Sonographic features suggestive of amyloidosis in hemodialysis patients: Relations to serum beta2-microglobulin


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Received 20 March 2014; accepted 21 March 2014
Available online 26 April 2014

Abstract Aim of the work: To determine sonographic features suggestive of amyloidosis in hemodialysis patients complaining of shoulder pain, and to study their relations to serum beta2-microglobulin (β2M).

Patients and methods: Clinical examination, skeletal survey, musculoskeletal ultrasonography of the shoulder joints, and serum β2M were done for 32 patients with end stage renal disease, who were regular on hemodialysis.

Results: Serum β2M levels were markedly raised in all patients, and increased with increasing duration of dialysis (r = 0.91, p < 0.001). Twenty-five patients had a non homogeneous thickening of the supraspinatus tendon >7 mm, and thickening of the biceps tendon >4 mm, 30 had synovial deposits, 27 had subdeltoid bursa effusion, 25 had thickened subacromial bursa, 7 had supraspinatus tendon tear, and 17 had bony erosions. Serum β2M levels significantly correlated with thickened supraspinatus tendon (>7 mm) and supraspinatus tendon tear (r = 0.41, p = 0.03 and r = 0.42, p = 0.01 respectively). Long time on hemodialysis was the significant independent determinant for supraspinatus tendon tear and humeral head erosions (p = 0.001 for each).

Conclusion: Elevated serum β2M levels and sonographic features suggestive of dialysis-related amyloidosis (DRA) were found in all hemodialyzed patients complaining of shoulder pain either with or without clinical and/or radiological features suggestive of DRA. So, for diagnosis of DRA, sonographic features should correspond to the presence of clinically or radiologically evident β2M amyloid, and we should exclude other causes of non-amyloid changes.

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1. Introduction

Dialysis-related amyloidosis (DRA) is a disorder caused by tissue deposition of beta2-microglobulin (β2M) as amyloid fibrils [1]. The tissue deposition of amyloid detected histologically occurs much earlier than any clinical or radiographic manifestations of the illness. A prospective postmortem study found joint amyloid deposition in 21% in patients receiving hemodialysis for less than 2 years, 50% at 4–7 years, 90% at 7–13 years, and 100% at more than 13 years [2].

The pathogenesis and pathophysiology of DRA are probably multifactorial and have been associated with the duration of renal failure, patient’s current age, patient’s age at initiation of hemodialysis, duration of hemodialysis, bioincompatibility of dialysis membranes [3], less residual renal function, and the coexistence of high-turnover renal osteodystrophy [4].

Heparin is widely used as an anticoagulant in hemodialysis. Yamamoto et al. [5] have suggested that heparin could exert a subtle effect on the development of β2M amyloidosis under some clinical conditions. Also, Uji et al. [6] have suggested a possible association of β2M with glycosaminoglycans (GAGs) containing a sulfate moiety, including heparin, in hemodialyzed patients.

Dialysis-related amyloidosis is characterized by painful stiff joints, usually first involving the shoulder, and less commonly the hands, wrists, knees, and other large joints. Involvement tends to be bilateral and symmetrical, and is frequently associated with the carpal tunnel syndrome (CTS) and tenosynovitis [7].

As clinical symptoms of DRA are nonspecific, they may easily be misinterpreted as other joint diseases [8]. Typical specific radiological amyloid bone cysts are a late event. Different studies observed that capsulosynovial swelling precedes the development of characteristic bone cysts [9,10]. They are hypothesizing that amyloid infiltration of synovial membrane and tendons [11–13] might be accessible to ultrasonographic assessment [14].

Ultrasonography (US) has been suggested as the modality of choice in evaluating DRA of the shoulder. Thickening of the supraspinatus tendon greater than 7 mm and thickening of the long head of the biceps tendon greater than 4 mm, both in the appropriate clinical setting, have correlated excellently with DRA of the shoulder [9,15,16].

Biopsy is nearly always required for definitive diagnosis. However, because histologic confirmation is not always possible and increased serum β2M level is not diagnostic, imaging findings combined with history and clinical findings are usually used for the assessment of musculoskeletal involvement by DRA [17].

The aims of the study were to determine sonographic features suggestive of amyloidosis in hemodialysis patients complaining of shoulder pain, and to study their relation to serum β2M.

