Nephrol Dial Transplant (2005) 20: 1721–1725
doi:10.1093/ndt/gfh890
Advance Access publication 14 June 2005

Nephrology Dialysis Transplantation

Brief Report

Clinicopathological and epidemiological analysis of amyloidosis in Turkish patients

Cüneyt Ensari[1], Arzu Ensari[2], Necmiye Tümer[3] and Ergun Ertug[4]

[1]Atatürk Hospital Nephrology and Dialysis Unit, [2]Department of Pathology, [3]Department of Pediatric Nephrology and [4]Department of Nephrology, University of Ankara Medical School, Ankara, Turkey

Downloaded from https://academic.oup.com/ndt/article/20/8/1721/1922151 by Ankara University Library (ANK) user on 11 December 2021

Abstract

Background. The aim of the present study was to assess the correlation of immunohistochemical subtyping with clinical diagnosis in order to achieve useful epidemiological data regarding amyloidosis in Turkish patients.

Method. We carried out immunohistochemical studies on 128 biopsies from various sites of 111 patients with biopsy-proven amyloidosis and, based on the results, classified the patients. We assessed the correlation of immunohistochemical subtype with clinical diagnosis and gathered epidemiological data.

Results. The sites most biopsied were kidney and rectum, followed by the testicle, liver, small intestine and bladder. Amyloid deposits showed positive staining with a single antibody in 120 biopsies. Pure amyloid A (AA) positivity was seen in 113 biopsies; six biopsies were positive for amyloid λ (AL) and one for β_2-microglobulin (β2MG). The clinical diagnoses of 81 patients (98 biopsies all AA positive) were suggestive of familial Mediterranean fever (FMF). Also AA positive were eight patients with tuberculosis, seven patients with rheumatoid arthritis, four patients with bronchiectasis and one patient with Crohn's disease. The biopsies from seven patients clinically suspected to have plasma cell dyscrasias were AL positive. One patient undergoing haemodialysis was β2MG positive. Two patients without definite diagnoses showed double or triple positivity, which could not be interpreted and classified immunohistochemically.

Conclusions. This study demonstrates that the predominant association of AA amyloidosis is with FMF. It also suggests that the routine immunohistochemical study of patients with amyloidosis who are of certain ethnic backgrounds suffices for classifying the subtype of amyloid fibril protein and the related disease.

Keywords: amyloidosis; classification; epidemiology; immunohistochemistry

Introduction

Amyloidosis is characterized by the generalized or localized extracellular deposition of amyloid, a proteinaceous fibrillar material, in various tissues and organs. In the past, the presence of amyloid was established by staining tissue with Congo red and inspecting it under polarized light—without attempting to determine the subtypes of the deposits. The application of immunohistochemical methods, however, helps to identify the major protein component of an amyloid fibril, which allows determination with certainty of the disease or diseases causing amyloidosis. Although many types of fibril proteins have been defined, amyloidosis is mainly classified into primary (AL) or secondary (AA) groups according to the biochemical nature of the protein forming the fibril. However, the aetiology of amyloidosis comprises a heterogeneous group of diseases. Previously published reports on amyloidosis, including autopsy series, strongly suggested that the prevalence and types of amyloidosis and related diseases could vary significantly in different patient populations [1–4].

Despite the widespread use of immunohistochemical methods to classify cases of amyloidosis, difficulties frequently arise in the interpretation of the results obtained [5,6]. There is also not much information in the literature about how accurately immunohistochemical subtypes relate to clinical diagnoses [5–7], though classifying primary amyloidosis by immunohistochemistry has been reported to be more difficult compared with secondary amyloidosis [8].

Correspondence and offprint requests to: Cüneyt Ensari, MD, Konutkent II, B4 Blok, C-2, Çayyolu 06530, Ankara, Turkey.
E-mail: aensari@ato.org.tr

Published by Oxford University Press on behalf of ERA-EDTA [2005]

The aims of the present study, therefore, were to classify amyloidosis by immunohistochemically subtyping amyloid fibril proteins and to assess the correlation of immunohistochemical information with clinical diagnoses, in order to retrieve useful epidemiological data.

