Detection of serum Cys C and Hcy as well as urine mindin and NAG contents in patients with diabetic nephropathy and the value for early diagnosis of disease

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ABSTRACT

Objective: To study the serum Cys C and Hcy as well as urine mindin and NAG contents in patients with diabetic nephropathy and the value for early diagnosis of disease. Methods: Patients with diabetic nephropathy and patients with diabetes alone were selected for study, serum was collected to detect Cys C, Hcy, PGF-2α, MDA, AOPP, SOD, GSH-Px and VitE contents, and urine was collected to detect mindin, NAG, MST1, JNK, Foxos, Caspase-3 and Caspase-12 contents. Results: mindin, NAG, MST1, JNK, Foxos, Caspase-3 and Caspase-12 contents in urine as well as Cys C, Hcy, PGF-2α, MDA and AOPP contents in serum of patients with diabetic nephropathy were significantly higher than those of patients with diabetes alone, and SOD, GSH-Px and VitE contents in serum were significantly lower than those of patients with diabetes alone; the higher the CKD stage, the higher the mindin, NAG, MST1, JNK, Foxos, Caspase-3 and Caspase-12 contents in urine as well as Cys C, Hcy, PGF-2α, MDA and AOPP contents in serum, and the lower the SOD, GSH-Px and VitE contents in serum; mindin and NAG contents in urine were positively correlated with MST1, JNK, Foxos, Caspase-3 and Caspase-12 contents; Cys C and Hcy contents in serum were positively correlated with PGF-2α, MDA and AOPP contents, and negatively correlated with SOD, GSH-Px and VitE contents. Conclusion: Cys C and Hcy in serum as well as mindin and NAG in urine of patients with diabetic nephropathy begin to increase from CKD1 stage, are closely related to cell apoptosis and oxidative stress injury, and help early diagnosis of the disease.

1. Introduction

Diabetic nephropathy (DN) is the most common microvascular complication of diabetes, its pathological features are glomerular capillary damage, tuberous sclerosis and mesangial proliferation, and clinical manifestations are persistent proteinuria and progressive renal damage. Diabetic nephropathy is the most common cause of end-stage renal disease and also the main cause of death in patients with diabetes. At present, the pathogenesis of diabetic nephropathy has not been fully elucidated, and early diagnosis of disease also needs unified indicators. Existing research believes that oxidative stress, cell apoptosis, inflammation and so on are all associated with the occurrence of diabetic nephropathy[1-3]. In the research, serum Cys C and Hcy as well as urine mindin and NAG were selected and analyzed, and the change of above four indicators in patients with diabetic nephropathy and the value for early diagnosis of disease were specifically assessed.

2. Case, materials and methods

2.1. Included cases

Patients with type 2 diabetes treated in our hospital from January 2012 to December 2015 were retrospectively analyzed and screened according to the medical records, 24 h urinary albumin excretion > 30 mg was used as the standard of diabetic nephropathy, and 30 cases of patients with diabetes alone and 80 cases of patients with diabetic nephropathy were selected for study. Patients with
diabetic nephropathy included 20 cases of CKD1 stage, 20 cases of CKD2 stage, 20 cases of CKD3 stage and 20 cases of CKD4 stage. Informed consent was obtained from all patients.

2.2. Research materials

Conventional experimental consumables were purchased from Axygen Company, enzyme-linked immunosorbent assay kits were purchased from Shanghai Xitang Company, xanthine oxidase, thiobarbituric acid and immunoturbidimetric assay kits were purchased from Nanjing Jiancheng Company, and fluorescence quantitative PCR kits were from Beijing Tiangen Company. Microplate reader was from Bio-tek Company, spectrophotometer was from Eppendorf Company and PCR apparatus was from Bio-rad Company.

2.3. Research methods

2.3.1. Sample collection

Sample collection was finished within 3 d after patients were admitted to hospital, collected urine is morning urine and 5 mL was collected and directly preserved in low-temperature freezer; collected blood was fasting venous blood, which was centrifuged to collect upper serum and preserve it in low-temperature freezer.

2.3.2. Indicator detection

Cys C, mindin, NAG and PGF-2 α were detected by enzyme-linked immunosorbent assay, SOD and GSH-Px were detected by xanthine oxidase, thiobarbituric acid and immunoturbidimetric assay kits were purchased from Nanjing Jiancheng Company, and fluorescence quantitative PCR kits were from Beijing Tiangen Company. Microplate reader was from Bio-tek Company, spectrophotometer was from Eppendorf Company and PCR apparatus was from Bio-rad Company.

2.4. Statistical processing

SPSS 16.0 software was used for data processing, comparison among groups was by variance analysis, and reference standard was \( P<0.05 \).