2. Patients and methods

2.1. Study subjects

The study was conducted at the renal unit of Minia University Hospital, Minia governorate, Egypt. All patients with end stage renal disease (ESRD), who were regular on hemodialysis were included. All patients had persistent shoulder pain lasting for more than 6 weeks. They were 32 patients, 18 males (56.2%) and 14 females (43.8%). Fifteen healthy age and sex matched subjects served as controls. Informed consent was taken from all participants in the study. The study was approved by the ethics committee of the Faculty of Medicine, Minia University.

All study patients were undergoing hemodialysis with low-flux hemodialyzers. All patients were dialyzed 3 times/week. The duration of each session was 4 h.

Patients having any evidence of autoimmune deficiency syndrome, lymphoproliferative disorders, inflammatory disorders like systemic lupus erythematosus, rheumatoid arthritis, Crohn’s disease, and liver cirrhosis, which lead to rise in β2M were excluded from the study. Patients with chronic infections like tuberculosis, chronic osteomyelitis and patients with primary amyloidosis were also excluded.

2.2. Clinical diagnosis

Patients were examined clinically to show if they completed the characteristic triad of DRA or not. The triad included flexor tenosynovitis of the hand with diminished extension of the fingers, signs or symptoms of CTS and shoulder pain with diminished range of motion [18,19].

2.3. Laboratory investigations

Serum β2M was estimated by ELISA technique (pre hemodialysis) using ORG 5BM beta2-microglobulin kit, the normal range: 0–3.0 μg/ml [20]. Serum parathyroid hormone (PTH) was assayed in all patients by the Intact Parathyroid Hormone ELISA Kit, normal range: 15–55 pg/ml [21].

Blood urea and creatinine were measured in all patients. The normal plasma concentration of urea was 20–40 mg/dl, and the normal range of creatinine was 0.6–1.2 mg/dl for male and 0.5–1.1 for female.

2.4. Imaging

Skeletal survey involving X-rays of the shoulders, pelvis, cervical spine, lumbar spine, knees and wrists were taken to look for subchondral bone erosions and cysts, bone fractures, destructive arthropathy and spondyloarthropathy – characteristic of amyloidosis [22]. A radiological diagnosis of renal osteodystrophy was made if any of the following were present: subperiosteal resorption, tuft resorption, distal clavicular erosions, Rugger–Jersey spine or other typical bony sclerosis, or coarse bony trabeculation [23].

All patients underwent musculoskeletal ultrasonography of the shoulder joints to assess features suggestive of amyloidosis. Ultrasonograms were obtained and interpreted by one researcher (F.A., rheumatologist) using conventional greyscale ultrasound machine with a 7.5–12 MHz linear transducer. In the shoulders: tendons were examined, evaluating changes of their sonographic appearance, alterations of their margins, modifications of their thickness and the presence of peritendinous fluid collection; sonographic signs of bursitis were searched for [24]. Bony erosion should be specifically noted during shoulder sonography as a sign of arthropathy [16]. The normal supraspinatus tendon thickness was
considered from 3 to 6 mm [16] and the normal biceps tendon thickness from 2.7 to 3.5 mm [25].

Statistical analysis: The statistical analysis was performed using SPSS 16.0. Descriptive statistics were done by number and percent as well as mean and SD. ANOVA test was used to compare the difference between more than two group means in interval and ordinal variables. Correlations were calculated using Pearson’s correlation coefficient. A backward/stepwise multiple linear regression model was used for detection of independent predictors of ultrasonographic features characteristic of amyloidosis of the shoulder. The level of statistical significance was set at a $p$ level <0.05.

3. Results

3.1. Characteristics of the study population

Demographic data, causes of ESRD and type of heparin used for studied patients on hemodialysis are shown in Table 1. The cause of ESRD was not identified in 11 patients, while hypertension was the second cause (9 patients). Among all patients, 18 were using low-molecular weight heparin (LMWH), and 14 were using unfractionated heparin (UFH) in dialysis.

3.2. Clinical and radiological findings

All patients had shoulder pain with restriction of movement in 5 patients. CTS was found in 18.8%, and flexor tenosynovitis was found in 15.6%. But only 5 cases (15.6%) with their dialysis duration $\geq$10 years completed the clinical triad suggestive of DRA.

Radiological findings suggestive of amyloidosis were found in 9 patients only (28.1%), two of them had the clinical triad. Typical radiographic changes of renal osteodystrophy were found in 9 patients (28.1%).

