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BMC *Musculoskeletal Disorders*

Dear Dr. Lee,

Thank you very much for your evaluation and advice on my manuscript. The following manuscript has now been reviewed extensively and changes made on a point-by-point basis according to your requirement. This revision had two copies; one with showing the changes by track tool and another without it.

**Prevalence of Amyloid Deposition in Longstanding Rheumatoid Arthritis in Iranian Patients by Abdominal Fat Pad Biopsy and Assessment of Clinical and Laboratory Characteristics.**

Sincerely yours

Ahmad Salimzadeh, M..D.
Assistant Professor of Rheumatology
Sina Hospital, Tehran University of Medical Sciences, Imam Khomeini Avenue, Tehran, Iran.
Telefax:: +98-21-66716545
E.mail : salimzad@tums.ac.ir
Prevalence of Depositon Amyloid in Long standing Rheumatoid Arthritis in Iranian Patients by Abdominal Fat Pad Biopsy and Assessment of Clinical and Laboratoratory Characteristics

G. Hussein Alishiri1§, Ahmad Salimzadeh2§, M. Bagher Owlia3, Jafar Forghanizadeh4, Roya Setarehshenas5, Nasrin Shayanfar5, Jafar Forghanizadeh4, Roya Setareshenas4, Nasrin Shayanfar4;

1Assistant Professor of Medicine, Department of Rheumatology, Baqiyatallah University of Medical Sciences, Tehran, Iran.
2Assistant Professor of Medicine, Department of Rheumatology, Tehran University of Medical Sciences, Tehran, Iran.
3Assistant Professor of Medicine, Department of Rheumatology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

4Professor of Medicine, Department of Rheumatology, Iran University of Medical Sciences, Tehran, Iran.
5Assistant Professor of Medicine, Department of Pathology, Iran University of Medical Sciences, Tehran, Iran.

§Corresponding Author
GHA: ghalishiri@yahoo.com
AS: salimzad@tums.ac.ir
MBA: mbolia2@yahoo.com
JF: j_forghani@yahoo.com
RS: royasetarereshenas@hotmail.com
NS: nasrinshayanfar@yahoo.com
ABSTRACT

Background: The To study was aimed at determining the prevalence of secondary amyloidosis in a series group of Iranian patients with Rheumatoid Arthritis (RA) and to assess ment of its correlation how it correlates with the clinical and laboratory findings and data.

Method: A total number of 220 patients (167 female, 53 men) with a minimum five years history of minimal 5 years of RA were selected (155 female, 50 men). Abdominal subcutaneous fat pad biopsy (ASFB) was performed and specimens were treated by-with Congo red staining. All of the specimens were examined for apple-green birefringence under polarized light microscope. Clinical and laboratory characteristics of the patients were assessed. Chi-square test and unpaired student’s t-test were done for intergroup comparison.

Results: Deposition of amyloid test was positive in 15 patients (6.8%) by ASFB. The 13 patients had minimal amyloid deposit. In clinical significant characteristics 9 patients had proteinuria (60%) and in 7 patients presented with sever significant constipation (46.6%).

Conclusion: It was concluded that these Iranian patients with RA have a low prevalence of amyloid deposits with clinical manifestations and in half of them had no clinical manifestation. Follow up studies are needed to determine—investigate
whether this subclinical amyloidosis will develop into clinically significant amyloidosis.

**Background**

Reactive or secondary amyloidosis is a well known and an important late complication of chronic inflammatory disease, especially rheumatoid arthritis (RA)\(^1\). In this condition, large amounts of amyloid molecules (of the AA type) are deposited over a wide area. The main clinical manifestation of secondary amyloidosis (SA) is marked proteinuria and gastrointestinal symptoms; generally, it carries which has a poor prognosis and causes death in 2-9% of cases\(^2-3\).

