urge a healthy eating behavior, decreased energy intake, orderly and steady physical activity [6]. T2DM develops as a result of a decrease in the sensitivity of insulin receptors. Largely, these diabetic patients have uncontrolled blood sugar levels, obesity and cardiovascular disease. Metabolic correction such as proper diet and scientific supplementation has been suggested as a suitable approach to improve clinical parameters [7]. In addition, attempts as precise metabolic, blood pressure and lipid management is aimed to slow down the loss of renal function. Therefore, to develop new methods to improve the complications of T2DM has become a priority for research. Despite the rapid progress of information on the molecular mechanisms of these complications, effective new treatments are still missing [8]. Despite recent scientific innovations, diabetic nephropathy continues to be a therapeutic problem. Diabetes management, targets a retarded progression of renal function loss by rigorous blood sugar, blood pressure and lipid control. At the same time, diabetes treatment is necessary to be individualised. Meticulous, tight glycemic control reduces microvascular complications in patients with diabetes. On the other hand, meticulous glycemic control has a limited effect on the macrovascular complications or it may lead to increased risk of major

Correspondence to: Harun Akar, University of Health Sciences, Izmir Tepecik Health Research and Application Center, Department of Internal Medicine, Izmir, Turkey; E-mail: harunakar.md@gmail.com

cardiovascular events [9]. By the way, smoking is a risk factor for the progression of diabetic nephropathy in patients with type 1diabetes [10]. So, one of the most important initiatives that can be done at this point may be to quit smoking.

Conventional treatments and renal and cardiovascular complications

Insulin and glyburide were observed to be associated with an increased risk of major cardiovascular events. Lachin *et al*. examined differences among rosiglitazone, metformin and glyburide over 5 years [11]. Albumin/creatinine ratio, eGFR, and blood pressure were examined in the patients. The albumin/creatinine ratio was found to slowly increase with metformin. Metformin was found as ineffective in lowering albuminuria and in microvascular protection in the short and long term, respectively. The albumin/creatinine ratio was found to slightly decrease with glyburide during the first 2 years, then increased slowly over time.

Strict control of blood sugar and blood pressure at desired levels prevent the formation and progression of microvascular complications. Albuminuria reducing effects of renin-angiotensin system inhibitors are greater than those of calcium channel blockers. In the ADVANCE study, a combined touch of blood pressure reducing and meticulous glucose control by gliclazide resulted in significant reductions in major renal events and all-cause mortality [12]. During 4.3 years of follow-up, risks of macrovascular and microvascular events, renal events, and death were assessed yearly in this study.

There was no report about the possible role of alpha-glucosidase inhibitors on the progression of diabetic nephropathy. Positive effects of metformin have been shown in the recently diagnosed T2DM with obesity [13]. Metformin lowers both mortality and morbidity in T2DM patients probably due to its cardioprotective property that is independent of its hypoglycemic effect, and not produced by sulphonylureas or insulin [14]. Despite this positive cardiovascular effects in patients with T2DM, there is no evidence of nephropathy reducing effect of metformin.

Abdominal obesity, metabolic syndrome, type 2 diabetes, cardiovascular disease and microvascular diabetic complications are all closely connected with chronic low grade inflammation. Likewise, glucose-lowering agents could additionaly contribute to improved outcomes via their anti-inflammatory effects [15]. Since both agents are considered as insulin-sparing or insulin sensitizers, most of the studies have used metformin and pioglitazone for this purpose. Both agents seem to have greater anti-inflammatory activity than sulphonylureas or glinides. Metformin and pioglitazone act as AMP-activated protein kinase (AMPK) activator and peroxisome proliferator-activated receptor-gamma (PPARγ) agonists, respectively. Pioglitazones were associated with very low tissue and serum inflammation. The effect of α-glucosidase inhibitors on inflammatory markers seems quite small. PPAR-γ agonists such as thiazolidinediones make insulin resistance better and contribute to diabetes control in theory.

Externally supplied insulin may also have anti-inflammatory effects. It is necessary to differentiate anti-inflammatory effects due to meticulous glucose control from anti-inflammatory class effect.

The classic nomenclature "diabetic nephropathy" has altered to "diabetic chronic kidney disease" (DCKD) [16]. In the diabetic environment, both metabolic and hemodynamic disequilibriums, principally such as activation of the renin-angiotensin system, create a chain of events. Tubular epithelial cells, podocytes, and mesangial cells can fabricate profibrotic cytokines which contribute to increasing proteinuria during the formation and progression of DCKD [17]. The benefits of intensive glycemic control in type 1 and 2 DM were delaying the commencement and progression of DCKD and decreasing the cardiovascular event rates [17,18]. There is no detailed information specific to renal protective properties of insulin therapy other than the benefits arising from rigorous blood glucose control [17]. On the other hand, as a more advanced technology insulin pump therapy was found to be associated with lesser cardiovascular mortality than multiple insulin injection in type 1 diabetes mellitus [19].

New pharmacological strategies and renal and cardiovascular complications

Dipeptidyl peptidase-4 inhibitors (DPP4-I) reduce blood glucose levels by preserving glucagon-like peptide-1 (GLP-1) from enzymatic breakdown, thereby repairing insulin release induced by food. Since DPP-4 inhibitors have some non-incretin substrates, such as cytokines, chemokines, and neurohormones, there may be some desired and unanticipated vascular effects due to stabilization of these substrates by DPP4-I.

As a result of experimental studies, inhibition of DPP4 is thought to improve inflammation, endothelial function, blood pressure, and lipid metabolism [20]. Potentially beneficial effects were reported on diabetic microvascular complications as a result of the inhibition of DPP4 in experimental studies [21]. Although early clinical knowledge support the protective role of DPP4 inhibitors in diabetic microangiopathy, there is inadequate information claiming that this class of drugs directly prevents or reduces microangiopathy independent of glucose control in humans [21]. Experimental plus early clinical knowledge propose that DPP4 inhibition has the potential to interfere with the onset and progression of microangiopathy [21,22]. DPP4 inhibitors were found to set against vascular smooth muscle cell proliferation which alleviates neointimal hyperplasia in experimental and early clinical studies [21]. Although

experimental and early clinical studies propose that DPP4-I can provide protective effects on vasculature, results of placebo-controlled phase IV trials have yet shown no decline in cardiovascular outcomes [21]. Zheng *et al*. demonstrated that increased plasma DPP4 activities were vigorously associated with type 2 diabetic nephropathy. They suggested these associations do not suggest causality [15]. DPP-4 inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists seem more encouraging in terms of their anti-inflammatory effects [15]. These incretin-based therapies carry out pleiotropic effects.

Inhibitors of sodium-glucose cotransporters type 2 (SGLT2) recommend a new route for the handling of T2DM [23]. These promising drugs diminish hyperglycemia by increasing urinary glucose excretion. Ensuing decline in glucotoxicity rehabilitates beta-cell sensitivity to glucose and tissue insulin sensitivity. So, SGLT2 inhibitors meaningful and permenantly lower glycated hemoglobin, with the least hypoglycemia risk. The amelioration of glucose control is similar or even slightly better than metformin, sulfonylureas or sitagliptin, with the add-on value by providing meaningful reductions in body weight and blood pressure. Inhibition of SGLT2 by dapagliflozin has moderate and marked effects on uric acid elimination and reduces plasma uric acid levels. Although the inhibition of SGLT2 increases uric acid excretion, the osmotic effect of glucose significantly dilutes the urine, increases the urine flow rate and reduces the likelihood of nidus formation [24]. Inhibitors of sodium-glucose transporter-2 (SGLT-2) may have a "potentially nephroprotective" effect by a number of mechanisms such as restoring the tubuloglomerular feedback mechanism and reducing glomerular hyperfiltration, reducing hyperglycemic inflammation and fibrotic markers, and limiting renal injury. Attention has been suggested in frail geriatric patients and patients with chronic kidney disease. It shows an increased risk of genital fungal infections. Concern about an unforeseen risk of euglycemic ketoacidosis has been lately reported. As an interesting finding, a striking reduction in cardiovascular mortality was reported with empagliflozin which is an example of a SGLT2 inhibitor. Canagliflozin usage in elderly patients and in patients with renal impairment was found to be associated with a decreased efficiency and an increased risk of unwanted effects such as hypotension, further deterioration of renal function, hyperpotassemia, hypoglycemia, genital fungal infections, and elevation in the low-density lipoprotein levels [25]. There are no clinical studies about anti-inflammatory effects of sodium-glucose cotransporter type 2 (SGLT2) inhibitors. Although SGLT2 inhibitors may have indirect effects due to reduced glucotoxicity, they do not seem to have a systemic anti-inflammatory activity.

Tight blood pressure and glucose control is evenly important. Renin-angiotensin-aldosterone system (RAAS) inhibitor medications only partially protect against the development and progression of diabetic nephropathy. In contrast, RAAS inhibitors seem to fail as primary prevention therapy in type 1 diabetes [26]. Examples for hopeful targets for neurohormonal activation (inhibition of alternative components of RAAS and neprilysin), tubuloglomerular feedback mechanisms (sodium glucose cotransporter 2 inhibitors and incretin-based therapy) and renal tissue inflammation and fibrosis. DPP4 activity was demonstrated as a predictor for the onset of inflammation and microalbuminuria in Chinese patients without diabetes and it was suggested that this finding might have important implications in understanding the proinflammatory role of DPP4 in microalbuminuria pathogenesis [27]. There was a limited number of reports on the assessment of different antihypertensive agents in preventing nephropathy among type 1 hypertensive diabetics. Many studies display the profit of an ACEI or ARB in preventing or delaying the onset of nephropathy in patients with type 2 diabetes and hypertension [28]. Antiproteinuric effects of RAS combination therapy do not seem to augment the prevention of renal disease progression. Combined use of the RAS inhibiting agents was found to be associated with an increased rate of serious adverse events [29].

Discussion

The common point of microvascular complications is glucose-induced damage. Factors that lead to the progression of diabetic complications may be the formation of oxidative stress, inflammation and advanced glycation end products. Carotenoids (such as lutein, zeaxanthin, lycopene, and astaxanthin) as part of the antioxidant system may contribute to attenuate free radical injury, and reduce inflammation, and they may be used as part of an approach to inhibit the complications associated with diabetes [30]. Recent molecules, such as bardoxolone methyl, pentoxifylline, inhibitors of protein kinase C (PKC), sulodexide, pirfenidone, endothelin receptor antagonists, vitamin D supplements, and phosphate binders have been associated with controversial consequences or significant side effects [29].

GLP-1R agonists have impact over glucose control that may have an indirect role in nephroprotection. GLP-1R agonists induce fullness and reduce body weight. Body weight was not affected by DPP-4 inhibitors. DPP4 inhibitors improve renal microvascular complications to a lesser extent than GLP-1R agonists [31]. Incretin-based therapies may have positive effects on renal hemodynamic, metabolic and inflammatory parameters involved in the pathogenesis of diabetic nephropathy [31]. Both incretin-based drugs have been reported to inhibit renal tubular sodium reabsorption in experimental models and in humans, as well as to reduce glomerular pressure and albuminuria [31]. DPP4 inhibitor linagliptin was found to be associated with a significant reduction

in clinically relevant kidney disease end-points in patients with type 2 DM in a large-scale study [32].
Both the angiotensin converting enzyme and dipeptidyl peptidase-4 are the main enzymes involved in the degradation of bradykinin and substance P, theoretically increasing the risk of angioedema (AO), especially due to the accumulation of vasoactive kinins, when both are pharmacologically inhibited. It has been reported that the use of concurrent ACEI and DPP-4 inhibitor may be associated with a five-fold increase in the risk of AO when compared with ACEI alone. Patients with a known predisposition for angioedema should be carefully considered and monitored when treating [33].

Conclusions

The available early information on the potential cardiorenoprotective role of SGLT2 inhibition in patients with diabetes are supportive. Strong enough trials with renal outcomes are needed to assess the renal protective effects of antihyperglycemic drugs, especially in the cases of dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 receptor agonists and sodium-glucose cotransporter 2 inhibitors. Since glucose, blood pressure and lipid levels are paramount parameters of vascular load, new approaches are needed to adjust these factors vigorously together in T2DM. There is still a long way to go in the field of DN research.

Acknowledgements. No potential conflicts of interest relevant to this article were reported. All authors contributed to acquiring and interpreting data, reviewed/edited the manuscript and are taking responsibility for the contents of the article.

Conflict of interest statement. None declared.

References

1. Hołynska A, Kucharska A, Sinska B, Panczyk M. [The level of nutrition knowledge versus dietary habits of diabetes patients treated with insulin]. [Article in Polish] *Pol Merkur Lekarski* 2015; 39 (233): 292-296.
2. Simpson KA, Mavros Y, Kay S, *et al*. Graded Resistance Exercise And Type 2 Diabetes in Older adults (The GREAT2DO study): methods and baseline cohort characteristics of a randomized controlled trial. *Trials* 2015; 16(1): 512.
3. Breen C, Ryan M, Gibney MJ, O'Shea D. Diabetes-related nutrition knowledge and dietary intake among adults with type 2 diabetes. *Br J Nutr* 2015; 114(3): 439-447.
4. Parker AR, Byham-Gray L, Denmark R, Winkle PJ. The effect of medical nutrition therapy by a registered dietitian nutritionist in patients with prediabetes participating in a randomized controlled clinical research trial. *J Acad Nutr Diet* 2014; 114(11): 1739-1748.
5. Rossen J, Yngve A, Hagstromer M, *et al*. Physical activity promotion in the primary care setting in pre- and type 2 diabetes-the Sophia step study, an RCT. *BMC Public Health* 2015; 15: 647.
6. Franz MJ, Boucher JL, Rutten-Ramos S, VanWormer JJ. Lifestyle weight-loss intervention outcomes in overweight and obese adults with type 2 diabetes: a systematic review and meta-analysis of randomized clinical trials. *J Acad Nutr Diet* 2015; 115(9): 1447-1463.
7. Miranda-Massari JR, Gonzalez MJ, Fernando AS, *et al.* Metabolic Correction as a tool to improve diabetes type 2 management. *Bol Asoc Med Puerto Rico* 2015; 107(2): 54-59.
8. Pryor R, Cabreiro F. Repurposing metformin: an old drug with new tricks in its binding pockets. *Biochem J* 2015; 471(3): 307-322.
9. Bell DS, Patil HR, O'Keefe JH2. Divergent effects of various diabetes drugs on cardiovascular prognosis. *Rev Cardiovasc Med* 2013; 14(2-4): e107-e122.
10. Feodoroff M, Harjutsalo V, Forsblom C, *et al.* Smoking and progression of diabetic nephropathy in patients with type 1 diabetes. *Acta Diabetol* 2016; 53(4): 525-533.
11. Lachin JM, Viberti G, Zinman B, *et al.* ADOPT Study Group. Renal function in type 2 diabetes with rosiglitazone, metformin, and glyburide monotherapy. *Clin J Am Soc Nephrol* 2011; 6(5): 1032-1040.
12. Zoungas S, de Galan BE, Ninomiya T, *et al.* Combined effects of routine blood pressure lowering and intensive glucose control on macrovascular and microvascular outcomes in patients with type 2 diabetes: New results from the ADVANCE trial. *Diabetes Care* 2009; 32(11): 2068-2074.
13. Buhse S, Muhlhauser I, Lenz M. The 'Old' Anti-Diabetic Agents: A Systematic Inventory. *Endocr Dev* 2016; 31: 28-42.
14. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet* 1998; 352(9131): 854-865.
15. Zheng T, Liu Y, Qin S, *et al*. Diab Vasc. Increased plasma dipeptidyl peptidase-4 activities are associated with high prevalence of diabetic nephropathy in Chinese patients with newly diagnosed type 2 diabetes: A cross-sectional study. *Dis Res* 2016; 13(2): 127-136.
16. Martinez-Castelao A, Navarro-Gonzalez JF, Gorriz JL, de Alvaro F. Review the Concept and the Epidemiology of Diabetic Nephropathy Have Changed in Recent Years. *J Clin Med* 2015; 4(6): 1207-1216.
17. Gorriz JL, Nieto J, Navarro-Gonzalez JF, *et al*. Nephroprotection by Hypoglycemic Agents: Do We Have Supporting Data? *J Clin Med* 2015; 4(10): 1866-1889.
18. Nathan DM, Cleary PA, Backlund JY, *et al.* Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 2005; 353(25): 2643-2653.
19. Steineck I, Cederholm J, Eliasson B, *et al.* Swedish National Diabetes Register. Insulin pump therapy, multiple daily injections, and cardiovascular mortality in 18,168 people with type 1 diabetes: observational study. *BMJ* 2015; 350: h3234.
20. Avogaro A, Fadini GP. The effects of dipeptidyl peptidase-4 inhibition on microvascular diabetes complications. *Diabetes Care* 2014; 37(10): 2884-2894.
21. Fadini GP, Albiero M, Avogaro A. Direct effects of DPP-4 inhibition on the vasculature. Reconciling basic evidence with lack of clinical evidence. *Vascul Pharmacol* 2015; 73: 1-3.
22. Scheen AJ, Esser N, Paquot N. Antidiabetic agents: Potential anti-inflammatory activity beyond glucose control. *Diabetes Metab* 2015; 41(3): 183-194.
23. Scheen AJ. SGLT2 inhibition: efficacy and safety in type 2 diabetes treatment. *Expert Opin Drug Saf* 2015; 14(12): 1879-1904.
24. Thomas MC. Renal effects of dapagliflozin in patients with type 2 diabetes. *Ther Adv Endocrinol Metab* 2014; 5(3): 53-61.

25. Toderika Y1, Ferguson N. Canagliflozin: a new class of anti-diabetic agent targeting the sodium-glucose cotransporter. *Cardiol Rev* 2014; 22(2): 97-104.
26. Lytvyn Y, Bjornstad P, Pun N, Cherney DZ. New and old agents in the management of diabetic nephropathy. *Curr Opin Nephrol Hypertens* 2016, Feb 17.
27. Zheng T, Baskota A, Gao Y, *et al*. Increased plasma dipeptidyl peptidase 4 activities predict new-onset microalbuminuria in association with its proinflammatory effects in Chinese without diabetes: a four-year prospective study. *Nephrol Dial Transplant* 2015; 30(3): 460-466.
28. Isaacs AN, Vincent A. Antihypertensive therapy for the prevention of nephropathy in diabetic hypertensive patients. *J Clin Pharm Ther* 2016; 41(2): 111-115.
29. Dounousi E, Duni A, Leivaditis K, *et al*. Improvements in the Management of Diabetic Nephropathy. *Rev Diabet Stud* 2015; 12(1-2): 119-133.
30. Murillo AG, Fernandez ML. Potential of Dietary Non-Provitamin A Carotenoids in the Prevention and Treatment of Diabetic Microvascular Complications. *Adv Nutr* 2016; 7(1): 14-24.
31. Muskiet MH, Smits MM, Morsink LM, Diamant M. Review The gut-renal axis: do incretin-based agents confer renoprotection in diabetes? *Nat Rev Nephrol* 2014; 10(2): 88-103.
32. Cooper ME, Perkovic V, McGill JB, *et al*. Kidney Disease End Points in a Pooled Analysis of Individual Patient-Level Data From a Large Clinical Trials Program of the Dipeptidyl Peptidase 4 Inhibitor Linagliptin in Type 2 Diabetes. *Am J Kidney Dis* 2015; 66(3): 441-449.
33. Scott SI, Andersen MF, Aagaard L, *et al*. Dipeptidyl peptidase-4 inhibitor induced angioedema-an overlooked and potentially lethal adverse drug reaction? *Curr Diabetes Rev* 2017, Feb 14.

BANTAO Journal 2017; 15(1): 6-9

BJ

BANTAO Journal

Review

Resistant Hypertension and Cardiorenovascular Risk

Ingrid Prkacin[1,2], Petra Vrdoljak[2], Gordana Cavric[3], Damir Vazanic[4], Petra Pervan[5] and Visnja-Nesek Adam[6]

[1]Department of Internal Medicine, Merkur Clinical Hospital, Zagreb, [2]University of Zagreb, School of Medicine, Zagreb, [3]Intensive Unit, Merkur Clinical Hospital, Zagreb, [4]Croatian Institute of Emergency Medicine, Zagreb, [5]Public Health Centre Zagreb-Center, Zagreb, [6]Department for Anesthesiology, Resuscitation and Intensive Care, University Hospital Sveti Duh, Zagreb; University of Osijek, School of Medicine Osijek, Croatia

Abstract

Studies have documented independent contribution of sympathetic activation to the cardiovascular disease continuum. Hypertension is one of the leading modifiable factors. Most if not all the benefit of antihypertensive treatment depends on blood pressure lowering, regardless how it is obtained. Resistant hypertension is defined as blood pressure that remains uncontrolled in spite of the concurrent use of three antihypertensive drugs of different classes. Ideally, one of the three drugs should be a diuretic, and all drugs should be prescribed at optimal dose amounts.

Poor adherence to antihypertensive therapy, undiscovered secondary causes (e.g. obstructive sleep apnea, primary aldosteronism, renal artery stenosis), and lifestyle factors (e.g. obesity, excessive sodium intake, heavy alcohol intake, various drug interactions) are the most common causes of resistant hypertension.

Cardio(reno)vascular morbidity and mortality are significantly higher in resistant hypertensive than in general hypertensive population, as such patients are typically presented with a long-standing history of poorly controlled hypertension. Early diagnosis and treatment is needed to avoid further end-organ damage to prevent cardiorenovascular remodeling.

Treatment strategy includes lifestyle changes, adding a mineralocorticoid receptor antagonist, treatment adherence in cardiovascular prevention and, in case of failure to control blood pressure, renal sympathetic denervation or baroreceptor activation therapy. The comparative outcomes in resistant hypertension deserve better understanding. In this review, the most current approaches to resistant hypertension and cardiovascular risk based on the available literature evidence will be discussed.

Keywords: resistant hypertension, cardiorenovascular risk

Introduction

Despite numerous treatment methods, a significant number of patients does not achieve optimal blood pressure levels. Resistant hypertension (RH) is diagnosed when treatment strategy including lifestyle changes and use of three antihypertensive drugs (one of them being a diuretic) at recommended doses fails to lower systolic blood pressure (SBP) and diastolic blood pressure (SBP) values to <140 and <90 mmHg (<140/85 mmHg for diabetic patients), or when patients use four or more antihypertensive drugs regardless of blood pressure control [1]. The prevalence of RH in general hypertensive population is 10-15% [2,3]. RH can be real or apparent (pseudoresistant). The most common causes of pseudoresistance are:

1) poor adherence to antihypertensive therapy;
2) white-coat effect;
3) inaccurate measurement of blood pressure;
4) pseudohypertension, i.e. arterial stiffening caused by extensive calcifications which prevents occlusion of the brachial artery; it is more common in elderly patients [4].

Poor adherence to antihypertensive therapy is the most important cause of unsuccessful blood pressure control. Retrospective analyses show that approximately 40% of patients will not continue their antihypertensive medications during the first year after diagnosing RH. During 5-10 years of follow-up, those numbers reach 60% [5]. Inaccurate blood pressure measurement is not uncommon; it occurs when patients are not instructed to sit calmly and quietly, and when the cuff is too small [6]. Keeping that in mind, pseudoresistant hypertension should not be confused for real one in order to avoid unnecessary diagnostic procedures and treatments. Successful treatment involves the physician (correct pharmacological approach, reduce therapeutic inertia) and the patient (to regulary take medications that have been proven effective and well tolerated).

