Usefulness of Serum Hepatocyte Growth Factor for the Diagnosis of Amyloidosis

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Abstract

Objective The diagnosis of amyloidosis still relies on biopsy, but there has been a growing demand for the development of a specific noninvasive diagnostic technique. Hepatocyte growth factor (HGF) acts on a variety of epithelial cells in multiple ways and is predominantly produced by mesenchymal cells and macrophages. In the present study, we measured the serum HGF level in patients with amyloidosis and investigated its usefulness for the diagnosis of this disease.

Methods The subjects were 18 patients diagnosed as having amyloidosis by biopsy. We also measured serum HGF in 47 patients with chronic glomerulonephritis, 32 patients on hemodialysis, and 24 healthy volunteers. The serum HGF level was measured using an HGF ELISA kit.

Results The serum HGF level of patients with amyloidosis was significantly increased compared with that of healthy volunteers, patients with chronic glomerulonephritis, and hemodialysis patients (2.26±2.73 ng/ml versus 0.20±0.04 ng/ml, 0.23±0.08 ng/ml, and 0.18±0.07 ng/ml respectively, p<0.0001). There was no significant difference between amyloid light-chain and amyloid A amyloidosis, but the serum HGF level of amyloidosis patients who died within 1 year of measurement was significantly higher than that of patients who lived for more than 1 year (2.83±2.85 ng/ml versus 0.49±0.26 ng/ml, p<0.01).

Conclusions The serum HGF level was significantly elevated in both amyloid light-chain and amyloid A amyloidosis and was a very useful indicator of suspected amyloidosis as well as a potential prognostic indicator. The serum HGF level may become a useful indicator for diagnosing amyloidosis.

Key words: prognosis, kidney disease, rheumatoid arthritis

Introduction

Amyloidosis is a disorder characterized by deposition in the extracellular tissues of fibrils composed of various proteins, such as amyloid light chains (AL) in primary (AL) amyloidosis, amyloid A (AA) in secondary (AA) amyloidosis, and β2-microglobulin in dialysis-associated amyloidosis. These fibrils have a predominantly antiparallel β-pleated sheet configuration, and can be identified in biopsy specimens both by their characteristic appearance on electron microscopy, and by their ability to bind Congo red and thioflavine T (1). The diagnosis of amyloidosis still relies on biopsy and the pathological demonstration of typical deposits, with few other indicators available to suspect or support the diagnosis of this disease. Since biopsy is an invasive examination, a noninvasive and sensitive test for amyloidosis is needed (2, 3). The presence of a para-protein in serum or urine is useful in AL amyloidosis but it is also common in patients with multiple myeloma irrespective of the presence of amyloidosis.

Hepatocyte growth factor (HGF) was originally purified as a potent mitogen of primary cultured hepatocytes (4). HGF acts on a variety of epithelial cells in multiple ways, as an activator of mitogenesis, motogenesis, and morphogenesis (5-7). Both HGF and its receptor c-met exhibit cell type-specific expression, with HGF being predominantly produced by mesenchymal cells, whereas the c-met gene is expressed in epithelial cells. This pattern of mesenchymal sources adjacent to epithelial targets suggests that HGF is a prime candidate for mediating mesenchymal-epithelial interactions during the processes of embryogenesis, tissue repair, and organ regeneration (8). With respect to the kidney, HGF stimulates the proliferation of renal epithelial cells, and is a potent renotrophic factor involved in regeneration after acute renal failure (9-12). There have been no previous investigations of the relationship between amyloidosis and HGF. In the present study, we measured the serum HGF level in patients with amyloidosis and investigated its usefulness for making diagnosis of this disease.
Table 1. Clinical Characteristics and Serum HGF Levels of 18 Patients with Amyloidosis