The prevalence of SA in RA patients in western countries varies between 5 and 78%, depending on the ethnicity of the subject, the method of amyloid detection, and other variables in the patient’s condition\(^4\). In a published study on 120 RA cases, 26.5% tested positive in aspiration test\(^5\).

The simplest, most reliable method for amyloid deposition screening which is considered and gold standard procedure for amyloid deposition is subcutaneous abdominal fat aspiration\(^6\) with Congo red staining is used in the method\(^6-9\). The abdominal fat aspiration method has a sensitivity of between 54-26.5 and 82% for detection of amyloid deposits\(^7-8-9\).

Clinical manifestations of RA vary according to the race and genetic predisposition of the subjects, as well as to environmental factors. It can, therefore, be concluded that the occurrence of the late complication of RA differs according to the above-mentioned factors in Iranian subjects.
Various procedures other than ASFA named biopsy have been tried to develop a more sensitive test for detecting amyloid deposits. Barile et al. showed that true cut needle biopsy has a sensitivity of 78%. They did not find any correlation between amyloid deposits and the clinical manifestations of the disease. Breedveld found 59% positive test results using the biopsy method rather than the aspiration method in a diverse inflammatory arthritis group and with samples taken from different sites. In the two studies mentioned, the selected patients were too diverse in clinical picture and/or too few for any conclusion to be drawn from the test results that were obtained. It would therefore be quite reasonable to assume that the biopsy method affords greater sensitivity and yields more reliable results in the detection of amyloid deposits.

Different studies have been conducted to evaluate the prevalence of secondary amyloidosis in RA patients, yielding varying results depending on the type of geographic population studied and the diagnostic method used. The data, published as case series or case report, show that the prevalence of secondary amyloidosis in the Middle Eastern region, and in the neighboring countries of Iran, is low. Another more specific method that has recently been developed, and which has not been given much publicity, is called abdominal fat-pad biopsy (AFPB) method.

Our study, which is the first of its kind, was aimed at directly investigating the prevalence of amyloid deposition in Iranian patients by using fat-pad biopsy method, and determining whether and establishing how it correlates with clinical and laboratory findings and data.

**Participants and methods:**

The subjects were selected from among patients referred to a pool of two outpatient clinics of Rasool Akram and Baqiyatollah hospitals in Tehran, within the period of December 2001 through October 2003.
**Inclusion:** All the patients met the 1988-1987 American College of Rheumatology (ACR) classification criteria for RA, which stipulate a mean positive value of 4.6. They were included in the study consecutively. The duration of the disease for all the subjects was longer than 5 years and their disease was still active. All the patients had been referred to our clinic by specialists.

**Exclusion:** Patients with an onset of disease earlier than 16 years of age, coexisting chronic disease itself capable of independently inducing amyloidosis (e.g. chronic infection), or those otherwise declining to take part in the study were excluded. A total of 220 patients took part in the study, all of whom giving written informed consent prior to entering the study. The approval of the Ethics Committee of the Research Department was sought and obtained.

**Method:** Small samples (minimum size 3*3mm) of abdominal fat pad were obtained through a small periumbilical incision under local induced anesthesia by 1 ml lidocaine 1%. The specimens were fixed in 10% formalin. On the same occasion, an 18 gauge needle connected to a 10 ml syringe was inserted into the periumbilical fat tissue. A suction force was applied to aspirate fat samples, which were thereby mounted on 3 microscope slides and left to air-dry before being fixed using formalin as fixative. Only one doctor was assigned the task of performing the procedure on all the subjects. The samples were subsequently stained with Congo red stain and examined underly--__polarized light microscope. Two independent pathologists blindly examined the specimens. The intensity of the staining was assessed by visual estimation in all the 3 samples for each patient. The amounts of amyloid deposits found were rated as: negative = no detectable amyloid deposits in a small isolated area, (+) = little, less than 10 %, (++) = moderate, for amyloid deposition between 10 and 60 %, and (+++) = severe, involvement of more than 60% involvement of the test area. Should there occur any conflict between the examiners’ test results, the smaller value was considered.

