Screening for amyloid in subcutaneous fat tissue of Egyptian patients with rheumatoid arthritis: clinical and laboratory characteristics


Objective: To screen for amyloid and to assess associated clinical and laboratory characteristics in Egyptian patients with rheumatoid arthritis (RA).

Methods: Abdominal subcutaneous fat aspirates were consecutively collected from 112 patients (103 women, nine men) having RA for five years or more. To detect amyloid, fat smears were stained with Congo red and the concentration of amyloid A protein in fat tissue was measured. Clinical, radiological, and laboratory characteristics of the patients were assessed.

Results: Amyloid was detected in eight (7%) of the fat smears stained with Congo red. Compared with the Congo red stain, the sensitivity for detecting amyloid by measurement of amyloid A protein in fat tissue was 75% and the specificity was 100%. The amount of amyloid found was small for both methods. The median disease duration of the eight amyloid patients was significantly longer (17 years) than that of the non-amyloid patients (10 years). Bronchopulmonary disease and constipation were more common, whereas proteinuria and chronic renal insufficiency were not. The number of swollen joints and the number of red blood cells were significantly lower in the amyloid group.

Conclusions: Quantification of amyloid A protein and staining with Congo red are strongly concordant methods of screening for amyloid in fat tissue. The prevalence of amyloid in Egyptian patients with RA is 7%. Proteinuria is not a discriminating feature, whereas long disease duration, constipation, bronchopulmonary symptoms, and a moderate to low number of red blood cells may help to identify the arthritic patients with amyloid.

A amyloidosis is a well known complication of chronic inflammatory diseases, such as rheumatoid arthritis (RA).

The prevalence of AA amyloidosis in RA varies considerably among different geographical populations. In Egypt and Jordan, only familial Mediterranean fever is reported as a cause of AA amyloidosis and has been described in 11 and seven patients with renal failure respectively. Although amyloid is also a well known complication in children with juvenile chronic arthritis (JCA), no amyloid was reported in Arab patients with JCA in a study from Kuwait and another from Saudi Arabia. Only one case of a Saudi patient with JCA and amyloid has been reported. It was speculated that Arabs might differ genetically from others in their ability to develop amyloid. However, another explanation for the almost complete absence of amyloidosis in Arab patients with arthritis may be a concealed presentation of its clinical features.

Common clinical features of AA amyloidosis are proteinuria, loss of renal function, and gastrointestinal disorders. Although AA amyloid can be detected sometimes in patients with arthritis in the absence of clinical features of amyloidosis, the clinical importance of such “silent” deposits remains to be determined. The presence or absence of risk factors, such as longstanding disease, may also influence the development of AA amyloid. Whether Arab patients with longstanding RA do not develop AA amyloidosis or differ in the way of presenting clinical features is unknown.

To determine the prevalence of AA amyloid in RA, tissue should be examined for the presence of amyloid. The gold standard for detecting amyloid is by aspirating abdominal subcutaneous fat tissue, which has a sensitivity ranging between 54% and 82%. Measurement of amyloid A protein in fat tissue may be an alternative method of detecting AA amyloid and the value of the Congo red method may even be improved by combining both methods.

Therefore this study aimed at screening for amyloid in fat tissue of Egyptian patients with RA by two different methods in order to assess the prevalence of amyloid, possible risk factors for its development, and associated clinical characteristics.

PATIENTS AND METHODS

Study design

Abdominal subcutaneous fat tissue was screened for the presence of amyloid by examination of Congo red stained smears and by quantification of the amyloid A protein concentration of the tissue involved. Patients identified as having amyloid were compared with non-amyloid patients. Because of the expected longer duration of the RA in amyloid patients, every amyloid patient was matched with three non-amyloid patients for disease duration, age, and sex. Clinical, radiological, and laboratory characteristics of the groups were compared.