Correspondence to: Ingrid Prkacin, Clinical Hospital Merkur, Department of Internal Medicine, I. Zajca 19, Zagreb, Croatia; Phone: 00 385 1 23 53 470; Fax: 00 385 1 24 31 393; E-mail: ingrid.prkacin@gmail.com

We need diagnosis-based approach that takes into consideration not only a person's blood pressure but also the overall cardiovascular risk (CVD) [7].

Predictors of resistant hypertension

Studies of RH are limited by high cardiovascular (CV) risk in this population; comorbidities (e.g. diabetes, chronic kidney disease, obstructive sleep apnea) and their associated medical therapies which can be confounding factors; and the inability to include a large number of study participants [8]. However, studies show that BP is usually not regulated because of persistently elevated SBP [9]. In early adulthood, SBP is higher in men than in women, whereas after the age of 60, it is higher in women. DBP values also increase until the age of 55, and later steadily lower. Consequently, pulse pressure (the difference between SBP and DBP) increases. Other predicting factors are old age, obesity, and chronic kidney disease [9,10].

Etiology

RH has an extreme phenotype, so it would be reasonable to expect that genetic factors may play a great role. Although the genetic researches are limited, a certain number of genes has been associated with impaired response to antihypertensive treatment. However, environmental factors contribute to the development of RH, making it a multifactorial disease [4].
Lifestyle factors that are associated with RH are obesity (BMI>30 kg/m2), excessive dietary sodium intake, heavy alcohol intake, and use of drugs such as nonnarcotic analgesics, especially nonsteroidal antiinflammatory agents (NSAIDs) [4].
Secondary causes include unrecognized/untreated obstructive sleep apnea (OSA), primary aldosteronism, chronic parenchimal kidney disease, renal artery stenosis, and diabetes. Uncommon causes are pheochromocytoma, Cushing's disease, aortic coarctation, and intracranial tumors [8].

Diagnosis

To determine true hypertension, the French Society of Hypertension gave the following recommendations [11]:

1) To avoid poor blood pressure measurement, a standardized device and an appropriate cuff-size should be used;
2) White-coat effect should be eliminated by ambulatory (ABPM) or home (HBPM) blood pressure measurement. Thresholds for uncontrolled hypertension are: HBPM≥135/85 mmHg, 24-h ABPM ≥130/80 mmHg, day-time ABPM ≥135/85 mmHg, night-time ABPM ≥120/70 mmHg;
3) It is necessary to determine if the optimal triple-drug therapy is prescribed;
4) Poor patient compliance should be assessed using a questionnaire, urine drug analysis and pill-count;
5) It is suggested to search for factors that could influence treatment resistance (e.g. obesity, excessive dietary sodium intake, alcohol, drug interactions).

After true resistant hypertension is confirmed, evaluation should include identification of the underlying cause, and assessment of cardiovascular risk and end-organ damage [12]. While secondary causes are relatively rare in general hypertensive population, they are frequently found in RH. Medical history can be useful: loud snoring, daytime sleepiness and witnessed apnea indicate OSA; a history of peripheral or coronary atherosclerotic disease and worsening kidney funcion are suspicious for renal artery stenosis; labile hypertension followed by palpitations and diaphoresis indicate the possibility of pheochromocytoma. In physical examination, carotid, abdominal or femoral bruits indicate renal artey stenosis; moon facies, abdominal striae and central obesity suggest Cushing's disease; diminished femoral pulses and difference between arm and thigh blood pressures are suspicious for aortic coarctation or aortoiliac disease. Biochemical evaluation should include routine metabolic profile (sodium, potassium, chloride, bicarbonate, glucose, blood urea nitrogen, creatinine, albumin/creatinine ratio, eGFR), urine proteins, plasma aldosterone and renin so as their ratio can be calculated (high aldosterone/renin ratio indicates primary aldosteronism), metanephrine and normetanephrine in 24-hour urine, cortisol in 24-hour urine. Doppler of renal arteries, MR or MSCT angiography are recommended to assess the anatomy before RDN [12]. Lastly, screening for possible end-organ damage is extremely important [8].

Device therapies for resistant hypertension

In recent decades, the use of antihypertensive drugs has revolutionised the therapy of hypertension. Despite the available pharmacological inhibition of the sympathetic nervous system, about 50% of patients show suboptimal control and pharmacotherapy does not provide adequate effects in clinical practice. A hyperactivity of the sympathetic nervous system is a condition that confers a high cardiovascular risk in the patient [12,13]. New devices like Renal sympathetic denervation, Baroreceptor activation therapy and Continuous positive airway pressure were developed to interrupt the cardiovascular disease continuum, the leading cause of death globally.

Renal sympathetic denervation

Renal sympathetic denervation delivers energy to the renal nerves to help control blood pressure. Many observational studies have shown that RDN is a safe method of reducing office BP in patients with RH, with an additional positive effect on blood glucose metabo-

lism, obstructive sleep apnea and signs of hypertensive end-organ damage [12,14]. The reasons for the rapid introduction of RDN in the therapy of rHT were the reported high efficiency and safety of the procedure. The effectiveness was demonstrated in the studies Symplicity HTN-1 and HTN-2, and in the EnligHTN-1 Study (by using special RF ablation catheters). According to the results of different trials, including Symplicity HTN-3 (this study did not show differences in SBP reduction between treatment and control groups, but in the context of the study characteristics and the way it was conducted, there are several concerns about inexperienced doctors in the field of RDN, the study population and the medical treatment), RDN is safe and procedure-related complications of catheter-based RDN were very rare [12].

Baroreceptor activation therapy

Baroreceptor activation therapy (BAT) or baropacing can be applied in patients with treatment resistant hypertension. When baroreceptors sense an icrease in carotid transmural pressure, they respond by inhibiting sympathetic and stimulating parasympathetic centres in the brainstem [15]. As a result, any increase in blood pressure (BP) will return to its initial level. Most studies on BAT took only office BP as criterion for efficacy but only one study in which the effect on 24-hour was assessed showed that BP had fallen by 8/5 mmHg afer 6 months of therapy which was statistically significant [15].

Continuous positive airway pressure

Nasal continuous positive airway pressure (CPAP) ventilation is considered the treatment for obstructive sleep apnea (OSA) of moderate to severe degree [16]. The effects of CPAP on BP levels have been shown to be variable, but in some subgroups of patients, those with severe OSA or/and with RH, more substantial effects of CPAP have been reported [16].

Discussion

Cardiovascular diseases were the leading cause of mortality in 2012, comprising 31% of all deaths and killing 17.5 million people. Hypertensive disease of the heart, blood vessels, brain and kidney is frequently found in patients with RH. RH is a consequence of different pathophysiologic processes (e.g. increased stimulation of renin-angiotensin system and aldosterone production, arterial stiffness, atherosclerotic disease) that are associated with high cardiovascular risk [8].

CV morbidity and mortality is noted in patients with both high and relatively low levels of blood pressure (110-115 mmHg SBP and 70-75 mmHg DBP). CV risk doubles with increase of 20 mmHg SBP and 10 mmHg DBP [7]. SBP is a better predictor than DBP after the age of 50, and it seems that in older patients pulse pressure plays a significant role; that is indicated by particularly high CV risk in patients with high SBP and normal or low levels of DBP [17]. Other risk factors that are usually related with RH have a synergistic effect in development and worsening of RH.

Studies have shown 47% higher risk of cardiovascular morbidity and mortality in RH patients [18,19]. Significant worsening of heart and blood vessel disease and higher mortality has been noted in subpopulations with preexistant ischemic heart disease [20]. Studies indicate a notably higher CV risk in patients with RH and diabetes or chronic kidney disease compared to general hypertensive population [21,22]. Compared with the non-RH population, the RH population had a greater prevalence of comorbid conditions including diabetes mellitus (DM, 48% vs. 30%), CKD (45% vs. 24%), ischemic heart disease (41% vs. 22%), cerebrovascular disease (16% vs. 9%; $P<0.001$ for all) [23]. Compared with controlled RH patients (cRH)-individuals with uncontrolled RH (uRH; 61.7%) were at a greater risk for cerebrovascular (CVA) and end-stage renal disease (ESRD). The risk of ESRD and CVA were 25% and 23% greater, respectively, in uRH compared with cRH, supporting the linkage between blood pressure and both outcomes [23].

Patients with diabetes and/or chronic kidney disease (CKD) have sympathetic nervous system hyperactivation that leads to fluid overload, aggravation of hypertension and further deterioration and loss of renal function, and it has been demonstrated that RDN is associated with stabile kidney function [12]. The most obvious explanation relating effect of stabile kidney function could be that after RDN treatment an increased renal blood flow will result in increase in GFR. Renal sympathetic denervation (RDN) produce multilevel inhibition of the sympathetic nervous system, and trigers additional positive metabolic effects [13,14].

The effectiveness of all therapeutic approaches should be tested in diverse settings of uRH. Carefully designed studies (some ongoing, such as SPYRAL HTN OFF and ON-MED) will provide the evidence that RDN is also an efficacious therapy [14]. Further meta analysis will evaluate the importance of new devices.

Conclusions

It is widely accepted that hypertension is associated with a number of other CV risk factors (metabolic syndrome, endothelial dysfunction, arterial stiffness or kidney disease) that makes it a part of a multifactorial process in disease development. Poor adherence is a major cause of lack of blood pressure control and it can be misleading in further diagnostics and treatment with detection of drugs in blood and/or urine. Among chronic kidney disease (CKD) patients, those with RH have been reported to experience a two-fold greater

risk for both cardiovascular events and ESRD, compared with those without resistant hypertension.
RH population may benefit from more individualized care rather than broad, general recommendations

Acknowledgements. No potential conflicts of interest relevant to this article were reported. All authors contributed to acquiring and interpreting data, reviewed/edited the manuscript. I.P. contributed to interpreting data and wrote, reviewed/edited the manuscript. G.C. and V.N.A reviewed/ edited the manuscript. P.V., P.P. and D.V. are taking responsibility for the contents of the article.

Conflict of interest statement. None declared.

Reference

1. Vongpatanasin W. Resistant hypertension: a review of diagnosis and management. *JAMA* 2014; 311: 2216-2224.
2. Egan BM, Zhao Y, Axon RN, *et al*. Uncontrolled and Apparent Treatment Resistant Hypertension in the U.S. 1988–2008. *Circulation* 2011; 124(9): 1046-1058. doi: 10.1161/CIRCULATIONAHA.111.030189.
3. Sim JJ, Bhandari SK, Shi J, *et al.* Characteristics of Resistant Hypertension in a Large Ethnically Diverse Hypertension Population of an Integrated Health System. *Mayo Clinic Proceedings* 2013; 88(10): 1099-1107.
4. Prkacin I, Balenovic D, Jurina A, *et al*. Underestimated frequency of resistant hypertension in predialysis nondiabetic patients. *Acta Med Croatica* 2012; 66 (3): 229-233.
5. Pimenta E. Update on Diagnosis and Treatment of Resistant Hypertension. *Iran J Kidney Dis* 2011; 5(4): 215-227.
6. Bilo G, Sala O, Perego C, *et al*. Impact of cuff positioning on blood pressure measurement accurancy: may a special designed cuff make a difference? *Hypertension Research* 2017; 40(6): 573-580. doi: 10.1038/hr.2016.184.
7. Whelton PK, Reboussin DM, Fine LJ. Comparing SPRINT and the HOPE-3 blood pressure trial. *JAMA Cardiol* 2016; 1(8): 857-858. DOI:10.1001/jamacardio.2016.2051.
8. Calhoun DA, Jones D, Textor S, *et al.* Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Circulation* 2008; 117(25): e510-e526.
9. Gupta AK, Nasothimiou EG, Chang CL, *et al*. Baseline predictors of resistant hypertension in the Anglo-Scandinavian Cardiac Outcome Trial (ASCOT): a risk score to identify those at high-risk. *Hypertension* 2011; 29(10): 2004-2013. doi: 10.1097/HJH.0b013e32834a8a42.
10. Prkacin I, Ozvald I, Cavric G, *et al*. Importance of urinary NGAL, serum creatinine standardization and estimated glomerular filtration rate in resistant hypertension. *Coll Antropol* 2013; 37 (3): 821-825.
11. Denolle T, Chamontin B, Doll G, *et.al.* Management of resistant hypertension: expert consensus statement from the French Society of Hypertension, an affiliate of the French Society of Cardiology. *J Hum Hypertens* 2016. doi: 10.1038/jhh.2015.122.
12. Prkacin I, Vrhovec B, Legovic A, *et.al*. Renal denervation and resistant hypertension: back to the future. *Acta Med Croatica* 2016; 70(2): 41-45.
13. Prkacin I, Balenovic D, Djermanovic-Dobrota V, *et al*. Resistant hypertension and Chronotherapy. *Mater Sociomed* 2015; 27(2): 118-121.
14. Kandzari DE, Kario K, Mahfoud F, *et al*. The SPYRAL HTN Global Clinical Trial Program: Rationale and design for studies of renal denervation in the absence (SPYRAL HTN OFF-MED) and presence (SPYRAL HTN ON-MED) of antihypertensive medications. *Am Heart J* 2016; 171: 82-91.
15. Walbach M, Lehnig LY, Schroer C, *et al*. Effect of baroreflex activation therapy on ambulatory blood pressure in patients with resistan hypertension. *Hypertension* 2016; 67: 701-709.
16. Feldstein CA. Blood pressure effects of CPAP in nonresistant and resistant hypertension associated with OSA. A systematic review of randomized clinical trials. *Clin Exp Hypertens* 2016; 38: 337-346.
17. Vishram JK, Borglykke A, Andreasen AH, *et al*. Impact of Age on the Importance of Systolic and Diastolic Blood Pressures for Stroke Risk: The MOnica, Risk, Genetics, Archiving and Monograph (MORGAM) Project. *Hypertension* 2012; 60: 1117-1123.
18. Daugherty SL, Powers JD, Magid DJ, *et al*. Incidence and prognosis of resistant hypertension in hypertensive patients. *Circulation* 2012; 125(13): 1635-1642.
19. Bangalore S, Fayyad R, Laskey R, *et al*. Prevalence, predictors, and outcomes in treatment-resistant hypertension in patients with coronary disease. *Am J of Med* 2014; 127(1): 71-81.
20. Smith SM, Gong Y, Handberg E, *et al.* Predictors and outcomes of resistant hypertension among patients with coronary artery disease and hypertension. *Hypertension* 2014; 32(3): 635-643.
21. De Nicola L, Gabbai FB, Agarwal R, *et al.* Prevalence and prognostic role of resistant hypertension in chronic kidney disease patients. *J Am College of Cardiology* 2013; 61(24): 2461-2467.
22. American Diabetes Association. Cardiovascular disease and risk management. Sec 8. In Standards of Medical Care in Diabetes. *Diabetes Care* 2016; 39(1): S60-S71.
23. Sim JJ, Bhandari SK, *Shi* J, *et al*. Comparative Risk of Renal, Cardiovascular, and Mortality Outcomes in Controlled, Uncontrolled Resistant, and Nonresistant Hypertension. *Kidney Int* 2015; 88(3): 622-632.

BANTAO Journal 2017; 15(1): 10-15

BJ

BANTAO Journal

Original article

Evaluation of Clinical and Pathological Characteristics of Patients with IgA Nephropathy Based on Oxford Classification System: Should Crescents be Included?

Sibel Ersan[1], Omur Gokmen Sevindik[2], Caner Cavdar[1], Sibel Ada[1], Aykut Sifil[1], Ali Celik[1], Sulen Sarioglu[3] and Taner Camsari[1]

[1]Dokuz Eylul University Hospital, Department of Internal Medicine, Division of Nephrology, [2]Dokuz Eylul University Hospital, Department of Internal Medicine, [3]Dokuz Eylul University Hospital, Department of Pathology, Izmir, Turkey

Abstract

Introduction. None of the classification systems in immunoglobulin A (IgA) nephropathy has been widely agreed or implemented by clinicians or pathologists. In order to meet this need, "Oxford Classification System", which is highly reproducible and predictive for clinical course, was developed in 2009. In the present study, we investigated clinical and pathological characteristics of patients with IgA nephropathy based on current classification and the predictivity of crescent presence on prognosis.

Methods. The study comprised 40 patients with diagnosis of primary IgA nephropathy on renal biopsy. The biopsy findings and follow-up parameters of patients were retrospectively re-evaluated. Pathological findings were examined based on the Oxford classification system. The presence of crescent formation in the specimens was noted.

Results. The presence of crescent formation was predictive of poor prognosis regarding the glomerular filtration rate (eGFR), the level of proteinuria, and mean arterial pressure (MAP).

Conclusion: Considering the importance of crescent formation in prediction of the clinical course and need for immunosuppressive therapy, it is suggested that crescent presence can be included in this classification system.

Keywords: glomerulonephritis, IgA nephropathy, Oxford classification, crescents

Introduction

IgA nephropathy is the most prevalent glomerular disease all over the world [1-3]. Although it usually displays a benign course, 10-40% of patients progresses to end-stage renal disease within 10 to 20 years [4-6]. Therefore, exposing clinical and pathological parameters that would predetermine the course of disease is important. Studies revealed that clinical signs such as hypertension, proteinuria and low baseline glomerular filtration rate (eGFR) independently influence prognosis [7-9]. IgA nephropathy is pathologically characterized by minimal lesions to diffuse proliferative glomerulonephritis under light microscope, and IgA deposition in the glomerular mesangium by immunofluorescence [10]. Certain histopathological characteristics can predict clinical course and numerous classification systems have been developed under the light of these studies [11-13]. These classification systems did not solely intend to determine prognosis but also served for the development of a common language among pathologists and clinicians to characterize the disease [14]. Within this context, the classification systems approved until today include those developed by Lee, Haas *et al.* and by the World Health Organization for lupus nephritis [11,12]. However, these classification systems could not find an extensive and common field of usage among either nephrologists or pathologists due to weak reproducibility and weak correlation with clinical course [14].

In 2009, the clinicians who were the members of International Network for IgA Nephropathy, and pathologists together evaluated the data of 265 patients from 15 centers, and they consequently created "Oxford Classification System" that included pathological parameters with high reproducibility and demonstrated relation with clinical course. The classification system consisted of mesangial hypercellularity (M), endocapillary proliferation (E), segmental sclerosis (S) and tubular atrophy-interstitial fibrosis (T) and "M E S T" scoring system was developed according to their relation with clinical course. Although they are reproducible, parameters such as crescent formation and necrosis, of which the relation with clinical course has been definitely exposed in the previous studies, have not been included in the

Correspondence to: Sibel Ersan, Dokuz Eylul University Hospital, Nephrology Division, Izmir, Turkey; Phone: +90 232 46 96 969; Fax: +90 232 45 71 984; E-mail: ersansibel1@gmail.com

classification system as they were less prevalent in the current cohort [15].

The present study was aimed to investigate clinical and pathological characteristics of patients, as well as whether predictive character of the classification could be enhanced with the inclusion of some important parameters excluded from the classification. It was suggested that the data obtained could contribute to the literature in terms of follow-up and management of patients with IgA nephropathy.

Materials and methods

The Ethics Committee approval was obtained from Non-invasive Clinical Trials Evaluation Committee of our University under the protocol number 46-IOÇ/2010 for the study.

The study was conducted retrospectively by obtaining data from patients' records and it comprised 40 adult biopsy-proven IgA patients followed-up in our Clinic for at least six months. Renal biopsy specimens of the patients were re-evaluated by a nephro-pathologist using the "Oxford Classification System" criteria [15] and were recorded in accordance with the classification. Crescent formations and presence of necrosis in the biopsy specimens were also recorded. Presence of crescent was defined as the presence of crescent formations in more than 50% of the glomeruli.

The gender and age of the patients, date of biopsy, indications for biopsy, estimated glomerular filtration rate (eGFR) calculated according to the MDRD [16] formula, presence of macroscopic or microscopic hematuria, amount of 24-hour urinary protein excretion (g/day), systolic and diastolic blood pressures, mean arterial blood pressure (which is calculated by diastolic blood pressure + (systolic blood pressure-diastolic blood pressure)/3 formula), presence of hypertension (arterial blood pressure higher than 140/90 mmHg), whether they had been currently receiving antihypertensive or immunosuppressive drugs and, if so, the name of these drugs, and whether they had chronic renal insufficiency at admission and, if any, the degree of renal insufficiency [17] were derived from patient data obtained at admission and during biopsy and were recorded.

Based on all these data obtained, end-points such as final glomerular filtration rate, mean follow-up glomerular filtration rate, difference between the initial and final glomerular filtration rates and need for use of immunosuppressive drugs were obtained. Effects of clinical and histopathological parameters on end-points were investigated by statistical methods.

Statistical analyses

Distributions of the variables were assessed by Shapiro-Wilk normality tests and using Q-Q plots. Taking sample sizes and distribution of variables into account, Student-t, ANOVA, Mann-Whitney U and Kruskal Wallis tests were used for between group comparisons; whereas, differences between matched groups were compared using paired Samples T test and Wilcoxon test. Bonferroni test was used as a post hoc test. Relation between parametric variables was evaluated by Pearson's correlation analysis; whereas, relation between non-parametric or ordinal variables was evaluated by Spearman correlation analysis. Categorical variables were analyzed using Chi-square test and Fisher's exact test. In order to determine the relations with the end-points, linear regression analysis was used for continuous dependent variables, whereas logistic and cox regression analyses were used for categorical dependent variables. Parametric data were presented as mean ± standard deviation, whereas non-parametric data were presented as median, and categorical data were presented as percentages. Statistical analyses were done using the SPSS program for Windows (SPSS version 11.0., Chicago, IL). Statistical significance was considered as $p<0.05$.

Results

Of the 40 patients enrolled in the study, 42.5% were female and the mean age of the patients at diagnosis was 39.45±12.07 years. The mean glomerular filtration rate (eGFR) at diagnosis was 79.49±31.22 ml/min/1.73m^2. Four patients had clinical symptoms of acute kidney injury (AKI) at diagnosis. Twelve of the 40 patients had hypertension and 6 of these patients had been regularly receiving renin-angiotensin system blockers (RASB). There was no patient receiving steroid or any other immunosuppressive drug at baseline. The median value for 24-hour urinary protein excretion was 1.12 g/day, with minimum excretion rate of 0.13 g/day and maximum excretion rate of 9.9 g/day. The average value for mean arterial pressure (MAP) was 92.74± 13.85 mmHg.

Considering total duration of follow-up, median follow-up period was 40.93 months with minimum follow-up period of 6 months and maximum follow-up period of 201 months (Table 1).

Twenty-five percent of the patients had macroscopic hematuria. The most common indication for biopsy was microscopic hematuria with 60%. Clinical symptoms of nephrotic syndrome were present in 7.5% of the patients and clinical symptoms of acute renal insufficiency were present also in 7.5% of the patients. Proteinuria accompanied the macroscopic hematuria in 15% and microscopic hematuria in 47.5% of the patients. Pathological level of proteinuria was present in a total of 77.5% of the patients.