Patient variables including age, duration of disease, age at onset of disease, presence or absence of constipation, and use of disease modifying anti-rheumatic drugs (DMARDs) such as gold and D-penicillamine were noted and taken into account.
Muscle strength was measured by 5 degree force score normally applied during routine physical examination. Functional disability status was evaluated by revised criteria for the classification of global functional status. Radiographic damage was evaluated according to the general criteria for the classification for the progression of rheumatoid arthritis. Precise physical examination as well complete blood count, ESR, IgM rheumatoid factor, and C reactive protein values were also taken into account. Urinalysis results and 24-hour urine protein values, where indicated, were also included. Plain hands X ray obtained for all patients.

Statistical analysis was performed with SPSS version ll.5. The chi-squared test and unpaired student t test were done for intergroup comparisons. A p value less than 0.05 was considered significant. Clinical amyloidosis was established in RA patients with proteinuria and confirmation of amyloidosis by positive AFPB.

Results:
Fat tissue specimens were obtained from all the 220 patients. Deposition of amyloid was found positive in 15 out of 220 patients. Thirteen of the specimens showed minimal (+) amyloid deposits, moderate (++) amounts (+++) of amyloid deposits were found in two specimens, but none showed abundant (+++) amyloid deposits. Ten out of 15 cases that tested positive for amyloid by biopsy method also tested positive for amyloid by fat aspiration method. Eight Nine out of 15 (60%) patients had clinical amyloidosis, and presented with marked proteinuria, with between 300 mg to 800 mg in 24-hour urine collection (P<0.05). No patient had pedal edema and none was taking gold and D penicillamine therapy. Proteinuria was also present in 17 out of 205 patients (8%) who tested negative for amyloidosis with the ASFB method. In these patients the intensity of proteinuria was less than 500 mg/24h. After one year follow up none of asymptomatic patient developed renal abnormality.
Mild proteinuria (<500 mg/day) was present in 17 cases. Constipation was present more frequently in the patients with amyloid (P<0.05).
There was no significant difference was found related to such in the variables including as age, sex, and duration of the disease between the patients tested positive with amyloid and those tested negative the others (table 1). More of the patients who tested positive for amyloid presented with lower muscle strength and higher functional disability class and greater progression stage than the group who tested negative for amyloid (P<0.05). There was no significant difference in the laboratory findings for data between the patients with amyloid and the ones who tested negative for amyloid deposition others as shown in table 1.

**Discussion:** Secondary amyloidosis is a well known complication of RA. The prevalence of secondary amyloidosis in RA patients varies considerably depending on the geographical population studied and the diagnostic method used. Its prevalence in western countries varies widely (8% - 78%)7,10.18. Recent studies using ASFA method in Asian and North African countries have also showed different results. Wakhlu et al showed in a study that 30 out of 113 (26.5%) adult Asian RA patients from northern India patients tested positive for amyloid deposits by ASFA method9. Mansouri et al detected amyloid deposits in 8 out of 112 (7%) of the ASFA smears from Egyptian RA patients8. The prevalence of secondary amyloidosis in the Middle Eastern region and the neighboring countries of Iran has not been determined with certainty. Published data are available as case series or case reports. Ozdogan reported the characteristics of 147 Turkish patients with juvenile chronic arthritis retrospectively13. A 10% incidence of secondary amyloidosis was found in the group studied. Only a single case of juvenile chronic arthritis and amyloid deposition was reported in a Saudi patient14.

