Prevalence of dialysis-related amyloidosis in diabetic patients

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Abstract It has recently been shown that $\beta_2$-microglobulin isolated from amyloid deposits in dialysis patients is modified by advanced glycation (AGE). In this context it appeared of interest to examine in a cross-sectional multicentre study whether dialysis-related amyloidosis, as evaluated by X-ray assessment of cysts in the metacarpal bones, was different in diabetic patients on maintenance haemodialysis for more than 5 years time compared with matched non-diabetic controls. We evaluated the hand skeleton of 75 diabetic patients (9 type I, 66 type II; 35 male, 40 female; median age 64 years, range 31-86; median duration of dialysis 7 years, range 5-17). They were compared with 150 patients without diabetes mellitus who were matched for age, gender and duration of dialysis. Hand X-rays were centrally evaluated by one radiologist unaware of the underlying clinical diagnosis. The overall frequency of amyloid cysts was 9/75 (12%) in diabetic patients (95% confidence interval 4.6-19.3%) and 28/150 (19%) in matched controls (95% confidence interval 12.4-24.9%). The results indicate that diabetes mellitus does not confer an increased risk of dialysis-related amyloid cysts. The results are of interest with respect to the mechanism of amyloid formation.

Key words: $\beta_2$-microglobulin amyloidosis; uraemia; haemodialysis; dialysis-related amyloidosis; glycated proteins; advanced glycation

Introduction

Formation of dialysis-related amyloid, secondary to precipitation of modified $\beta_2$-microglobulin ($\beta_2$-M) molecules in osteoarticular tissue, is one of the major unresolved problems of long-term haemodialysis treatment. Recently Miyata et al. [1] demonstrated that $\beta_2$-M isolated from amyloid deposits was modified by AGE (advanced glycation end product) transformation. Such AGE-modified material had potent effects with respect to induction of human monocyte chemotaxis and macrophage secretion of TNF$\alpha$ and IL-1 [2]. The issue has been raised [3] whether AGE transformation occurred in situ after precipitation of insoluble $\beta_2$-M amyloid fibrils had occurred or whether AGE transformation was a precondition for amyloidogenesis to occur. Recently, AGE-transformed $\beta_2$-M molecules have been shown in the circulation [4], but clearcut evidence for precipitation of such transformed $\beta_2$-M material as amyloid fibrils has not been provided.

Nevertheless it appeared to be of interest to examine whether amyloidosis was more frequent in patients with diabetes mellitus. The rationale for this consideration was the observation of Makita et al. [5] that AGE are particularly elevated in diabetic patients with terminal renal failure, although elevation was also noted in non-diabetic patients. The clinical impression is that dialysis-related amyloidosis is infrequent in diabetic patients. The issue is confounded, however, by the known short life expectancy of diabetic patients on maintenance haemodialysis [6]. According to van Ypersele [7] the cumulative risk of amyloidosis increases dramatically with duration of dialysis, beginning approximately after the fifth year of maintenance haemodialysis. Advanced age is an important factor as well [7-9], and constitutes an independent risk factor when assessed by multivariate analysis [7].

To collect a sufficient number of elderly diabetic patients with more than 5 years duration of maintenance haemodialysis, we conducted a multicentre study in 39 German dialysis units. Diabetic patients were compared with age and gender-matched non-diabetic patients in the respective centres who had been dialysed for a comparable number of months. The end point...
of the study was the frequency of X-ray evidence of metacarpal cysts which were diagnosed by one examiner who was unaware of the underlying diagnosis.

Patients and methods

Patients

In 39 German dialysis centres, 75 diabetic patients were included who had been on dialysis for more than 5 years. They had been dialysed throughout this time with cuprophane membranes only, with the exception of one centre, which provided nine diabetic and 18 control patients who had been on high flux polysulfone membranes for the whole time. According to the National Diabetes Data Group criteria [10] nine patients were of type I and 66 of type II. Of the type II diabetic patients, 29 patients were on insulin, 11 were on oral hypoglycaemic agents and 26 were on dietary treatment alone.

In each centre each diabetic patient was matched with two non-diabetic control patients who had comparable age, gender and duration of dialysis. The underlying diseases were glomerulonephritis (n=32), chronic 'pyelonephritis' (n=22), analgesic nephropathy (n=16), polycystic kidney disease (n=14), sundry diagnoses (n=14) and unidentified primary cause (n=52). Residual diuresis was present in 19 out of 75 diabetic patients (median 200 ml/day, range 50–1000) and 30 out of 150 controls (median 250 ml/day, range 20–1000).