Table 1. Baseline demographic and clinical characteristics of patients

Age at diagnosis (years ± SD)	39.45±12.07	
Gender		
Male	23(57.5%)	
Female	17(42.5%)	
Body Mass Index		26.45±3.75
Follow-up duration (median month)		40.93(6-201)
Mean Arterial Pressure (mmHg)	92.74±13.85	
Baseline eGFR (ml/min/1.73m^2)	79.49±31.22	
Baseline Proteinuria (median, g/day)	1.12(0.13-9.9)	
CKD stage		
Stage 1 (eGFR>=90)	3(7.5%)	
Stage 2 (eGFR 89-60)	11(27.5%)	
Stage 3 (eGFR 59-30)	13(32.5%)	
Stage 4 (eGFR 29-15)	3(7.5%)	
AKI	4(10%)	
Hypertension	12(30%)	

Table 2. Characteristic pathological features in the study group

Mesangial Hypercellularity Score		**Frequency (%)**
	M 0 (≤0.5)	9(22.5%)
	M 1 (>0.5)	31(77.5%)
Endocapillary Hypercellularity		
	E 0 (No)	28(70%)
	E 1 (Yes)	12(30%)
Segmental Glomerulosclerosis		
	S 0 (No)	13(32.5%)
	S 1 (Yes)	27(67.5%)
Interstitial Fibrosis -Tubular Atrophy (IFTA)		
	T0 (0-25%)	20(50%)
	T1 (26-50%)	14(35%)
	T2 (>50%)	6(15%)
Crescent formation		
	No	30(75%)
	Yes	10(25%)
Necrosis		
	No	33(82.5%)
	Yes	7(17.5%)

Frequency of the pathological findings in each specimen is shown in Table 2. Mesangial hypercellularity was the most prevalent finding (77.5%) followed by segmental sclerosis (67.5%), whereas crescent formation and necrosis were only present in 25% and 17.5%, respectively. Total chronicity scores were moderate and severe in 50%.

During follow-up it was observed that 35% of the patients had new-onset hypertension and the prevalence of hypertensive patients in the group reached 65%, of whom 92.5% were given a RAS blocker. The mean final eGFR was 86.51±40.95 ml/min/1.73m^2 and the mean follow-up eGFR was 82.33±36.27 ml/min/1.73m^2. The median amount of urinary protein excretion at the end of follow-up period was 0.23 g/day (minimum 0.05, maximum 4.20); the median amount of the mean urinary protein excretions during follow-up period (the mean of all protein excretion during follow-up period)

Table 3. The relationship between pathological parameters and clinical follow-up parameters

	Mean follow-up eGFR (ml/min/1.73m^2) p value		**Mean follow-up Proteinuria (g/day) p value**		**Mean follow-up MAP (mmHg) p value**		**Final eGFR (ml/min/1.73m^2) p value**	
Mesengial hypercellularity								
M0	121.39±26.14	**0.001***	0.18 (0.15-0.32)	**<0.001***	85.00±8.08	**0.001***	126.88±26.64	**0.002***
M1	70.99±30.61		0.69 (0.10-5.73)		103.53±15.07		74.79±36.87	
Endocapillary hypercellularity								
E0	96.34±33.29	**<0.001***	0.30 (0.10-3.98)	**<0.001***	96.04±17.25	**0.011***	101.19±38.60	**<0.001***
E1	49.63±16.64		1.14 (0.59-5.73)		107.11±7.75		52.26±21.41	
Segmental glomerulosclerosis								
S0	108.85±36.15	**0.002***	0.21 (0.15-5.63)	**0.007***	85.18±12.57	**<0.001***	113.12±38.69	**0.004***
S1	69.55±29.13		0.69 (0.10-5.73)		106.19±12.34		73.70±36.05	
IFTA								
T0	107.84±29.34	**<0.001***	0.24 (0.10-1.41)	**<0.001***	93.20±12.69	**0.007***	115.57±32.00	**<0.000***
T1	64.02±20.94		0.69 (0.29-1.88)		103.48±19.11		65.45±25.47	
T2	40.00±12.63		3.59 (0.59-5.73)		110.28±6.40		38.79±12.13	
Crescent								
C0	92.22±34.69	**0.002***	0.35 (0.10-5.64)	**0.002***	95.62±14.18	**0.017***	97.29±39.83	**0.004***
C1	52.66±21.38		1.15 (0.29-5.73)		110.57±15.72		54.17±24.57	
Necrosis								
N0	85.41±33.37	**0.122**	0.44 (0.10-5.73)	**0.247**	97.30±14.26	**0.169**	90.54±38.51	**0.140**
N1	67.78±48.09		0.71 (0.29-1.88)		109.05±20.10		67.53±49.78	

***p<0.05**

was 0.58 g/day (minimum 0.05, maximum 4.20). The mean arterial pressure (MAP) during follow-up was 99.36±15.79 mmHg.

When the patients were evaluated individually for pathological parameters, no significant difference was determined between the groups in terms of relation of presence and absence of parameters with age and gender, with only exception that female gender was significantly more prevalent in the patient group with necrosis versus without necrosis (85.7% vs. 33.3%; p=0.011). Although the patient group was not homogeneous for age and gender, there was no difference between the groups in terms of age and gender.

The assessment of relationship between clinical follow-up characteristics and the presence or absence of pathological parameters revealed that all adverse pathological parameters except for the presence of necrosis were significantly associated with low mean follow-up and final eGFR; high mean follow-up and final proteinuria; and also high follow-up MAP (Table 3).

Glomerular filtration rates decreased as the severity of tubular atrophy-interstitial fibrosis increased. Various degrees of clearance loss were recorded at the end of follow-up in the patients with endocapillary hypercellularity, tubular atrophy-interstitial fibrosis, and crescent formation. The lowest baseline glomerular filtration rate was found in the patients with severe tubular atrophy and interstitial fibrosis; this also applied to follow-up and final glomerular filtration rates (Figure 1).

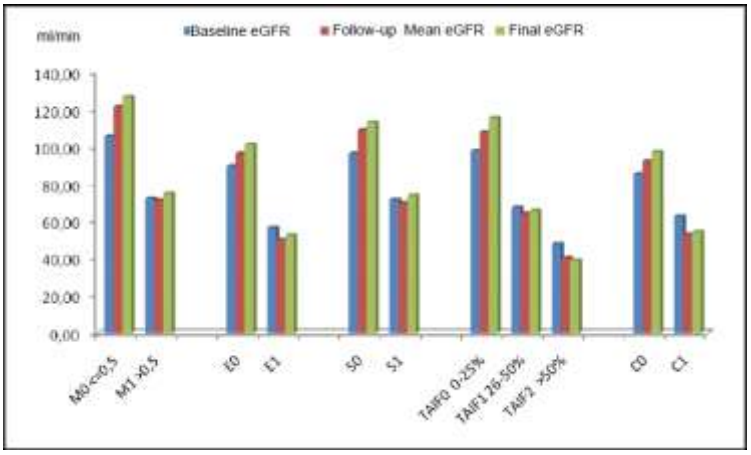

Fig. 1. Pathological parameters and baseline, follow-up and final eGFRs

With regard to all groups, decrease in the amount of follow-up and final proteinuria was conspicuous. The amounts of baseline, follow-up and final proteinuria were higher in the patients with severe tubular atrophy-interstitial fibrosis as compared to all other groups. The lowest amounts of baseline, follow-up and final proteinuria were recorded in the group without mesangial hypercellularity. Presence of all pathological parameters was associated with increased amount of proteinuria. Presence of endocapillary hypercellularity, tubular atrophy-interstitial fibrosis and crescent was associated with higher amount of proteinuria than the presence of other features (Figure 2).

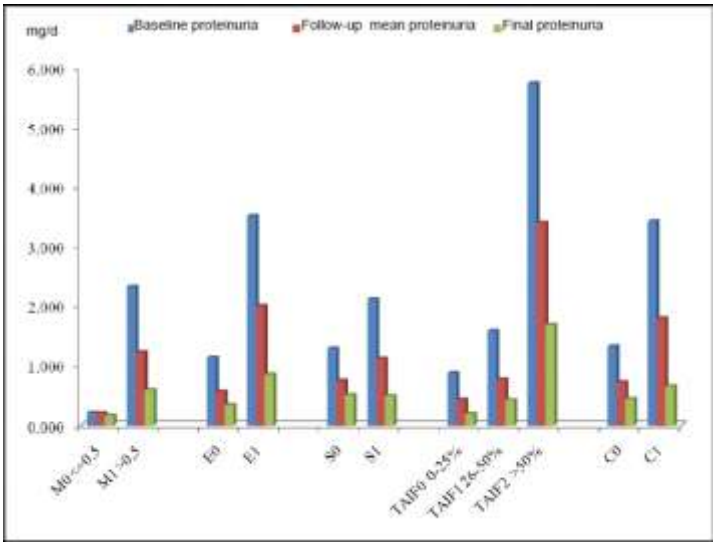

Fig. 2. Pathological parameters and baseline, follow-up and final proteinuria

Evaluation of pathological parameters by univariate regression analysis in terms of final eGFR and follow-up eGFR, which were the end-points, revealed that presence of each pathological parameter is individually a significant predictor. Evaluation of pathological parameters by univariate regression analysis for the difference in glomerular filtration rate, which was the end-point, revealed that only the presence of tubular atrophy-interstitial fibrosis and crescent formation were significant predictors (Table 4).

Table 4. Predictivity of Presence of Pathological Parameters in Clinical Course (Univariate Analysis)

	Final eGFR (ml/min/1.73m^2)		Mean follow-up eGFR (ml/min/1.73m^2)		GFR Difference (ml/min/1.73m^2)	
	β (s.d)	p	β (s.d)	p	β (s.d)	p
Mesangial Hypercellularity	-52.09(13.24)	**<0.001***	-50.40(11.26)	**<0.001***	-18.47(9.29)	0.054
Endocapillary Hypercellularity	-48.93(11.91)	**<0.001***	-46.71(10.16)	**<0.001***	-15.71(8.52)	0.073
Segmental Glomerulosclerosis	-39.43(12.46)	**0.003***	-39.30(10.64)	**0.001***	-14.56(8.38)	0.090
IFTA	-41.11(6.09)	**<0.001***	-36.22(5.43)	**<0.001***	-14.75(5.08)	**0.006***
Crescent	-43.13(13.43)	**0.003***	-39.56(11.78)	**0.002***	-20.41(8.81)	**0.026***

***p<0.05**

Discussion

In the present study, the "Oxford Classification System", which was developed by the member clinicians and pathologists of the network for IgA nephropathy and is the newest and most promising system, was investigated in our patient group in terms of reproducibility and validity for IgA nephropathy, which has yet no internationally accepted and high reproducible classification system in terms of clinical and pathological parameters although it is the leading cause of glomerulonephritis. In addition, crescent formations and necrosis, which have not been included in the system, were also evaluated and relation of pathological parameters with both clinical end-points and drug usage was explored [15,18].

Analyses clearly exposed the validity and reproducibility of the classification system and, in addition, demonstrated the relation of crescent formation, which has yet not found a place in the classification system, with poor clinical course.

In the present study, all patients that had at least 6-month follow-up data were included in the analyses without establishing any exclusion criteria in order to investigate the classification system, which is thought to be applied in all patients with IgA nephropathy, in all aspects. In this respect, it can be said that the present study is fictionally superior to the reference study of the classification system, which started with many exclusion criteria and therefore was designed in the way to exclude a group rich in acute and rapid crescent formations.

Evaluating the demographic data obtained at the end of the study, it was observed that the mean age at diagnosis was 39.75 years and there was a weak male predominance. Relatively advanced mean age determined in the present study for the patients with Ig A nephropathy, which is known to involve usually the ages of 20-to-30 years, was attributed to the absence of routine screening programs in Turkey and biopsy being saved for patients with concomitant proteinuria or loss of clearance. Literature review revealed no large-scale study that reflects the characteristics of Turkish population in terms of demographic data such as prevalence and gender distribution of IgA nephropathy, which showed significant racial difference.

The most common reason for biopsy was long-lasting and persistent microscopic hematuria accompanied or not by proteinuria (60%). Although literature suggests that macroscopic hematuria is the leading presenting symptom, this did not apply to our study group. Only 25% of the patients had macroscopic hematuria accompanied or not by proteinuria. In our patient group, the prevalence rates of baseline pathological proteinuria (77.5%) and baseline low clearance (75%) were substantially high.

The most common pathological parameter was the presence of mesangial hypercellularity (77.5%). This finding was similar with the patient population that formed the basis for the recent classification system [15]. Presence of crescent, which could have not found a place in the classification system, was observed in 25% of biopsy samples, whereas necrosis was observed in 17.5%. This rate was much lower in the patient group that formed the basis for the development of the classification system. This difference between the present patient group and the patient group that formed the basis for the classification system can be explained by exclusion of the patients with less than 1-year follow-up period and accordingly exclusion of the patients with more severe course or acute onset.

Low baseline glomerular filtration rate, high amount of baseline urinary protein excretion, and high baseline mean arterial pressure were individually associated with poor outcomes. This also applied to the data adjusted for age and gender. These findings display strong similarity with large-scale studies in the literature conduc-

ted on the clinical course of IgA nephropathy. D'Amico *et al*. published a review in 2004, which evaluated large-scale prognosis studies, and they similarly determined that high baseline proteinuria, low baseline glomerular filtration rate and high baseline blood pressure were associated with poor renal prognosis [1,2,15,19].

With regard to the contribution of pathological parameters to the clinical end-points and their predictive values, all pathological parameters excluding necrosis were found to be associated with poor end-points in univariate analyses. This was independent of age and gender of patients. This finding clearly supports the validity of the classification system. In the literature, it is clearly stated that presence of each pathological variable negatively influences the prognosis. The fact that presence of these parameters, individually or together, unfavorably influences the prognosis has also been shown by the Oxford Classification System. Endocapillary hypercellularity, the effect of which to the clinical end-points in the classification system could have not been demonstrated clearly, was among the parameters determined to unfavorably influence the clinical course, as demonstrated by Haas M. *et al*. [13] and D'Amico *et al*. [1].

Tubular atrophy-interstitial fibrosis and crescent have been underlined as independent predictors of end-points. There are many studies supporting crescent formations to be poor prognostic factors [20-23]. In the present study, definite findings about poor clinical course are an important step in revealing that classification system should be re-evaluated in terms of crescent formations.

Conclusion

The present study is one of the pioneer studies that explore the reproducibility and validity of the Oxford Classification System in adult patient group. The importance of crescent formation in predicting clinical end-points has been determined. The limitations of the study are: firstly, the study included a limited cohort of patients (40 subjects with IgA nephropathy) with a short follow-up in some patients; secondly, the number of patients with crescents represented too small subset of patients to allow clear conclusion statistically; thirdly, the lack of relation with types of crescents and clinical outcomes, and finally the relationship between the serum IgA/C3 ratio and severity of histological lesions needs to be addressed.

The results of the present study suggest that crescent formation can be included in the classification system when supported by large scale prospective studies.

Conflict of interest statement. None declared.

References

1. D'Amico G. Natural history of idiopathic IgA nephropathy and factors predictive of disease outcome. *Semin Nephrol* 2004; 24(3): 179-196.
2. Barratt J, Feehally J. IgA nephropathy. *J Am Soc Nephrol* 2005; 16(7): 2088-2097.
3. D'Amico G. The commonest glomerulonephritis in the world: IgA nephropathy. *Q J Med* 1987; 64(245): 709-727.
4. Schena, FP. A retrospective analysis of the natural history of primary IgA nephropathy worldwide. *Am J Med* 1990; 89(2): 209-215.
5. Floege, J, Feehally J. IgA nephropathy: recent developments. *J Am Soc Nephrol* 2000; 11(12): 2395-2403.
6. Geddes CC, Rauta V, Gronhagen-Riska C, *et al*. A tricontinental view of IgA nephropathy. *Nephrol Dial Transplant* 2003; 18(8): 1541-1548.
7. Feehally J. Predicting prognosis in IgA nephropathy. *Am J Kidney Dis* 2001; 38(4): 881-883.
8. Bartosik LP, Lajoie G, Sugar L, Cattran DC. Predicting progression in IgA nephropathy. *Am J Kidney Dis* 2001; 38(4): 728-735.
9. Rauta V, Finne P, Fagerudd J, *et al*. Factors associated with progression of IgA nephropathy are related to renal function--a model for estimating risk of progression in mild disease. *Clin Nephrol* 2002; 58(2): 85-94.
10. Galla JH. IgA nephropathy. *Kidney Int* 1995; 47(2): 377-387.
11. Haas M. Histologic subclassification of IgA nephropathy: a clinicopathologic study of 244 cases. *Am J Kidney Dis* 1997; 29(6): 829-842.
12. Lee HS, Lee MS, Lee SM, *et al*. Histological grading of IgA nephropathy predicting renal outcome: revisiting H. S. Lee's glomerular grading system. *Nephrol Dial Transplant* 2005; 20(2): 342-348.
13. Haas M, Rahman MH, Cohn RA, *et al*. IgA nephropathy in children and adults: comparison of histologic features and clinical outcomes. *Nephrol Dial Transplant* 2008; 23(8): 2537-2545.
14. Roberts IS, Cook HT, Troyanov S, *et al*. The Oxford classification of IgA nephropathy: pathology definitions, correlations, and reproducibility. *Kidney Int* 2009; 76(5): 546-556.
15. Cattran DC, Coppo R, Cook HT, *et al*. The Oxford classification of IgA nephropathy: rationale, clinicopathological correlations, and classification. *Kidney Int* 2009; 76(5): 534-545.
16. Levey AS, Greene T, Beck GJ, *et al*. Dietary protein restriction and the progression of chronic renal disease: what have all of the results of the MDRD study shown? Modification of Diet in Renal Disease Study group. *J Am Soc Nephrol* 1999; 10(11): 2426-2439.
17. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; 39(Suppl 1): S1-266.
18. Feehally J, Barratt J, Coppo R, *et al*. International IgA nephropathy network. Clinico-pathological classification of IgA nephropathy. *Contrib Nephrol* 2007; 157: 13-18.
19. Coppo R, D'Amico G. Factors predicting progression of IgA nephropathies . *J Nephrol* 2005; 18(5): 503-512.
20. Freese P, Norden G, Nyberg G. Morphologic high-risk factors in IgA nephropathy. *Nephron* 1998; 79(4): 420-425.
21. Hogg RJ, Silva FG, Wyatt RJ, *et al*. Prognostic indicators in children with IgA nephropathy-report of the Southwest Pediatric Nephrology Study Group. *Pediatr Nephrol* 1994; 8(1): 15-20.
22. Boyce NW, Holdsworth SR, Thomson NM, Atkins RC. Clinicopathological associations in mesangial IgA nephropathy. *Am J Nephrol* 1986; 6(4): 246-252.
23. Roufosse CA, Cook HT. Pathological predictors of prognosis in immunoglobulin A nephropathy: a review. *Curr Opin Nephrol Hypertens* 2009; 18(3): 212-219.

BANTAO Journal 2017; 15(1): 16-19

BJ

BANTAO Journal

Original article

Influence of Hemodialysis Treatment on Biochemical Markers of Bone Disease

Aida Coric[1], Halima Resic[1], Goce Spasovski[2], Orhan Lepara[3], Selma Ajanovic[1], Nermina Klapuh[4], Nejra Prohic[1] and Amela Beciragic[1]

[1]Clinic of Hemodialysis, Clinical Center University of Sarajevo, Bosnia and Herzegovina, [2]Department of Nephrology, Faculty of Medicine, University of Skopje, Macedonia, [3]Department of Human Physiology, Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina, [4]Organizational Unit - Clinical Chemistry and Biochemistry, Clinical Center University of Sarajevo, Bosnia and Herzegovina

Abstract

Introduction. Bone disease is a chronic complication of chronic kidney disease and major clinical problem in hemodialysis (HD) patients. The aim of our study was to assess the influence of treatment longevity on biochemical parameters of mineral and bone metabolism in HD patients, and to identify the most important parameters.
Methods. The research was observational and retrospective, involved 70 patients, mean age 58.69±12.54, divided into groups in respect to the duration of dialysis treatment (Group I-5 years, Group II-5-10 years and Group III-over 10 years).
Results. Serum phosphorus was increased, but the values tend to increase along with dialysis duration - (Group I: 1.93±0.45; Group II: 1.97±0.50; Group III: 2.01±0.37; p>0,05). Calcium values were also not significantly increased based on the duration of treatment [Group I: 2.3 (2.2-2.41); Group II: 2.46 (2.15-2.6), Group III: 2.35 (2.10-2.52)]. Dialysis and PTH correlated positively in the first group of patients (Rho=0.470, p=0.013). The values of calcium and alkaline phosphatase correlated positively in all patients (Rho=0.351, p=0.003). PTH was significantly higher in the second and third compared to the first group (p=0.009 and p=0.038, respectively), and there was no significant difference between the second and the third group. Interestingly, parathyroidectomized patients had higher PTH values compared to those without parathyroidectomy (557 vs. 359 pg/ml).
Conclusion. The most reliable marker for clinical monitoring of bone disease in dialysis patients is PTH. The values of calcium and phosphorus are highly variable and not reliable parameters for bone disease follow-up.

Keywords: hemodialysis duration, mineral and bone metabolism, PTH

Introduction

Chronic kidney disease is defined as kidney failure that persists for at least 3 months, caused by structural or functional kidney disorder, manifested by histological abnormalities or disorders characterized by blood, urine or kidney appearance, with decrease in glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 for over 3 months [1,2]. It is characterized by different level of uremia but also by changes in the volume and content of body fluids and electrolytes, and imbalance of numerous hormones. In the progression of CKD the disorder in the metabolism of calcium (Ca) and phosphorus (P), and parathyroid hormone (PTH) and vitamin D3 occurs at an early stage [2], according to some studies already with the glomerular filtration rate (GFR) of 60 ml/min/ 1.73m^2, that is in the second stage of CRF [3]. This disorder deteriorates from stage to stage and is therefore significantly represented in patients on dialysis. Complicated causal effects in this electrolyte-hormone imbalance can be explained by the kidney tissue prolapse which results in decrease of active metabolite of vitamin D3 (1.25(OH)2D3) synthesis [4]. It results from the phosphate retention along with a decreased absorption of Ca, and both hyperphosphatemia and hypocalcemia stimulate parathyroid glands with the development of secondary hyperparathyroidism and disordered Ca and PTH regulation. If accompanied by bone resistence to PTH effect, this metabolic imbalance leads to significant disorder in bone metabolism, especially in patients on dialysis [3-6]. Progressive bone abnormalities in patients with CKD are traditionally qualified as renal osteodystrophy (ROD). However, the National Kidney Foundation (NKF) provided new recommendations according to which the term ROD is used to explain modification of bone structure morphology in patients with CKF, based exclusively on histological results obtained from bone biopsy. Clinical, bioche-

Correspondence to: Aida Coric, University Clinical Center Sarajevo, Bolnicka 25, 71000 Sarajevo, Bosnia and Herzegovina; Phone: + 387 33 269 070; E-mail: idacoric@yahoo.com

mical and radiological abnormalities associated with ROD should be treated as elements of wider clinical syndrome; Chronic Kidney Disease-Mineral and Bone Disorder, i.e. CKD-MBD Syndrome [5-7]. In dialysis patients the CKD-MBD syndrome is characterized by:

- abnormality in metabolism of Ca, P, PTH and vitamin D3, and/or
- abnormalities in bone turnover, mineralization, volume and bone growth, and/or
- vascular calcifications and calcifications of other soft tissues.

According to some authors, bone biopsy is a "golden" standard in diagnosing and monitoring of bone abnormalities [5-7], but having in mind the invasive character of the procedure, it has been recommended only in cases which from the clinical-biochemical perspective are not clear enough (e.g. in patients with high PHT values and low concentration of alkaline phosphatase).
Clinical symptoms of mineral-bone metabolism occur at a rather late stage of CKD. Some studies have reported that clinical symptoms are present in at least 10% of patients, and histomorphological changes in 35-90% of cases [8]. Given that the mineral-bone metabolism can be diagnosed based on laboratory findings and before the occurrence of the initial symptoms, it is important to primarily monitor the level of parathyroid hormone, but also the level of alkaline phosphatase, Ca, P, and vitamin D, in order to timely administer the appropriate therapy and prevent more severe consequences. It is evident that extended HD treatment can only deteriorate these problems if not timely detected and appropriately treated. With regard to chronology of the problem related to calcium and phosphate metabolism in HD patients, we were interested in selection of the most significant parameters that changed over the time spent on hemodialysis, and could have influenced the bone disease development. The aim of the study was to assess the influence of HD treatment longevity on biochemical parameters of mineral and bone metabolism in HD patients, and to identify the most important monitoring parameters.