The patients were divided into two groups: group A; included patients with clinical and/or radiological features suggestive of DRA ($n = 12$), and group B; included patients without clinical and/or radiological features suggestive of DRA ($n = 20$).

3.3. Laboratory investigations

Predialysis serum $\beta_2$M levels were markedly raised in both groups of patients with a significant difference in comparison to their levels in the control group ($p < 0.001$) as shown in Table 2, and increased with increasing duration of dialysis ($r = 0.91, p < 0.001$) (Fig. 1). There was no significant difference in levels of $\beta_2$M between patients using LMWH and those using UFH in dialysis.

Serum parathyroid hormone, blood urea and creatinine levels were raised in both groups of patients with a significant difference in comparison to their levels in the control group ($p < 0.001$) (Table 2).

3.4. Ultrasonographic findings

Table 3 summarizes the relevant sonographic features of this study and shows that the features were more in hemodialyzed patients than controls. Twenty-five patients (9 in group A, 16 in group B) had a nonhomogeneous thickening exceeding 7 mm of the supraspinatus tendon (Figs. 2 and 3) and thickening of the biceps tendon in excess of 4 mm (Fig. 4). Synovial deposits were detected in 30 patients (11 in group A, 19 in group B) and appeared as hyperechoic polypoid clusters in the subdeltoid bursa (Figs. 5 and 6). Subdeltoid bursa effusion was found in 27 patients (11 in group A, 16 in group B). Thickened subacromial bursa was found in 25 patients (10 in group A, 15 in group B). Seven patients (4 in group A, 3 in group B) had supraspinatus tendon tear (Figs. 2 and 6). Bony erosions were found in 17 patients (9 in group A, 8 in group B) (Fig. 3). There was no significant difference in the previous findings between patients using LMWH in dialysis and those using UFH.

3.5. Relationship between serum $\beta_2$M level and features suggestive of amyloidosis

Serum $\beta_2$M levels did not correlate with any clinical or radiological features suggestive of amyloidosis, but

| Table 1 Demographic data, causes of end stage renal disease and type of heparin used for patients on hemodialysis. |
|---------------------------------|---------------------------------|
| **Patients on hemodialysis ($n = 32$) Range (mean ± SD) | **Patients on hemodialysis ($n = 32$) Range (mean ± SD) |
| Age (years) | 19–70 (40.6 ± 13.4) |
| Dialysis duration (years) | 0.5–23 (6.8 ± 5.1) |
| Age at dialysis onset (years) | 11–68.5 (33.8 ± 14.3) |
| **Number (%) | **Number (%) |
| Sex | Males 18 (56.2) |
| Causes of ESRD | Females 14 (43.8) |
| Diabetes | 6 (18.8) |
| Hypertension | 9 (28.1) |
| Glomerulonephritis | 2 (6.2) |
| Chronic pyelonephritis | 3 (9.4) |
| Chronic interstitial nephritis | 2 (6.2) |
| Renal stone | 2 (6.2) |
| Hyperuricemia | 1 (3.1) |
| Unknown | 11 (34.4) |
| Type of heparin used in dialysis | LMWH 18 (56.2) |
| UFH | 14 (43.8) |

ESRD, end stage renal disease, LMWH, low-molecular weight heparin, UFH, unfractionated heparin.
significantly correlated with thickened supraspinatus tendon (>7 mm), supraspinatus tendon tear and humeral head erosions \( (r = 0.41, p = 0.03 \text{ and } r = 0.42, p = 0.01 \text{ and } r = 0.44, p = 0.01 \text{ respectively}) \) as shown in Table 4. Other laboratory investigations (serum PTH, urea, creatinine) did not correlate with any feature.

### 3.6. Relationship between demographic data, clinical, radiological, and sonographic features suggestive of amyloidosis

Demographic data did not correlate with any of clinical or radiological features suggestive of amyloidosis, while duration of hemodialysis significantly correlated with supraspinatus
tendon tear and sonographic humeral head erosions ($r = 0.46$, $p = 0.008$ and $r = 0.48$, $p = 0.005$ respectively).

There were significant correlations between supraspinatus tendon tear and radiological humeral head cysts and erosions ($r = 0.36$, $p = 0.03$ and $r = 0.47$, $p = 0.004$ respectively).

