DOI 10.1515/pterid-2013-0030 — Pteridines 2013; 24(3-4): 219–223

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Evaluation of dihydropteridine reductase activities in patients with kidney failure

Abstract: End-stage renal disease (ESRD) is the inability of the kidneys to remove waste products from the blood. The most important factors causing ESRD that require hemodialysis are diabetes and hypertension. There are limited numbers of studies to evaluate tetrahydrobiopterin pathway in these patients. The aim of the study was to evaluate tetrahydrobiopterin pathway by measuring its important components, biopterin to creatinine concentrations and dihydropteridine reductase activities in diabetes and hypertension patients treated with/without hemodialysis. The patients undergoing hemodialysis were classified as diabetic nephropathy (n=21), hypertensive nephropathy (n=20) and others (n=30), while the controls consisted of healthy subjects (n=21), diabetic subjects (n=23) and hypertensive subjects (n=22) without any renal disorder. It was found that urinary biopterin to creatinine concentrations significantly increased in kidney failure patients undergoing hemodialysis compared to the healthy control group ($p<0.05$). Additionally, there were significant differences in urinary biopterin to creatinine concentrations between diabetes or hypertension patients and their hypertensive or diabetic control counterparts (both $p<0.05$). Our results indicated an alteration in tetrahydrobiopterin pathway in ESRD, and in the presence of secondary pathologies such as diabetes and hypertension in the patients undergoing hemodialysis, more considerable changes are observed in the pathway.

Keywords: biopterin; dihydropteridine reductase; hemodialysis; tetrahydrobiopterin.

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Introduction

Chronic kidney disease (CKD), also called end-stage renal disease (ESRD), is increasingly recognized as a public health problem [1–3]. Cardiovascular diseases, diabetes, hypertension and obesity are major risk factors for CKD [3, 4]. According to the Kidney Disease Outcomes Quality Initiative of the US National Kidney Foundation, kidney failure is defined as having a glomerular filtration rate (GFR) below 15 mL/min per 1.73 m^2 for ≥3 months or requiring renal replacement therapy at higher GFR levels to sustain his/her life [1]. Diabetes and hypertension are the most leading causes of CKD, and early identification of CKD is needed to prevent disease progression and reduce the risk of cardiovascular morbidity and mortality [3].

One of the most important unconjugated pteridines, 5,6,7,8-tetrahydrobiopterin (BH_4), is the essential cofactor of not only phenylalanine hydroxylase, but also tyrosine and tryptophan hydroxylases, the rate-limiting enzymes in the biosynthesis and, therefore, in the regulation of important hormones and neurotransmitters – catecholamines, serotonin and melatonin [5–7]. Hence, a block in the BH_4 pathway leads to alterations in the production of neurotransmitters [8]. Besides these, nitric oxide synthase (NOS) and alkylglycerol monooxygenase are also BH_4-dependent enzymes [9]. BH_4 provides electrons, and, as a consequence, it is transformed into 4-α-hydroxytetrahydrobiopterin and reduces molecular oxygen. Biopterin is synthesized by BH_4 oxidation, and it is mostly excreted by urine in reduced (BH_2-dihydro) or oxidized form [5]. BH_4 is converted to quinonoid dihydrobiopterin and can then be regenerated by the enzyme dihydropteridine reductase (DHPR). Thus, DHPR has a key role in the maintenance of BH_4 in the body [10, 11] and that is why it is an essential enzyme in the phenylalanine, tyrosine and tryptophan hydroxylating systems [5, 12, 13].

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Imbalance of tetrahydrobiopterin homeostasis triggers important alterations in the pathway, and this may play a role in the etiology of some diseases or some pathologies such as diabetes and hypertension [10, 12, 13]. Moreover, any deficiency or decrease in DHPR enzyme activity can cause a profound effect on the metabolism of neurotransmitters and leads to neurotransmitter abnormalities. It is known that patients may develop signs of neurological disturbances including confusions, since the enzymatic activity in blood appears to reflect cerebral activity [5, 12–14]. In the literature on this field, there are just a few studies showing alteration in the tetrahydrobiopterin pathway in chronic renal failure. Therefore, the aim of the present study was to investigate the changes in tetrahydrobiopterin pathway by blood DHPR activities and urine biopterin to creatinine concentrations in ESRD patients undergoing hemodialysis and also to evaluate the effect of diabetes and hypertension existence on the homoeostasis of the pathway.