Materials and methods

This was an observational, retrospective and cross-sectional study conducted at the Clinic of Hemodyalisis, University Clinical Center Sarajevo (UCCS), from January to December 2015. The study included 70 regular (three times a week) HD patients. The respondents were divided into groups based on hemodialysis treatment duration: Group I-HD duration <5 years; Group II-HD duration from 5-10 years; Group III-HD duration >10 years.
The study did not include patients with malignant and liver diseases, acute infections and septic syndrome. All patients used dialysis solution with 1.25 mmol /L calcium concentration. Also, all patients used the same phosphate binder, calcium carbonate. In the local UCCS laboratory, the following biochemical parameters were regularly monitored: serum calcium, serum phosphate and alkaline phosphates (four times a year), at analyzer Vitros 5600 Integrated System Microslide Technology, using Principles of Spectrophotometry. Determination of intact PTH was performed in the laboratory of the Clinic of Nuclear Medicine at apparatus Cobas 6000 and Cobas 411 (Roche), using an immunoassay analyzer. The reference values were set based on the recommendations of good clinical practice of the American Initiative for the successful hemodialysis outcome (K/DOQI Clinical Practice Guidelines): 150-400 pg/ml for PTH, 2.10-2.60 mmol/l for calcium, from 1.13-1.78 mmol/l for phosphorus and for Ca x P <4.40 $mmol^2/l^2$ [9,10].
Statistical analysis was performed with the SPSS 16 software (version 16.0, SPSS Inc, Chicago, Illinois, USA). Division of variables was tested by the Kolmogorov-Smirnov or Shapiro-Wilk test. The data was presented as median, mean value and interquartile range. For continuous variables, the comparison between the groups was made using the Kruskal-Wallis or Mann-Whitney U-test. Correlation between the continuous variables was tested by Spearman's correlation analysis. P-values less than 0.05 were considered statistically significant [11].

Results

Of the total number of 70 patients, 27 were females (38.5%) with mean age of 59.91±14.73 years, ranging from 19-87 years. The most common basic disease of the patients was pyelonephritis-in 26 patients (37.1%), followed by glomerulonephritis in 14 patients (20%), hypertension in 13 patients (18.5%), diabetes mellitus in 15.7%, polycystic kidney disease in 7.1% and lupus nephritis in 1.6% of patients.

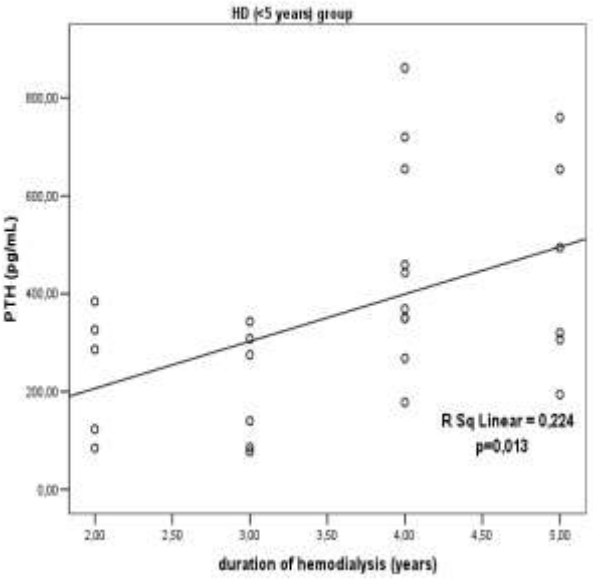

Fig. 1. Correlation of PTH levels and duration of hemodialysis in Group I patients with HD duration <5 years (Rho=0.470; p=0.013)

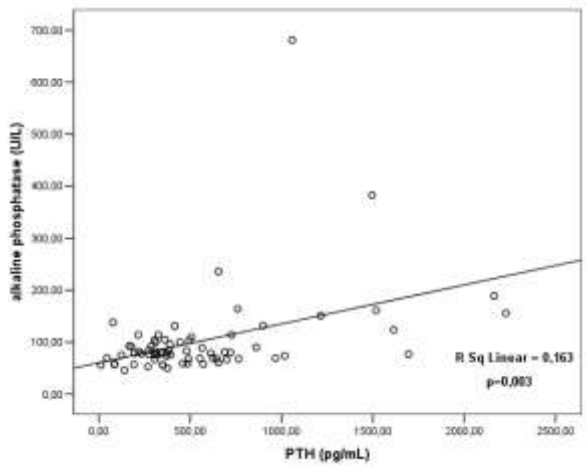

Fig. 2. PTH and alkaline phosphatase correlated positively in all patients (Rho=0.351; p=0.003)

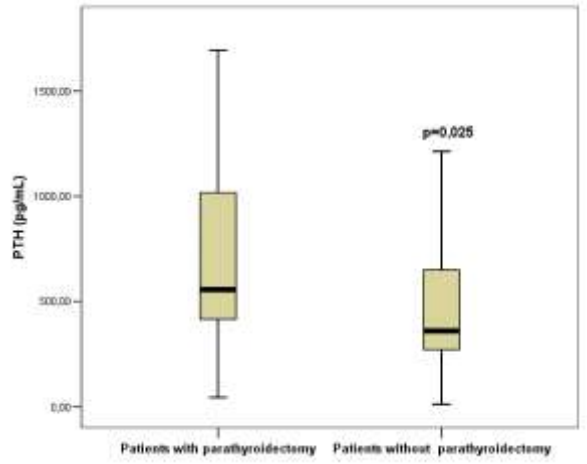

Fig. 3. PTH values in patients with and without parathyroidectomy

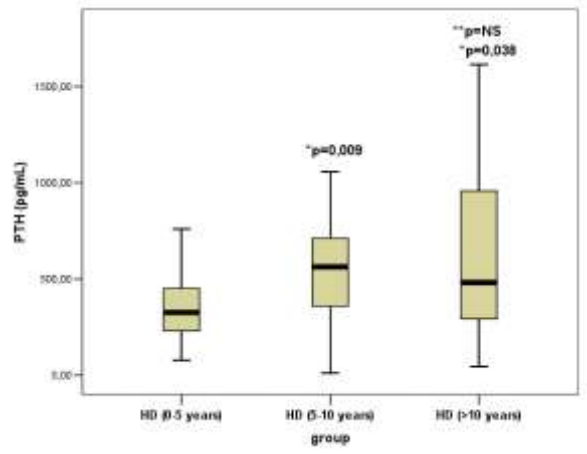

Fig. 4. PTH was significantly higher in the second and third compared to the first group (p=0.009 and p=0.038, respectively), with no significant difference between the second and third group

The highest proportion of patients was in Group I-40%, followed by Group III-31.42% and Group II-17.1%.
There was no significant difference in phosphate levels between the groups (Group I: 1.93±0.45; Group II: 1.97±0.50; Group III: 2.01±0.37; p=ns). The same pattern with similar levels between the groups was observed for calcium [(Group I: 2.3 (2.2-2.41); Group II: 2.46 (2.15-2,6), Group III: 2.35 (2.10-2.52): p=ns].
The duration of hemodialysis treatment and PTH levels correlated positively in Group I, Rho=0.470, p= 0.013 (Figure 1).
PTH and alkaline phosphatase correlated positively (Rho= 0.351, p=0.003) in all patients (Figure 2).
Interestingly, PTH level was higher in parathyreoidectomized patients compared to the others without parathyroidectomy (557 vs 359,5 pg/ml, p=0.025) (Figure 3).
PTH was significantly higher in the second and third compared to the first group (p=0.009 and p=0.038, respectively), while there was no significant difference between the second and third group (Figure 4).

Discussion

Abnormalities of mineral-bone metabolism are frequent both in predialysis patients with CKD and in HD patients. The effective clinical approach to these patients implies the control of phosphate retention and prevention of hyperphosphatemia in order to preserve the serum Ca level within the reference ranges and prevent proliferation of parathyroid gland cells, and subsequent increase in PTH [12].
Of the total of 70 patients, 14(20%) were with parathyroidectomy.
The growth trend of PTH was mainly monitored in Group II (duration of dialysis from 5 to 10 years), as well as increased PTH values mainly in patients with parathyroidectomy. Phosphorus, as the most important parameter of mineral-bone metabolism control, had increased values in all groups of patients, which indicates the possibility of bone disease complications, bone deformities and increased cardiovascular morbidity and mortality.
Although all our patients were on calcium-based phosphate binder therapy, satisfactory phosphate control during hemodialysis treatment was not achieved. There could be various reasons thereof, but the most common are inadequate phosphate intake and irregular treatment with phosphate binders.
Increased production of calcium and phosphorus (increased values of calcium and phosphate) during hemodialysis lead to an increased risk of intravascular calcifications and subsequent increase in cardiovascular morbidity and mortality of these patients [13-16].
Given the high PTH values in the group of patients with parathyroidectomy, there is a question related to the existance of ectopic parathyroid tissue, hyperactivity of other parathyroid glands, which are postoperatively repeatedly connected with longer duration of dialysis.
Some studies allege that bone specific AF (bAF) shows a high statistically significant interdependency with PTH in monitoring the progression of bone disease. It is also alleged that there is statistically significant corre-

lation between the total AF and PTH but to a lesser extent [17,18].

Taking into account that disorder of the mineral-bone metabolism leads to a significant deterioration in quality of life, morbidity, and also mortality, primarily of cardiovascular system, the recommendations of good clinical practice of the American Initiative for successful dialysis outcome (K/DOQI Clinical Practice Guidelines) are that levels of PTH and AF are determined every three months in patients on dialysis, and levels of Ca and P each month, and even more frequently in patients on therapy, [19] in order to ensure adequate control, slow down the progression and improve the quality of life for dialysis patients.

Conclusion

The examination of biochemical markers of mineral-bone metabolism has shown that PTH is the most reliable marker for clinical monitoring of bone disease, as it correlates well with the values of alkaline phosphates and calcium, whereas the levels of calcium and phosphate are highly variable and not reliable indicator of bone metabolism in patients on hemodialysis. Given the high PTH values in the group of patients with parathyroidectomy, there is a question related to the existance of ectopic parathyroid tissue, hyperactivity of other parathyroid glands, which are postoperatively repeatedly connected with longer duration of dialysis (20% of patients were with parathyroidectomy). Increased levels of calcium and phosphates during hemodialysis treatment lead to an increased risk of intravascular calcificates and subsequent increase of cardiovascular morbidity and mortality of these patients. American Initiative for successful dialysis outcome (K/DOQI Clinical Practice Guidelines) recommended that levels of PTH and AF are determined every three months in patients on dialysis, and levels of Ca and P each month, and even more frequently in patients on therapy, in order to ensure adequate control, reduce the progression of bone disease and improve the quality of life for dialysis patients.

Conflict of interest statement. None declared.

References

1. Natural Kidney Fondation. Evaluation, Classification and Stratification. *Am J Kidney Dis* 2002; 39: Sl-S226.
2. D'Haese PC, Spasovski G, Sikole A, *et al*. A multicenter study on the effects of lanthanum carbonate (Fosrenol™) and calcium carbonate on renal bone disease in dialysis patients. *Kidney International* 2003; 63: S73-S78.
3. Locatelli F, D'Amilo M, Eznesevski H. The epidemiology of and stage disease in Baluts countries. *Nephrol Dial Transplant* 2001; 16: 42-1338.
4. Brandi L. 1alpha(OH)D3 One-alpha-hydroxy-chole-calciferol-an active vitamin D analog. Clinical studies on prophylaxis and treatment of secondary hyperparathyroidism in uremic patients on chronic dialysis. *Dan Med Bull* 2008; 55(4): 186-210.
5. Tsukamoto Y. Disorders of bone and mineral metabolism in CKD: CKD-MBD as a new entity. *Clin Calcium* 2007; 17(5): 660-664.
6. Gal-Moscovici A, Popovtzer MM. New worldwide trends in presentation of renal osteodystrophy and its relationship to parathyroid hormone levels. *Clin Nephrol* 2005; 63(4): 284-289.
7. Moe S, Drueke T, Cunningham J, *et al*. Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2006; 69: 1945-1953.
8. Fauci AS, Braunwald E, Kasper DL, *et al*. Harrison's Principles of Internal Medicine. 17th ed. USA: Publisher: McGraw-Hill Professional 2008.
9. Committee on Examination of the Evolving Science for Dietary Supplements. Food and Nutrition Board, Institute of Medicine. "Evolution of Evidence for Selected Nutrient and Disease Relationships". Washington, D.C.: National Academy Press 2002.
10. Wei M, Taskapan H, Esbaei K, *et al*. K/DOQI guideline requirements for calcium, phosphate, calcium phosphate product, and parathyroid hormone control in dialysis patients: can we achieve them? *Int Urol Nephrol* 2006; 38(3-4): 739-743.
11. SPSS: Data Analysis with Comprehensive Statistics Software "computer program". Version 16.0. SPSS Inc; 2004.
12. Spasovski G, Bervoets A, Geert J, *et al*. Spectrum of renal bone disease in end-stage renal failure patients not yet on dialysis. *Nephrol Dial Transplant* 2003; 18: 1159-1166.
13. Jesri A, Braitman LE, Pressman GS. Severe mitral annular calcification predicts chronic kidney disease. *Int J Cardiol* 2008; 128(2): 193-196.
14. Spasovski G. New aspects of treatment of renal bone disease in dialysis patients. *Contributions, Sec Biol Med Sci* 2007; 28(1): 205-213.
15. Jonjic N, Djordjevic V, Djordjevic N, Ljubenovic S. Effect of the hemodialysis duration, age and gender on the emergence of the renal osteodystrophy symptoms. *Acta Medica Medianae* 2008; 47(4): 19-24.
16. Goodman GW. Medical management of secondary hyperparathyroidism in chronic renal failure. *Nephrol Dial Transplant* 2003; 18: 2-8.
17. Jorge C, Gil C, Possante M, *et al*. Bone alkaline phosphatase besides intact parathyroid hormone in hemodialysis patients-any advantage? *Nephron Clin Pract* 2005; 101(3): cl22- cl27.
18. Cavalier E, Delanaye P, Collette J, *et al*. Evaluation of different bone markers in hemodialyzed patients. *Clin Chim Acta* 2006; 371(1-2): 107-111.
19. Arenas MD, Alvarez-Ude F, Gil MT, *et al*. Application of NKF-K/DOQI Clinical Practice Guidelines for Bone Metabolism and Disease: changes of clinical practices and their effects on outcomes and quality standards in three haemodialysis units. *Nephrol Dial Transplant* 2006; 21(6): 1663-1668.

BANTAO Journal 2017; 15(1): 20-23

BJ

BANTAO Journal

Original article

Effect of Melatonin Administration on Prevention of Contrast-Induced Nephropathy following Coronary Angiography

Morteza Qaribi[1], Ali Abdolrazaghnejad[2], Reza Shahmirzaei[3] and Abdolghader Pakniyat[4]

[1]Department of Emergency Medicine, Valiasr Hospital, Arak University of Medical Sciences, Arak, [2]Department of Emergency Medicine, Sina Hospital, Tehran University of Medical Sciences, Tehran, [3]Cardiology Department, Ghods Hospital, Arak University of Medical Sciences, Arak, [4]Student Research Committee, Arak University of Medical Sciences, Arak, Iran

Abstract

Introduction. Contrast-induced-nephropathy (CIN) is a common complication during angiography that may lead to long-term complications. This study was conducted to investigate the effect of melatonin administration on prevention of CIN in patients who underwent coronary angiography with intra-arterial contrast agents.
Method. This is single-blind randomized clinical trial that was performed over 100 patients with indication for coronary angiography. Patients are randomly assigned to two equal groups. All patients in the 12 hours before and 12 hours after the procedure, were received adequate intravenous hydration with normal saline and for the intervention group in addition to hydration, the day before angiography and immediately after angiography 3 mg melatonin was administered. For all patients, serum level of creatinine (Cr), blood urea nitrogen (BUN) and glomerular filtration rate (GFR) before and 48 hours after the procedure were measured. Data were analyzed using SPSS 18 software.
Results. Totally 100 participants with the mean age of 64.0±8.2 years were enrolled (63% male). There was no significant difference between intervention and control groups in baseline and demographic characteristics (P> 0.05). Although the mean serum Cr and BUN level increased in both groups, but the mean Cr, BUN and GFR before and after coronary angiography was not statistically significant. Based on the definition of CIN in the current study, 3(6%) patients from intervention group and 2(4%) patients from control group were affected by CIN (P = 0.243).
Conclusion. It is likely that, melatonin administration has no significant effect on prevention of CIN following coronary angiography.

Keywords: acute kidney injury, contrast media, coronary angiography, melatonin, prevention and control

Introduction

Contrast induced nephropathy (CIN) is the third most common cause of in-hospital acute kidney injury, after hypotension and surgical intervention [1,2]. In other words, CIN is responsible for 5-30% of acute kidney injuries in inpatient setting, and after aminoglycoside agents, it is the second most common cause of nephrotoxicity [3-5]. CIN is defined as increased creatinine of at least 0.5 mg/dL or 25% increase in serum creatinine level after contrast administration. Elevation of serum creatinine occurs within 48-72 hours after the contrast imaging performance and usually returns to normal levels within 7-10 days [6-8]. Based on the evidence from previous studies, the prevalence of CIN varies from 0.6 to 2.3% in the general population to 20% in some patients with underlying cardiovascular disease [9]. Although the probability of kidney function recovery from CIN is high, in 10-25% of the cases dialysis was required. It could increase duration of hospital stay and mortality by 5 times that may lead to higher risk of complications such as respiratory failure, sepsis and hemorrhage [9-12]. According to the prior studies, patients with a history of kidney injury, especially those with underlying diabetes mellitus, congestive heart failure, hypotension, concomitant use of nephrotoxic agents or administration of high volume contrast agents are at higher risk of kidney injury [13]. The mechanism of kidney injury caused by contrast agents is unclear, may be it is related to the toxic effects on renal epithelial cells and oxidative stress [14,15]. Due to the devastating effects of intravenous contrast agents on kidney function in some patients, various materials have been used to prevent this destruction [9]. Nowadays, use of low osmolality contrast agents, adequate hydration before and after contrast administration, discontinuation of diuretics and metformin, reducing the dosage of contrast agent and administration of vasodilator drugs are applied to prevent CIN [16]. In several studies in order to prevent harmful effects of contrast agents on kid-

Correspondence to: Ali Abdolrazaghnejad, Department of Emergency Medicine, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran; Phone: +989 12 71 41 399; E-mail: ali.abdorazzagh@gmail.com

ney, other substances such as theophylline contrast agents, sodium bicarbonate, HMG-COA reductase inhibitors, ascorbic acid, dopamine and N-acetyl cysteine (NAC) were used in various clinical trials [17]. Melatonin (N-acetyl-5-methoxytryptamine) is a hormone that is secreted from the pineal gland, which has effects besides adjusting sleep-wake cycles such as blood pressure mediator, sedation and analgesic effects [18,19]. According to the previous studies, one of the important effects of melatonin is its antioxidant effect that removes free radicals [20,21]. Previous studies also addressed about minimal side effects of melatonin and also it is rapidly absorbed after oral administration and its performance begins immediately [20,21]. Considering the assumption of melatonin's antioxidant effect and its impact on the process of kidney injury and also due to the high availability and the role of oxidative stress in contrast induced nephropathy, some animal studies support prophylactic and therapeutic effects of melatonin in CIN [20-25]. This study was conducted to investigate the effect of melatonin administration on prevention of contrast induced nephropathy in patients who underwent coronary angiography with contrast agents.

Materials and methods

This is a single blind randomized clinical trial conducted in Arak, Iran. The research protocol was approved by the ethical committee of Arak University of Medical Sciences. The researchers followed the tenets of the Declaration of Helsinki throughout the study. Informed consent was obtained from the participants.

All patients with more than 18 years old that candidate for performing coronary artery angiography were enrolled to the study using convenience sampling method. The patients were excluded if any known history of allergy to melatonin or contrast agents, pregnancy and lactation, gastrointestinal malabsorption problem, recent infectious diseases, history of chronic systemic disease, recent melatonin administration at any dose, and concomitant use of medications that may have an impact on renal function such as theophylline, dopamine, mannitol, furosemide.

The sample size was calculated to be 50 persons for each group with respect to the prevalence of CIN in similar studies (α=0.05). Participants in two groups were matched in terms of age, sex, history of diabetes mellitus and hypertension, serum level of creatinine (Cr), blood urine nitrogen (BUN) and glomerular filtration rate (GFR). Levels of serum Cr and BUN were measured and GFR were calculated using Cockraft-Gault Formula. This formula was selected following an expert consult with a nephrologist.

Baseline and demographic information including age, gender, history of diabetes mellitus and hypertension, serum Cr and BUN and the level of GFR were recorded in pre-prepared checklist.

Participants were randomized at a ratio of 1:1 to either intervention group (50 patients) or control group (50 patients). Both groups of patients were hydrated with 1 ml/kg/hour normal saline 9%, from 12 hours before angiography through to 12 hours after that. In the intervention group melatonin was also administered; 1 tablet (3 mg) a day before angiography and 1 tablet (3 mg) immediately after angiography. Since melatonin has not been used for CIN in human studies before, and based on expert opinion, minimum dose of melatonin was used.

Patients were followed by the clinical information checklist before angiography, and 48 hours after that. Their serum Cr and BUN and GFR was measured as well. According to the prior studies, CIN was defined as 25% increase in serum creatinine level or increased creatinine of at least 0.5 mg/dL, 48 hours after angiography.

There is no reported side effect due to melatonin administration, still due to the effects of sleep regulation by melatonin, patients were recommended not to drive or carry on work that requires full mental concentration within 12-24 hours after taking melatonin.

Evaluation and recording the clinical response (based on laboratory measurements) of patients was done by another doctor who had no knowledge of prescription drugs and patients' group.

Data were analyzed using SPSS version 18 (version 18, SPSS Inc, Chicago, IL) and statistical methods to determine the frequency of variables. In order to analyze the quantitative variables, Student t-test was used and X2 test was used for qualitative variables. The p-value < 0.05 was considered as significant level.

Results

The mean age of the participants was 64±8.2 years and 63% were male. Demographic and baseline characteristics of patients are presented in Table 1. Based on the findings, 74(74%) and 81(81%) patients had diabetes mellitus and hypertension respectively. The baseline mean creatinine, BUN and GFR level was 0.83±0.92

Table 1. Demographic and baseline characteristics of studied patients

Variable	Case group	Control group	p
Age (year)	63.6±7.7	66±7.3	0.451
Sex			
Male	44	39	0.343
Female	26	32	0.462
Past medical history			
Diabetes mellitus	46	48	0.246
Hypertension	52	49	0.091
Bassline renal function tests			
Creatinine (mg/dl)	0.19±0.4	0.49±0.4	0.29
Blood urine nitrogen (mg/dl)	18.7±6.8	19.03±9.6	0.64
Glomerular filtration rate (ml/min)	84.04±25.2	86.4±30.1	0.64

mg/dl, 18.86±8.7 mg/dl, and 85.32±27.9 ml/min respectively. There was no significant difference between intervention and control groups in baseline clinical and demographic characteristics (P>0.05).

Table 2 shows mean Cr, BUN and GFR before and 48 hours after angiography in both studied groups. The mean serum Cr and BUN level raised in both groups, and the mean GFR decreased in result. But the difference between the two groups was not statistically significant.