It appears that using the biopsy method yields more positive results as compared with those obtained by the aspiration method. Barile performed an abdominal subcutaneous fat biopsy (ASFA) with a tru-cut needle in 50 Mexican patients in order to investigate the presence of secondary amyloidosis in RA patients. Amyloid deposits were found in 78% of the patients, but no correlation was found between
amyloid deposits and the clinical manifestations of disease. The largest series studied was that by Kobayashi. Of the 407 Japanese patients studied, 54 (13.3%) were found to have gastrointestinal amyloidosis using gastroduodenal endoscopy. In our study, a group of consecutive RA patients were evaluated using SAFB method. We found a prevalence of 6.8% of amyloid deposits in the 220 patients, with a mean disease duration of 9 years. It is a lower incidence than that obtained in the Mexican and Japanese series using the biopsy method. This can be attributed to lower prevalence of secondary amyloidosis in the specific geographic population studied or in similar geographic populations.

Now it needs to be determined whether there is any correlation between the differing test results obtained as regards the presence of clinical amyloidosis and the amyloid test used.

In a Spanish survey of 313 RA patients with a history of the disease longer than five years, amyloid deposition was detected in 16% of the cases. Common clinical features of nephropathy were present in 25% of these amyloid-positive patients. In asymptomatic amyloid positive patients, long term follow-up did not show overt amyloidosis in the majority of the cases. This was found to be the case not only at the time of the ASFA test, but also after a long follow-up period.

In our study 60% of amyloid positive patients presented with clinical proteinuria. None of the 220 patients had nephrotic or malabsorption syndromes. Although a statistical difference was found in the severity of the proteinuria (up to 800 mg/24h) between amyloid-positive and amyloid-negative groups, its clinical significance is uncertain.

A correlation was suggested between the presence of clinical amyloidosis and the intensity of amyloid deposition. Kobayashi et al found that the clinical manifestations suggestive of systemic amyloidosis in gastroduodenal mucosa was more frequent in the marked amyloid deposits group than in the slight deposits group (47% vs 14%, P<0.05). Casanovas et al also found that the frequency of marked
deposits was significantly higher in the group of patients with visceral amyloidosis (57% vs. 22%).

In our study; thirteen of the positive specimens showed minima and two of them showed moderate amyloid deposits, but none displayed abundant amyloid deposits. Eight out of 15 patients had clinical amyloidosis, and presented with proteinuria. No correlation was found between the intensity of amyloid deposits and clinical amyloidosis. It is contrary to Kobayashi and Casanovas’s findings.

Significant correlation was suggested between the amyloid deposits and the duration of the disease. In the present study, no significant difference was found, as related to duration of disease, between the amyloid-positive and the amyloid-negative groups.

Although the number of the patients tested is too small to draw firm conclusions from the results, it seems that constipation and proteinuria were prominent features of the Iranian patients.

A greater number of patients who tested positive for amyloid presented with more functional disability class and progression stage than the ones who tested negative for amyloid (P<0.05). No significant difference was found in the laboratory findings between the amyloid-positive and the amyloid-negative group, as shown in table 1.

Amyloid deposits were present in 6.8 percent of the patients with RA with mean disease duration of 9 years. Of those, 60 percent presented with clinical renal involvement.

Amyloidosis has a high prevalence rate of 75% using tru-cut abdominal biopsy and 15-20% at autopsy. When fat pad aspiration method is used, sensitivity of the test is 25% and 7% in Indian and Egyptian patients respectively. Other sites such as gastroduodenal mucosa a sample in a group of 407 Japanese patients with RA were tested. The presence of amyloid was 13.3% of all.
Using fat pad aspiration method, a correlation between clinical amyloidosis and intense amyloid deposit was suggested. Aspiration of abdominal fat tissue is easy to perform in an out-patient clinic but further clinical tests are necessary to confirm the presence of amyloid deposits, especially in weakly positive cases. Significant correlation was established between amyloid deposits and the duration of disease.

In the present study, no significant difference was found, as related to duration of disease, between amyloid positive and amyloid negative groups (table 1). To compare the result of present study with similar ones, proteinuria looks higher but it isn’t conclusive because of small number of positive amyloid patients as shows in table 2.

Is there any correlation for clinical amyloidosis and amyloid test? None of 220 patient had nephrotic or malabsorption syndrome. Although a statistical difference is found in proteinuria (up to 800 mg/24h) in two groups, but its clinical significance is uncertain. It is true especially where amyloid deposit is weak.

