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Urinary Neopterin in Patients with Diabetes Mellitus and Foot Ulcers

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Abstract

Increased urinary or serum concentrations of neopterin have been reported in disorders associated with systemic immune activation, e.g. viral infections, cancer, or autoimmune diseases, but the data on neopterin concentrations in patients with diabetes mellitus are limited. The aim of the present study was to investigate urinary neopterin concentrations in patients with diabetes mellitus and with or without diabetic foot. Urinary neopterin was determined by high-performance liquid chromatography in 51 patients with diabetes mellitus, including 19 patients with diabetic foot, and 18 healthy controls. Neopterin concentrations were significantly increased in patients with diabetes mellitus compared to controls (mean $\pm$ standard deviation; 221 ± 149 vs. 139 ± 66 $\mu\text{mol/mol}$ creatinine; Mann-Whitney U test, $p = 0.003$). Urinary neopterin concentrations in patients with or without diabetic foot were identical (224 ± 113 vs. 219 ± 169 $\mu\text{mol/mol}$ creatinine). No significant difference was also observed between patients with type 1 and type 2 diabetes mellitus, between patients treated with insulin compared to patients not treated with insulin, patients with or without diabetic retinopathy, with or without diabetic neuropathy, with or without diabetic nephropathy, with or without ischemic heart disease, with or without ischemic disease of lower extremities, and with or without ischemic cerebrovascular disease. A trend of higher neopterin concentrations was observed in patients with any complication of diabetes compared to no complications (234 ± 164 vs. 176 ± 73 $\mu\text{mol/mol}$ creatinine), and compared to controls, urinary neopterin was significantly increased only in patients with complications. No significant correlation was observed between urinary neopterin and glycosylated hemoglobin ($r_s = -0.14$, $p = 0.39$) or the duration of diabetes ($r_s = 0.25$, $p = 0.09$). In conclusion, present results demonstrate that urinary neopterin is increased in patients with diabetes mellitus. No differences were observed in urinary neopterin concentrations based on type of diabetes, therapy or presence of diabetic foot, and urinary neopterin did not correlate with the glycosylated hemoglobin levels or duration of diabetes. Increased urinary neopterin concentrations in patients with diabetes mellitus is associated with the presence of complications and may reflect systemic immune activation associated with atherosclerosis.

Key words: diabetes mellitus; diabetic foot; glycosylated hemoglobin; neopterin

Introduction

Increased urinary or serum neopterin concentrations has been reported originally in patients with malignant tumors and viral infections (1). Subsequently, increased neopterin concentrations were observed in different disorders associated with systemic immune activation, including patients with type 1 diabetes mellitus (2, 3).

Diabetes mellitus is one of the most prevalent chronic diseases. Most of the morbidity and mortality associated with diabetes is caused by late sequelae,

majority of which is linked to atherosclerosis. The term diabetic foot denotes a condition characterized by poorly healing ulcers of the lower extremity associated with vascular complications of diabetes, diabetic neuropathy and disordered leukocyte function (4). The information on urinary or serum neopterin concentrations in patients with diabetes mellitus and its complications is limited (2, 3).

The aim of the present study was to investigate in patients with diabetes mellitus and with or without diabetic foot.

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Patients and Methods

Fifty-one patients with diabetes mellitus, 32 males and 19 females, mean age ($\pm$ standard deviation) 56 ± 15 (range 22 - 83) years were included in the study. Fifteen patients had type 1 diabetes mellitus and 36 patients had type 2 diabetes mellitus. Thirty-one patients were treated with insulin and 20 patients were not treated with insulin. Nineteen patients had diabetic foot, while 32 patients were without this complication. Eighteen healthy subjects, 10 males and 8 females, aged 52 ± 15 (range 25 - 88) years served as control group.

Urine sample were collected at regular visits, and stored at -20°C until analysis. After centrifugation (5 minutes, 1300 g) and diluting 100 μl of urine specimens with 1.0 ml of mobile phase containing 2 g of disodium-EDTA per liter, samples were injected onto a column, and neopterin was determined using high performance liquid chromatography systems LC1084A with HP 79850 A integrator (Hewlett-Packard, Palo Alto, CA, USA) as described (5). Neopterin was identified by its native fluorescence (353 nm excitation, 438 nm emission) and quantified by external standard method. Creatinine was determined by Jaffé reaction after dilution of the sample 1:50 on Hitachi 716 analyzer (Tokyo, Japan) using a commercial kit (Boehringer, Mannheim, Germany) according to the manufacturer's instructions, and neopterin concentrations were expressed as neopterin/creatinine ratio ($\mu\text{mol/mol}$ creatinine).

