AA Amyloid Quantification in Biopsy Samples from the Stomach

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Abstract. Amyloidosis is usually diagnosed through the histological examination of biopsy samples. However, its quantitative evaluation can be difficult. In this study, we immunochemically measured amyloid A (AA) proteins in biopsy samples taken from the stomachs of patients with AA amyloidosis. Samples were treated with guanidine and were subjected to an enzyme immunoassay for serum amyloid A. The results were compared with histological findings. All patients who tested negative for amyloid deposits had low values that clearly distinguished them from amyloid-positive patients. Among the amyloid-positive patients, the AA values correlated significantly with histological findings. This method may be useful for the quantitative evaluation of AA amyloidosis.

Key words: amyloidosis, biopsy, AA protein, quantification

Introduction

Systemic amyloidosis is a condition in which insoluble fibrillar aggregates accumulate in the extracellular spaces of organs [1]. The disease has long been believed to be progressive and difficult to treat. Recently, however, approaches effective at reducing amyloid deposits have been developed. Here, the focus was on AA amyloidosis, which involves chronic inflammatory disorders such as rheumatoid arthritis (RA).Degradation products of the acute phase reactant serum amyloid A (SAA) constitute amyloid deposits. Suppression of the synthesis of SAA by anti-inflammatory regimens in amyloidosis patients was shown to reduce the number of amyloid deposits [2]. Therefore, it is necessary to establish methods for evaluating the kinetics of amyloid deposition.

In the study mentioned above, the load of amyloid was evaluated by scintigraphy utilizing a labeled serum amyloid P component that has a high affinity for all amyloid deposits [3]. This method is useful for evaluating the amyloid load in a whole body, but has not been widely adopted because of commercial unavailability. Consequently, amyloid deposits are usually examined histologically in biopsy samples. The gastroduodenum is an appropriate site for examination [4]. However, histological evaluation has some disadvantages. For example, amounts of amyloid deposits can vary among sections even from small tissues. A quantitative evaluation of amyloid deposits is often difficult.

In this study, we quantified AA proteins in gastric biopsy samples and found that the method complements histological examinations.

Materials and Methods

Patients. 36 rheumatoid arthritis (RA) patients participated in this study. RA patients undergoing a gastroduodenal endoscopy in Dohgo Spa Hospital were investigated. There were 34 females and 2 males, aged 58 to 84 years (mean: 64.4). RA was diagnosed according to the American College of Rheumatology’s 1987 criteria [5]. The duration of RA ranged from 3 to 52 years (mean: 15.1). Among the patients, 14 received a biopsy for the first time to screen for amyloidosis (screening group). The other 22 had already been diagnosed with AA amyloidosis and received a biopsy to evaluate the disease activity (follow-up group). The study protocol...
was approved by the ethics committee of Jichi Medical University.

**Evaluation of amyloid load.** Two samples were obtained from the antrum of the stomach. One was treated with 10% formalin and subjected to histological examination including Congo red staining. The other was kept frozen at -30 °C until further analysis. Congo red staining was graded from 0 to 3 (amyloid score) based on the area of amyloid deposits.

The frozen materials were weighed and rinsed briefly in saline to avoid contamination. Those were then sonicated for 1 min in 4M guanidine-HCl, 0.05M Tris, pH 8.5 and left overnight at room temperature. After a brief spin, the supernatant was diluted more than 1:100 in phosphate-buffered saline containing 1% bovine serum albumin and subjected to a sandwich enzyme immunoassay for SAA as described previously [6]. The results are shown as ng of AA protein (equivalent to SAA) per mg of tissue weight.

To confirm that the biopsy samples contained degraded SAA (AAs) rather than intact, some were randomly assigned to immunoblot analyses according to previously described procedures [6].

The relationship between quantified AAs and amyloid scores is shown in **Figure 2**. Score 0 was clearly distinguishable from the positive scores. AA values in positive patients increased with the score, but not all differences were statistically significant. All 13 cases in the screening group tested negative in the histological evaluation and had AA concentrations below 10ng/mg of tissue, clearly lower than those of the positive patients (Figure 2). There was an overall significant correlation between histological scores and AA quantification (r=0.829; p<0.0001).

**Results**

Immunoblot analyses of the biopsy samples showed that the main immunoreactive bands were the AA76 monomer (the amino terminal 76 residues of SAA and the most common form of AAs in the deposits) and its dimer (Figure 1). Polymerized forms of AA, intact SAA, and AAs smaller than AA76 were also observed in smaller quantities. Therefore, the method mostly detects AA proteins from the deposits.

**Discussion**

Removal of SAA derived from the circulation by rinsing the samples with saline can affect the quantification of AA. However, the washing out of saline–soluble fractions by sonication and spinning before guanidine extraction resulted in the loss of a considerable amount of amyloid-derived AAs (unpublished observation). Therefore, the present method utilized a brief spinning prior to guanidine extraction.
A faint band corresponding to intact SAA was observed on the immunoblot. ELISA detected AAs in amyloid-negative materials, but at a very low level (below 10 ng/mg of tissue). This may be due to the minimal contamination of plasma, the involvement of intact SAA during fibril formation in amyloidotic materials, or SAA expressed normally in extra-hepatic sites [7]. In any case, invalid results of AA values did not exceed 10 ng/mg of tissue as justified by the results from amyloid negative samples.

Immunoblotting demonstrated the presence of the most common form of AA, AA76, in the amyloid deposits. According to our own experience, AA76 can be detected only in solid tissue amyloid materials, and not in any of non-amyloid material. Thus, the presence of AA76 may confirm the diagnosis of amyloidosis.

Although this is the first report of AA being quantified in biopsy samples from the stomach, abdominal fat taken by needle aspiration has also been used to quantify AA [8]. In that report, the amount of AA was successfully measured, but several samples positive by Congo red staining showed no significant increase in AA values. Our method did not miss samples found to be positive by histological examination. Using 10 ng/mg of tissue of AA as cut-off value, sensitivity and specificity was 100% and 76.5%, respectively, based on the results of histological findings. However, 4 samples showing AA values above the cut-off in samples with histology score 0 were from patients with already diagnosed amyloidosis. Two samples showing low values (13.4 and 14.1 ng/mg of tissue) may be from patients whose amyloid loads were significantly reduced but still remained at low levels. In the other two samples showing clearly elevated values (82 and 850 ng/mg of tissue), it is likely that sections cut for histology might not happen to contain amyloid deposits.

The absence of significant differences in AA values between amyloid positive scores may have resulted from the small number of subjects studied and/or a variation in the quantity of amyloid deposits among the sections. In the latter case, quantification of AA may supplement histological examinations. The overall significantly positive correlation between histological scores and AA quantification underscores the utility of our method. Importantly, negative results of AA quantification can rule out amyloidosis, based on our work. In addition, quantities or changes in amyloid deposits may be evaluated more accurately.

In conclusion, the quantification of AA in biopsy samples is useful for the screening of AA amyloidosis and can be used for follow-up of the disease.
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