3. Results

3.1. mindin and NAG contents in urine

Analysis of mindin and NAG contents in urine of patients with diabetic nephropathy and patients with diabetes alone was as follows: mindin and NAG contents in urine of patients with CKD1 stage, CKD2 stage, CKD3 stage and CKD4 stage diabetic nephropathy were significantly higher than those of patients with diabetes alone; analysis of mindin and NAG contents in urine of patients with different CKD stage diabetic nephropathy was as follows: the higher the CKD stage, the higher the mindin and NAG contents in urine, shown in Table 1.

<table>
<thead>
<tr>
<th>Diabetic nephropathy</th>
<th>Mindin (μmol/L)</th>
<th>NAG (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKD1 stage</td>
<td>216.98±33.96</td>
<td>24.57±4.12</td>
</tr>
<tr>
<td>CKD2 stage</td>
<td>305.62±40.39</td>
<td>39.44±6.48</td>
</tr>
<tr>
<td>CKD3 stage</td>
<td>388.23±44.12</td>
<td>46.22±6.41</td>
</tr>
<tr>
<td>CKD4 stage</td>
<td>527.49±58.33</td>
<td>69.87±7.79</td>
</tr>
<tr>
<td>Diabetes alone</td>
<td>143.62±23.95</td>
<td>18.48±2.97</td>
</tr>
</tbody>
</table>

3.2. Cys C and Hcy contents in serum

Analysis of Cys C and Hcy contents in serum of patients with diabetic nephropathy and patients with diabetes alone was as follows: Cys C and Hcy contents in serum of patients with CKD1 stage, CKD2 stage, CKD3 stage and CKD4 stage diabetic nephropathy were significantly higher than those of patients with diabetes alone; analysis of Cys C and Hcy contents in serum of patients with different CKD stage diabetic nephropathy was as follows: the higher the CKD stage, the higher the Cys C and Hcy contents in serum, shown in Table 2.

<table>
<thead>
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<th>CysC (mg/L)</th>
<th>Hcy (μmol/L)</th>
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<tr>
<td>CKD1 stage</td>
<td>1.54±0.28</td>
<td>21.33±2.57</td>
</tr>
<tr>
<td>CKD2 stage</td>
<td>1.98±0.30</td>
<td>28.91±3.71</td>
</tr>
<tr>
<td>CKD3 stage</td>
<td>2.77±0.36</td>
<td>34.42±5.13</td>
</tr>
<tr>
<td>CKD4 stage</td>
<td>4.03±0.56</td>
<td>42.21±5.91</td>
</tr>
<tr>
<td>Diabetes alone</td>
<td>1.18±0.22</td>
<td>16.88±2.89</td>
</tr>
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</table>

3.3. mRNA contents of apoptotic cells in urine and their correlation with mindin and NAG contents

mRNA contents of MST1, JNK, Foxos, Caspase-3 and Caspase-12 in urine of patients with diabetic nephropathy were significantly higher than those of patients with diabetes alone, and the higher the CKD stage, the higher the mRNA contents of MST1, JNK, Foxos, Caspase-3 and Caspase-12 in urine. Analysis of the correlation between mindin, NAG contents in urine and mRNA contents of apoptotic molecules showed that NAG and mindin contents were positively correlated with MST1, JNK, Foxos, Caspase-3 and Caspase-12 contents, shown in Table 3.

<table>
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3.4. Serum oxidative stress indicators

PGF-2α, MDA and AOPP contents in serum of patients with diabetic nephropathy were significantly higher than those of patients with diabetes alone, and SOD, GSH-Px and VitE contents were significantly lower than those of patients with diabetes alone; the higher the CKD stage, the higher the PGF-2α, MDA and AOPP contents in serum, and the lower the SOD, GSH-Px and VitE contents. Analysis of the correlation between Cys C, Hcy contents in serum and oxidative stress indicators showed that Cys C and Hcy contents in serum were positively correlated with PGF-2α, MDA and AOPP contents, and negatively correlated with SOD, GSH-Px and VitE contents, shown in Table 4.
4. Discussion

Serum creatinine and blood urea nitrogen contents are commonly used indicators of clinical assessment of renal function, but because the kidney has its own compensation, there will be no significant change in the early renal damage, which is not conducive to early diagnosis of disease and the evaluation of disease. In the progression of diabetic nephropathy, there is change in glomerular filtration and renal tubular re-absorption function, it can cause the change in contents of a variety of molecules in the blood and urine, and the determination of related parameters in blood and urine can provide reference and basis for the early diagnosis and the evaluation of diabetic nephropathy. N-acetyl-β-D glucosaminidase (NAG) is a kind of lysosomal acid hydrolase mainly located in the proximal tubule, and in cases of renal tubular injury, NAG expression significantly increases and it enters into the urine[4,5]. Mindin is a kind of extracellular matrix protein, it is the pattern recognition molecule necessary to start the nonspecific immune inflammatory response and also the integrin ligand on the podocyte foot process and it can cause podocyte damage[6]. Excessive generation of Mindin in local renal glomerulus can cause kidney damage, and Mindin generated in the urine also increases accordingly. Analysis of the contents of above two molecules in urine in the research showed that NAG and mindin contents in urine of patients with diabetic nephropathy were significantly higher than those of patients with diabetes alone, and the higher the CKD stage, the higher the NAG and mindin contents in urine. It indicated that NAG and Mindin contents in urine began to change from CKD1 stage diabetic nephropathy and were conducive to early diagnosis of disease.