3.7. Predictors of ultrasonographic features suggestive of amyloidosis

The supposed predictors for ultrasonographic features suggestive of amyloidosis (including; age at onset of dialysis, dialysis duration, serum β2M level, serum urea and creatinine, presence of renal osteodystrophy and type of heparin used in dialysis) were analyzed by backward/stepwise multiple regression model, and it was found that:

Dialysis duration was the only significant independent determinant for supraspinatus tendon tear ($p = 0.001$), while
patients’ age at onset of dialysis and dialysis duration were the significant independent determinants for the humeral head erosions ($p = 0.001$ for each of which) and therefore retained and included in the regression equation, while other factors did not have significant contributions in the presence of retained variables and therefore were excluded.

### 4. Discussion

Beta2-microglobulin amyloid fibrils primarily accumulate in the osteoarticular tissues, causing musculoskeletal symptoms, including polyarthralgia, CTS and bone cysts [26].

In the present study, all patients had shoulder pain with restriction of movement in 5 cases. CTS was found in 18.8%, and flexor tenosynovitis was found in 15.6%. But only 5 cases (15.6%) complete the clinical triad suggestive of DRA. Radiological findings suggestive of amyloidosis were found in 9 patients (28.1%). Radiographic erosions have been confused with those of hyperparathyroidism [27], since most long-term dialysis patients also have the secondary form of this.

Serum concentrations of β2M are substantially higher in patients undergoing hemodialysis than in healthy individuals. However, serum β2M levels are similar in hemodialysis patients with and without β2M amyloidosis [28]. In the current study, serum β2M was found to be 1.9 ± 0.3 mg/L in the control group which raised to 84.9 ± 9.1 mg/L in patients with clinical and/or radiological features suggestive of DRA, and 75.1 ± 14.8 in those without (about 60-80 times higher than that of normal control subjects) as the hemodialysis was performed with low flux dialysis membranes that were impermeable to β2M. Similar high levels were observed by previous studies [29–32], but Gejyo et al. [33] reported no relation between onset of DRA and serum level of β2M, while Dember and Jaber [1] postulated that the onset of DRA may be after accumulation of β2M with long duration of dialysis treatment.

Elevated blood levels of β2M are not diagnostic because virtually all patients with end stage renal disease have elevated levels and the magnitude of the elevation does not correlate with the presence of clinical disease [4]. Radiography is not a sensitive method for the early detection of β2M deposits. In fact, asymptomatic deposits may antedate symptoms and be observed early after dialysis onset (within 1 year) [34] or even before start of dialysis treatment [35].

Among the new imaging methods, ultrasound will probably be of help in the early detection of soft tissue changes. US of joint capsules and tendons has been proposed as a potentially useful means for diagnosis of DRA [9]. In the present study, both groups of patients had ultrasonographic features suggestive of amyloidosis in their shoulders. The results of previous studies [9,15,16,36,37] have been confirmed by our finding. Shoulder sonography has been shown to be useful for the diagnosis of amyloidosis not only in patients on chronic dialysis but also in patients with early dialysis.

Thickness of supraspinatus tendon and biceps tendon is associated with DRA [37]. In 1993, Jadoul and colleagues [9] confirmed that thickened supraspinatus tendons (greater than 7.8 mm) were markers for DRA. In our study, a nonhomogeneous thickening of the supraspinatus tendon > 7 mm and thickened biceps tendon > 4 mm were found in 9 patients in group A and 16 patients in group B. Increased thickness of the subacromial bursa also was identified in patients with DRA [16]. In our study, it was found in 10 patients in group A and in 15 patients in group B. Amyloid deposition appears to be the most likely explanation for thickening of tendons and synovial tissues, although associated inflammation also may contribute [15].

Kiss et al. [17] have reported that, the measured structures (supraspinatus tendon, biceps tendon and subacromial bursa), however, may be transiently edematous; tendinitis and synovitis are the main causes of nonamyloid swelling of the supraspinatus or biceps tendon. Measurement should, therefore, be obtained in the absence of clinical symptoms suggestive of these conditions or, if not possible, repeated 1 month later. In our study, we cannot exclude causes of nonamyloid swelling of tendons especially in group B.