The relevant demographic and biochemical data are given in Table 1.

The presence of clinical signs or symptoms diagnosed as evidence of the carpal tunnel syndrome were evaluated from patient records.

Data for the patients were collected by one of two investigators (H.L. or C.J.). X-rays of the hand skeleton were taken using standard techniques. X-rays were evaluated centrally by one radiologist (I.M.) who was unaware of the clinical diagnoses.

Table 1. Demographic and biochemical data (median and range)

<table>
<thead>
<tr>
<th></th>
<th>Diabetes (n = 75)</th>
<th>Control (n = 150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 (31–86)</td>
<td>63 (31–84)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>35/40</td>
<td>70/80</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>16 (4–43)</td>
<td>–</td>
</tr>
<tr>
<td>Predialytic blood glucose (mg/dl)</td>
<td>145 (70–502)</td>
<td>–</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 (15.9–31.8)</td>
<td>22.8 (14.4–33.9)</td>
</tr>
<tr>
<td>Predialytic serum creatinine (mg/dl)</td>
<td>8.8 (4.2–11.7)</td>
<td>9.6 (4.0–15.3)</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.51 (1.07–1.69)</td>
<td>1.56 (1.04–1.9)</td>
</tr>
<tr>
<td>1,84-PTH (pmol/l)</td>
<td>21.6 (1.6–117)</td>
<td>20.8 (1.1–408)</td>
</tr>
<tr>
<td>Alkaline phosphatase (U)</td>
<td>132 (70–1634)</td>
<td>137 (57–268)</td>
</tr>
<tr>
<td>Serum Ca (mmol/l)</td>
<td>2.38 (2.1–2.82)</td>
<td>2.41</td>
</tr>
<tr>
<td>β2-M (mg/l)</td>
<td>27.8 (8.6–60.3)</td>
<td>31.5 (9.4–74.8)</td>
</tr>
<tr>
<td>Duration of dialysis</td>
<td>7 (5–17)</td>
<td>7 (5–18)</td>
</tr>
<tr>
<td>Weekly duration of dialysis (h)</td>
<td>12 (9–15)</td>
<td>12 (9–18)</td>
</tr>
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</table>

Study design

The study was designed as an unbalanced comparison of one diabetic patient with two matched non-diabetic controls. The primary predefined end point of the study was the presence of metacarpal cysts characterized according to van Ypersele [7] as bone defects of the wrist with a diameter of at least 5 mm with a normal joint space adjacent to the subchondral bone defect so as to exclude subchondral bone cysts of osteoarthritic origin.

Ancillary measurements

1,84-PTH was measured with the Nichols assay. β2-M concentrations in predialytic samples were measured using the LIA-mat® β2-microglobulin test [11].

Statistical design

Values are given as median and range. A frequency of metacarpal cysts of 20% was assumed in the control population. The study was designed to have 80% power to detect a 38% frequency in the group of diabetic patients (Mantel–Haenszel test).

Results

Characteristics of the patient population

These long-term haemodialysis patients had only exceptionally some minimal diuresis. They were reasonably well dialysed according to predialytic serum creatinine and Kt/V values. On average, predialytic blood glucose at the time of the study was reasonably controlled. Severe hyperparathyroidism, defined as 5-fold elevation of 1,84-PTH, i.e. > pmol/l, was seen in 21 out of 75 (28%) of the diabetic and 53 out of 150 (35%) of the non-diabetic patients. Two of the diabetic and eight of the non-diabetic patients with 5-fold elevation of 1,84-PTH had metacarpal cysts. Advanced osteitis fibrosa defined by acroosteolysis of the terminal phalanx and/or subperiosteal resorption zones on the radial aspect of the middle phalanx was seen in only four non-diabetic patients, however. In patients with metacarpal cysts, 1,84-PTH concentrations were not significantly different from those without cysts.

Frequency of metacarpal cysts

The primary study end point was X-ray evidence of metacarpal cysts. One or more metacarpal cysts were seen in nine out of 75 diabetic patients (12%; 95% confidence interval 4.6–19.3%), compared to 28 out of 150 non-diabetic controls (19%; 12.4–24.9%). Two of the 32 diabetic patients below age 60 (6.3%) and seven of 43 patients above age 60 (16.3%) had metacarpal cysts. The respective figures in the non-diabetic controls were five out of 64 (7.8%) in patients below age 60 and 23 out of 86 (26.7%) in patients above age 60. Figure 1 gives the cumulative frequency of metacarpal cysts as a function of duration of dialysis. The
frequency clearly increased with duration of dialysis in patients with diabetes as well as in non-diabetic controls.