Patients

Egyptian patients with RA fulfilling the criteria of the American College of Rheumatology were gathered consecutively from two outpatient clinics of El-Minia and Cairo University Hospital in the period from January until August 1999. Only

Abbreviations: ELSA, enzyme linked immunosorbent assay; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; JCA, juvenile chronic arthritis; RA, rheumatoid arthritis; RBC, red blood cells; RF, rheumatoid factor; SAA, serum amyloid A.
patients with disease duration of five years or more were included in the study. Patients with disease onset before 16 years of age were excluded. No other exclusion criteria were used. The total number of patients was 112 (103 women, 9 men). Informed consent was obtained from all patients and the principles of the Declaration of Helsinki were followed. The median disease duration was 10 years, ranging from five to 27 years. The median age was 48 years, ranging from 23 to 75 years.

**Clinical and radiological assessment**

Disease and drug history, routine physical and musculoskeletal examinations were performed. Disability was measured by the Health Assessment Questionnaire (HAQ) index and functional capacity according to Steinbrocker’s classification. A numerical analogue scale was used for the patient’s global assessment of the pain. Clinical disease activity was scored as the Ritchie articular index (maximum score 78), the number of swollen joints, and the number of deformed joints (both maximum number 38). Chest x ray examinations of all patients were carried out as well as plain radiographs of the hands, graded according to Steinbrocker.

**Laboratory investigations**

Routine laboratory tests were carried out in all patients—namely, the erythrocyte sedimentation rate (ESR), complete blood count, liver and kidney function tests, urine analysis, and microscopic sediment. The acute phase reactants serum amyloid A protein (SAA) and C reactive protein were measured by enzyme linked immunosorbent assay (ELISA). Reference basal values are <4.2 mg/l and <2.3 mg/l, respectively. Serum amyloid P component levels were measured by ELISA (normal median 30.5 mg/l for men and 22.5 mg/l for women, range 10–48 mg/l). The IgM rheumatoid factor (RF) levels were measured by ELISA (reference normal values are <10 IU/ml).

**Abdominal fat aspiration**

Abdominal fat tissue was aspirated with a needle of 16 gauge connected to a syringe of 10 ml. A simple modification by adding maintained negative pressure (suction force) made the procedure easier. The aspirated fat was treated in two different ways. Fat smears on three glass slides were stained with alkaline Congo red and investigated with polarised light under an Olympus BX 50 microscope, 100 W. Two independent investigators (TMEM and BPCH) scored blindly the severity of amyloid deposition of three smears of every patient. Severity was assessed by visual estimation of the percentage of the smear area affected by amyloid deposition in polarised light: negative (−) was no detectable amyloid, little (+) was less than 10%, moderate (++) was between 10% and 60%, and abundant (+++) was more than 60%. The remaining fat tissue was stored at −20°C until the amyloid A protein concentration was measured by the Health Assessment Questionnaire (HAQ) index and functional capacity according to Steinbrocker’s classification. A numerical analogue scale was used for the patient’s global assessment of the pain. Clinical disease activity was scored as the Ritchie articular index (maximum score 78), the number of swollen joints, and the number of deformed joints (both maximum number 38). Chest x ray examinations of all patients were carried out as well as plain radiographs of the hands, graded according to Steinbrocker.

**Statistical methods**

Statistical analysis was performed with the statistical package Graph Pad Prism, version 3.02 (Graph Pad Software Inc, San Diego, CA, USA). The Mann-Whitney test was used for detecting differences between two unpaired groups. The Spearman rank correlation was used for correlations. The χ² test for trend was used to detect a trend and Fisher’s exact test was used in the 2×2 tables. A two tailed p value <0.05 was considered significant.

**RESULTS**

**Detection of amyloid in fat tissue specimens**

Fat tissue was obtained from all 112 patients for making smears. After this, ample fat tissue of each patient (median weight 176 mg, range 42–460 mg) was available for measurement of amyloid A protein. Apart from minor bruising in 24 patients (21%) and a mild self limiting subcutaneous infection in one patient (1%), no adverse reactions were reported.

Eight of 112 (7%) fat tissue specimens were positive for amyloid in the Congo red stain. Small deposits (+) were found in seven specimens, moderate deposition (++) in only one specimen, whereas abundant amyloid (+++) was not seen at all. In one of the specimens only a small area of one of the three slides was positive for amyloid, whereas in each of the other seven specimens all three slides were positive.