Table 2. The mean creatinine, blood urea nitrogen and glomerular infiltration rate before and 48 hours after angiography in two studied groups

Variable	Case group	Control group	p
Creatinine (mg/dl)			
Pre Angiography	0.19±0.4	0.49±0.4	0.29
48 hours Later	0.39±0.4	0.89±0.3	0.18
p	0.25	0.08	
Blood urine nitrogen (mg/dl)			
Pre Angiography	18.7±6.8	19.03±9.6	0.64
48 hours Later	20.3±3	22.2±6.2	0.57
p	0.24	0.16	
Glomerular filtration rate (ml/min)			
Pre Angiography	84.04±25.2	86.4±30.1	0.64
48 hours Later	80.2±30.1	81.7±32.3	0.57
p	0.1	0.07	

Based on the definition of CIN in the current study, 3(6%) patients from intervention group and 2(4%) patients from control group were affected by CIN. This difference was not statistically significant (P=0.243).

Discussion

This study showed that melatonin as an adjunct therapy for inhibiting CIN does not have greater advantage over use of rehydration alone. According to the current evidence regarding the benefits of anti-oxidative agents in prevention of kidney injury process, it was assumed that the melatonin may be effective in this regard [14, 20-22]. However, based on the results of current study, the melatonin was not significantly effective on CIN prevention. Nasri *et al*. showed that melatonin can significantly prevent and treat CIN in rat [22]. In a study by Gazi *et al*. it was reported that both preventive and treatment administration of melatonin were lead to significant improvement of kidney function in rats [26]. In the study of Kilic *et al*. serum Cr and BUN level in the mice that had been using combination of melatonin and cisplatin, were significantly lower than that those who were only using cisplatin [27]. The study of Zararsiz *et al*. showed that melatonin can significantly prevent the oxidative damage in the kidney that arises from formaldehyde [28]. Lee *et al*. addressed that melatonin can significantly prevent the gentamycin induced oxidative injury based on histopathologic analysis [29]. According to the results of previous animal studies, it seemed that melatonin could be beneficial in prevention of CIN. Nevertheless, the results of our survey was not consistent with the result of these studies [22,24,25,30].

The limitations of our study included small sample size and lack of long-term evaluation in patients that may suffered from CIN. To the best we know, no clinical human study has been conducted regarding assessing the role of melatonin administration on prevention of CIN and also there is few animal model, further studies with larger sample size and further fallow up is recommended. There are some other formulas such as Modification of Diet in Renal Disease (MDRD), and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) in terms of GFR measurement that could be used instead of Cockcroft-Gault [31]. Considering these formulas in future research would be valuable.

Conclusion

It is likely that, melatonin administration has no significant effect on prevention of CIN following coronary angiography.

Acknowledgments. This paper has been excerpted from thesis of Dr. Ali Abdolrazaghnejad for Emergency Medicine Residency at Arak University of Medical Sciences, Arak, Iran. There is no doubt that conduction of the present study might not be feasible without cooperation of the patients, therefore we express our high gratitude and acknowledgement to the aforementioned persons and organizations and other colleagues in this researching project.

Authors' Contribution:

All the authors have contributed to drafting/revising the manuscript, study concept. All of the authors declared their accountability for all parts of the article.

Conflict of interest statement. None declared.

Funding/Support:

The current research has been executed with sponsorship from Arak University of Medical Sciences**.**

References

1. Tepel M. Acetylcysteine for the prevention of radiocontrast-induced nephropathy. *Minerva cardioangiologica* 2003; 51(5): 525-530.
2. O'sullivan S, Healy D, Moloney MC, *et al*. The Role of N-Acetylcysteine in the Prevention of Contrast-Induced Nephropathy in Patients Undergoing Peripheral Angiography A Structured Review and Meta-Analysis. *Angiology* 2013; 64(8): 576-582.
3. Barrett BJ, Parfrey PS. Preventing nephropathy induced by contrast medium. *New England Journal of Medicine* 2006; 354(4): 379-386.
4. Abushouk AI, Taheri MS, Pooransari P, *et al*. Pregnancy Screening before Diagnostic Radiography in Emergency Department; an Educational Review. *Emergency* 2017; 5(1): 60.

5. Hashemi B, Safari S, Hosseini M, *et al.* A Systematic Review of Iranian Experiences in Seismo-Nephrology. *Archives of trauma research* 2016; 5(2): e28796.
6. Stacul F, van der Molen AJ, Reimer P, *et al.* Contrast induced nephropathy: updated ESUR contrast media safety committee guidelines. *European radiology* 2011; 21(12): 2527-2541.
7. Thomsen H, Morcos S. Contrast media and the kidney: European Society of Urogenital Radiology (ESUR) guidelines. *The British journal of radiology* 2003; 76(908): 513-518.
8. Persson PB, Hansell P, Liss P. Pathophysiology of contrast medium-induced nephropathy. *Kidney international* 2005; 68(1): 14-22.
9. Mehran R, Nikolsky E. Contrast-induced nephropathy: definition, epidemiology, and patients at risk. *Kidney international Supplement* 2006; (100): S11-S15.
10. Saitoh T, Satoh H, Nobuhara M, *et al*. Intravenous glutathione prevents renal oxidative stress after coronary angiography more effectively than oral N-acetylcysteine. *Heart and vessels* 2011; 26(5): 465-472.
11. Safari S, Yousefifard M, Hashemi B, *et al*. The value of serum creatine kinase in predicting the risk of rhabdomyolysis-induced acute kidney injury: a systematic review and meta-analysis. *Clinical and experimental nephrology* 2016; 20(2): 153-161.
12. Safari S, Yousefifard M, Hashemi B, *et al*. The Role of Scoring Systems and Urine Dipstick in Prediction of Rhabdomyolysis-induced Acute Kidney Injury: a Systematic Review. *Iranian journal of kidney diseases* 2016; 10(3): 101-106.
13. Goldenberg I, Shechter M, Matetzky S, *et al*. Oral acetylcysteine as an adjunct to saline hydration for the prevention of contrast-induced nephropathy following coronary angiography. A randomized controlled trial and review of the current literature, 2004.
14. Weisbord SD, Palevsky PM. Radiocontrast-induced acute renal failure. *Journal of Intensive Care Medicine* 2005; 20(2): 63-75.
15. McCullough PA. Contrast-induced acute kidney injury. *Journal of the American College of Cardiology* 2008; 51(15): 1419-1428.
16. Stevens M, McCullough P, Tobin K, *et al*. A prospective randomized trial of prevention measures in patients at high risk for contrast nephropathy: results of the PRINCE Study. Prevention of Radiocontrast Induced Nephropathy Clinical Evaluation. *Journal of the American College of Cardiology* 1999; 33(2): 403-411.
17. Allaqaband S, Tumuluri R, Malik A, *et al.* Prospective randomized study of N-acetylcysteine, fenoldopam, and saline for prevention of radiocontrast-induced nephropathy. Catheterization and cardiovascular interventions. *Official journal of the Society for Cardiac Angiography & Interventions* 2002; 57(3): 279-283.
18. Wilhelmsen M, Amirian I, Reiter R, *et al*. Analgesic effects of melatonin: a review of current evidence from experimental and clinical studies. *Journal of pineal research* 2011; 51(3): 270-277.
19. Peres M. Melatonin, the pineal gland and their implications for headache disorders. *Cephalalgia: an international journal of headache* 2005; 25(6): 403-411.
20. Sezgin G, Ozturk G, Guney S, *et al*. Protective effect of melatonin and 1, 25-dihydroxyvitamin D3 on renal ischemia-reperfusion injury in rats. *Renal failure* 2012; 35(3): 374-379.
21. Hara M, Yoshida M, Nishijima H, *et al*. Melatonin, a pineal secretory product with antioxidant properties, protects against cisplatin-induced nephrotoxicity in rats. *Journal of pineal research* 2001; 30(3): 129-138.
22. Nasri H, Tavakoli M, Ahmadi A, *et al.* Ameliorative effect of melatonin against contrast media induced renal tubular cell injury. *Pakistan journal of medical sciences* 2014; 30(2): 261-265.
23. Murphy SW, BARRETT BJ, Parfrey PS. Contrast nephropathy. *Journal of the American Society of Nephrology* 2000; 11(1): 177-182.
24. Bailey SR. Past and present attempts to prevent radiocontrast nephropathy. *Reviews in cardiovascular Medicine* 2000; 2: S14- S18.
25. Bagshaw S, Ghali W. Theophylline for prevention of contrast-induced nephropathy: a systematic review and meta-analysis, 2005.
26. Gazi S, Altun A, Erdogan O. Contrast-induced nephropathy: preventive and protective effects of melatonin. *Journal of pineal research* 2006; 41(1): 53-57.
27. Kilic U, Kilic E, Tuzcu Z, *et al.* Melatonin suppresses cisplatin-induced nephrotoxicity via activation of Nrf-2/HO-1 pathway. *Nutrition & metabolism* 2012; 10(1): 7-.
28. Zararsiz I, Sarsilmaz M, Tas U, *et al*. Protective effect of melatonin against formaldehyde-induced kidney damage in rats. *Toxicology and industrial health* 2007; 23(10): 573-579.
29. Lee I, Kim S, Lee S, *et al*. Melatonin attenuates gentamicin-induced nephrotoxicity and oxidative stress in rats. *Archives of toxicology* 2012; 86(10): 1527-1536.
30. Millea P. N-acetylcysteine: multiple clinical applications. *American family physician* 2009; 80(3): 265-269.
31. Michels WM, Grootendorst DC, Verduijn M, *et al.* Performance of the Cockcroft-Gault, MDRD, and New CKD-EPI Formulas in Relation to GFR, Age, and Body Size. Clinical Journal of the American Society of Nephrology. *CJASN* 2010; 5(6): 1003-1009.

BANTAO Journal 2017; 15(1): 24-28

BJ

BANTAO Journal

Original article

Monitoring of Renal Allograft Function with Different Equations: What are the Differences?

Irena Rambabova Bushljetikj[1], Gjulsen Selim[1], Olivera Stojcheva Taneva[1], Sasho Dohchev[2], Oliver Stankov[2], Sotir Stavridis[2], Skender Saidi[2], Kocho Dimitrovski[3], Beti Zafirova Ivanovska[4], Nikolina Basic Jukic[5] and Goce Spasovski[1]

[1]University Clinic of Nephrology, [2]University Clinic of Urology, [3]Institute of Transfusion Medicine, [4]Institute of Epidemiology, Statistics and Medical Informatics, Medical Faculty, University of Skopje, Macedonia, [5]Department of Nephrology, Arterial Hypertension, Dialysis, and Transplantation, University Hospital Centre Zagreb and School of Medicine, University of Zagreb, Croatia

Abstract

Introduction. Monitoring of graft function by creatinine concentrations in serum and calculated glomerular filtration rate (GFR) is recommended after kidney transplantation. KDIGO recommendations on the treatment of transplant patients advocate usage of one of the existing mathematical equations based on serum creatinine. We compared clinical application of three equations based on serum creatinine in monitoring the function of transplanted kidney.

Methods. A total number of 55 adult patients who received their first renal allograft from living donors at our transplant center in between 2011-2014 were included into the study. Renal allograft GFR was estimated by the Cockroft-Gault, Nankivell and MDRD formula, and correlated with clinical parameters of donors and recipients.

Results. The mean age of recipients was 35.7±9.5 (range 16-58), and the mean age of donors was 55.5±9.0 (34-77) years. Out of this group of 55 transplant patients, 50(90.91%) were on hemodialysis (HD) prior to transplantation. HD treatment was shorter than 24 months in 37(74%) transplant patients. The calculated GFR with MDRD equation showed the highest mean value at 6 and 12 months (68.46±21.5; 68.39±24.6, respectively) and the lowest at 48 months (42.79±12.9). According to the Cockroft&Gault equation GFR was the highest at 12 months (88.91±24.9) and the lowest at 48 months (66.53±18.1 ml/min). The highest mean level (80.53±17.7) of the calculated GFR with the Nankivell equation was obtained at 12 months and the lowest (67.81±16.7 ml/min) at 48 months. The values of Pearson's correlation coefficient between the calculated GFR and the MDRD at 2 years after transplantation according to donor's age of r=-0.3224, correlation between GFR and the Cockfroft & Gault at 6 and 12 months and donor's age (r=-0.2735 and r=-0.2818), and correlation between GFR and the Nankivell at 2 years and donor's age of r=-0.2681, suggested a conclusion that calculated GFR was lower in recipients who had an older donors.

Conclusion. Our analysis showed difference in the calculated GFR with different equations at the same time points. Using one mathematical equation during the total post-transplantation period would be a recommended method in order to eliminate the discrepancy in determining the stage of kidney failure.

Key words: renal transplantation, glomerular filtration rate, estimation, Cockroft-Gault, Nankivell, MDRD, outcome

Introduction

The increased number of patients with chronic kidney disease (CKD) is one of the challenges encountered by nephrologists worldwide. CKD is a global public health problem that affects 5-10% of the population in western countries, with economic implications on health funds. Decrease in glomerular filtration rate (GFR) lower than 60 ml/min has also been emphasized [1,2]. Patients with kidney diseases are at higher risk of death, especially from cardiovascular events as well as at higher risk of progressive exacerbation of the kidney function leading to development of end-stage renal disease (ESRD), frequent hospitalizations and poor quality of life [3]. Treatment modalities of ESRD include dialysis (peritoneal and hemodialysis) and kidney transplantation. Increasing prevalence of ESRD on one hand, and stagnant or declined organ donation on the other hand prolong waiting time for kidney transplantation. Thus, the age of potential transplant recipients and comorbidities of patients as a result of dialysis treatment seem to be increasing over time [4]. Many factors have impact on the function of transplanted kidney. Some of them are modifiable and thus are of particular importance [5].

Correspondence to: Irena Rambabova Bushljetikj, [1]University Clinic of Nephrology, Medical Faculty, University of Skopje, Macedonia; irambabova@yahoo.com

Monitoring of graft function by creatinine concentrations in serum and calculated GFR is recommended after transplantation. KDIGO recommendations on the treatment of transplant patients advocate usage of one of the existing mathematical equations based on serum creatinine [6].

We compared clinical application of three equations based on serum creatinine in monitoring the function of transplanted kidney.

Material and methods

A total number of 55 adult patients who received their first renal allograft from the living donor at our transplant center in between 2011 to 2014 were included into the study. Donor data included sex, age of the donor, type of donation (related or unrelated donor), and data that refer to the patient were sex, age, length of hemodialysis (HD) treatment prior to transplantation, primary kidney disease, type of immunosuppressive therapy. Clinical and biochemical variables, serum creatinine, serum urea, protein status, 24 hours proteinuria, body weight and height were analyzed at 3, 6, 12, 24, 36 and 48 months after transplantation.

The estimated GFR was calculated with three equations.

1. Cockcroft–Gault equation

$$[(140\text{-age}_{(years)}) \text{ x weight}_{(kg)}/(0.814\text{serum creatinine}_{(\mu mol/l)}](\text{x}0.85, \text{ for females}).$$

2. Nankivell equation

$$6.7/(\text{serum creatinine}_{(mmol/l)}+0.25\text{xweight}_{(kg)}\text{-}0.5 \text{ x urea(mmo/L)-}100/\text{height}_{(m)}^{2}+35(25 \text{ for females})$$

3. MDRD study equation:

$$170 \times (\text{serum creatinine}_{(mg/dl)})^{-0.999} \times (\text{age}_{(years)})^{-0.176} \times (0.762 \text{ if patient is female}) \times (1.18 \text{ if patient is black}) \times (\text{serum urea nitrogen concentration}_{(mg/dl)})^{-0.170} \times (\text{serum albumin concentration}_{(g/dl)})^{0.318}.$$

Results

The mean age of recipients was 35.7±9.5 (range 16-58), and the mean age of donors was 55.5±9.0 (34-77) years. Out of this group of 55 transplant patients, 50 (90.91%) were on HD prior to transplantation, while pre-emptive transplantation was performed in 5 patients. HD treatment was shorter than 24 months in 37 (74%) transplant patients.

Table 1. Serum creatinine levels

Creatinine		Descriptive Statistics	
µmol/L	N	mean±SD	min - max
3 months	55	116.47±55.6	70-421
6 months	55	114.69±53.9	56-451
12 months	55	109.71±39.7	65-320
24 months	55	124.51±57.3	55-470
36 months	52	124.31±35.3	58-240
48 months	51	137.35±43.4	59-290

Table 1 presents the mean values, minimal and maximal levels of serum creatinine at 3, 6, 12, 24, 36 and 48 months after transplantation; gradual increase in the level was registered by the 48th month.

Table 2. Estimated GFR (glomerular filtration rate) based on three equations, MDRD (Modification of Diet in Renal Diseases), Cockroft-Gault and Nankivell

MDRD		Descriptive Statistics	
	N	mean ± SD	min - max
3 months	55	67.82±23.1	10.86-133.67
6 months	55	68.39±24.6	10.13-164.69
12 months	55	68.46±21.5	14.96-143.08
24 months	55	49.97±14.6	7.5-98.68
36 months	52	47.21±12.7	19.13-92.33
48 months	51	42.79±12.9	18.18-89.61
Cockroft-Gault			
3 months	55	86.41±149.9	17.29-149.96
6 months	55	86.62±24.1	16.14-161.5
12 months	55	88.91±24.9	22.75-145.79
24 months	55	76.23±21.2	14.52-115.0
36 months	52	72.52±18.8	25.27-111.15
48 months	51	66.53±18.1	27.15-104.0
Nankivell			
3 months	55	78.58±18.1	16.36-114.4
6 months	55	78.93±18.5	14.31-128.68
12 months	55	80.53±17.7	22.73-117.67
24 months	55	73.82±18.4	12.2-18.45
36 months	52	72.11±16.1	28.55-124.6
48 months	51	67.81±16.7	23.75-122.9

We used these values for estimation of allograft function by three equations (Table 2).

The calculated GFR with the MDRD equation showed the highest mean value at 6 and 12 months (68.46±21.5; 68.39±24.6, respectively) and the lowest at 48 months (42.79±12.9 ml/min). According to the Cockroft & Gault equation GFR was the highest at 12 months (88.91±24.9) and the lowest at 48 months (66.53±18.1 ml/min). The highest mean level (80.53±17.7) of the calculated GFR with the Nankivell equation was obtained at 12 months and the lowest (67.81±16.7 ml/min) at 48 months.

A negative correlation was registered between GFR calculated with the MDRD and HD treatment during the total follow-up. However, the correlation was statistically significant only at the end of the follow-up, 48 months post-transplantation (R=-0.316; p=0.03). GFR calculated with the Cockroft & Gault and Nankivell equations showed a negative statistically insignificant correlation with HD treatment duration.

The values of Pearson's coefficient on the correlation between the calculated GFR and the MDRD at 2 years after transplantation according to donor's age of r=-0.3224, correlation between GFR and the Cockfroft & Gault at 6 and 12 months and donor's age of r=-0.2735 and r=-0.2818, correlation between GFR and the Nankivell at 2 years and donor's age of r=-0.2681, suggest a conclusion that calculated GFR was lower in recipients who

had older donors.
The results obtained showed that grafts from younger donors had a better function analyzed with the MDRD equation during the total analyzed period after transplantation, but a statistically significant difference was confirmed at 24 months after transplantation (p=0.033). The function of the graft calculated with the Cockroft &Gault equation was better in those obtained from younger donors in all analyzed time points, but a statistically significant difference was confirmed at 6 (p=0.029), at 12(p=0.011), and at 24 months (p=0.028) after transplantation. Higher levels of GFR calculated with the Nankivell equation were registered for the graft from younger donors in the completely analyzed period, but the mean GFR levels between grafts obtained from donors younger than 60 years and grafts from donors aged 60 years and over did not reach statistical significance (Student t-test).
The function of the graft analyzed with the Cockroft-Gault equation was better in the groups with glomerulopathies as a primary disease compared to the group with other kidney diseases, being statistically different at 24, 36 and 48 months after transplantation ($p<0.05$).

Discussion

We have analyzed the function of the graft by using three mathematical equations based on serum creatinine concentration and factors of influence during the first 48 months after transplantation.
KDIGO recommendations for kidney transplant recipients advocate estimation of GFR during post-transplantation follow-up by determination of serum creatinine level. None of the equations demonstrated their superiority. Our study compared GFR calculated with three equations, Cockroft-Gault and Nankivell.
Cockroft-Gault is the oldest equation, most frequently applied in the clinical practice, but insufficiently analyzed for its precision and accuracy in transplant recipients [7]. Nankivell equation was constructed especially for transplant patients [8]. Levy *et al*. constructed a predictive equation from a group of 1628 patients included in the Modification of Diet in Renal Diseases (MDRD). This equation has been incorporated in majority of studies including transplant patients [9].
In our study the highest levels of calculated GFR were obtained with the Cockroft-Gault equation. Conducted studies have shown that this equation overestimate GFR. The unique component that is being changed is serum creatinine, which in the post-transplantation period depends on the improved nutritional status and corticosteroid therapy. The lowest GFR levels were obtained with the MDRD equation, that includes other biochemical variables such as serum albumin, urea, as well as other categorical variables. However, the best results for precision and specificity regarding the directly determined GFR were obtained with the MDRD equation [17].
Regarding the factors of influence, we analyzed the graft function in patients with different HD treatment duration before transplantation. We registered a negative correlation during the total follow-up between GFR calculated with the MDRD equation and HD length of treatment. The level of GFR rate with the MDRD equation decreased with the increase of HD duration. The correlation was statistically significant solely at the end of the follow-up, i.e. at 48 months post-transplantation. Using the other two equations, we also observed a negative correlation but without statistical difference.
Studies have demonstrated negative association between length of HD and post-transplantation outcome regarding survival of the graft and recipients. American Renal Data System shows advantage not only of the preemptive transplantation on graft survival, but the period of HD treatment may be also a risk factor for graft loss and mortality of recipients both for living and deceased donor transplantation [10]. This analysis has revealed that HD treatment for 6-12 months has a 37% long-term impact on graft loss in comparison with preemptive transplantation regardless the duration of the underlying disease (diabetes, glomerular diseases). Another analysis has shown that the length of HD treatment has an impact on the graft loss only in transplant patients from living donors [11]. Studies have supported the acceptance of even marginal donors if the waiting time for transplantation was very long. In the study where kidneys from the same donor were transplanted to recipients with different HD treatment duration, less than 6 months and longer than 24 months, it was shown that the long-term ten-year graft survival was statistically significant by 63% vs. 29% respectively for the mentioned groups [12].
In our study dialysis vintage longer than 24 months resulted in poorer graft function. Complications associated with HD treatment include traditional risk factors such as older age, dyslipidemia, DM, left-ventricular hypertrophy, as well as non-traditional risk factors such as albuminuria, anemia, impaired metabolism of calcium and phosphorus, malnutrition and oxidative stress, that lead to development of cardiovascular diseases. These complications are a result of the poorer function of the transplanted kidney in this group of patients.
Regarding the age of donors as a factor influencing the graft function, our analysis done with the Pearson's coefficient of linear correlation on the association of the calculated GFR with the MDRD equation at 2 years, Cockcroft & Gault equation at 6 and 12 months, and with Nankivell equation at 2 years suggested that the calculated GFR was lower in kidney recipients who had older donors.
The function of the graft analyzed with the three equations regarding the age of the donors showed a higher GFR at all time points in younger donors, with statistical difference when using the MDRD equation at 24

months post-transplantation (p=0.033), with the Cockcroft &Gault equation at 6 months (p=0.029), at 12 months (p=0.011), and at 24 months (p=0.028), and with the Nankivell equation being at borderline at 24 months (p=0.051).