Sommer et al. [37] reported hyperechoic polypoid clusters in the subdeltoid bursa, subdeltoid bursa effusion, and supraspinatus tendon tears in dialyzed patients. In our study, polypoid-shaped subdeltoid bursa was found in 11 patients in group A and 19 patients in group B; and subdeltoid bursa effusion was found in 11 patients in group A and 16 patients in group B, while supraspinatus tendon tear was found in 4 patients in group A patients and 3 patients in group B.

In our patients, serum β2M levels increased with increasing duration of dialysis ($r = 0.91$, $p < 0.001$) which was previously reported by Fry et al. [38]. No direct relationship between age and β2M level was seen in the present study which was similarly reported by Jeloka et al. [29]. Serum β2M levels did not correlate with any clinical or radiological findings suggestive of amyloidosis, but significantly correlated with thickened supraspinatus tendon (> 7 mm), supraspinatus tendon tear and humeral head erosions ($r = 0.41$, $p = 0.03$ and $r = 0.42$, $p = 0.01$ and $r = 0.44$, $p = 0.01$ respectively), indicating that these sonographic features had relation to absolute level of β2M. Long time on hemodialysis was the significant independent determinant for supraspinatus tendon tear and humeral head erosions ($p = 0.001$ for each).

Low-molecular weight heparins as well as unfractionated heparin are used as anticoagulation during hemodialysis therapy. Still commonly used is UFH, followed by LMWH preparations with distinct advantages [39]. Ariga et al. [40] reported that LMWH can reverse the process of amyloidosis; inhibit fibril formation by blocking the formation of β-plated structures. A possible therapeutic approach using LMWHs to interfere with the interaction between proteoglycans and amyloid βproteins and to arrest or prevent amyloidogenesis is suggested.

### Table 4 Relationship between serum β2M level and ultrasonographic features suggestive of amyloidosis.

<table>
<thead>
<tr>
<th>Ultrasonographic features</th>
<th>Serum β2M</th>
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<tr>
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<td>$r$</td>
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<tr>
<td>Thickened supraspinatus tendon (&gt; 7 mm)</td>
<td>0.41</td>
</tr>
<tr>
<td>Thickened biceps tendon (&gt; 4 mm)</td>
<td>0.19</td>
</tr>
<tr>
<td>Polypoid-shaped subdeltoid bursa</td>
<td>0.05</td>
</tr>
<tr>
<td>Subdeltoid bursa effusion</td>
<td>−0.14</td>
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<tr>
<td>Thickened subacromial bursa</td>
<td>−0.15</td>
</tr>
<tr>
<td>Supraspinatus tendon tear</td>
<td>0.42</td>
</tr>
<tr>
<td>Humeral head erosions</td>
<td>0.44</td>
</tr>
</tbody>
</table>

* Significant $p$-value < 0.05.
In the present study, we cannot judge which type of heparin used in dialysis has a role in the process of amyloidosis, but mostly UFH was responsible for this as all the patients were using UFH at the beginning of dialysis then after a period of time 18 patients were changed to LMWH. Accordingly, we had no significant differences in the levels of B2M and the frequency of ultrasonographic features characteristic of amyloidosis between dialysis patients using LMWH and those using UFH.

Diagnosis of B2M amyloidosis is suggested primarily by the characteristic triad: shoulder pain, carpal tunnel syndrome, and flexor tenosynovitis in the hand. Radiographic findings corroborate the diagnosis. Ultrasonography of the shoulder provides a relatively sensitive and highly specific adjunct to the clinical diagnosis especially if it reveals either of two characteristic changes: rotator cuff thickness greater than 8 mm or echogenic pads between muscles of the rotator cuff [41].

This study has some limitations. The number of patients with clinical and/or radiological features suggestive of DRA is small, and large series may be needed to estimate sensitivity and specificity of findings. Sonography is an operator dependant technique, and high frequency range of machine may be needed.

In conclusion, elevated serum B2M levels and sonographic features suggestive of DRA were found in all hemodialyzed patients complaining of shoulder pain either with or without clinical and/or radiological features suggestive of DRA. So, sonographic features should correspond to the presence of clinically or radiologically evident B2M amyloid, and we should exclude other causes of non-amyloid sonographic changes. Our study determines the relation between serum B2M and thickened supraspinatus tendon (> 7 mm) and supraspinatus tendon tear. Longer hemodialysis duration was considered as a predictor factor for supraspinatus tendon tear and humeral head erosions. Further studies are needed to confirm the results and to determine the role of heparin in development of amyloidosis.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References