The results of quantification of amyloid A protein in fat tissue were evaluated by using the Congo red stain as the gold standard. As shown in fig 1, amyloid A protein concentrations of all 104 Congo red negative specimens were below the upper limit of a group of Dutch controls without AA amyloidosis (<1.3 µg/mg protein), resulting in a specificity of 100% (with a 95% confidence interval of 97 to 100%). Six of the eight specimens positive for amyloid in the Congo red stain had raised concentrations of amyloid A protein (ranging from 2.4 to 92 µg/mg protein), resulting in a sensitivity of the quantitative method of 75% (with a 95% confidence interval of 35 to 97%). A seventh Congo red positive specimen had a borderline concentration (1.1 µg/mg protein), whereas the concentration in the eighth specimen was negative (0.06 µg/mg protein).

One of the six specimens with an increased amyloid A protein concentration (5.4 µg/mg protein) was the specimen with a small Congo red positive area in only one of the three slides.

**Clinical characteristics of the patients with amyloid**

All eight patients with amyloid were women who had a longer disease duration (median 17 years) than the 104 patients without amyloid (median 10 years). Table 1 shows their main clinical characteristics. All 112 patients were white subjects. The patients with amyloid did not differ in religious background, occupation, and marital status from the other patients. No differences were detected in type or mode of onset of the arthritis, in the course of the disease, in the frequency of joint surgery, in the use of non-steroidal anti-inflammatory drugs, and in the use of disease modifying anti-rheumatic drugs such as n-penicillamine, sulfasalazine, glucocorticoids, antimalarial drugs, and cyclophosphamide. Past use of parenteral gold was more common and present use of methotrexate less common in the patients with amyloid (table 1).

No difference was found between the patients with amyloid and the others in fatigue, morning stiffness, grip strength,
HAQ, functional capacity, and the frequency of extra-articular manifestations. The Ritchie articular index showed no difference, but the number of swollen joints was lower and the number of deformed joints was higher in the amyloid group. Radiological severity according to Steinbrocker showed a trend towards more severe disease in the patients with amyloid (see table 1). The frequency of other symptoms, such as abdominal pain, diarrhoea, nausea, vomiting, symptoms or signs of ischaemic heart disease, hypertension, pitting oedema, and neuropathic symptoms, did not differ from the other patients. Constipation, a history of proteinuria, and bronchopulmonary disease were seen more frequently in the patients with amyloid (table 1).

Table 2 presents the laboratory measurements. The results of the ESR, haemoglobin, and the number of red blood cells (RBC) are presented and were analysed separately for both sexes because of differences in the normal reference ranges between the sexes. Blood was not available from one of the amyloid patients and eight of the non-amyloid patients. The ESR values of the amyloid group tended to be higher than those of the non-amyloid group. Both SAA and RBC of the amyloid group were lower than in the non-amyloid group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Amyloid (n=7)</th>
<th>Non-amyloid (n=96)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR F: 0–30 mm/1st h</td>
<td>90</td>
<td>60</td>
<td>0.05</td>
</tr>
<tr>
<td>M: 0–20 mm/1st h</td>
<td>45</td>
<td>25–92</td>
<td></td>
</tr>
<tr>
<td>CRP &lt;2.3 mg/l</td>
<td>9.0</td>
<td>23</td>
<td>0.08, NS</td>
</tr>
<tr>
<td>SAA &lt;4.2 mg/l</td>
<td>25</td>
<td>1.0–455</td>
<td></td>
</tr>
<tr>
<td>SAP 10–48 mg/l</td>
<td>26</td>
<td>3–60</td>
<td></td>
</tr>
<tr>
<td>RF &lt;10 kIU/l</td>
<td>72</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Hb F: 115–155 g/l</td>
<td>101</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>M: 140–180 g/l</td>
<td>114</td>
<td>102–138</td>
<td></td>
</tr>
<tr>
<td>RBC F: 3.5–5.0 ×10¹²/l</td>
<td>3.86</td>
<td>4.45</td>
<td></td>
</tr>
<tr>
<td>M: 4.3–5.9 ×10¹²/l</td>
<td>4.69</td>
<td>3.95–5.74</td>
<td></td>
</tr>
<tr>
<td>Platelets 130–400 ×10⁹/l</td>
<td>320</td>
<td>310</td>
<td></td>
</tr>
<tr>
<td>Leucocytes 3.2–9.8 ×10⁹/l</td>
<td>8.1</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>Urea 3.0–6.5 mmol/l</td>
<td>11.4</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>Creatinine 62–106 µmol/l</td>
<td>78</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Albumin 34–47 g/l</td>
<td>37</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>

p values below 0.1 are shown; NS, not significant.