Analyses about the transplantation outcome have shown that the quality of donated kidney has a leading role in the function and long-term survival of the graft. On the other hand, long waiting lists for transplantation, aging of potential kidney recipients and more rapid deterioration of the health condition during hemodialysis stimulate donation of organs from individuals older than 60 years [13]. The number of glomeruli and mean glomerular volume are in negative correlation with age after the sixth decade of life. The number of sclerotic glomeruli after the age of 60 is 30-50% as result of glomerulosclerosis, microvascular lesions and total loss of nephrons. The number of functional nephrons is smaller in grafts from older donors than from younger donors [14,15]. The systematic analysis on the transplantation outcome that included function of the graft and survival of the recipients in the period 1980-2008 showed that kidney recipients from donors older than 60 years had a poorer 5-year outcome regarding graft function and survival of the recipients than those from younger donors [16].

In some countries, as shown in the Norwegian registry, 16% of living donors are older than 60 years, and 7.7% are over the age of 65, whereas in Canada 6% of donors are older than 60 years [18,19]. The American RDS shows no upper age limits regarding acceptance of donors [20].

The results obtained in the recent analysis of United Network for Organ Sharing (UNOS) database from 1994 to 2012 that made comparison of living donors >60 years, living donors <60 years, showed equal graft survival and overall survival in older donors compared to SCD (standard criteria donor), better than ECD (expanded criteria donor), but worse than grafts obtained from younger living donors [21-23]. In comparison with other studies, our study has shown a poorer function of grafts from older donors in the early period post-transplantation, at 6 months and 2 years, with statistical difference during total follow-up of 48 months. However, the function of grafts from older donors is satisfactory and by the end of the analysis supports donation of organs from expanded criteria donors, especially in conditions of insufficiently developed cadaveric transplantation.

Our patients were divided in two groups regarding the graft function and the underlying, baseline disease. The first group included patients with glomerulonephritis and diabetic nephropathy as basic disease, and the second group included patients with obstructive nephropathy, polycystic kidney disease and undifferentiated diseases. Higher GFR levels were registered in the first group with the three used equations at all time points, and the statistical difference was observed with the C&G equation at 24, 36 and 48 months. Glomerular diseases as a cause for development of end-stage kidney disease account for 30-50% of transplant population. These patients are at risk of recurrence of the underlying disease and graft loss. The new immunosuppressive medications influence the rate of acute rejection as well as reduce the chronic allograft nephropathy [24]. The study of Briganti *et al*. comprising a total of 1505 patients with performed biopsy of the native and transplanted kidney demonstrated that recurrent GN as a cause of graft loss is on the third place of all defined causes. The risk of graft loss in the first year was 0.6% and at 10 years 8.4% [25]. Diabetes as the underlying disease can eventually develop recurrence of diabetic nephropathy, but to a different clinical degree. Eighty to hundred percent of patients with DM as an underlying disease develop histological changes within diabetic nephropathy. The time of onset of DN in the graft is 6 years after transplantation. On the other hand, the incidence for development of DN as a cause for graft loss has been insufficiently clinically examined and is considered to be rare [26].

Of note, our results have shown better function of the grafts in patients with diagnosed glomerular diseases and diabetic nephropathy versus those with undifferentiated diseases. This might be a result of the optimal doses of immunosuppressive medications, which consequently lead to a smaller number of episodes of acute rejection and decrease in calcineurin toxicity, in the period of 4 years follow up after transplantation. In order to make comparison with world trends that show poorer functioning of the graft in a long-term follow-up up to 10 years, further analyses are required. Absence of the correct etiological diagnosis pre-transplantation imposes the need of performing kidney biopsies so as to predict the post-transplantation course and to determine the most optimal immunosuppressive therapy.

Conclusion

Our analysis has shown difference in the calculated GFR with different equations at the same time points. Using a single mathematical equation during the total post-transplantation period would be a recommended method in order to eliminate the discrepancy in determining the stage of graft failure.

With regard to the factors of influence, longer HD treatment and expanded criteria donors have a negative impact on the graft function. Matching of adult groups and etiological assessment of the causes of end-stage renal disease aimed at optimization of immunosuppressive therapy are recommended with respect to the transplantation procedure outcome.

Conflict of interest statement. None declared.

Reference

1. JhaV, Garcia-Garcia G, Iseki K, *et al*. Chronic kidney disease: Global dimension and perspectives. *Lancet* 2013; 382: 260-272.
2. Levey AS, Atkins R, Coresh J, *et al*. Chronic kidney diseaseas a global publichealth problem: Approaches and initiatives-A position statement from Kidney Disease Improving Global Outcomes. *Kidney Int* 2007; 72: 247-259.
3. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, *et al*. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. *Lancet* 2013; 382(9889): 339-352.
4. Tonellia M, Wiebea N, Knollb G, *et al*. Systematic Review: Kidney Transplantation Compared With Dialysis in Clinically Relevant Outcomes. *Am J Transplant* 2011; 11: 2093-2109.
5. Legendre C, Canaud G, Martinez F. Factors influencing long-term outcome after kidney transplantation. *Transplant Int* 2014; 27(1): 19-27.
6. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009; 9(3): S1-S157.
7. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16: 31-41.
8. Nankivell BJ, Gruenewald SM, Allen RD, *et al*. Predicting glomerular filtration rate after kidney transplantation. *Transplantation* 1995; 59: 1683-1689.
9. Levey AS, Bosch JP, Lewis JB, *et al.* A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; 130: 461-470.
10. Meier-Kriesche HU, Port FK, Ojo AO, *et al*. Effect of waiting time on renal transplant outcome. *Kidney Int* 2000; 58: 1311-1317.
11. Mange KC, Joffe MM, Feldman HI. Effect of the use or nonuse of long-term dialysis on the subsequent survival of renal transplants from living donors. *N Engl J Med* 2001; 344: 726-731.
12. Meier-Kriesche HU, Kaplan B. Waiting time on dialysis as the strongest modifiable risk factor for renal transplant outcomes-a paired donor kidney analysis. *Transplantation* 2002; 74: 1377-1381.
13. Gill JS, Tonelli M, Johnson N, *et al.* The impact of waiting time and comorbid conditions on the survival benefit of kidney transplantation. *Kidney Int* 2005; 68(5): 2345-2351.
14. Nyengaard JR, Bendtsen TF. Glomerular number and size in relation to age, kidney weight, and body surface in normal man. *Anatomical Record* 1992; 232(2): 194-201.
15. Kappel B, Olsen S. Cortical interstitial tissue and sclerosed glomeruli in the normal human kidney, related to age and sex. A quantitative study. *Virchows Archiv* 1980; 387: 271-2773.
16. Iordanous Y, Seymour N, Young A, *et al*. Recipient outcomes for expanded criteria living kidney donors: the disconnect between current evidence and practice. *Am J Transplant* 2009; 9(7): 1558-1573.
17. Issa N, Meyer KH, Arrigain S, *et al*. Evaluation of Creatinine-Based Estimates of m Glomerular Filtration Rate in a Large Cohort of Living Kidney Donors. *Transplantation* 2008; 86: 223-230.
18. Oien CM, Reisaeter AV, Leivestad T, *et al*. Living donor kidney transplantation: The effects of donor age and gender on short- and long-term outcomes. *Transplantation* 2007; 83: 600-606.
19. United States Renal Data System (USRDS). Annual Data Report Atlas. Online 2008, Available at: http://www.usrds.org/atlas.htm.
20. Mandelbrot DA, Pavlakis M, Danovitch GM, *et al*. The medical evaluation of living kidney donors: A survey of US transplant centers. *Am J Transpl* 2007; 7: 2333-2343.
21. Englum BR, Schechter MA, Irish WD, *et al*. Outcomes in kidney transplant recipients from older living donors *Transplantation* 2015; 99(2): 309-315.
22. Frei U, Noeldeke J, Machold-Fabrizii V, *et al*. Prospective age-matching in elderly kidney transplant recipients: a 5-year analysis of the Eurotransplant Senior Program. *Am J Transplant* 2008; 8: 50-55.
23. Boesmuller C, Biebl M, Scheidl S, *et al*. Long-term outcome in kidney transplant recipients over 70 years in the Eurotransplant Senior Kidney Transplant Program: a single center experience. *Transplantation* 2011; 92: 210-216.
24. Choy BY, Chan MH, Lai KN. Recurrent Glomerulonephritis After Kidney Transplantation. *Am J Transplant* 2006; 6: 2535-2542.
25. Briganti EM, Russ GR, McNeil JJ, *et al*. Risk of renal allograft loss from recurrent glomerulonephritis. *N Engl J Med* 2002; 347:103-109.
26. Bhalla V, Nast CC, Stollenwerk N, *et al*. Recurrent and de novo diabetic nephropathy in renal allografts. *Transplantation* 2003; 75(1): 66-71.

BANTAO Journal 2017; 15(1): 29-32

BJ

BANTAO Journal

Short communication

Plasma Calcium Concentration Modifies the Blood Sodium During Hemodialysis: Lessons from Hard Water Syndrome

Davide Viggiano[1,2] and Pietro Anastasio[1]

[1]Department of Cardio-Thoracic and Respiratory Science, Second University of Naples, [2]Deptartment of Medicine and Health Sciences, University Molise, Campobasso, Italy

Abstract

Introduction. Extracellular sodium (Na^+) concentration is maintained within a tight physiological range due to hormonal control, that mainly modulates thirst, Na^+ and water renal excretion. Extra-renal regulation of Na^+ and water homeostasis is only partially understood. Recently it has been debated whether the osmotically inactive Na^+ storage is fixed or variable.
Methods. In the present study, fourteen End-Stage Renal Disease (ESRD) patients treated by chronic hemodialysis underwent by accident to a sharp increase in plasmatic calcium (Ca^{+2}) levels due to the failure of the water control system, leading to the so-called hard water syndrome. The levels of plasmatic Ca^{+2} after 1 hr of hemodialysis were correlated with urea, Na^+, potassium (K^+) and creatinine levels. Eleven ESRD patients treated with hemodialysis under similar conditions were used as controls.
Results. The hard water syndrome resulted in hypercalcemia, while mean plasma levels of Na^+, K^+ and urea were not different compared to controls. Plasma creatinine levels were slightly but significantly higher that control. A correlation analysis on the measured variables has showed a positive correlation between plasma Ca^{+2} and Na^+ levels (Pearson=0.428, p=0.032), and the absence of any correlation with K^+, creatinine and urea concentration.
Conclusions. Our study suggests that acute changes in plasmatic Ca^{+2} levels may affect Na^+ concentration in the absence of renal function; it is possible that hypercalcemia may trigger Na^+ release from the osmotically inactive storage. These data further support previous observations on the interplay of sodium and calcium at extrarenal sites.

Keywords: calcium, hemodialysis, hard water syndrome, hypercalcemia, natremia

Introduction

The extracellular sodium represents one of the main determinants of plasma osmolarity. A fine regulation of Na^+ plasmatic levels is guaranteed by a strict hormonal control, which mainly acts on the regulation of thirst, Na^+ and water renal reabsorption [1]. Several evidences suggest that a large reservoir of osmotically inactive sodium is represented by the extracellular matrix of bone, skin and muscles [2]. The circumstances under which this reservoir may vary are under active debate. It has been recently suggested that there is a balance between the osmotically active and inactive Na^+ pool; thus, upon slow changes in body fluids, the composition of inactive Na^+ pool changes accordingly. In rats, long-term salt deprivation is accompanied by a decrease in the charge density of skin GAGs, and the consequent mobilization of osmotically inactive Na^+. This finding suggests that the skin and the connective tissues may serve as a Na^+ storage, capable to release Na^+ in response to reduced intake by changing its polyanionic character [3].
In addition, several studies have recently shown that in both humans and animal models, chronic hyponatremia is associated with bone resorption and osteoporosis [4]. One possibility is that chronic hyponatremia may lead to sodium loss from the bones with consequent bone demineralization [5]. It remains unclear how this large Na+ reservoir is regulated. Clearly, since the extracellular proteins can bind both calcium and Na^+, some extrarenal form of interaction between the two ions might exist. For instance, it is well known that many cells express a Na^+- Ca^{+2} Exchanger (NCX), and that there is a perfect coupling between Na^+ and calcium transcellular fluxes based on their extracellular concentrations [6,7].
The exact knowledge of this interplay is important because it may provide an additional mechanism of sodium regulation, independent of the kidney function; in addition, this regulation might have clinical impact particularly in patients undergoing dialysis (who lack

Correspondence to: Davide Viggiano, Dept. of Medicine and Health Sciences, Univ. Molise, 86100-Campobasso, Italy Now at: Department of Cardio-Thoracic and Respiratory Science, Second University of Naples, Italy; Phone: +39 081 56 66 650, +39 348 49 75 413; Fax +39 081 56 66 821; E-mail: davide.viggiano@unimol.it

kidney regulation for extracellular sodium and rely on the dialysis system).

Under physiological conditions, the extrarenal Na^+/ Ca^{+2} interplay cannot be easily studied, because of the tight control of the kidney itself of these ions, which masks any extrarenal [8].

On the other hand, in patients with the absence of kidney function, such as in patients under dialysis treatment, the Na^+ and calcium levels are also tightly controlled by the dialysis.

Therefore, it seems impossible in human subjects to test the relation between calcium and sodium levels in the absence of kidney compensation.

Recently, we have had the possibility to study the effects of an extracellular calcium increase on Na^+ levels in subjects undergoing chronic hemodialysis treatment. These subjects underwent by accident to a sharp increase in calcium levels due to a failure in the water control system (Hard Water Syndrome).

These subjects experienced a rapid increase in blood pressure and malaise, and therefore the dialysis process has been interrupted after 1 hr. This unfortunate and irreproducible accident represented also a unique occasion to test the extra-renal effects of calcium on plasma sodium in human subjects without kidney compensation.

The hypothesis is that in presence of a large increase of extracellular calcium, the latter should compete with the Na^+ bound to the extracellular matrix, thus leading to a linear increase of (unbound) plasmatic Na^+.

We also expect that this effect is dampened by the buffering effect of the dialysis process on Na^+ ions; however, a correlation between calcium levels and the Na^+ levels should yet be detected as the rate equilibrium of Na^+ would be slower if a greater amount of Na^+ is mobilized by a larger amount of calcium.

We discuss the findings under the hypothesis of a sodium-calcium interplay at extrarenal sites, which has already received a large support based on animal models (see also the recent review by Sterns) [9].

Materials and methods

Fourteen patients under chronic hemodialysis treatment three times a week for end-stage renal disease (ESRD) have received, by accident, unsoftened tap water in their dialytic process.

Analyses were conducted retrospectively using deidentified patient data; thus this study was deemed exempt from the requirement of ethical approval by the institutional review board. We adhered to the Declaration of Helsinki; informed consent was not required.

The water conductivity in samples derived from tap water reached 483 mS/cm, while the treated water had a mean value of 15 mS/cm. The dialysis treatment was stopped after 60 minutes due to the emergence of severe headache, vomiting, hypertension, tachycardia and nausea, typical for the "Hard Water Syndrome" [10]. Water samples for chemical surveillance were collected from the tubes serving the dialysis: the problem in the water filters was recognized and subsequently solved. All patients subsequently fully recovered from the accident and no patient suffered any health problem due to it.

For comparison, blood samples from 11 patients under regular dialysis were taken 60 minutes after the start of the treatment.

Hemodialysis was delivered using a Fresenius HD machine FX 5800 (Fresenius FMC, Germany) programmed to provide a dialysis flow rate of 500 ml/min at a temperature of 36°C. Standard bicarbonate buffered dialysate concentrate (Fresenius 335) was used to yield a dialysis fluid containing the following concentrations: bicarbonate 32 mmol/L, glucose 5.5 mmol/L, calcium 1.5 mmol/L, K^+ 2.0 mmol/L, Na^+ 142 mmol/L. Blood flow was set in the range 200-360 ml/min.

All patients had arteriovenous fistulas and arterial blood samples were collected from the fistula at the end of the dialysis process. All patients received seleparin 3000-4000U as anticoagulation each dialysis session.

At the end of the 60-min dialysis process, the blood samples were collected to measure plasma sodium, potassium, calcium, urea and creatinine.

Statistical analysis

Multivariate ANOVA was used to verify significant differences between the two groups. Multiple post-hoc was performed to identify significant differences. Correlation analysis between the measured variables was conducted to observe relationships among the serum ions. The rejection value was set at $p<0.05$.

Results

As shown in Table 1, after 1 hr of dialysis treatment, the control and hypercalcemic patients were comparable for urea, K^+ and Na^+ levels. Conversely, in all patients

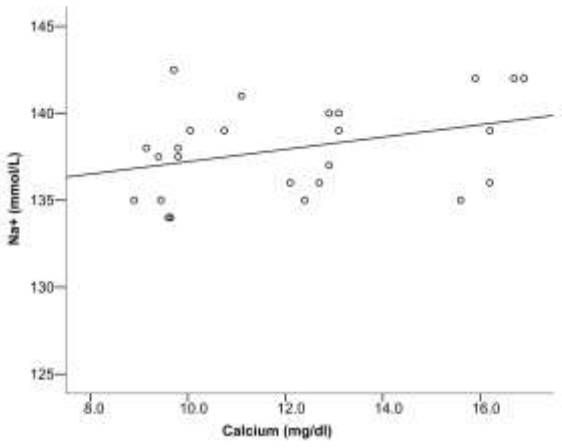

Fig 1. Correlation between calcium (horizontal axis) and sodium levels (vertical axis)

treated with hard water the most remarkable result was the presence of an increase by 45% of blood calcium levels ($p<0.01$, test t for non-paired data). Moreover, hypercalcemic patients also showed a small increase (13%), but statistically significant, of creatinine levels ($p=0.011$, t-test for non-paired data).

A correlation analysis on the measured variables after dialysis is shown in Figure 1. Specifically, calcemia was not correlated with potassium, creatinine and urea levels, whereas a positive correlation was noted with the Na^+ levels (Pearson=0.428, $p=0.032$). Figure 1 also shows the linear regression analysis between calcium and Na^+.

Discussion

The present study shows that, in the absence of renal function, natremia linearly depends on calcium levels. This observation went previously unnoticed because the strength of the relationship was quite weak (for 1 mg/dl of change in calcemia, the natremia changed by only 0.27 mmol/L). Therefore, to observe this relationship two conditions must be met: first, large modifications in the calcemia must occur to induce a quantifiable modification in natremia. This condition was met in our setting when a population of subjects with hard water syndrome was studied. Second, linear correlation methods should be used rather than classification of subjects in two or more calcemic groups. Indeed, even in our settings, if the population was subdivided in only two groups (normal versus high calcium), the power of the test would be insufficient to establish a significant difference in natremia (Table 1).

Table 1. Characteristics of controls and hypercalcemic patients (post-dialysis refers to values after 1 hr)

	Dialysis Controls (mean ±SEM)	**Dialysis hypercalcemic (mean ±SEM)**	**p (t-test for non-paired data)**
n	11	14	
Creatinine (mg/dl)	5.2±1.3	5.9±0.3	P=0.058
Urea (mg/dl)	61.9±15	54.6±13.2	NS
Na^+ (mEq/L)	137.23±2.5	138.5±2.6	NS
K^+ (mEq/L)	4.39±0.47	4.29±0.6	NS
Ca^{+2} (mg/dl)	9.66±0.48	14±2	P<<0.01

NS: p> 0.05

The fact that the subjects were ESRD patients under chronic hemodialysis clearly excludes the role of kidney function in the pathogenesis of this phenomenon. However, our observation can be explained by more than one mechanism. The first possibility is that high calcium concentration in the dialysate may affect the dialysis membrane permeability. This hypothesis is unlikely, because the state of hypercalcemia seems not to affect the diffusion of other electrolytes such as potassium, as indicated by the absence of any difference in K^+ plasma levels between patients and controls.

Another possibility is that the increased calcium concentration in the dialysate, leading to an increased osmolality of the dialysis solution, may alter the osmotic gradient, thus producing water and solute removal.

Theoretically, a hypertonic dialysis solution is supposed to diminish the efficiency of water and solute removal. In fact, by the 1980-ties, after the advent of blood pumps and dialyzers with large surface area, the use of hypotonic dialysate solutions (that cause a severe dialysis disequilibrium syndrome) were considered no longer crucial to obtain dialysis salt and water removal, and therefore, dialysate solutions containing more physiological concentration of Na^+ have been used subsequently [11]. However, the increased osmolality of the dialysate caused by the accidental increased calcium content is supposed to diminish urea, water and other solute removal. Interestingly, besides the increased plasmatic calcium levels, and urea, the other measured electrolytes did not differ significantly among patients and controls.

Moreover, we cannot exclude that the elevated amount of extracellular calcium modifies the plasma membrane permeability, leading to a cell loss of Na^+. However, several factors are against this hypothesis:

1) the intracellular amount of sodium is much lower than extracellular fluids, so diffusion or facilitated process is unlikely to occur;
2) as the trend toward an increased plasma concentration is confined to the Na^+, a not-specific and generalized permeability of the plasma membrane is unlikely to happen?

Therefore, we speculate that this phenomenon might be explained by the buffering activity of the extracellular matrix in response to an increased extracellular calcium concentration, leading to release of Na^+ in exchange with calcium (Figure 2).

Major limitations of the study are: (i) the limited sample size (ii) the limited amount of available data (unfortunately, due to the acute setting of the accident, other important hematologic parameters were not measured at the time), (iii) the impossibility to directly measure the amount of osmotically inactive Na^+ before and after the accident. However, given the rarity of the hard water syndrome, this information still retains its validity in human beings.

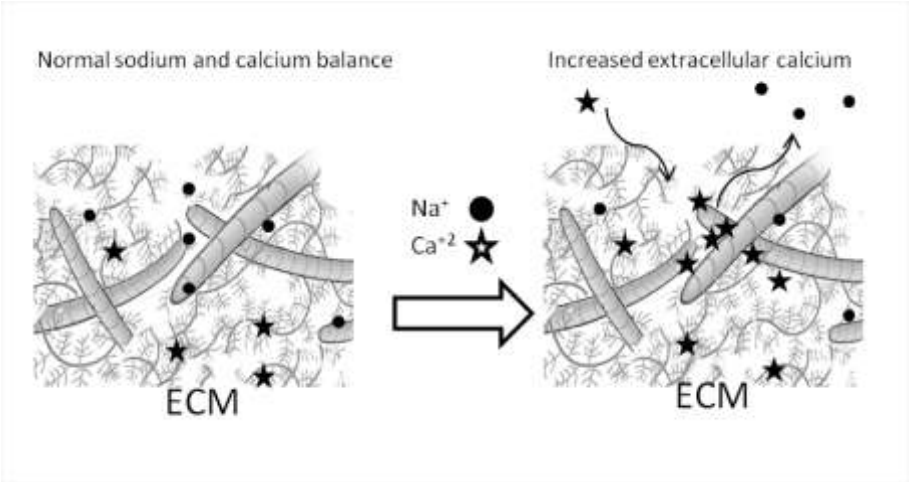

Fig. 2. Schematic representation of sodium/calcium interplay at the level of the extracellular matrix

In fact, the repetition of the study is practically impossible, because it derives from an unfortunate accident during a dialysis treatment. However, the data are of large interest and might foster further studies in the field.

Conclusion

Our data suggest that in the absence of kidney function, an extracellular increase of calcium induced by high calcium levels into the dialysate is accompanied by a trend toward increased plasma Na^+ levels. Speculatively, hypercalcemia might foster sodium release from the extracellular matrix. This might represent an additional form of sodium homeostasis, which does not necessitate kidney intervention. The conclusion of the study has a number of limitations. First, a larger amount of data should be necessary to lend further support to the hypothesis of an extrarenal handling of sodium. However, this is simply impossible, given the incidental nature of our observations, which make them also very precious and rare. Therefore, the observation of a correlation between calcium and sodium in this case is simply a confirmation of the hypothesis of an extrarenal interplay of the two molecules, due to the large extracellular reservoir of sodium [9].