Glycosylated hemoglobin was determined by high-performance liquid chromatography using Variant II Turbo System (Bio-Rad, Hercules, CA, USA) according to the instruction of the manufacturer and expressed according to the IFCC reference system (6).

Urinary neopterin concentrations of different groups of patients and controls were compared by Mann-Whitney U test. The correlations were examined with Spearman rank correlation coefficient. The decision on statistical significance was based on $p = 0.05$ level. The analyses were performed using NCSS software (Number Cruncher Statistical Systems, Kaysville, Utah, USA).

Results

Compared to controls, neopterin concentrations were significantly increased in patients with diabetes mellitus (mean $\pm$ standard deviation; 139 ± 66 , range 55 - 293 vs. 221 ± 149 , range 89 - 1038 $\mu\text{mol/mol}$ creatinine, $p = 0.003$). The increase of urinary neopterin concentrations was significant, but, in general, mild, and only 9 of the 51 (18 %) patients had urinary

neopterin above 271 $\mu\text{mol/mol}$ creatinine (mean $\pm$ 2 standard deviation of healthy controls).

Nineteen patients had diabetic foot while 32 patients did not have this complication. Urinary neopterin concentrations in patients with or without diabetic foot were almost identical (224 ± 113 , range 89 - 584 vs. 219 ± 169 , range 95 - 1038 $\mu\text{mol/mol}$ creatinine; Figure 1). No significant difference was also

Figure 1. Dot plot of urinary neopterin measurements in patients without diabetic foot (1), patients with diabetic foot (2), and healthy controls (3); horizontal lines indicate mean values.

observed between patients with type 1 and type 2 diabetes mellitus (230 ± 229 vs. 216 ± 104 $\mu\text{mol/mol}$ creatinine), between patients treated with insulin compared to patients not treated with insulin (237 ± 178 vs. 196 ± 88 $\mu\text{mol/mol}$ creatinine), patients with or without diabetic retinopathy (235 ± 191 vs. 214 ± 112 $\mu\text{mol/mol}$ creatinine), with or without diabetic neuropathy (205 ± 95 vs. 241 ± 198 $\mu\text{mol/mol}$ creatinine), with or without diabetic nephropathy (226 ± 83 vs. 221 ± 182 $\mu\text{mol/mol}$ creatinine), with or without ischemic heart disease (205 ± 90 vs. 232 ± 177 $\mu\text{mol/mol}$ creatinine), with or without ischemic disease of lower extremities (242 ± 123 vs. 213 ± 165 $\mu\text{mol/mol}$ creatinine), or with or without ischemic cerebrovascular disease (281 ± 173 vs. 215 ± 152 $\mu\text{mol/mol}$ creatinine). Only 11 patients had no sign of any complication of diabetes. While lower neopterin concentrations in patients without any complications compared to patients with complications (176 ± 73 vs. 234 ± 164 $\mu\text{mol/mol}$ creatinine) had not reached statistical significance, urinary neopterin concentrations were significantly increased compared to controls only in the latter

group.

In 41 patients, glycosylated hemoglobin was determined simultaneously to neopterin measurement. The mean glycosylated hemoglobin was 6.7 ± 2.3 % (range 2.0 - 13.2 %). No significant correlation was observed between urinary neopterin and glycosylated hemoglobin ($r_s = -0.14$, $p = 0.39$). The mean duration of diabetes was 11 ± 7 (range 1 - 30) years. No significant correlation was observed between urinary neopterin and the duration of diabetes ($r_s = 0.25$, $p = 0.09$).

Discussion

The present data demonstrate the presence of systemic immune activation, reflected in increased urinary neopterin concentrations, in patients with diabetes mellitus, irrespective of the type of diabetes or therapy. Originally, we have hypothesized that urinary neopterin concentrations will be increased in patients with diabetic foot compared to patients without this complication, but urinary neopterin concentrations in patients with or without diabetic foot were almost identical. No correlation was also observed between urinary neopterin and glycosylated hemoglobin indicating, in agreement with an earlier report (7), that long-term control of diabetes is not directly related to systemic immune activation.