RA, rheumatoid arthritis; chronic infection‡, any kind of past chronic infection (such as tuberculosis, chronic urinary tract infections, chronic bronchitis, and other infections); bronchopulmonary, symptoms, signs, or radiological changes suggestive of bronchopulmonary disease.
and RBC were negatively correlated (Spearman’s $r_s=-0.41$, $p<0.0001$). Haemoglobin and RBC were positively correlated ($r_s=0.39$, $p<0.0001$). Proteinuria was present in three patients with amyloid and in 20 patients without amyloid (38% v 19%); this difference was not significant.

Comparison with matched non-amyloid patients

Some of the differences detected between the patients with amyloid and the other patients may be explained by the longer duration of the arthritis. Therefore, each amyloid patient was matched with three non-amyloid controls for disease duration, age, and sex. In this way the group of non-amyloid patients was divided into two subgroups: 24 matched controls and 80 remaining non-amyloid patients (median disease duration eight years).

Table 1 shows the results of the comparison of the amyloid patients and the matched non-amyloid patients. Constipation and bronchopulmonary disease tended to be more common in the amyloid group, but the difference did not reach statistical significance. Figure 2A shows that the number of swollen joints was lower ($p<0.05$) in the amyloid group than in the matched group. Figure 2B shows that the number of deformed joints was higher both in the amyloid group ($p<0.005$) and in the matched group ($p<0.001$) than in the remaining group of non-amyloid patients.

Figure 2C shows the serum SAA levels. These tended to be lower in the amyloid patients than in the matched controls, although this difference did not reach significance ($p=0.08$). The ESR did not differ between the amyloid patients (median 90, range 17–130) and the matched patients (median 65, range 23–130). Figure 2D shows that the RBC count was lower in the amyloid patients than in the matched controls ($p<0.01$).

Individual characteristics of the eight patients with amyloid

Table 3 shows some characteristics of the individual patients with amyloid. The patient classified as (+++) in Congo red stained slides had the highest concentration of amyloid A protein in fat tissue. This patient had proteinuria (+) with an onset less than six months before the investigation. Patients number 2 and number 7 had a history of proteinuria for more

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**Table 3** Laboratory and clinical characteristics of the eight patients with amyloid.

The patients are arranged according to the amyloid A protein concentration in fat tissue.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45</td>
<td>55</td>
<td>41</td>
<td>60</td>
<td>61</td>
<td>44</td>
<td>53</td>
<td>65</td>
</tr>
<tr>
<td>Arthritis duration (years)</td>
<td>23</td>
<td>15</td>
<td>19</td>
<td>12</td>
<td>15</td>
<td>25</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Congo red score</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amyloid A in fat (µg/mg protein)</td>
<td>92</td>
<td>67</td>
<td>21</td>
<td>5.4</td>
<td>2.4</td>
<td>2.2</td>
<td>1.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Serum SAA (mg/l)</td>
<td>50</td>
<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
<td>2.0</td>
<td>ND</td>
<td>14</td>
<td>3.0</td>
</tr>
<tr>
<td>Serum CRP (mg/l)</td>
<td>22</td>
<td>6.0</td>
<td>4.0</td>
<td>14</td>
<td>9.0</td>
<td>ND</td>
<td>14</td>
<td>9.0</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>88</td>
<td>190</td>
<td>83</td>
<td>75</td>
<td>70</td>
<td>77</td>
<td>64</td>
<td>78</td>
</tr>
<tr>
<td>Proteinuria (by stick)</td>
<td>+</td>
<td>+++</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bronchopulmonary disease</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Constipation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

SAA, serum amyloid A protein; CRP, C reactive protein; ND, not done.
The latter patient had a different type of amyloid (AL or ATTR) in the quantitative method. Although the possibility that another Congo red positive specimen was definitely negative method is high (100%), whereas the sensitivity is only 75%. One Congo red positive specimen had a borderline value, compared with the Congo red stain, the specificity of the quantitative method definitely has a place in the detection of AA amyloidosis. However, this relation did not reach statistical significance. Contrary to other reports, in this study antimalarial drugs, chronic urinary tract infections, or the absence of RF are not associated with an increased risk of developing amyloidosis.