Homocysteine (Hcy) is a kind of amino acid with microvascular damage effect, and abnormal glycolipid metabolism in patients with diabetes can cause excessive generation of Hcy, which induces oxygen free radical generation and causes microvascular oxidative stress damage[7,8]. Cystatin C (CysC) has the effect of cysteine protease inhibitor, its content rises correspondingly with the increase of homocysteine level, and it can play the role of its own compensation to a certain extent and inhibit further increase of homocysteine level[9]. In addition, CysC also has the effect of inducing granulocyte chemotaxis, migration and infiltration, and can enhance inflammatory response and lead to glomerular microvascular damage[10]. CysC in circulating blood was all through glomerular filtration, and re-absorbed CysC in renal tubule is completely catabolized and will not re-enter the blood. Therefore, serum Hcy and CysC contents can accurately reflect the glomerular filtration function. Analysis of Hcy and CysC contents in serum of patients with diabetic nephropathy and patients with diabetes alone in the research showed that Hcy and CysC contents in serum of patients with diabetic nephropathy were significantly higher than those of patients with diabetes alone, and the higher the CKD stage, the higher the Hcy and CysC contents in serum. It indicated that Hcy and CysC contents in serum began to change from CKD1 stage of diabetic nephropathy and were conducive to early diagnosis of disease.

Increase of NAG and Mindin contents in urine are mainly associated with renal tubular damage, and cell apoptosis is a key link causing renal tubular damage. Apoptosis is programmed cell death mediated by a variety of signaling pathways and executed by a variety of apoptotic molecules[11]. Caspase protease is the most important family of proteins initiating and executing apoptosis, there are a total of 11 Caspase molecules known in human, and those associated with renal tubular damage include Caspase-3 and Caspase-12. Caspase molecule activation is regulated by multiple upstream signaling pathways, and excessive activation of the signaling pathway mediated by mammalian sterile 20-like kinase 1 (MST1) is closely related to renal tubular damage. After phosphorylation, MST1 can activate the downstream JNK pathway and, Foxos pathway, thus activating a variety of Caspase molecules and causing cell apoptosis[12]. Analysis of apoptosis-related molecule expression in urine in the research showed that mRNA contents of MST1, JNK, Foxos, Caspase-3 and Caspase-12 in urine of patients with diabetic nephropathy were significantly higher than those of...
patients with diabetes alone, and the higher the CKD stage, the higher the mRNA contents of MST1, JNK, Foxos, Caspase-3 and Caspase-12 in urine. Further analysis of the correlation between mindin, NAG contents in urine and mRNA contents of apoptotic molecules showed that NAG and mindin contents were positively correlated with MST1, JNK, Foxos, Caspase-3 and Caspase-12 contents. It indicated that excessive generation of NAG and mindin in urine was closely related to cell apoptosis.

Increased contents of homocysteine and cystatin C in the blood are mainly involved in the production of oxygen free radicals and activation of oxidative stress response. Damage caused by oxidative stress is directly related to impaired glomerular filtration function, matrix reconstruction, interstitial fibrosis and so on[13]. PGF-2 α will be produced when oxygen free radicals attack phospholipid composition in the glomerular capillary basement membrane, which results in increased glomerular permeability, thickened basement membrane and declined filtration function; lipid and protein composition in local cells will generate MDA and AOPP respectively by the effect of oxygen free radicals. SOD and GSH-Px are important antioxidant enzymes in the body that can neutralize the excessively generated oxygen free radicals[14]; vitamin E (VitE) is the intermediate product in the generation process of superoxide free radicals, which can remove oxygen free radicals through non-enzyme mechanism[15]. In the continuous generation process of oxygen free radicals, SOD, GSH-Px and VitE are constantly consumed, which is characterized by reduced serum antioxidant contents. Analysis of serum oxidative stress indicators in the research showed that PGF-2 α, MDA and AOPP contents in serum of patients with diabetic nephropathy were significantly higher than those of patients with diabetes alone and positively correlated with Cys C, Hcy contents in serum, and SOD, GSH-Px and VitE contents were significantly lower than those of patients with diabetes alone and negatively correlated with Cys C and Hcy contents in serum. It indicated that excessive generation of Cys C and Hcy in serum was closely related to the activation of oxidative stress response.

Based on above discussion, it can be concluded that Cys C and Hcy in serum as well as mindin and NAG in urine of patients with diabetic nephropathy begin to increase from CKD1 stage, are closely related to cell apoptosis and oxidative stress injury, and help early diagnosis of the disease.

References