Materials and methods

Subjects

Seventy-one patients undergoing regular hemodialysis therapy in the hemodialysis center of the nephrology unit of the university hospital were recruited for the study. The patients were classified into three groups according to their primary renal disorders: (i) patients with diabetic nephropathy (n=21); (ii) patients with hypertensive nephropathy (n=20); and (iii) others, including patients with reflux nephropathy or interstitial nephritis, and patients with renal insufficiency depending on other causative factors (n=30). All patients were dialyzed on a 4-h, thrice-weekly schedule.

A total of 66 people were recruited as controls; they consisted of (i) healthy volunteers (n=21); (ii) controls with diabetes mellitus (n=22); and (iii) controls with hypertension (n=23) who were admitted to the Internal Medicine and Endocrinology Clinic. None of the controls had renal insufficiency, retinopathy or albuminuria at the pathologic level.

All subjects participated in the study voluntarily, and all of them provided written consent before blood samples were drawn. The study was approved by the local Ethics Committee of the university in accordance with the Helsinki Declaration of 1981.

Collection of samples

Urine and blood samples were collected from the dialysis patients on a mid-week dialysis day at the beginning of the treatment session. Because most of the hemodialysis patients were anuric, urine samples could be collected only from 49 of 71 patients. Peripheral venous blood samples from the subjects were drawn and dropped on a filter paper. DHPR enzyme assay was performed on these dry blood spots. All samples were kept away from direct light and stored at 20°C until assay day.

Measurements of biopterin

Biopterin and creatinine concentrations in each urine sample were analyzed by high-performance liquid chromatography (HP Agilent 1100, Hewlett-Packard, Vienna, Austria), without an oxidation step, as described before [15]. A column (25 cm×4.6 mm) containing octyldodecyl silica gel C18 (5 µm particle size; Hichrom, Berkshire, UK) was used. Biopterin was quantified by using a fluorescence detector (λ_{ex}: 353 nm, λ_{em}: 438 nm) with potassium dihydrogen phosphate buffer containing 2.5% methanol (v/v) (pH 7.0) as a mobile phase. Creatinine concentrations were determined simultaneously by using an ultraviolet detector (HP Agilent 1100, Vienna, Austria) at a wavelength of 235 nm. The biopterin levels were expressed as micromoles of biopterin per mole of creatinine.

Determination of DHPR activity

Dry blood spots were extracted with 0.15 mol/L of cold KCl solution. This elute was used in the assay of DHPR enzyme activity. Enzyme activity was measured spectrophotometrically at 550 nm wavelength (Shimadzu UV160, Shimadzu, Tokyo, Japan) by following the BH_4-dependent reduction of ferricytochrome c in the presence of NADH [10, 15, 16]. The enzyme activity was expressed as nanomoles of cytochrome c reduced per minute relative to the 6-mm diameter of blood spots.

Statistical analysis

All of the results are expressed as the mean±standard error of the mean. The software SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. The Mann-Whitney U test and Pearson correlation test were performed. Furthermore, the results were confirmed with a multivariate regression model. $p<0.05$ was considered statistically significant.

Ethics statement

The study was approved by the Clinical Research Ethics Committee of Atatürk University (Ethics Committee approval date: 2006/4/1, protocol number: 19).

Results

As shown in Figure 1, biopterin to creatinine concentrations in total hemodialysis patients were higher than those of the healthy controls (p=0.002). Additionally, biopterin to creatinine concentrations in diabetic and hypertensive controls were significantly higher compared

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to healthy controls (both $p<0.05$). Furthermore, diabetic and hypertensive nephropathy patients also had significantly higher biopterin to creatinine concentrations than healthy controls (both $p<0.05$). The mean values of the urinary biopterin to creatinine concentrations of the hypertensive nephropathy and diabetic nephropathy groups were higher than those of the hypertensive control and diabetic control groups, respectively. However, the differences were not significant. The highest urinary biopterin to creatinine concentrations were found in patients with diabetic and hypertensive kidney-failure groups. However, there was not any difference between the hypertensive and the diabetic nephropathy groups ($p>0.05$).

The results of the DHPR activities in the study groups are presented in Figure 2. It was found that the enzyme activity was only significantly lower in hypertensive nephropathy patients compared to the hypertensive control group ($p=0.007$).

Among the compared groups, no statistical correlations were found between blood DHPR activities and urine biopterin levels (all $p>0.05$).