Subjects and methods

We included in this study 128 amyloid-positive biopsies from 111 patients whose paraffin blocks were available for immunohistochemical analysis. The histopathological diagnosis was made on Congo red-stained specimens viewed under polarized light, and the subtyping of amyloidosis was made by immunohistochemical analysis. The mean patient age of the cohort (70 male, 41 female) was 34.2 years (range 6–65).

Paraffin sections, 4 μm thick, were taken from the 128 biopsy specimens, which were from the following locations: 69 renal, 36 rectal, 15 testicular, five hepatic, two small intestinal and one bladder biopsy. We selected an antibody panel that included monoclonal antibodies to amyloid A (1:100), λ (1:50) and κ (1:50) light chains, transthyretin (1:100) and β_2-microglobulin (β2MG) (1:50) (DAKO) (but we left out the extremely rare forms of amyloid fibrils). An avidin–biotin/peroxidase technique was employed, which used AEC as chromogen with Mayer's haematoxylin counterstaining. The interpretation of immunohistochemically stained slides was done by a pathologist who was unaware of the clinical conditions of the biopsied patients.

Positive controls were prepared separately for each antibody: rectal biopsy from a patient with proven familial Mediterranean fever (FMF)-related amyloidosis for amyloid A (AA), reactive lymph node for amyloid λ (AL) and amyloid κ (AK), pancreatic tissue for amyloid transthyretin (ATTR) and normal skin for β2MG. Primary antisera were replaced by phosphate-buffered saline (PBS) and served as negative controls.

All patients' files were reviewed and their clinical histories were noted.

Results

The subtypes of amyloid deposits were established immunohistochemically in 128 biopsies taken from 111 patients. In 17 cases, biopsies had been taken from multiple organs: 10 patients had had renal and rectal biopsies; seven patients had had renal and testicular biopsies. The results of the immunohistochemical analysis of all biopsies are presented in Table 1. Amyloid deposits were positive to a single antibody in 120 biopsies (93.8%); eight biopsies (6.2%) stained positive to more than one antibody.

Positivity only for AA was seen in 113 of 128 biopsies (88.3%); six biopsies (4.7%) were positive only for AL and one biopsy (0.8%) only for β2MG. None of the biopsies stained positive exclusively for ATTR. Immune staining by more than one antibody was present in eight biopsies (6.2%). Among them, there were three biopsies expressing AA + ATTR positivity, three AA + AL positivity, one AA + β2MG positivity and one AA + AL + ATTR positivity. In six biopsies, however, only one of these antibodies demonstrated a homogenous and strong expression within the deposits. AA was strongly and more homogenously positive than other antibodies in five of these; one renal biopsy that showed dual positivity for AA and AL was interpreted as AL, since staining with this antibody was stronger and more widespread than with AA. In the one biopsy showing triple positivity and in another showing double staining with AA and AL, all antibodies were evenly distributed and expressed equally strongly, and thus were interpreted as 'unclassified'.

Correlation with clinical diagnosis

The search through the 111 patients' files revealed that 81 (73%) had family histories or clinical diagnoses of FMF, or both. From these 81 patients, 95 biopsies were taken, consisting of 48 renal, 36 rectal, 10 testicular, two hepatic, one small intestinal and one bladder; and all but three showed pure AA positivity (Figure 1a–d). The remaining three biopsies showed double staining with AA and with AL, ATTR or β2MG, wherein AA was the predominant antibody. All multiple organ biopsies that were consistently AA positive had been taken from patients with FMF. The renal biopsies of eight patients (7.2%) with diagnoses of tuberculosis (Tbc) and of the four patients (3.6%) with diagnoses of bronchiectasis were positive for AA only. Of the renal biopsies of seven patients (6.3%) with rheumatoid arthritis (RA), five showed pure AA positivity and two stained positive to both