Most Egyptian amyloid patients detected in this screening study do not have the common clinical features of AA amyloidosis. Although the three patients with proteinuria probably had amyloid deposition in the kidneys, this remains speculative because no renal biopsies were carried out. Proteinuria and chronic renal insufficiency are not significantly more common in these patients. The most prominent symptom of the amyloid patients is constipation (in 63%), which may be more discriminating than diarrhoea in this particular group. Constipation may be caused by gastrointestinal or autonomic nervous system involvement. The SAA levels tended to be lower and the ESR higher in the amyloid patients, although both differences lose statistical significance when the amyloid patients are compared with matched controls. The low number of swollen joints and the possible low SAA levels, both rather unexpected, seem to reflect a low level of inflammatory activity of the arthritis of these patients at the moment of investigation. The low number of RBC of the amyloid patients may explain the raised ESR in this situation of low inflammatory activity.

As may be expected in a screening study, the amount of amyloid in fat tissue is low, both in the semiquantitative Congo red stain and in the quantitative amyloid A protein determination. Strong concordance is present between the quantification of amyloid A protein and the Congo red method. The upper limit of the amyloid A protein concentration in fat tissue of RA patients without amyloid appears to be similar to that described for a group of Dutch patients. The sensitivity of detecting amyloid in rectal tissue (80–90%) may be somewhat higher than in fat tissue, aspiration of fat tissue is nowadays the preferred way to screen patients for the presence of amyloid. Aspiration of fat is easy to perform in an outpatient clinic and requires no specialty consultation or technical expertise, has a high yield, and has only minimal side effects for the patients.

DISCUSSION

Amyloid is present in some Egyptian patients with RA. The prevalence of amyloid is 7% in the group of patients with five years’ or more duration of RA, and increases to 16% in the group with disease duration of more than 10 years. Although the sensitivity of detecting amyloid in rectal tissue (80–90%) may be somewhat higher than in fat tissue, aspiration of fat tissue is nowadays the preferred way to screen patients for the presence of amyloid. The higher radiological severity score, and the more frequent use of gold drugs in the past by the amyloid patients. A possible second risk factor for amyloid in this group may be comorbidity in the form of concurrent bronchopulmonary disease. Because the number of patients with amyloid is rather small, the comparison with the non-amyloid patients should be interpreted with caution. Long disease duration (median 17 years) is the most important risk factor for the development of amyloid in our Egyptian patients with RA. Long disease duration is also related to the higher number of deformed joints, the higher radiological severity score, and the more frequent use of gold drugs in the past by the amyloid patients. A possible second risk factor for amyloid in this group may be comorbidity in the form of concurrent bronchopulmonary disease.

To conclude, amyloid is present in some Egyptian patients with RA. The prevalence of amyloid is about 7% and increases...
Screening for amyloid in subcutaneous fat tissue

to 26% in a selected group of patients with a combination of a long disease duration and a moderate to low number of RBC. In our opinion this selected group represents those patients particularly at risk of having amyloid. Comorbidity in the form of concurrent bronchopulmonary disease may be an additional risk factor to look for. Quantification of amyloid A protein concentration is a highly specific method for amyloid detection in fat tissue and the combination of this method with the Congo red method makes it more objective and convenient to detect small amounts of amyloid. The amount of amyloid in this screening group is low, and the renal function (that is, serum creatinine and urine protein) seems not to be affected by the amyloidosis. Although the number of amyloid patients is too small to draw firm conclusions, and the results should therefore be interpreted with caution, it seems that constipation, a small number of swollen joints, a low acute phase response (that is, low SAA levels), and a small number of red blood cells are prominent features of these Egyptian patients. Follow up studies are needed to investigate whether this “silent” amyloidosis will eventually develop into clinically significant amyloidosis.

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