Discussion

Imbalance of tetrahydrobiopterin homoeostasis leads to excessive production of its oxidized form, biopterin, and high biopterin may be involved in the production of reactive oxygen species. Thus, the balance between oxidant and antioxidant status is disrupted in favor of oxidative stress, and this condition may underlie the pathology of such diseases as diabetes and hypertension. It is known that there is a relationship between BH_4 and diabetes mellitus/hypertension [5]. In contrast, the precise role of elevated biopterin levels has not been explained in the vascular and/or nonvascular renal system so far. Additionally, the effects of presence and/or absence of the above-mentioned pathologies in kidney failure have not been studied in detail.

Here, it was found that the only significant difference in DHPR activities among the study groups was between hypertensive nephropathy patients and their control counterparts. Lower DHPR activity in hypertensive nephropathy patients indicates that the enzyme cannot maintain the homeostasis at the regeneration step. This is also confirmed by the higher excretion of urine biopterin to creatinine concentrations in this group. Decreasing activity of DHPR in this group may indicate that the enzyme can be affected by nephropathy. Altmann et al. [12] measured the concentrations of biopterin derivatives in 38 patients on hemodialysis who had no clinical evidence of encephalopathy. They found that serum concentrations of biopterin derivatives were markedly elevated [12]. Yokoyama et al. [17] evaluated the oxidized and reduced forms of biopterin in hemodialysis patients and found the BH_4/BH_2 ratio to be decreased significantly.

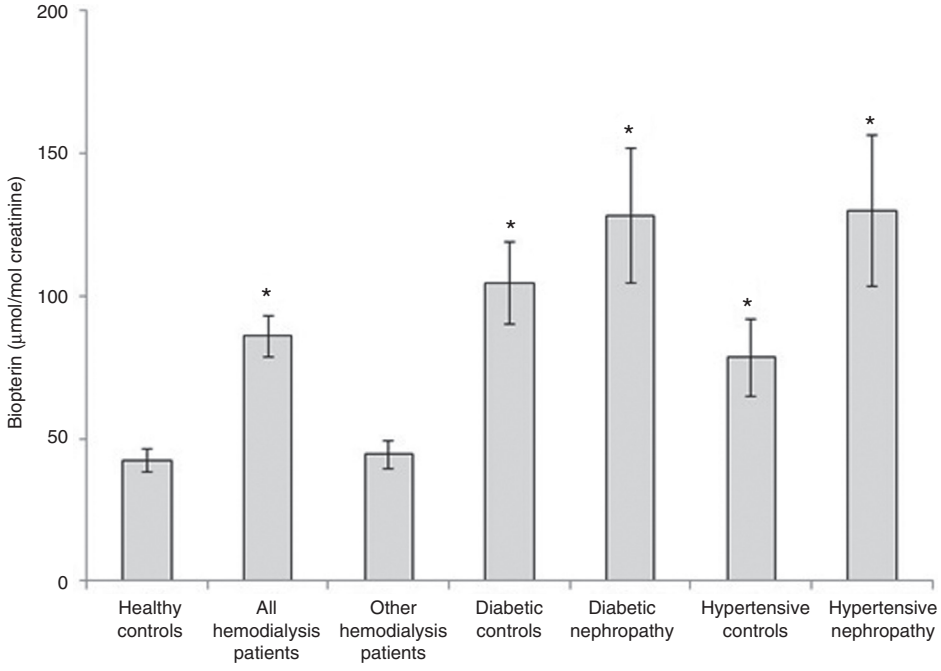

Figure 1 Comparison of urinary biopterin levels (µmol/mol creatinine) in the study groups. Values of the urinary biopterin levels are shown as mean±standard error of the mean. *$p<0.05$, vs. healthy controls.