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>Amyloid precursor</th>
<th>Serum HGF (ng/ml)</th>
<th>Plasma creatinine (mg/dl)</th>
<th>Proteinuria</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HS</td>
<td>48</td>
<td>F</td>
<td>AL (primary)</td>
<td>0.59</td>
<td>dialysis</td>
<td>3+</td>
<td>5 years, alive</td>
</tr>
<tr>
<td>2. NH</td>
<td>43</td>
<td>M</td>
<td>AL (primary)</td>
<td>5.58</td>
<td>1.0</td>
<td>4+</td>
<td>died after 8 months</td>
</tr>
<tr>
<td>3. MK</td>
<td>82</td>
<td>M</td>
<td>AL (primary)</td>
<td>0.49</td>
<td>1.2</td>
<td>4+</td>
<td>died after 3 months</td>
</tr>
<tr>
<td>4. SI</td>
<td>56</td>
<td>F</td>
<td>AL (primary)</td>
<td>0.15</td>
<td>1.4</td>
<td>4+</td>
<td>3 years, alive</td>
</tr>
<tr>
<td>5. HS</td>
<td>60</td>
<td>M</td>
<td>AL (primary)</td>
<td>1.65</td>
<td>0.7</td>
<td>3+</td>
<td>died after 8 months</td>
</tr>
<tr>
<td>6. WH</td>
<td>63</td>
<td>F</td>
<td>AL (primary)</td>
<td>5.42</td>
<td>0.4</td>
<td>2+</td>
<td>died after 7 months</td>
</tr>
<tr>
<td>7. SK</td>
<td>61</td>
<td>M</td>
<td>AL (primary)</td>
<td>0.72</td>
<td>3.1</td>
<td>3+</td>
<td>1 year, alive</td>
</tr>
<tr>
<td>8. TM</td>
<td>72</td>
<td>M</td>
<td>AL (primary)</td>
<td>0.67</td>
<td>dialysis</td>
<td>3+</td>
<td>died after 2 months</td>
</tr>
<tr>
<td>9. FA</td>
<td>79</td>
<td>F</td>
<td>AL (primary)</td>
<td>7.15</td>
<td>0.9</td>
<td>3+</td>
<td>3 months, alive</td>
</tr>
<tr>
<td>10. HA</td>
<td>50</td>
<td>M</td>
<td>AL (primary)</td>
<td>8.3</td>
<td>6.9</td>
<td>3+</td>
<td>died after 10 months</td>
</tr>
<tr>
<td>11. AT</td>
<td>65</td>
<td>F</td>
<td>AL (multiple myeloma)</td>
<td>0.43</td>
<td>dialysis</td>
<td>3+</td>
<td>3 years, alive</td>
</tr>
<tr>
<td>12. TM</td>
<td>65</td>
<td>F</td>
<td>AA (rheumatoid arthritis)</td>
<td>0.33</td>
<td>1.6</td>
<td>3+</td>
<td>1 year, alive</td>
</tr>
<tr>
<td>13. SY</td>
<td>53</td>
<td>F</td>
<td>AA (rheumatoid arthritis)</td>
<td>1.08</td>
<td>dialysis</td>
<td>3+</td>
<td>died after 5 months</td>
</tr>
<tr>
<td>14. KD</td>
<td>64</td>
<td>F</td>
<td>AA (rheumatoid arthritis)</td>
<td>0.79</td>
<td>dialysis</td>
<td>4+</td>
<td>died after 10 months</td>
</tr>
<tr>
<td>15. SS</td>
<td>61</td>
<td>F</td>
<td>AA (rheumatoid arthritis)</td>
<td>0.55</td>
<td>dialysis</td>
<td>1+</td>
<td>1 year, alive</td>
</tr>
<tr>
<td>16. FT</td>
<td>61</td>
<td>F</td>
<td>AA (rheumatoid arthritis)</td>
<td>0.30</td>
<td>1.4</td>
<td>4+</td>
<td>2 years, alive</td>
</tr>
<tr>
<td>17. HY</td>
<td>68</td>
<td>F</td>
<td>AA (rheumatoid arthritis)</td>
<td>0.37</td>
<td>2.4</td>
<td>3 months, alive</td>
<td></td>
</tr>
<tr>
<td>18. YK</td>
<td>58</td>
<td>F</td>
<td>AA (rheumatoid arthritis)</td>
<td>0.90</td>
<td>dialysis</td>
<td>3+</td>
<td>3 years, alive</td>
</tr>
</tbody>
</table>

AL: amyloid light-chain, AA: amyloid A.

Subjects and Methods

The subjects were 18 patients who were diagnosed as having amyloidosis by biopsy of the kidney or gastrointestinal tract. Their clinical characteristics are shown Table 1. Amyloidosis was diagnosed based on positive Congo red or Dylon staining. The differential diagnosis of AL and AA amyloidosis was done using anti-human lambda light-chain antibody and anti-amyloid A protein or the clinical features. All patients had proteinuria and/or renal dysfunction, but did not have serious infection, acute renal failure, or severe liver dysfunction. Since all patients had obvious renal dysfunction, we also measured the serum HGF level in 47 patients with chronic glomerulonephritis (CGN), 32 patients on hemodialysis, and 24 healthy volunteers. All of the dialysis patients had a short duration of hemodialysis (less than 10 years) and the cause of renal failure was primary glomerulonephritis.