Urinary neopterin was used in the present study to assess systemic immune activation. Neopterin is produced from guanosine triphosphate by macrophages stimulated with interferon- γ , and determination of neopterin concentrations may be used to monitor macrophage activity and systemic immune response (1). In earlier studies, urinary neopterin excretion was reported to be significantly increased in patients with different disorders, including solid tumors, hematologic malignancies, inflammatory bowel disease, autoimmune disorders, such as systemic lupus erythematosus (1), and also in diabetes mellitus (2, 3, 8). However, the data published so far on serum or urinary neopterin in patients with diabetes are limited. Manna *et al.* (2) investigated children and young adults with diabetes and found increased urinary neopterin in patients with newly diagnosed diabetes, while normal concentrations were observed in subjects more than 1 year after diagnosis. In another study from the same group, urinary neopterin was significantly increased in patients with newly diagnosed type 1 diabetes mellitus irrespective of the presence of islet cell antibodies, but in patients with chronic disease, urinary neopterin was increased only in patients positive for islet cell antibodies (3). Among 259 patients with critical limb ischemia, serum neopterin was significantly increased in diabetic patients, and among patients with diabetes,

significantly higher serum neopterin concentrations were observed in the presence of ulcer or gangrene (8). Bodlaj *et al.* (9) found in a study of patients with end-stage renal disease, no difference in serum neopterin in patients with or without diabetes. However, a marked increase of serum neopterin in both groups as a result of renal failure may have superseded any increase associated with diabetes. In another study, increased serum neopterin was found to be predictive of progression of diabetic nephropathy (10). Weitgasser *et al.* (7) reported in a cohort of 138 patients with type 1 diabetes a correlation between serum neopterin and duration of diabetes or serum cholesterol. A trend of higher neopterin was also observed in patients with diabetic retinopathy and microalbuminuria. Gottsater *et al.* (11) observed an increase in serum neopterin after the infusion of insulin.

In contrast to a previous report (7), no correlation was found between duration of diabetes and urinary neopterin concentrations. A mild, but significant increase of urinary neopterin concentrations in patients with diabetes mellitus was observed in the present study that was independent of the type, therapy or presence of foot complications. However, urinary neopterin concentrations above normal range, defined as mean plus 2 standard deviations of the control group, were observed only in a minority of patients, and in most patients urinary neopterin was only moderately increased. The patients were investigated in the chronic phase of diabetes, therefore no differences were observed in neopterin concentrations based on autoimmune etiology. The moderately increased urinary neopterin concentrations in patients with diabetes mellitus probably reflect systemic immune activation associated with atherosclerosis. In fact, atherosclerosis has been shown to be associated with increased neopterin levels in numerous studies (12 - 16), and systemic immune activation may play a crucial role in the progression of atherosclerosis (17). The findings of identical urinary neopterin concentrations in patients with or without diabetic foot are in contrast with a recently published report by Bertz *et al.* (8). However, neopterin was measured in that report in serum rather than urine, and differences in serum neopterin could be linked to differences in serum creatinine. In the present study patients also probably had less severe limb ischemia compared to the study reported by Bertz *et al.*, and most patients without diabetic foot in the present study had other complications of diabetes. Lower neopterin concentrations were observed in patients without any complication, although this difference was not significant, probably because of limited number of patients without complications. Moreover compared to controls, urinary neopterin was increased only in patients with complications, and not in patients with-

out any complication. Thus, present data suggest that increased urinary neopterin in patients with diabetes mellitus is associated with the presence of complications. Future studies should address the prognostic significance of increased urinary neopterin concentrations in patients with diabetes and correlation with other parameters of atherosclerosis. Because of few events, the prognostic significance of neopterin was not evaluable in the present cohort.

In conclusion, present results demonstrate that urinary neopterin is significantly increased in patients with diabetes mellitus. No differences were observed in urinary neopterin concentrations based on type of diabetes, therapy or presence of diabetic foot, and urinary neopterin did not correlate with the glycosylated hemoglobin levels. Increased urinary neopterin concentrations in patients with diabetes mellitus were associated with the presence of complications and may reflect systemic immune activation associated with atherosclerosis.

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