Table 1. The results of immunohistochemical analysis

Biopsy site	No. of biopsies	AA	AL	AK	ATTR	β2MG	Mixed
Kidney	69	65					4 (2 AA + AL and 2 AA + ATTR)
Rectum	36	35					1 (AA + ATTR)
Testicle	15	10	4				1 (AA + AL + ATTR)
Liver	5	0	2			1	2 (1 AA + AL and 1 AA + β2MG)
Small intestine	2	2					
Bladder	1	1					
Total	128	113	6	0	0	1	8

Downloaded from https://academic.oup.com/ndt/article/20/8/1721/1922151 by Ankara University Library (ANK) user on 11 December 2021

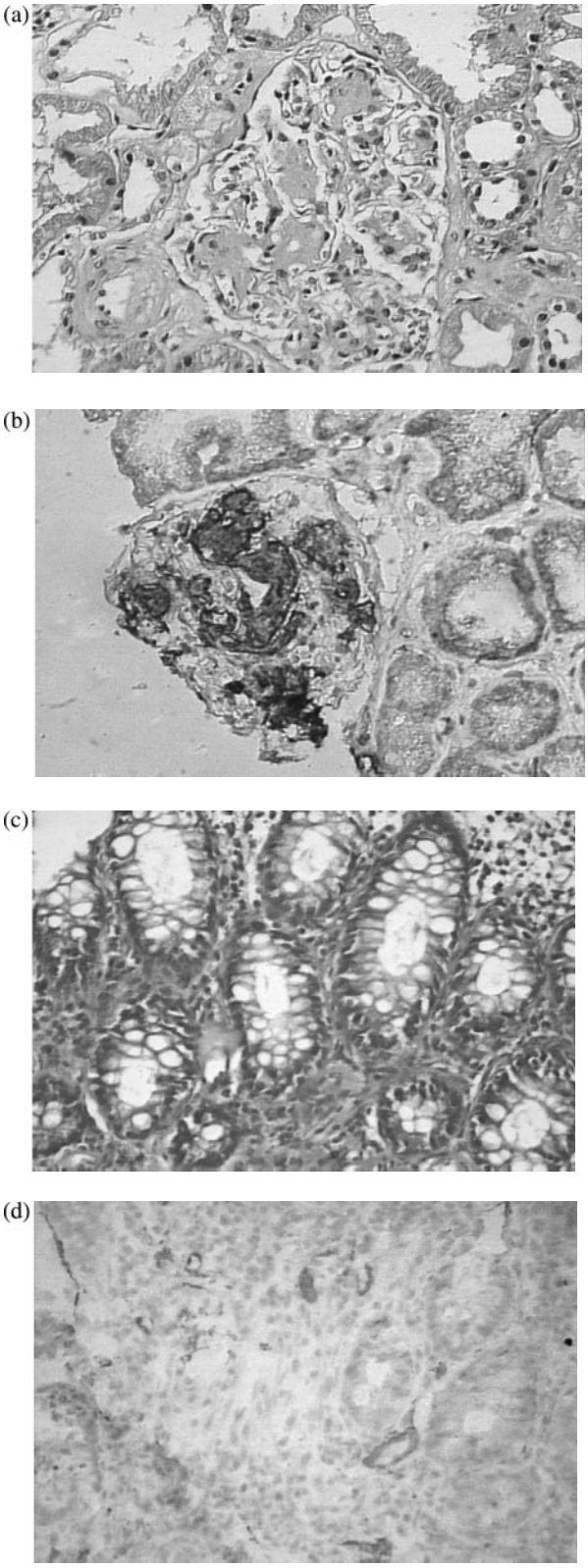


Fig. 1. (**a**) Renal biopsy showing one glomerulus with pale eosinophilic nodular expansions (H&E; ×400). (**b**) Renal biopsy showing one glomerulus with AA-positive amyloid deposition (anti-amyloid A; ×400). (**c**) Rectal biopsy showing thick-walled capillaries within the lamina propria (H&E; ×400). (**d**) Rectal biopsy showing capillaries with AA-positive amyloid deposition (anti-amyloid A; ×400).