The serum HGF level was measured using an HGF ELISA kit (Otsuka Assay Co., Ltd., Tokyo), which is widely employed for the diagnosis of fulminant hepatitis in Japan. This kit has an antihuman HGF monoclonal antibody as the solid phase and an antihuman HGF rabbit polyclonal antibody as the liquid phase. The monoclonal antibody only reacts with a disulfide-linked heterodimer of the α- and β-chains. The reference serum HGF level for this kit was 0.19±0.05 ng/ml and the serum HGF level that we found in our 24 healthy volunteers was 0.20±0.04 ng/ml. This was the same as had been obtained using the kit in fundamental studies. The detection limit of the kit was 0.1 ng/ml. To confirm the reproducibility of serum HGF data, blood samples were collected at different days from some of the patients with amyloidosis, and the reproducibility of measurement was shown to be good (Table 2).

Data were subjected to statistical analysis using analysis of variance and further analysis was performed using Fisher's protected least significant difference test. We also used Mann-Whitney U test to compare two groups. Results were expressed as the mean±SD and the level of significance was set at <0.05.

Results

Figure 1 shows the serum HGF levels in the healthy volunteers, CGN patients, hemodialysis patients, and the patients with amyloidosis. The CGN patients were divided into two groups according to their serum creatinine levels. In patients with amyloidosis, serum HGF was significantly increased compared with the level in healthy volunteers, patients with mild CGN (serum creatinine level <3.0 mg/ml), patients with severe CGN (serum creatinine level ≥3.0 mg/ml), and hemodi-
HGF for Diagnosing Amyloidosis

Figure 1. The serum HGF levels of healthy volunteers, CGN patients, hemodialysis patients, and patients with amyloidosis.

Figure 2. Serum HGF level in patients with AL amyloidosis or AA amyloidosis.

Figure 3. Serum HGF level and prognosis in patients with amyloidosis.

Discussion

It is difficult to diagnose amyloidosis because there are few specific symptoms. AL amyloidosis should be considered in the differential diagnosis of all patients over the age of 40 who have otherwise unexplained marked severe proteinuria (13, 14). In patients with rheumatoid arthritis, marked severe proteinuria can be induced by membranous nephritis due to gold, penicil-
are only diagnosed at autopsy. Treatment of this disease is often difficult, because there are few specific therapies available. However, it has been reported that the prognosis of amyloidosis can be improved by appropriate early treatment, for example using melphalan (15), colchicine (15, 16), and 4'-iodo-4'-deoxydoxorubicin (17). Thus, there has been a growing demand for the development of a specific noninvasive diagnostic technique.

The present study showed that the serum HGF level was significantly elevated in both AL and AA amyloidosis patients irrespective of the precursor protein, and HGF was shown to be a very useful indicator for suspecting amyloidosis. The mean HGF level was 10-fold higher compared with that in healthy controls. Some previous studies have detected an increased serum HGF level in renal diseases (18–20). In CGN patients, the serum HGF level was shown to be high compared with that of healthy controls, but the difference was small and not significant. In patients with rheumatoid arthritis, it was reported that the HGF level in synovial fluid was significantly increased, while serum HGF was not high (21, 22). In patients with multiple myeloma, it was reported that the HGF level at the time of diagnosis was significantly high, while serum HGF decreased by the treatment to nearly the normal level (23). Our patient with multiple myeloma (case 11) was well controlled by the treatment. The elevation of the mean serum HGF level in our patients with amyloidosis was extreme and was unlike that seen in other diseases. Even when the normal range was set at 0.39 ng/ml (mean ±4SD), the sensitivity of this examination was still 78% and the specificity was 100%. In addition, the serum HGF level may become a prognostic indicator. The serum level in patients who died within 1 year was significantly higher than that in patients who had a longer survival. Although this was a limited study, we clearly showed that the serum HGF level is often abnormally high in patients with amyloidosis.

The measurement of serum HGF must be reproducible if HGF is to be used for diagnosing amyloidosis; in the present study the measurements were shown to be highly reproducible when investigating some amyloidosis patients.

There have been no previous reports on the relationship between HGF and amyloidosis. HGF is mainly produced by mesenchymal cells and macrophages in response to strong stimulation by IL-1β and TNF-α (24). Infection is one of the important cause that stimulates the production of IL-1β and TNF-α. Serum HGF levels have been reported to increase in acute infection (25), but the extent of the increase is less than in amyloidosis; most of our patients had no obvious infection. The present study showed that the serum HGF level was elevated irrespective of the amyloid precursor protein in the patients with AL or AA amyloidosis. These results suggested that HGF may increase as a result of extracellular amyloid deposition leading to the stimulation of macrophages and mesenchymal cells and the elevation of serum HGF may be correlated with the amount or extension of amyloid. There is also a possibility that HGF is concerned with the pathogenesis of amyloid deposition. The relationship between amyloidosis and HGF is still unclear. However, it appears useful to measure serum HGF as an indicator for the diagnosis of amyloidosis and may be a prognostic indicator.

References

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