Conflict of interest statement. None declared.

References

1. Pontes RB, Girardi AC, Nishi EE, *et al*. Crosstalk between the renal sympathetic nerve and intrarenal angiotensin II modulates proximal tubular sodium reabsorption. *Exp Physiol* 2015; 100: 502-506.
2. Titze J, Dahlamnn A, Lerchl K, *et al*. Spooky sodium balance. *Kidney Int* 2014; 85: 759-767.
3. Schafflhuber M, Volpi N, Dahlmann A, *et al*. Mobilization of osmotically inactive Na^+ by growth and by dietary salt restriction in rats. *Am J Physiol Renal Physiol* 2007; 292: F1490-F1500.
4. Ayus JC, Negri AL, Kalantar-Zadeh K, Moritz ML. Is chronic hyponatremia a novel risk factor for hip fracture in the elderly? *Nephrol Dial Transplant* 2012; 27: 3725-3731.
5. Titze J, Shakibaei M, Schafflhuber M. Glycosaminoglycan polymerization may enable osmotically inactive Na^+ storage in the skin. *Am J Physiol Hear Circ Physiol* 2004; 287: H203-H208.
6. Molinaro P, Viggiano D, Nistico R *et al*. Na^+-Ca^{+2} Exchanger (NCX3) Knock-Out Mice Display an Impairment in Hippocampal Long-Term Potentiation and Spatial Learning and Memory. *J Neurosci* 2011; 31: 7312-7321.
7. Molinaro P, Cuomo O, Pignataro G, *et al*. Targeted disruption of Na^+/ Ca^{+2} exchanger 3 (NCX3) gene leads to a worsening of ischemic brain damage. *J Neurosci* 2008; 28: 1179-1184.
8. Zacchia M, Capasso G. Parvalbumin: a key protein in early distal tubule NaCl reabsorption. *Nephrol Dial Transplant* 2008; 23: 1109-1111.
9. Sterns RH. Disorders of Plasma Sodium-Causes, Consequences, and Correction. *N Engl J Med* 2015; 372: 55-65.
10. Freeman RM, Lawtoon RL, Chamberlain MA. Hard-water syndrome. *New Engl J Med* 1967; 276: 1113-1118.
11. Flanigan MJ. Role of sodium in hemodialysis. *Kidney Int Suppl* 2000; 76: S72-S78.

BANTAO Journal 2017; 15(1): 33-34

BJ

BANTAO Journal

Case report

Acute Peritonitis Caused by *Propionibacterium Acnes* in a Peritoneal Dialysis Patient

Nikolina Basic-Jukic, Vesna Furic-Cunko, Ivana Juric, Lea Katalinic, Ana Rukavina, Monika Kocman and Tamara Knezevic

Department of nephrology, arterial hypertension, dialysis and transplantation, University hospital centre Zagreb, Zagreb, Croatia

Abstract

Propionibacterium acnes is a gram-positive human skin commensal that is involved in the pathogenesis of acne and prefers anaerobic growth conditions. It has been considered as a low virulence pathogen in different clinical conditions. We present the case of acute peritonitis caused by *Propionibacterium acnes* in a peritoneal dialysis patient.

Keywords: acute peritonitis, peritoneal dialysis, Propionibacterium acnes

Introduction

Propionibacterium acnes is a gram-positive human skin commensal that is involved in the pathogenesis of acne and prefers anaerobic growth conditions. It has been considered as a low virulence pathogen in different clinical conditions [1]. We present a case of acute peritonitis caused by *Propionibacterium acnes* in a peritoneal dialysis patient.

Case report

A 50-year-old male with end-stage renal disease caused by autosomal dominant polycystic kidney disease had started with continuous ambulatory peritoneal dialysis in April 2014. In June 2016 he was admitted to hospital with a 2-week history of episodic cloudy dialysis fluid (once in 5 days) all of them with normal leucocytes in dialysis effluent and sterile cultures without pain or febrility. Broad investigations to determine the cause of the sterile peritonitis were planned, however, two weeks after the onset of intermittent changes in dialysis effluent appearance, *Propionibacterium acnes* was identified from the effluent culture in anaerobic culture conditions. On admission, his temperature was 36°C, heart rate 72 beats per minute and blood pressure 120/70 mmHg. The abdomen was not painful, with palpable polycystic kidneys. Skin on his back was covered by acnae. Peritoneal dialysis catheter exit-site was clear. Initial laboratory investigations revealed white blood count $6.9x10^9$/L with 69.7% neutrophils, hemoglobin 157 g/L, blood urea nitrogen 22.4 mmol/L, creatinine 1022 µmol/L and C-reactive protein 17.2 mg/L. Leucocytes in effluent were 0.1 $x10^9$/L with predominance of neutrophils. Chest X-ray was normal, as well as plain abdominal X-ray. Intraperitoneal vancomycin combined with ciprofloxacin was initiated and continued for 10 days with additional 3 doses of vancomycin 1 g each applied weekly. Patient responded promptly with L in effluent 0.0 /L and C-reactive protein 2.4 mg/L at discharge from the hospital and no further episodes of cloudy dialysis fluid over the next year.

Discussion

Improvements in microbiological technologies for identification of the pathogenic microorganisms may contribute to decrease number of "sterile" peritonitis episodes. *P. acnes* has been associated with focal intracranial infections [2], infections of the cerebrospinal fluid shunt [3], corneal infections [4], endophthalmitis [5], endocarditis of both prosthetic [6] and native aortic valves [7]. It has also been identified as a cause of several orthopedic, silicone breast prosthesis, and prosthetic joint infections [8-10]. It has also been found on peritoneal catheters from patients without signs of infection [11].
The tendency of *P. acnes* to form biofilm [8] suggests potential benefit of intraperitoneal application as well as for prolonged antibiotic treatment to avoid recurrent infection.
It is important to stress that according to the current criteria [12], the patient had only two of the proposed criteria for acute peritonitis. Apart from the cloudy dialysate and positive culture, the patient did not have abdominal pain, high fiver, nor increased leucocyte number in the peritoneal fluid. However, our case clearly demonstrate that even without clinical signs of peritonitis, such infection should occupy our attention and that therapy should be included with an aim to prevent possible con-

Correspondence to: Nikolina Basic-Jukic, Department of nephrology, arterial hypertension, dialysis and transplantation, University hospital centre Zagreb, Kišpatićeva 12, Zagreb, Croatia; E-mail: nina_basic@net.hr

sequences. Another point is that one should carefully examine the PD patients skin, which is often neglected in clinical examination.

Conclusion

In conclusion, *Propionibacterium acnes* is a rare cause of clinically important infection in peritoneal dialysis patients. Low virulence of *P. acnes* may be the main cause of the low initial leucocytes count in effluent culture, as well as of insidious and nonspecific clinical course. Tendency of biofilm formation requires prolonged antimicrobial treatment.

Conflict of interest statement. None declared.

References

1. Achermann Y, Goldstein EJ, Coenye T, Shirtliff ME. Propionibacterium acnes: from commensal to opportunistic biofilm-associated implant pathogen. *Clin Microbiol Rev* 2014; 27: 419-440.
2. Chu RM, Tummala RP, Hall WA. Focal intracranial infections due to Propionibacterium acnes: report of three cases. *Neurosurgery* 2001; 49: 717-720.
3. Thompson TP, Albright AL. Propionibacterium acnes infections of cerebrospinal fluid shunts. *Childs Nervous System* 1998; 14: 378-380.
4. Underdahl JP, Florakis GJ, Braunstein RE, *et al*. Propionibacterium acnes as a cause of visually significant corneal ulcers. *Cornea* 2000; 19: 451-454.
5. Clark WL, Kaiser PK, Flynn HW Jr, *et al*. Treatment strategies and visual acuity outcomes in chronic postoperative Propionibacterium acnes endophthalmitis. *Ophthalmology* 1999; 106: 1665-1670.
6. van Valen R, de Lind van Wijngaarden RA, Verkaik NJ, *et al*. Prosthetic valve endocarditis due to Propionibacterium acnes. *Interact Cardiovasc Thorac Surg* 2016; 23: 150-155.
7. Mohsen AH, Price A, Ridgway E, *et al*. Propionibacterium acnes endocarditis in a native valve complicated by intraventricular abscess: a case report and review. *Scandinavian Journal of Infectious Diseases* 2001; 33: 379-380.
8. Yu JL, Mansson R, Flock JI, Ljungh A. Fibronectin binding by Propionibacterium acnes. *FEMS Immunology and Medical Microbiology* 1997; 19: 247-253.
9. Tunney MM, Patrick S, Curran MD, *et al*. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. *Journal of Clinical Microbiology* 1999; 37: 3281-3290.
10. Shields MV, Abdullah L, Namdari S. The challenge of Propionibacterium acnes and revision shoulder arthroplasty: a review of current diagnostic options. *J Shoulder Elbow Surg* 2016; 25: 1034-1040.
11. Pihl M, Davies JR, Johansson AC, Svensater G. Bacteria on catheters in patients undergoing peritoneal dialysis. *Perit Dial Int* 2013; 33: 51-59.
12. Li PK, Szeto CC, Piraino B, *et al*. ISPD Peritonitis Recommendations: 2016 Update on Prevention and Treatment. *Perit Dial Int* 2016 9-10; 36(5): 481-508.

BANTAO Journal 2017; 15(1): 35-38

BJ

BANTAO Journal

Case report

Secret Underlying Unexplained Abdominal Pain, Neurological Symptoms and Intermittent Hypertension: Acute Intermittent Porphyria

Andac Komac[1], Elif Gram[1], Feray Gulec[2] and Harun Akar[1]

[1]University of Health Sciences, Izmir Tepecik Health Research and Application Center, Department of Internal Medicine, [2]University of Health Sciences, Izmir Tepecik Health Research and Application Center, Department of Neurology, Izmir, Turkey

Abstract

A 21-year-old female patient with abdominal pain, vomiting and constipation was admitted to the hospital with the possible diagnosis of diabetic ketoacidosis. Due to increased abdominal pain and constipation the patient underwent a surgery with the diagnosis of ileus. However, no pathological findings were found in the abdominal organs apart from serous fluid in the abdominal cavity. The patient became hypertensive, tachycardic and had an episode of seizures postoperatively. Neurological manifestations with unexplained abdominal pain indicated a diagnosis of acute intermittent porphyria (AIP). Acute intermittent porphyria diagnosis is based on elevated urinary δ-aminolevulinic acid (ALA) and porphobilinogen (PBG) levels as well as hydroxymethylbilane synthase (HMBS) IVS13-2 A>G heterozygous mutation. Familial Mediterranean Fever (FMF) gene mutations were not confirmed. Porphyria should be considered in the differential diagnosis of patients with recurrent abdominal pain, neurological symptoms and lack of FMF gene polymorphism.

Introduction

Porphyrias are a group of diseases caused by deficiency in the activity of enzymes related to heme biosynthesis [1]. The clinical manifestations of the disease are related to the accumulation of heme intermediates such as δ-aminolevulinic acid (ALA) and porphobilinogen (PBG) in various tissues [1]. Porphyria can be classified as cutaneous or hepatic [2]. Acute intermittent porphyria (AIP) represents the most common form of acute hepatic porphyria that depends on porphobilinogen deaminase deficiency and is manifested with increased urinary excretion of ALA and PBG [3,4,5]. Clinical signs usually appear after puberty and are more frequent in women [1]. Different stimuli such as hormonal factors, stress, prolonged fasting, infections, alcohol, porphyrinogenic drugs and reduced caloric intake may cause acute attacks. During attacks, abdominal pain, constipation, vomiting, hypertension, tachycardia, fever and various peripheral and central nervous system symptoms may be seen [4,5]. Hyponatremia due to inappropriate antidiuretic hormone secretion represents the most common electrolyte disorder in AIP.

Case

A 21-year-old female patient with a history of type 1 diabetes mellitus for nine years was admitted to the Emergency Department of the hospital complaining of vomiting, abdominal pain and constipation. The patient was treated with an intensive insulin regimen using aspart and glargine insulin. Upon further questioning, the patient stated that she had started cabbage and unbalanced diet (poor in carbohydrates and rich in proteins) one week prior to her admission in the hospital. She said that she was on regular insulin during this period. On physical examination blood pressure was 110/70 mm/Hg, heart rate 110 beats/minute and temperature 36.4ºC. The results of the laboratory tests in the emergency department are shown in Table 1.

The ultrasound scan of the abdomen showed no abnormalities and the patient was transferred to the Department of Internal Medicine with the possible diagnosis of diabetic ketoacidosis. Oral intake was interrupted and the patient was treated with intravenous insulin and saline infusion for 24 hours. Insulin infusion was stopped after ketones become negative in urine samples and intensive insulin therapy was restarted. As there were persistent complaints of abdominal pain and constipation for approximately 4 days during her hospitalization, the surgeons were called and the patient was transferred to the Department of Surgery. After a computed tomography (CT) scan of abdomen, the patient was operated with the diagnosis of ileus. However, apart from serous fluid collection in the abdominal cavity, no abnormal findings were observed. At the same time

Correspondence to: Andac Komac, Adalet neighborhood 1594/9 street, apartment no:93 5th floor Bayraklı/İzmir, Zip code:35000; E-mail: andac.d@hotmail.com

Table 1: Laboratory findings

Variables	Results	Reference values
Hemoglobin	10.8 gr/dl	12.2-16.2
Hematocrit	32.8 %	37.7-47.9
Mean Corpuscular Volume (MCV)	74.5 fL	80.0-97.0
Platelets	269 x10^3/uL	140-400
White blood cells	5.4 x10^3/uL	4.2-10.6
Neutrophil	2.8 x10^3/uL	2.0-6.9
Glucose	429 mg/dl	74-106
Urea	13 mg/dl	17-43
Creatinine	0.9 mg/dl	0.60-1.10
Aspartate Aminotransferase (AST)	68 U/L	0-35
Alanine Aminotransferase (ALT)	59 U/L	0-35
Lactate Dehydrogenase (LDH)	206 U/L	0-247
Albumin	3.3 g/dl	3.6-5.2
Sodium	128 mmol/L	136-146
Potassium	3.03 mmol/L	3.5-5.1
C-Reactive Protein (CRP)	0.434 mg/dl	0.000-0.800
Erythrocyte Sedimentation Rate (ESR)	8 mm/hour	0-20
Antinuclear antibody (ANA)	1/160 Nuclear stippling + 1/80 homogeneous	
Anti-Smooth-Muscle Antibody (ASMA)	Negative	
Antimitochondrial antibody (AMA)	Negative	
Glutamic acid decarboxylase (GAD) Antibody	7.93 IU/ml	>10 positive
Cortisol	17.19 µg/dL	6.2-.19.4
Toxoplasma gondii Antibodies, IgG	10.5 (positive)IU/mL	<7.2 Neg >8.8 Poz
Rubella IGG (the antibody)	152 (positive) IU/ml	< 9 Neg >11 Poz
IGG antibody to CMV)	99.4 (positive)IU/ml	< 0.4 Neg >0.6 Poz
Hepatitis B Surface Antigen	0.2 (negative) S/CO	
Hepatitis B Surface Antibody	> 1000.00 (positive) mIU/mL	
HIV Antibody	0.11 (negative)S/CO	
Hepatitis C virus (HCV) antibody	0.41 (negative)S/CO	
Aminolevulinic acid (ALA)	146 µmol/L	0-8 µmol/L
Porphobilinogen (PBG)	273 µmol/L	0-34 µmol/L

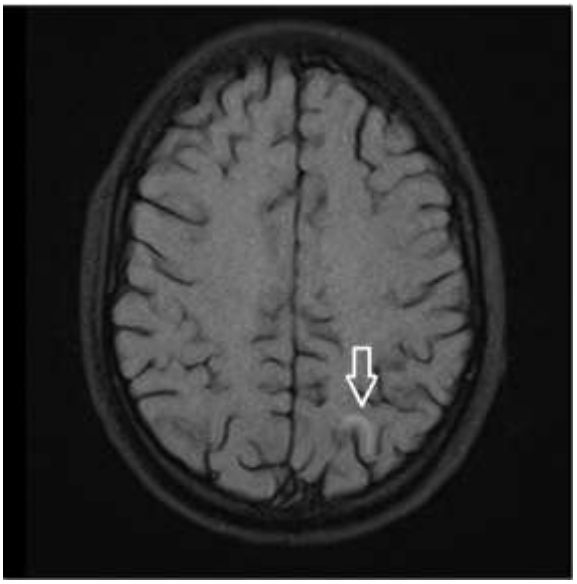

Fig. 1. Increased signal intensity in the occipital lobe as indicated by an arrow on the MRI image

gynecologists were also called, but again no gynecological abnormalities were found. During the first postoperative week, abdominal and back pains were repeated with increased intensity. The patient became hypertensive (160/100 mm/Hg) and tachycardic (150 pulse/ minute) on the 7th postoperative day and she developed tonicoclonic seizures that lasted for 3 minutes. A similar episode repeated twice. The patient was transferred to Intensive Care Unit because of deterioration of vital signs. Electroencephalography (EEG) showed paroxysmal abnormalities in the anterior cerebral hemisphere and magnetic resonance imaging (MRI) detected an increased signal intensity in the occipital lobe compatible with postictal edema (Figure 1). The laboratory results are shown in Table 1. Porphyria was considered in the differential diagnosis due to unexplained abdominal pain and neurological symptoms. ALA and PBG levels were measured in the urine. Urine ALA and PBG levels were found to be 146 µmol/L (reference values: 0-8 µmol/L) and 273 µmol/L (reference values: 0-34 µmol/L), respectively. Acute intermittent porphyria was diagnosed in the light of these results. AIP gene mutation were studied and hydroxymethylbilane synthase (HMBS) IVS13-2 A>G heterozygous mutation was found. Familial Mediterranean Fever (FMF) gene mutations were also studied but were not confirmed. Patients' symptoms regressed and vital signs were back to normal after administration of analgesics, beta blockers, insulin, intravenous dextrose and isotonic NaCl. Gabapentin was given in order to control seizures. After monitoring in the Intensive Care Unit she was transfe-

rred to the Department of Internal Medicine. Hyponatremia was considered secondary to SIADH, and sodium levels increased to normal with fluid restriction. Gabapentin was prescribed at a dose of 300 mg 2x1 daily. She was discharged with proper diet and instructions to avoid long-term fasting and porphyrinogenic drugs.

Discussion

AIP is a rare autosomal dominant metabolic disorder characterized by a deficiency of the enzyme PBG deaminase [6]. Its prevalence is 2-3 cases per 100000 persons per year [7].

Although the disease shows an autosomal dominant inheritance, genetic penetrance is low [8]. The most common presenting symptom of AIP is abdominal pain lasting from hours to days. Due to the characteristics of recurrent attacks of abdominal pain in acute intermittent porphyria, Familial Mediterranean Fever (FMF) should be considered in the differential diagnosis. FMF may be presenting with attacks of fever, abdominal pain, serositis and arthritis which are common manifestations in the Mediterranean Area, including our country. Furthermore, AIP should be considered in the differential diagnosis of patients with recurrent abdominal pain and with a negative FMF gene polymorphism. Patients with AIP might have symptoms that mimic acute abdomen which could lead to surgical intervention. In the case of suspected porphyria, after exclusion of possible causes of acute abdomen, urinary ALA and PBG levels should be measured [5]. Abdominal pain in patients with diabetic ketoacidosis is a common finding. In case of diabetic ketoacidosis, it is difficult to diagnose acute intermittent porphyria. On the other hand, ileus can be seen in patients with porphyria and ileus can cause abdominal pain. Porphyria should be considered in patients with unexplained abdominal pain or other characteristic symptoms. No clear association was defined between diabetes mellitus and AIP. One study suggests that there is a beneficial effect of diabetes on AIP [9]. There are no data indicating increased frequency of porphyria in type 1 diabetes. According to American Porphyria Foundation, some foods such as charcoal-broiled meats, cabbage, and Brussels sprouts contain chemical substances which can up-regulate hepatic ALA synthase 1. The amounts of such foods that could induce hepatic ALA synthase 1 have not been carefully studied [10]. AIP patients should be fed a balanced diet with moderate carbohydrate, protein and fat consumption and should avoid long periods of fasting or excessive diet. Dextrose infusion is recommended during an acute attack. Due to the diagnosis of type 1 diabetes, dextrose and insulin were administered at same time. Yang *et al*. have studied the types of HMBS mutations in Chinese patients with AIP and identified twenty-five HMBS mutations [11]. We found HMBS IVS13-2 A>G heterozygous mutation in our patient. AIP may affect the autonomic, peripheral and central nervous systems. Neurological symptoms of acute porphyrias include severe pain, paresis, peripheral neuropathy, muscle weakness, difficulty swallowing, other bulbar signs, confusion, delirium and seizures [12]. Posterior reversible encephalopathy syndrome (PRES) is a rare clinico-neuro-radiological entity and a rare presenting feature of AIP. PRES has distinct clinical and neuroimaging features and is characterized by sudden onset of headache, seizures, altered mental status and visual disturbances. MRI studies typically show edema involving bilateral white matter of posterior cerebral regions, especially the parieto-occipital lobes, and sometimes the frontal and temporal lobes. Other encephalic structures may also be involved [13]. Although AIP is associated with encephalopathy and epilepsy, few cases have been reported in the literature.

Conclusion

Our patient had severe muscle pain in legs and lumbar region. She developed tonicoclonic seizures that lasted for 3 minutes and repeated twice. These findings were attributed to neurological involvement of AIP and similar supportive therapy applied to AIP attacks was used. AIP should be kept in mind in the approach of patients with epileptic encephalopathy in the clinical practice. The choice of the antiepileptic drug is an important feature in preventing triggering of attacks as well as in treatment itself. In patients who have AIP and are admitted in emergency room with acute attack, the treatment should begin immediately. They should be treated with a high carbohydrate intake (at least 300 g/day), narcotic analgesics for the pain, beta blockers for tachycardia, intravenous hydration and electrolyte replacement if necessary. Fluid restriction should be considered in case of SIADH in patients with AIP. The most important part of treatment is to avoid any stimulus that can trigger an attack [14]. Porphyria needs to be considered in the differential diagnosis of patients with neurological symptoms, unexplained abdominal pain and intermittent hypertension.

Conflict of interest statement. None declared.

References

1. Sassa S. The porphyrias. *Photodermatol Photoimmunol Photomed* 2002; 18(2): 56-67.
2. Stein PE, Badminton MN, Rees DC. Update review of the acute porphyrias. *Br J Haematol* 2017; 176(4): 527-538.
3. Szlendak U, Bykowska K, Lipniacka A. Clinical, Biochemical and Molecular Characteristics of the Main Types of Porphyria. *Adv Clin Exp Med* 2016; 25(2): 361-368.
4. Puy H, Gouya L, Deybach JC. Porphyrias. *Lancet* 2010; 375(9718): 924-937.
5. Kauppinen R. Porphyrias. *Lancet* 2005; 365(9455): 241-252.

