Dialysis-related amyloidosis: visceral involvement and protein constituents

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Abstract. β2-M amyloidosis mainly concerns dialysis patients and typically presents with osteoarticular symptoms. In order to precise the incidence and gravity of visceral involvement, subcutaneous abdominal fat aspirates, skin and rectal biopsies, as well as echocardiograms were performed in 26 patients with severe β2-M amyloidosis. Visceral amyloidosis was confirmed in 58% and the numbers were even higher when including heart abnormalities suggestive of amyloidosis (81%). Clinical manifestations of visceral involvement were usually not severe and include odynophagia, gastrointestinal haemorrhage, intestinal obstruction, kidney stones, myocardial dysfunction and subcutaneous tumours. The removal and synthesis rates of β2-M were assessed during dialysis. Serum 131I-β2-M levels decreased by 5-10% with cuprophane and by 40-45% with polysulfone and polyacrylonitrile membranes. These reduction rates were higher than those found with unlabelled β2-M suggesting an increased synthesis or release during dialysis. The protein constituents of amyloid deposits were studied. Two different preparative methods to extract the proteins from amyloid deposits were used. TCA precipitation showed the presence of several proteins which were not observed with PBS homogenizing and resuspending in guanidine. The protein constituents of amyloid fibrils were studied by both, two dimensional gel electrophoresis (2D-gel) as well as protein sequencing after gel filtration. Similarly, the technical approach used for protein analysis greatly influenced the results. It was observed that 2D-gel displayed the presence of proteins which were missed by the gel filtration technique. Some of the proteins contained in amyloid deposits in addition to β2-M, were identified as globin chains, κ and λ light chains of immunoglobulins, and α2 macroglobulin. A putative participation of these other protein constituents on the pathogenesis of β2-microglobulin amyloidosis is discussed.

Key words: β2-microglobulin; dialysis related amyloidosis; protein constituents; visceral involvement

Introduction

Dialysis amyloidosis or β2-microglobulin (β2-M) amyloidosis, first described by the Tassin group [1], has become recognized as a frequent chronic complication and a new variety of systemic amyloidosis in long-term dialysis patients [2,3]. The incidence increases progressively with years on dialysis, reaching the majority of patients dialysed over 15 years [4]. The main clinical manifestations are rheumatological disorders including carpal tunnel syndrome, chronic synovitis, progressive bone destruction and pathological bone fractures, however systemic and visceral involvement have also been reported [5-7]. In 1985, β2-M was identified as the major constituent protein of this unique type of systemic amyloidosis [8,9]. β2-M has clearly been identified as playing a central role as the amyloidogenic protein and it has been shown that amyloid fibrils usually consist of intact molecules of β2-M [10]. However, the specific pathogenesis of β2-M amyloidosis remains unknown and it is believed that factors other than β2-M participate in the genesis of amyloid deposits [11]. In the present report we review the clinical characteristics of β2-M amyloidosis focusing on its visceral involvement and the pathogenic factors related to β2-M as well as the other protein constituents of amyloid deposits.

Clinical presentation

β2-M amyloidosis is predominantly localized to osteoarticular tissues, however systemic deposition and visceral organ involvement have been described with increasing frequency [5,12,13]. β2-M amyloid deposits have been found in the gastrointestinal tract, liver, heart, prostate, kidneys, endocrine glands and subcutaneous tissue. The visceral β2-M amyloid deposits are...
usually perivascular, of limited size and appear late in the evolution of the disease; in contrast, the articular and para-articular deposits are interstitial, widespread in deposition, and usually represent early manifestations of $\beta_2$-M amyloidosis. In order to investigate the extent of $\beta_2$-M amyloidosis, we studied a group of 26 dialysis patients affected with severe forms of $\beta_2$-M amyloidosis, performing in most of the patients subcutaneous abdominal fat aspirates, skin and rectal biopsies, echocardiogram and histological examination of surgical pieces. Histological confirmation of amyloid visceral involvement was demonstrated in 15 (58%) of the 26 patients studied, and when echocardiogram was considered the positive percentage increased to 81%. From this study we concluded that $\beta_2$-M amyloidosis has a systemic character and visceral involvement is frequently present [14].