The preferential distribution of the cysts was similar in diabetic patients and matched controls: lunate bone, five of all 16 cysts combined in the diabetes group (31%) vs 17 of all 48 cysts combined in the control group (35%); capitate bone, four of 16 (25%) vs six of 48 (13%); scaphoid bone, seven of 16 (44%) vs 21 of 48 (44%); others (hamate bone, triquetral bone), none of 16 vs four of 48 (8%). The maximal diameter of the cyst varied between 5 and 9 mm in diabetic and 5 and 10 mm in non-diabetic patients.

The presence of the carpal tunnel syndrome was not a predefined end point of the study. Clinical signs and symptoms of carpal tunnel syndrome were diagnosed by the physicians in charge in 10 out of 75 of the diabetic patients (13.3%) and 22 out of 150 of the non-diabetic controls (14.7%). Only two of the 10 diabetic and four of the 22 non-diabetic patients with carpal tunnel syndrome had metacarpal cysts by X-ray. This observation illustrates the poor sensitivity and specificity of the clinical diagnosis unless it is based on objective criteria and measurements. Patients with metacarpal cysts did not differ with respect to 1,84-iPTH concentrations and predialytic β2-M concentrations from patients without metacarpal bone cysts (data not given).

Discussion

The primary end point of the study was the frequency of metacarpal cysts in diabetic patients compared to matched non-diabetic dialysis patients. We found no excess frequency of metacarpal cysts that fulfilled the criteria established for cysts related to β2-M amyloidosis [7]. If anything, the frequency in diabetic patients was less, but the difference was not statistically significant, i.e. the frequency in diabetic patients was still within the 95% confidence interval of controls. Beta error analysis indicated that the study had sufficient power to detect a clinically relevant increase in frequency.

The study design excluded patients on dialysis for less than 5 years, since it is known that cysts are infrequent before the fifth year of dialysis. Since only diabetic patients with dialysis for more than 5 years were examined and compared to matched controls, the problem of high early attrition of diabetic patients [6] was circumvented. We cannot exclude the unlikely possibility that diabetic patients with worse glycemic control and at particularly high risk of β2-M amyloidosis selectively died off during the first 5 years of dialysis. With the exception of one centre, all patients had been dialysed using cellulosic membranes. This point is of importance, since lower cumulative frequency of metacarpal bone cysts was noted in patients dialysed on AN-69 membranes [7]. The overall conclusion of the study is not modified, however, if the patients from this one centre are excluded from analysis. The frequency of advanced hyperparathyroidism was notable. It is unlikely, however, that this caused misinterpretation of bone cysts, since only four non-diabetic patients had X-ray signs of osteitis fibrosa in the hand skeleton. Although the X-ray analysis was designed to be blind, blinding was incomplete to some extent, because dialysed diabetic patients could be identified from massive vascular calcification. The visibility of cysts is dependent, amongst other factors, on skeletal mineral content. We did not measure bone mineral density, but the prevalence of higher grades of demineralization, as qualitatively assessed from hand skeleton X-ray, was comparable in the two patient groups.

What are the potential implications of the results?

If diabetic patients had shown higher cumulative frequency of metacarpal bone cysts than non-diabetic patients, the study result would have provided a strong argument for a role of AGE-transformed β2-M in the genesis of amyloid fibrils. We emphasize that the above negative result does not exclude a pathogenetic role of AGE-transformed β2-M. The results of this study would be in line with the recent concept that more marked AGE transformation of proteins in renal failure is primarily a function of impaired renal excretion of products of the catabolism of AGE-modified proteins with accumulation of AGE peptides [5,12,13], and the role of glycaemia may be quantitatively less important than that of accumulation of AGE peptides. The presence of similar concentrations [14] of early [15] and late cross-linking [16] products of the AGE pathway in non-diabetic dialysis patients provides further evidence in this direction. Alternative explanations are not excluded, however. For instance, amyloidogenesis is influenced by a variety of predisposing or permissive factors [17]. It appears that local modifying factors, for instance glycosaminoglycans, activation of synoviocytes and macrophages with local generation of cytokines etc., play a major role. It is conceivable that diabetes mellitus interferes with such local factors and retards the development of amyloid-related lesions despite the (presumed) high concentrations of AGE-transformed β2-M molecules in the circulation [4].
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