6. Meyer UA, Strand LJ, Doss M, *et al*. Intermittent acute porphyria-demonstration of a genetic defect in porphobilinogen metabolism. *N Engl J Med* 1972; 286(24): 1277-1282.
7. Menegueti MG, Gil Cezar AT, Casarini KA, *et al*. Acute intermittent porphyria associated with respiratory failure: a multidisciplinary approach. *Crit Care Res Pract* 2011; 2011: 283690.
8. Karim Z, Lyoumi S, Nicolas G, *et al*. Porphyrias: A 2015 update. *Clin Res Hepatol Gastroenterol* 2015; 39(4): 412-425.
9. Lithner F. Beneficial effect of diabetes on acute intermittent porphyria. *Diabetes Care* 2002; 25(4): 797-798.
10. American Porphyria Foundation. Available online: http://www.porphyriafoundation.com/drug-database (accessed on 29 October 2014).
11. Yang CC, Kuo HC, You HL, *et al*. HMBS mutations in Chinese patients with acute intermittent porphyria. *Ann Hum Genet* 2008; 72(Pt 5): 683-686.
12. Bloomer JR. Bonkovsky HL. The porphyrias. *Dis Mon* 1989; 35: 1-54.
13. Hinchey J, Chaves C, Appignani B, *et al*. A reversible posterior leukoencephalopathy syndrome. *N Engl J Med* 1996; 334: 494-500.
14. Besur S, Hou W, Schmeltzer P, Bonkovsky HL. Clinically Important Features of Porphyrin and Heme Metabolism and the Porphyrias Metabolites. 2014; 4(4): 977-1006.

BANTAO Journal 2017; 15(1): 39-40

BJ

BANTAO Journal

Case report

Renal Allograft Dysfunction Possibly Caused by Amiodarone Nephrotoxicity: a Case-Report

Nikolina Basic-Jukic[1], Lea Katalinic[1], Marijana Coric[2], Monika Kocman[1], Branimir Krtalic[1] and Petar Kes[1]

[1]Department of nephrology, arterial hypertension, dialysis and transplantation, [2]Department of pathology, University hospital center, Zagreb, Croatia

Abstract

Amiodarone is a potent inhibitor of CYP3A4 and can increase serum concentrations of drugs that are substrates of this enzyme system. Immunosuppressive drugs are also metabolized through the cytochrome metabolic pathway what may lead to important drug-drug interactions.
A 60-year-old female received her second allograft from the deceased donor and was treated with tacrolimus, mycophenolate mofetil and steroids. Amiodarone was introduced for treatment of paroxysmal atrial fibrillation four days after the transplantation. One month after the discharge she was readmitted to hospital for evaluation of the creeping creatinine. Biopsy showed borderline acute rejection. She received 3 boluses of 6-methilprednisolone but creatinine continued to rise. Repeated biopsy was without signs of rejection with mild interstitial fibrosis/tubular atrophy, mild global glomerulosclerosis and moderate arterial sclerosis. However, tubular vacuolization was prominent. After careful revision of her therapy we decided to replace amiodarone with sotalol. One week later her creatinine fell from 350 to 220 µmol/l and remained stable. This case illustrates possible amiodarone nephrotoxicity in a renal transplant recipient. We suggest that patients who need amiodarone in combination with tacrolimus be closely monitored by both cardiologists and nephrologists, with frequent determinations of tacrolimus trough levels and serum creatinine measurements.

Key words: tacrolimus, amiodarone, interaction, renal transplantation, nephrotoxicity

Introduction

Amiodarone is an important drug indicated for the treatment of supraventricular and ventricular arrhythmias, such as atrial or ventricular fibrillation. It is a potent inhibitor of CYP3A4 and may increase serum concentrations of drugs that are substrates of this enzyme system. Additionally, amiodarone may inhibit the P-glycoprotein efflux pump in the intestines thus enhancing absorption of certain drugs. Immunosuppressive drugs are also metabolized through the cytochrome metabolic pathway [1], what may lead to important drug-drug interactions. Several case reports have described QT interval prolongation after concomitant use of amiodarone and tacrolimus [2,3]. Also, amiodarone nephrotoxicity has been demonstrated in animal and observational studies [4-6].
We report a case of kidney allograft dysfunction possibly caused by amiodarone nephrotoxicity precipitated by tacrolimus.

Case report

A 60-year-old woman with end-stage renal disease caused by chronic glomerulonephritis without biopsy received her second allograft from deceased donor in July 2014. First transplantation was performed in 1996, and graft functioned until 2005 when she had restarted with hemodialysis. Immunosuppression consisted of Thymoglobulin induction, tacrolimus (0.1 mg/kg), mycophenolate mofetil 2x1 g and steroids. Amiodarone was introduced 4 days after the transplantation for treatment of paroxysmal atrial fibrillation. She was discharged from the hospital with serum creatinine 212 umol/l 15 days after transplantation, in the sinus rhythm. Other drugs included pantoprazole, bisoprolol, valgancyclovire, trimetoprim-sulphometoxazol, vit D3, furosemid, minoxidil and moxonidine. She was readmitted to the hospital one month later for evaluation of allograft dysfunction with the "creeping" serum creatinine reaching 330 µmol/l. Tacrolimus trough level was 8.2 µg/L. Trimetoprim-sulphometoxazol was immediately omitted. Viruses were negative. Biopsy showed borderline acute rejection with negative C4d. Donor specific antibodies were negative. CML and ADCML were both positive, however, with evident cytotoxicity in the control test of autologous serum what may be consequence of drugs toxicity. She received 3 boluses (500 mg each) of 6-metilprednisolone with no effect. Serum creatinine continued to rise with

no changes indicative for acute rejection on repeated biopsy. Pathohistological finding included mild interstitial fibrosis/tubular atrophy, mild global glomerulosclerosis and arterial sclerosis. However, tubular vacuolization was present (Figure 1) indicating possible toxicity.

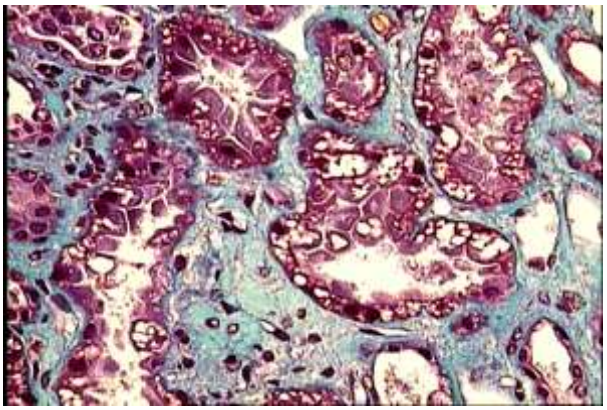

Fig. 1. Vacuolization of tubular epithelial cells. Hemalaun eosin, x 40

After careful revision of her therapy we suspected amiodarone nephrotoxicity and decided to replace amiodarone with sotalol for rhythm control. Seven days after removing amiodarone, serum creatinine fell from 350 to 220 µmol/l and remained stable during the follow-up of three years.

Discussion

We describe a case of a kidney transplant recipient with a possible amiodarone-tacrolimus interaction leading to amiodarone nephrotoxicity. Tacrolimus is metabolized by cytochrome CYP3A4 in liver and small bowel. It is also a substrate for P-glycoprotein, a drug transporter that decreases the absorption and increases excretion of substrates. Amiodarone inhibits both mechanisms and therefore may cause drug-drug interactions [1]. Amiodarone and tacrolimus interactions have been previously described [2,3]. Both are substrates at CYP3A for enzyme metabolism and could potentially be implicated in increasing the concentration of the other agent through competition for metabolism sites.

To our knowledge, this is the first report demonstrating amiodarone-tacrolimus interaction beyond the QT interval prolongation with normal trough levels of tacrolimus. It seems that underestimated drug-drug interaction ultimately induced amiodarone nephrotoxicity.

Previous case reports recommend frequent serum tacrolimus concentration monitoring and prospective tacrolimus dose reductions when the two drugs are given in combination. The amiodarone-induced inhibition of tacrolimus metabolism resulted in significantly lower doses of tacrolimus necessary to achieve proper therapeutic serum concentrations [3]. Much less is known about effects of tacrolimus on possible elevation of amiodarone concentration, which may be clinically important while both animal and observational studies suggested that amiodarone may cause renal impairment by reducing renal blood flow or inducing tubular alterations [4-6].

In conclusion, given the importance of the amiodarone-tacrolimus interaction, we suggest that patients who need amiodarone in combination with tacrolimus be closely monitored by both cardiologists and nephrologists, with frequent determinations of tacrolimus trough levels and serum creatinine measurements. If possible, an alternative agent for control of hearth rhythm in renal transplant recipients treated with tacrolimus should be discussed with cardiologists.

Conflict of interest statement. None declared.

Literature

1. Gill J, Heel RC, Fitton A. Amiodarone. An overview of its pharmacological properties, and review of its therapeutic use in cardiac arrhythmias. *Drugs* 1992; 43: 69-110.
2. Nalli N, Stewart-Teixeira L, Dipchand AI. Amiodarone-sirolimus/tacrolimus interaction in a pediatric heart transplant patient. *Pediatr Transplant* 2006; 10: 736.
3. Kisters K, Cziborra M, Funke C, *et al*. Amiodarone-tacrolimus interaction in kidney transplantation. *Clin Nephrol* 2008; 70: 563.
4. Jacobs MB. Serum creatinine increase associated with amiodarone therapy. *N Y State J Med* 1987; 87: 358-359.
5. Morales AI, Barata JD, Bruges M, *et al*. Acute Renal Toxic Effect of Amiodarone in Rats. *Pharmacol Toxicol* 2003; 92: 39-42.
6. Pollak PT, Sharma AD, Carruthers G. Creatinine elevation in patients receiving amiodarone correlates with serum amiodarone concentration. *Brit J Clin Pharmacol* 1993; 36: 125-127.

BANTAO Journal 2017; 15(1): 41-42

BJ

BANTAO Journal

Letter to the editor

Five Years of Renal Transplantations in Montenegro: Results and Challenges

Marina Ratkovic[1], Nikolina Basic-Jukic[2], Zeljko Kastelan[3], Danilo Radunovic[1], Vladimir Prelevic[1], Tvrtko Hudolin[3], Marija Grkovic[5], Jasna Stoic-Brezak[5] and Petar Kavaric[4]

[1]Department of nephrology and dialysis, Clinical Centre Montenegro, Podgorica, Montenegro, [2]Department of nephrology, arterial hypertension, dialysis and transplantation, University hospital Centre Zagreb, School of medicine University of Osijek and School of medicine University of Zagreb, Croatia, [3]Department of urology, University hospital centre Zagreb, School of medicine, University of Zagreb, Croatia, [4]Department of urology, Clinical centre Montenegro, Podgorica, Montenegro, [5]Department of anesthesiology, University hospital centre Zagreb, Zagreb, Croatia

Abstract

First renal transplantation in Montenegro was performed on September 25th, 2012. Since then, 32 transplantations have been performed. Only one was from deceased donor, the remaining were from living donors. 40.4% of all patients with end-stage renal disease currently live with the functioning renal allograft (190 patients on dialysis, 129 transplanted patients). There are 32 patients on the waiting list. Further efforts will be focused on development of the deceased donor program and introduction of the AB0 incompatible renal transplantations.

Keywords: renal transplantation, Montenegro, living donors

Dear Sir,

Until the establishment of the own renal transplant program citizens of Montenegro went for transplantations in neighboring countries of the former Yugoslavia if they had living donor or to countries with illegal market of organs or went for transplantation in the states with legal possibility for foreigners to be placed on the waiting list for kidney transplantation if they could financially afford transplantation (France, Russia). From 1990 until September 2012 92 transplantations were performed-4.14 per year. Doctors from Montenegro had to learn how to face numerous complications associated with this kind of transplantations. In order to stop transplant tourism and to provide the best option of renal replacement treatment, Montenegro decided to establish its own program despite the small overall number of dialysis patients which never exceeded 190.

Montenegro became a member of the Regional Health Development Center (RHDC), a part of the South East European Health Network (SEEHN), in February 2011. RHDC is an organization supported by the Council of Europe, with aim to establish all necessary conditions for the development of transplantation in the Southeastern Europe. At that time Croatian transplant results were enormous, placing Croatia on the top of the world with a record number of donors per million population and number of transplanted kidneys per million population. Connections and support from RHDC helped to establish collaboration between Montenegro and Croatia [1]. The first renal transplantation in Podgorica, Montenegro was performed on September 25th, 2012 in collaboration between the Croatian and the Montenegrin transplant teams. Since then, 32 transplantations have been performed-6.4 per year. Out of these 32 transplantations, 31 were from the living donor and only one from the deceased donor (Figure 1).

One patient developed thrombosis of the renal artery, and after the thrombendarterectomy had functioning allograft for additional two years when was retransplanted.

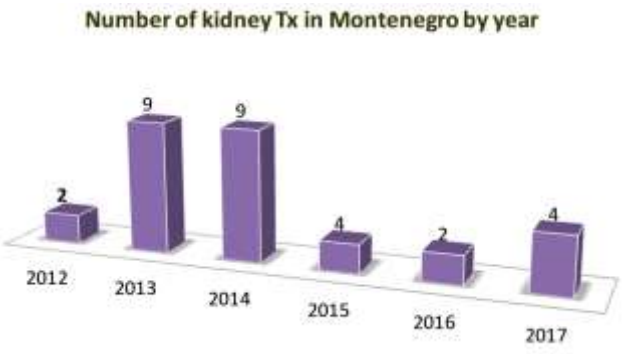

Fig. 1. Number of kidney transplantations performed at Clinical centre Montenegro since September 2012 by year of transplantation

Other patients are doing well without significant post-transplant complications, as well as all donors.

It is important to stress that 97 potential living donors were evaluated over the observed period. All of them were either family-related or emotionally-related potential donors. From this number, only 31(31.9 %) satisfied the criteria to become donors. Others had clinical contraindications for donation. Montenegro currently has 190 patients on dialysis in 11 centers dispersed throughout the country. The waiting list for renal transplantation was corrected; all patients were reevaluated and have been prepared for the eventual call for transplantation. Thus, 32 patients are currently on the waiting list (16.8% of dialysis population). 40.4% of all patients with end-stage renal disease are living with the functioning renal allograft.

Establishment of the national transplant program helped to increase number of transplantations per year. However, we have not solved the problem of patients without adequate living donor while we failed to develop deceased-donor transplantation program. Further steps include development of the AB0 incompatible program in order to increase number of potential living donors, and nationwide efforts to introduce organ donation from deceased donors.

In conclusion, huge efforts have been invested to promote deceased donor program in Montenegro. However, rejection rate is still extreme. It is obvious that families of potential donors still have no adequate knowledge to perceive transplantation. Thus, education of the medical personnel and of the community is mandatory if we want to improve our results.

Conflict of interest statement. None declared.

Reference

1. Goce Spasovski, Mirela Busic, Lydia Raley, *et al*. Current status of transplantation and organ donation in the Balkans-could it be improved through the South-eastern Europe Health Network (SEEHN) initiative? *Nephrol Dial Transplant* 2012; 27: 1319-1323.

INSTRUCTIONS TO AUTHORS

ALL ARTICLES MUST BE SUBMITTED ONLINE. Once you have prepared your manuscript according to the Instructions below, please send it as an attachment to the editor Goce Spasovski (spasovski.goce@gmail.com**).**

AIMS AND SCOPE

BANTAO Journal is the official publication of the **Balkan Cities Association of Nephrology, Dialysis, Transplantation and Artificial Organs.** The journals publish articles relating to clinical or laboratory investigations of relevance to nephrology, dialysis or transplantation. Papers relating to basic immunology, anatomy and physiology are welcomed if these relate to the kidney. Rapid communications, case reports, technical reports and letters to the Editor are also considered. Letters to the Editor do not necessarily express the views of the Editor, and may or may not be peer-reviewed. All material is assumed to be submitted exclusively unless otherwise stated, and must not have been published previously except in abstract form. Manuscripts related to the topic of the submitted manuscript which are in preparation or submitted to other journals, must be sent in together with the manuscript destined for *BANTAO Journal*.

Only papers in **UK/USA English** will be considered for publication. The text should be typed double spaced on A4 sized paper. If any tables, illustrations or photomicrographs have been published elsewhere, written consent to re-publication (in print and online) must be obtained by the author from the copyright holder and the authors, such permission being detailed in the cover letter.

Manuscripts should bear the full name and address, with telephone, fax, and email of the author to whom the proofs and correspondence should be sent (corresponding author). For all authors first name and surname should be written in full. In the covering letter the individual contribution of each co-author must be detailed. This letter must contain the statement: 'the results presented in this paper have not been published previously in whole or part, except in abstract form'. Should your manuscript be accepted for publication, you will be required to give signed consent for publication.

To accelerate publication only *one set of PDF proofs* is sent to the corresponding author by email. This shows the layout of the paper as it will appear in the Journal. It is, therefore, essential that manuscripts are submitted in their final form, ready for the printer. Proof-reading must be limited to the correction of typographical errors. Proofs must be returned by the author within 48 hours of receipt.

Authors are referred to the statement on uniform requirements for manuscripts submitted to biomedical journals prepared by an international committee of medical journal editors. (*Br Med J* 1982; 284: 1766-1770, *Ann Intern Med* 1982; 96: 766-771.)

CONFLICT OF INTEREST DECLARATION AND UNIFORM REQUIREMENTS OF THE INTERNATIONAL COMMITTEE OF MEDICAL JOURNAL

All manuscripts must conform to the Uniform Requirements of the International Committee of Medical Journal Editors (ICMJE), which can be found on http://www.icmje.org. These requirements include: disclosure of conflicts of interest, protections of patients' right to privacy, and conducting human and animal experiments in accordance with ethical standards.

All authors must disclose any potential conflict of interest which may affect their scientific judgment. Such conflicts of interest may be financial involvement, but may also be of different nature such as scientific competition, personal relationships or other. When disclosing conflicts of interest, sources of funding of the presented work must be declared. The corresponding author must include a Conflict of Interest Statement on behalf of all the authors at the end of the article. If no Conflict of Interest is declared this must be stated also.

When reporting experiments on human subjects, authors should indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

AUTHORS

Each author (written in full name and surname) should have participated sufficiently in the work to take public responsibility for the content. This participation must include:

1. Conception or design, or analysis and interpretation of data, or both.
2. Drafting the article or revising it.
3. Providing intellectual content of critical importance to the work described.
4. Final approval of the version to be published. (See Br Med J 1985; 291: 722-723.)

TEXT

The order of original articles should be as follows:

Title page giving details of all authors, including first or given name (see above).

On a separate page an abstract of ~250 words. It should consist of four paragraphs labelled, `Background', `Methods', `Results' and `Conclusions'. They should briefly describe, respectively, the problems being addressed in this study, how the study was performed, the salient results and what the authors conclude from the results.

Keywords not more than 6, in alphabetical order, characterizing the scope of the paper, the principal materials, and main subject of work. Authors are requested to supply an additional 10-15 keywords for electronic publication purposes.

Running title of not more than 75 letters and spaces.

On a new page: Introduction, Subjects and Methods, Results, Discussion, Acknowledgements, References (see below), Tables, Legends to Figures and Figures. All pages should be numbered consecutively commencing with the title page. Headings (Introduction; Subjects and Methods, etc) should be placed on separate lines. **It is most important that authors number their pages prior to submission as reviewers will refer to particular pages when providing their comments on the manuscript.**

Please ensure that your abstract and title page are included in the main body of your manuscript, as well as submitting it separately.

Any statistical method must be detailed in the Subjects and methods section, and any not in common use should be described fully or supported by references.
Authors should not use abbreviations in headings. Authors are advised to refrain from excessive use of uncommon abbreviations, particularly to describe groups of patients or experimental animals.
Up to 6 printed pages for an Original Article, 3 pages for a Case Report, Technical Report or Brief Report, and 2 page for a Letter were allowed. A printed page is ~850 words, but pro rata reductions in the length of the text must be made for Tables, Figures and illustrations. Images in Nephrology aims to publish 1 or 2 high quality pictures of great clinical interest accompanying by a minimal amount of text and 2-3 references, not exceeding 2 printed pages.

TABLES

Tables must be typed on separate pages and should follow the reference list. All Tables must be numbered consecutively and each must have a brief heading describing its contents. Any footnotes to Tables should be indicated by superscript characters. Tables must be referred to in the main text in order. All Tables must be simple and not duplicate information given in the text.

FIGURE PREPARATION

Please be aware that the requirements for online submission and for reproduction in the journal are different: (i) for online submission and peer review, please upload your Figures either embedded in the word processing file or separately as low-resolution images (.jpg, .tif, .gif or. eps); (ii) for reproduction in the journal, you will be required after acceptance to supply high-resolution .tif files (1200 d.p.i. for line drawings and 300 d.p.i. for colour and half-tone artwork) or high-quality printouts on glossy paper. We advise that you create your high-resolution images first as these can be easily converted into low-resolution images for online submission. The journal reserves the right to reduce the size of illustrative material. All micrographs must carry a magnification bar. Any photomicrographs, electron micrographs or radiographs must be of high quality. Wherever possible photographs should fit within the print area of 169 x 235 mm (full page) or within the column width of 82 mm. Photomicrographs should provide details of staining technique and a scale bar. Patients shown in photographs should have their identity concealed or should have given their written consent to publication. Normally no more than six illustrations will be accepted without charge.

TRADE NAMES

Non-proprietary (generic) names of products should be used. If a brand name for a drug is used, the British or International non-proprietary (approved) name should be given. The source of any new or experimental preparation should also be given.

REFERENCES

The references should be numbered in the order in which they appear in the text. At the end of the article the full list of references should give the name and initials of all authors unless there are more than six, when only the first three should be given followed by *et al*. The authors' names should be followed by the title of the article, the title of the Journal abbreviated according to the style of *Index Medicus*, the year of publication, the volume number and the first and last page numbers. References to books should give the title of the book, which should be followed by the place of publication, the publisher, the year and the relevant pages.
EXAMPLES
1. Madaio MP. Renal biopsy. *Kidney Int* 1990; 38: 529-543
Books:
2. Roberts NK. *The cardiac conducting system and the His bundle electrogram.* Appleton-Century-Crofts, New York, NY: 1981; 49-56
Chapters:
3. Rycroft RJG, Calnan CD. Facial rashes among visual display unit (VDU) operators. In: Pearce BG, ed. *Health hazards of VDUs.* Wiley, London, UK: 1984; 13-15
Note: It is the responsibility of the author to ensure the accuracy of the references in the submitted article. Downloading references direct from Medline is highly recommended.

COPYRIGHT

It is a condition of publication in the Journal that authors grant an exclusive licence to the Journal.

COLOUR ILLUSTRATIONS

Colour illustrations are accepted, but the authors will be required to contribute to the cost of the reproduction. Colour Figures will incur a printing charge of 50 euros. Illustrations for which colour is not essential can be reproduced as black and white images in the print journal and will remain in colour in the available PDF file on the web site of the journal.

EDITORIAL ENQUIRIES:

Editor in Chief:

GOCE SPASOVSKI
University Department of Nephrology
University of Skopje
Vodnjanska 17
Skopje, R. Macedonia
Email: spasovski.goce@gmail.com

BANTAO Journal
Published on behalf of:

Balkan Cities Association of Nephrology, Dialysis, Transplantation and Artificial Organs
www.bantao.org

BJ
BANTAO Journal

ERA-EDTA sponsored CME Activities
http://www.enp-era-edta.org/#/95/conferences/conferences

Chronic kidney diseases and kidney transplantation in the developing countries of south-eastern europe
October 7, 2017 – Sarajevo, Bosnia and Herzegovina

How can I improve the outcomes of kidney transplantation in my center?
October 13, 2017 – Antwerp, Belgium

Protein energy wasting and kidney disease; therapeutic challenges and opportunities
October 18-19, 2017 – Belek, Turkey

13th BANTAO Congress
October 4-8, 2017 – Sarajevo, Bosnia and Herzegovina