The visceral $\beta_2$-M amyloid deposits do not usually cause organ failure, although clinical manifestations are being described with increasing frequency. Some clinical manifestations recently reported for the visceral involvement of $\beta_2$-M amyloidosis include amyloid deposits in the tongue responsible for odynophagia, interstitial as well as vascular deposits in the intestine responsible for gastrointestinal haemorrhage and/or intestinal obstruction, kidney stones formed from $\beta_2$-M amyloid substance, myocardial dysfunction secondary to amyloid deposition in the heart, spontaneous rupture of the tendons (Achilles and quadriceps) and subcutaneous tumours in the buttocks, elbows, wrist and poplitic areas secondary to $\beta_2$-M amyloid accumulation [15–17]. These recent observations have thus raised concern that the development of $\beta_2$-M amyloidosis in dialysis patients may be a threat not only to their rheumatological health, but also to their life, although the frequency of life-threatening complications still appears to be minimal.

Pathogenesis

$\beta_2$-Microglobulin

$\beta_2$-M is a protein consisting of 99 amino acid residues and has a molecular weight of 11 800 Da. It was first isolated from the urine of patients with tubular proteinuria. $\beta_2$-M has 30% homology to the constant region domains of immunoglobulin. It is present on the surface of all nucleated cells as an integral part of the HLA class I antigen complex, but is also found in non-HLA-associated forms [18,19]. The synthesis rate of $\beta_2$-M has been estimated to be 50–200 mg/day; the cells with the greatest synthesis rate are lymphocytes, especially T-lymphocytes. Elevated plasma $\beta_2$-M may reflect an increased turnover of lymphatic cells in a variety of illnesses, including malignancies, AIDS, infectious diseases, rheumatic disease and probably in primary amyloidosis; or more frequently and importantly, the presence of renal failure with a deficiency of $\beta_2$-M clearance. The plasma concentration of $\beta_2$-M in normal subjects is less than 2 mg/l.

$\beta_2$-M is catabolized exclusively within the kidney and no other sites of catabolism have been demonstrated. $\beta_2$-M is freely filtered at the glomerulus and is completely reabsorbed and metabolized in situ by proximal tubule cells. It is calculated that approximately 150–250 mg of $\beta_2$-M is metabolized daily by normal kidneys. In patients with chronic renal failure, the serum $\beta_2$-M is massively increased more than 25–35 times the normal range. Residual renal function in dialysis patients could represent an important factor in the excretion of $\beta_2$-M, as serum $\beta_2$-M in these patients is lower than in patients without residual urine volume. In haemodialysed patients the mean serum concentration of $\beta_2$-M is on the order of 30–50 mg/l [20,21]. These levels appear rather stable in follow-up studies of 2–6 months, but in longer follow-up studies the concentration tends to increase with the time on dialysis. The reason for this has not been established although reduced renal excretion seems the most likely explanation. Some studies suggest an increased synthesis rate of $\beta_2$-M in patients receiving haemodialysis; this has not been confirmed by others.

The type of replacement therapy could be an important factor in $\beta_2$-M pathogenesis, especially concerning serum $\beta_2$-M. Conventional cellulosic membranes are not permeable to $\beta_2$-M and it is probable that serum $\beta_2$-M increases during dialysis with these types of dialysis membranes [22,23]. In contrast, synthetic membranes, such as polysulfone and polycrylonitrile, are permeable to $\beta_2$-M and decrease serum $\beta_2$-M. Other dialysis techniques such as haemofiltration or haemodiafiltration, in conjunction with these new synthetic dialysis membranes, achieve an even greater elimination rate of $\beta_2$-M. However, even daily haemofiltration does not result in normal serum $\beta_2$-M [24]. We investigated the clearance of $\beta_2$-M during haemodialysis using labelled $\beta_2$-M ($^{131}$I-$\beta_2$-M), and showed that during a haemodialysis session with the cuprophane membrane, serum $^{131}$I-$\beta_2$-M decreased 5–10% from its initial level, and with synthetic membranes (polysulfone and polycrylonitrile), the decrease ranged from 40 to 45% [25]. The decreases in labelled $\beta_2$-M were greater than those anticipated from unlabelled $\beta_2$-M studies, suggesting that there is probably synthesis of $\beta_2$-M during haemodialysis, with both types of dialysis membrane.

Peritoneal dialysis, despite the perfect biocompatibility of the peritoneal membrane, does not prevent $\beta_2$-M amyloidosis. Several cases of $\beta_2$-M amyloidosis have recently been reported in patients treated exclusively with peritoneal dialysis [26–28]. Serum $\beta_2$-M in patients on peritoneal dialysis is slightly lower than in haemodialysis, probably as a result of the relatively greater clearance of $\beta_2$-M with peritoneal dialysis techniques. Although recently a lesser incidence of $\beta_2$-M amyloidosis in peritoneal dialysis patients has been reported [29], whether peritoneal dialysis protects from or delays $\beta_2$-M amyloidosis when compared to conventional haemodialysis requires further studies.