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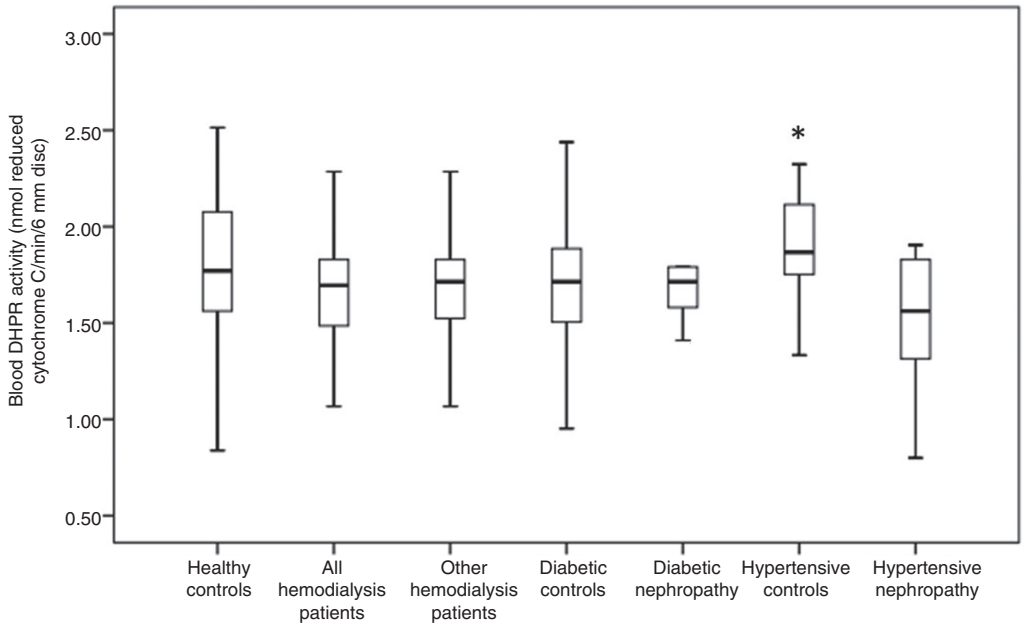

Figure 2 Box-plot graphs of blood DHPR activities in the control groups and patients undergoing hemodialysis. *p<0.05, vs. hypertensive controls.

They suggested that decreased reduction of DHPR during the conversion from BH_2 to BH_4 can cause the reduction in the form of BH_4. They concluded that the change in the state of reduction might be involved in the pathophysiology of renal failure [17]. To investigate whether changes in DHPR activity or biopterin to creatinine concentrations levels can be used as a biomarker in nephropathy, further detailed studies are required.

Since no oxidation step was performed in our study, measured biopterin levels were considered as total biopterin. Our results have apparently shown that renal failure may cause imbalance in the tetrahydrobiopterin pathway, since patients with end-stage renal failure have approximately two times higher biopterin to creatinine concentrations in comparison with healthy subjects. According to Yokoyama et al. [17], inducible NOS (iNOS) synthesis is increased with stimulation of macrophages and monocytes and, thus, unbalanced iNOS and endothelial NOS (eNOS) concentrations may be closely related to the disruption of pteridine metabolism in renal failure [17].

Tetrahydrobiopterin is an essential cofactor in the regulation of eNOS, and BH_4 is important for maintaining endothelium function and may be affected in type 2 diabetes in humans [18–21]. A variety of experimental approaches have been used to investigate BH_4 availability in vascular diseases including diabetes and hypertension in both animal models and clinical studies [18, 20, 21]. These studies suggested that decrease in BH_4 is caused by a decrease in the activity of GTP cyclohydrolase I (GTPCH I), which is a key enzyme of BH_4 synthesis [20, 21]. GTPCH I is activated with proinflammatory cytokines, which can also activate monocyte/macrophages and lead to increased neopterin levels in parallel to biopterin. Increasing neopterin levels can be confirmed by previous studies on hemodialysis patients [22]. Okumura et al. [23] found that renal BH_4 levels decreased in the type II diabetic rat model and that treatment with benidipine as an antihypertensive agent was effective in maintaining renal GTPCH I activity and BH_4 levels [23]. In the present study, the results obtained from either diabetes or hypertension patients have apparently shown an imbalance in the tetrahydrobiopterin cycle, as excessive amount of biopterin production was observed in patients with diabetes or hypertension, without nephropathy, compared to healthy controls. Biopterin to creatinine concentrations in diabetic or hypertensive nephropathy patients were threefold higher than those in healthy subjects. It can be speculated that the role of oxidative stress in these pathologies has been confirmed by increased biopterin to creatinine concentrations. Furthermore, when patients with renal failure are also diabetic or hypertensive, their biopterin to creatinine concentrations were observed to be higher than those of their control counterparts. It is well known that if these two pathologies are not kept under control, the development of nephropathy is inevitable. Finally, it can be said that detection of biopterin excretion needs

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to be monitored in the progression of nephropathy in order to investigate its use in the severity of pathologies and also in preventing nephropathy in diabetic or hypertensive patients. Since its cost and applicability are cheaper and easier than the common biomarkers such as β-2-microglobulin, it can be suggested that biopterin will be a good candidate for investigating its use in early determination of the development of nephropathy. However, this needs to be supported by broad comparable studies.

Acknowledgement: This study was supported by the Atatürk University Scientific Research Unit (BAP-2007/223).

Received September 25, 2013; accepted October 22, 2013; previously published online November 19, 2013

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