AA and ATTR. One patient (0.9%) with Crohn's disease had a small intestinal biopsy showing AA positivity. Overall, there were 101 (90.9%) patients diagnosed as having AA amyloidosis. Seven (6.3%) patients were being tested for plasma cell dyscrasias; five of them were diagnosed to have multiple myeloma, and the remaining two had an increase in blood marrow plasma cells. Two of the seven patients had had liver and one had had renal biopsies; four had had testicular biopsy. Except for the renal biopsy that showed dual staining with AA and AL (but with AL predominance), all of the biopsies in this group showed pure AL positivity. All seven patients were thus classified as AL amyloidosis. One patient (0.9%) who had chronic renal failure and was on haemodialysis had a liver biopsy that stained positively only with β2MG. Two patients (1.8%) were classified as 'unknown' because their clinical histories and symptoms, and histopathological examinations of their biopsies did not suggest a definite diagnosis. One of them showed positivity for AA and AL in her renal biopsy, which had been performed to rule out nephrotic syndrome. The other, who had had a testicular biopsy done in the work-up of infertility, was positive to three antibodies (AA + AL + ATTR). Clinical diagnoses and biopsy sites are presented in Table 2. The correlation of immunohistochemical subtyping and clinical diagnosis is summarized in Table 3.

Discussion

The classification of amyloidosis relies upon both clinicopathological findings and the epidemiological aspects of the underlying diseases. Although many studies have confirmed the value of immunohistochemically classifying amyloid [4,6], the interpretation of the results of immunohistochemistry can confound the clinicopathological diagnosis of amyloidosis. Lachmann *et al.* [8] reported that immunohistochemistry usually gives definitive results that identify reactive (AA) amyloidosis, while it frequently is not diagnostic of AL amyloidosis, due either to abnormal fibril conformation of light chain fragments or to the background tissue staining caused by normal immunoglobulins. In a recent study, immunofluorescence yielded negative staining in 35.3% of patients with AL amyloidosis proven by renal biopsies [9]. Since the vast majority (90.9%) of patients in our study presented with AA amyloidosis, the technical limitations of immunohistochemistry are not evident in them, except in two (1.8%) in whom we could not document any clinical condition related to amyloidosis. In these two cases, the absence of strong homogenous staining with a single antibody made the immunohistochemical classification of amyloidosis impossible. This technical failure could have resulted from an over-fixation of tissue in formalin and high-temperature paraffin embedding to which the archival specimens used in the present study may have been subjected. In contrast to the reports from Western countries where the

Downloaded from https://academic.oup.com/ndt/article/20/8/1721/1922151 by Ankara University Library (ANK) user on 11 December 2021

Table 2. The relationship of clinical diagnosis to biopsy site

Disease	No. of cases	No. of biopsies	Kidney	Rectum	Testicle	Liver	Small intestine	Bladder
FMF	81	98	48	36	10	2	1	1
Tuberculosis	8	8	8					
Bronchiectasis	4	4	4					
RA	7	7	7					
Crohn's disease	1	1					1	
Plasma CD	7	7	1		4	2		
Haemodialysis	1	1				1		
Unknown	2	2	1		1			
Total	111	128	69	36	15	5	2	1

FMF = familial Mediterranean fever; RA = rheumatoid arthritis; CD = cell dyscrasias.

Table 3. The correlation of immunohistochemical subtypes of amyloidosis and clinical diagnosis

Disease	Patients		AA		AL		AK		ATTR		β2MG		Mixed	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
FMF	81	73	78 + 3	73									3 (1 AA+AL, 1 AA + ATTR, 1 AA + β2MG)	
Tuberculosis	8	7.2	8	7.2										
Bronchiectasis	4	3.6	4	3.6										
RA	7	6.3	5 + 2	6.3									2 (2 AA + ATTR)	
Crohn's disease	1	0.9	1	0.9										
Plasma CD	7	6.3			6 + 1	6.3							1 (1 AL + AA)	
Haemodialysis	1	0.9									1	0.9		
Unknown	2	1.8											2 (1 AA + AL, 1 AA + AL + ATTR)	1.8
Total	111	100	101	91.0	7	6.3	0	0	0	0	1	0.9	2	1.8

FMF = familial Mediterranean fever; RA = rheumatoid arthritis; CD = cell dyscrasias.

majority of cases are AL amyloidosis [8,9], we had few patients with AL amyloidosis—all accurately diagnosed by means of immunohistochemistry.