In addition, recent reports of $\beta_2$-M amyloidosis occurring in patients with long-term chronic renal
failure even before starting dialysis treatment, have suggested that renal failure rather than dialysis treatment is the key to the pathogenesis of dialysis amyloidosis [30]. It is probable that dialysis treatment itself, without any capacity to decrease serum $\beta_2$-M, could play some role in accelerating $\beta_2$-M amyloidosis development.

Nevertheless, it seems that the increase in serum $\beta_2$-M is not sufficient to induce $\beta_2$-M amyloidosis. Serum $\beta_2$-M does not discriminate between dialysis patients with or without $\beta_2$-M amyloidosis, as some subjects require very long periods of time to develop $\beta_2$-M amyloidosis, while other do not, regardless of the serum $\beta_2$-M. However, since no case of $\beta_2$-M amyloidosis has ever been reported in subjects with normal or only slightly elevated $\beta_2$-M, it appears reasonable to postulate that at least some degree of retention is required for $\beta_2$-M amyloid deposition to occur. It is likely that other unknown amyloidogenic factors could interact with the increased $\beta_2$-M and accelerate the development of $\beta_2$-M amyloidosis.

Other protein constituents of $\beta_2$-M amyloidosis

Although great progress was made in $\beta_2$-M amyloidosis with the identification of $\beta_2$-M, it is noteworthy that other proteins are present in amyloid deposits. Since $\beta_2$-M does not seem to be the only factor influencing the occurrence of $\beta_2$-M amyloidosis, a role for these other proteins in amyloidogenesis has been hypothesized [11].

Technical approach

A first point to be discussed when analysing the protein constituents of a surgical specimen is the technical approach to be used, both for sample preparation and for protein analysis. The classical approach for amyloid preparation is based on the method described Pras et al. which uses water to resuspend amyloid fibrils [31]. However, although this method results in an increased concentration of amyloid fibrils, it is not precisely known which proteins are cleared during the procedure. The alternative approach is to precipitate most of the proteins contained in the amyloid deposits surgically obtained with 10% trichloroacetic acid [32]. We have analysed the amyloid deposits using both methods and found that $\beta_2$-M and another 170 kDa protein are consistently present in both preparations.

We have identified the latter as being $\alpha_2$-macroglobulin [33]. We have also identified some of the remaining proteins found with TCA precipitation as being globin chains, light chains of immunoglobulins, and also albumin, haemopexin and transferrin [32,34,35]. The second aspect is the technical approach to analyse the protein solution after preparation. Gejyo et al. used gel filtration and sequencing of the protein pooled in the most important peak. Although they were able to identify the most abundant protein ($\beta_2$-M), other compounds which could have been present at the start of the procedure might have been overlooked. We found that this could have been the case for globin chains [35].

Putative participation of the protein constituents in amyloidogenesis

In addition to the above proteins, glucosaminglycans [36], amyloid P component [10] and a novel protein called JUNO [37], as well as apolipoprotein E have also been identified in $\beta_2$-M amyloidosis deposits [38]. Whether all these proteins do participate in amyloid deposit formation and/or persistence, or they are merely contaminants, has not been elucidated at present. Nevertheless, even those compounds which are not properly forming part of the amyloid fibrils but they are related to them may participate in the disease.

When trying to evaluate the putative participation of the different proteins in amyloidogenesis in general, it seems important to look for a common mechanism for all types of amyloidosis. Thus, it has to be stressed that the amyloid P component is found in nearly all amyloidoses [39] and the same holds true for glucosaminglycans [36]. $\alpha_2$-Macroglobulin has also been found in other types of amyloidoses [40]. $\alpha_2$-Macroglobulin is known to be the major serum protease inhibitor and as a consequence we hypothesized that by modifying the protease–antiprotease balance, it could prevent amyloid fibrils from being degraded [11]. Indeed, other protease inhibitors have been found in $\beta_2$-M amyloidosis [41]. The antiprotease hypothesis is still under study.

Light chains of immunoglobulins are the fibrillar component of AL amyloidosis [42]. Brancaccio et al. have recently shown that $\kappa$ light chains could be a constituent of the previously described JUNO protein [37] and that they are space related to the amyloid fibrils [43]. Similar data have been reported for apolipoprotein E which is found in Alzheimer's disease [44] and in $\beta_2$-M amyloidosis [38]. Therefore, although no confirmation of a precise role for any of these proteins has been demonstrated, they are all good candidates to participate in amyloidogenesis. Research on $\beta_2$-M amyloidosis besides focusing on $\beta_2$-M and particularly the AGE-$\beta_2$-M, should be kept open to all the factors susceptible to influence this condition.

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References

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