**Conclusion:**

The study showed that a group of Iranian patients with RA presented with low prevalence of amyloid deposits (6.8%); about half of the patients had presented with subclinical disease. Although the number of amyloid patients was too small to merit drawing a firm conclusion, it seems quite likely for constipation and proteinuria to be a prominent clinical manifestation of the disease. With regard to the present study and the series mentioned, it seems that a positive amyloid test result per se cannot have much clinical significance, especially as Casanovas noted. Moreover, a positive test result for amyloid should not prompt the physician to adopt a more aggressive treatment choice in order to stop progression of the disease. Thus, in my view, it does not stand up to logic to assume that a test with such a low prognostic value as that of ASFB be adopted or recommended as a screen test.

A follow-up study is required to determine whether subclinical amyloidosis can eventually develop into full blown clinical amyloidosis as for higher prevalence of proteinuria.

The authors declare that they have no competing interests.
Aknowledgment:
The authors are greatly thanked to colleagues in Hazrat Rasoul Akram and Baqiatollah hospitals. This study was supported by Iran University of Medical Sciences.

References:


16. Hochberg MC, Chang RW, Dwosh I, Lindsey S, Pincus T, Wolfe F. The American College of Rheumatology 1991 revised criteria for the classification of
global functional status in rheumatoid arthritis.


Table 1. Comparison of clinical and laboratory variables of amyloid positive and negative patients with rhumatoid arthritis.

<table>
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<th>Amyloid positive</th>
<th>Amyloid Negative</th>
<th>P* value</th>
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<tr>
<td>- Total patient, no (%)</td>
<td>15(6.8)</td>
<td>205(93.2)</td>
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<tr>
<td>- Age, yrs, mean ± SD</td>
<td>55.2 ± 12(32-75)</td>
<td>51.6 ± 13.2 (22-80)</td>
<td>NS &gt;0.05</td>
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<td>- Male: Female, no</td>
<td>3:12</td>
<td>50: 155</td>
<td>NS &gt;0.05</td>
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<td>- Age at disease onset yrs. Mean ± SD</td>
<td>46.07 ± 7.33 (27-65)</td>
<td>40.18 ± 6.39 (17-70)</td>
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<td>- Disease duration, yrs. Mean ± SD</td>
<td>9.13 ± 4.67 (5-20)</td>
<td>11.41 ± 6.85 (5-39)</td>
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<td>- Constipation no (%)</td>
<td>7(46.6)</td>
<td>39(19)</td>
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<td>- Proteinuria, no. (%)</td>
<td>9(60)</td>
<td>17(8.3)</td>
<td>&lt;0.001</td>
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<td>- RF. Positive, no. (%)</td>
<td>10(66)</td>
<td>130(63.7)</td>
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<td>- CRP, *No, (%)</td>
<td>13(86)</td>
<td>156(67)</td>
<td>NS &gt;0.05</td>
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<td>- Receiving DMARD, no, (%)</td>
<td>12(80)</td>
<td>155(75.6)</td>
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<td>- Past use of gold, no (%)</td>
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<td>17(8.3)</td>
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*Class 1 No: 61; Class 2 No: 86; Class 3 No: 49; Class 4 No: 4*
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| RF: rheumatoid–Rheumatoid Factor; CRP: C-reactive protein; DMARD: disease modifying antirheumatic drug. *Normal value <2.3 mg/dl.

Table 2. Comparison of clinical and laboratory variables of Ag amyloid positive in different studies.
ASFA: Abdominal Subcutaneous Fat Aspiration, ASFB: Abdominal Subcutaneous Fat Biopsy.
RF: Rheumatoid Factor.
Additional files provided with this submission:

Additional file 1: Amyloidosis for BMC revised.doc: 137Kb
http://www.biomedcentral.com/imedia/1902644850812895/sup1.DOC