Amyloidosis may be diagnosed and classified in a sample from any affected tissue. Nevertheless, it is still important to define the most suitable site to biopsy and examine in order to detect early amyloid deposition. None of the studies performed so far [2,3] have established the preferred site to biopsy, although rectal biopsy seems to be most commonly used, mainly because of its easy accessibility [3,10]. Biopsies of another easily accessible site, abdominal fat, have been reported to be insensitive for diagnosing AA amyloidosis, while renal biopsy is becoming more popular in the diagnosis of amyloidosis—probably due to the increase in the numbers of well-trained nephrologists [4]. Our study also showed renal biopsy to be the most used intervention to diagnose amyloidosis. In practice, however, clinical presentation seems to be the main determinant of the biopsy site—a patient with proteinuria would undergo a renal rather than a rectal biopsy. Renal or rectal biopsies were common in our cohort, performed depending on the presenting clinical symptoms. Though a variety of anatomical sites have been used for the detection of amyloid [3,4], we found only a few reports mentioning testicular biopsy despite the high incidence of its use observed in our study [11,12]. One of these reports considers testicular biopsy valuable and more sensitive than rectal biopsy for diagnosing systemic amyloidosis [12].

In many developed countries, primary amyloidosis is the more common form of systemic amyloidosis [1–3], the incidence of secondary amyloidosis having been reduced in them with the virtual abolition of chronic infections. However, it is still encountered mainly in patients suffering from RA, followed in frequency by chronic infectious diseases and Crohn's disease; Tbc and FMF, on the other hand, were rarely associated with amyloidosis [5,13]. Secondary amyloidosis, however, is still the more common type of systemic amyloidosis in developing countries [1,3], where Tbc and other chronic infections are the leading causes of systemic amyloidosis, followed in frequency by RA, which is observed only in a small percentage of patients with amyloidosis [14,15]. In the Middle East, however, among patients of various ethnic origins, FMF leads the aetiologies of amyloidosis. This ethnically restricted genetic disorder mainly affects individuals of Mediterranean–Middle Eastern origins, such as Jews, Arabs, Armenians and Turks [16]. In a recent study, a lower incidence of amyloidosis was observed in a group of Italian patients with FMF [17]. Although the true figures for the country are not well documented, Turkey is known to have a high

Downloaded from https://academic.oup.com/ndt/article/20/8/1721/1922151 by Ankara University Library (ANK) user on 11 December 2021

prevalence of FMF and FMF-related amyloidosis [18,19].

Not surprisingly, secondary amyloidosis is the main form of systemic amyloidosis in our cohort, many individuals among which have a clinical diagnosis of FMF—followed by Tbc, RA and bronchiectasis in nearly equal frequencies. This finding seems concordant with previously reported findings from Turkey [19,20]. Although Tbc is still one of the major health problems in Turkey, especially in underdeveloped areas of the country, cases of Tbc and bronchiectasis are fewer in number in our cohort compared with numbers previously reported for this country [20]. Our study, however, was conducted in Ankara, the capital of Turkey, where better living standards and health care prevail than in various other regions of the country.

This retrospective study demonstrates that the population of patients with amyloidosis in Turkey is significantly different from those in Western countries. Our results indicate the predominant association of AA amyloidosis with FMF. They also suggest that the routine immunohistochemical analysis of amyloidosis in patients of certain ethnic backgrounds suffices for determining the subtype of amyloid fibril protein and the associated disease. However, detailed clinical information is mandatory; a definite diagnosis should not be made based solely on immunohistochemical observations. Moreover, since increasing immigration affects the ethnic structure of developed countries, to prevent long-term serious complications (such as amyloidosis) and to improve the quality of patients' lives, physicians need to be aware that certain diseases have higher prevalences in certain ethnic communities.

Conflict of interest statement. None declared.

References

1. Simms RW, Prout MN, Cohen AS. The epidemiology of AL and AA amyloidosis. *Baillieres Clin Rheumatol* 1994; 8: 627–634
2. Falk RH, Comenzo RL, Skinner M. The systemic amyloidoses. *N Engl J Med* 1997; 337: 898–909
3. Sipe JD, Cohen AS. Amyloidosis. In: Braunwald A, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL, eds. *Harrison's Principles of Internal Medicine*, 15th edn. McGraw-Hill, New York; 2001: 1974–1979
4. Röcken C, Sletten K. Amyloid in surgical pathology. *Virchows Arch* 2003; 443: 3–16
5. Hoshii Y, Takahashi M, Ishihara T, Uchino F. Immunohistochemical classification of 140 autopsy cases with systemic amyloidosis. *Am J Pathol* 1994; 4: 352–358
6. Röcken C, Schwotzer EB, Linke RP, Saeger W. The classification of amyloid deposits in clinicopathological practice. *Histopathology* 1996; 29: 325–335
7. Strege RJ, Saeger W, Linke RP. Diagnosis and immunohistochemical classification of systemic amyloidoses. *Virchows Arch* 1998; 433: 19–27
8. Lachmann HS, Booth DR, Booth SE *et al*. Misdiagnosis of hereditary amyloidosis as AL (primasy) amyloidosis. *N Engl J Med* 2002; 346: 1786–1791
9. Novak L, Cook WJ, Herrera GA, Sanders PW. AL-amyloidosis is underdiagnosed in renal biopsies. *Nephrol Dial Transplant* 2004; 19: 3050–3053
10. Lee JG, Wilson JA, Gottfried MR. Gastrointestinal manifestations of amyloidosis. *South Med J* 1994; 87: 243–247
11. Haimov-Kochman R, Prus D, Ben-Chetrit E. Azoospermia due to testicular amyloidosis in a patient with familial Mediterranean fever. *Hum Reprod* 2001; 16: 1218–1220
12. Ozdemir BH, Ozdemir OG, Ozdemir FN, Ozdemir AI. Value of testis biopsy in the diagnosis of systemic amyloidosis. *Urology* 2002; 59: 201–205
13. Joss N, McLaughlin K, Simpson K, Boulton-Jones JM. Presentation, survival and prognostic markers in AA amyloidosis. *Q J Med* 2000; 93: 535–542
14. Shah VB, Phatak AM, Shah BS, Kandalkar BM, Haldankar AR, Ranganathan S. Renal amyloidosis—a clinicopathologic study. *Indian J Pathol Microbiol* 1996; 39: 179–185
15. McAdam KP, Raynes JG, Alpers MP, Westermark GT, Westermark P. Amyloidosis: a global problem common in Papua New Guinea. *P N G Med J* 1996; 39: 284–296
16. Orbach H, Ben-Chetrit E. Familial Mediterranean fever—a review and update. *Minerva Med* 2001; 92: 421–430
17. Regina ML, Nucera G, Diaco M *et al*. Familial Mediterranean fever is no longer a rare disease in Italy. *Eur J Hum Genet* 2003; 11: 50–56
18. Yazici H, Özdogan H. Familial Mediterranean fever in Turkey. In: Sohar E, Gafni J, Pras M, eds. *Familial Mediterranean Fever*. Freund Publishing House Ltd, London; 1997: 66–71
19. Tuglular S, Yalcinkaya F, Paydas S *et al*. A retrospective analysis for etiology and clinical findings of 287 secondary amyloidosis cases in Turkey. *Nephrol Dial Transplant* 2002; 17: 2003–2005
20. Paydas S. Report on 59 patients with renal amyloidosis. *Int Urol Nephrol* 1999; 31: 619–631

Received for publication: 15.8.04
Accepted in revised form: 11.2.05

Downloaded from https://academic.oup.com/ndt/article/20/8/1721/1922151 by Ankara University Library (ANK) user on 11 December 2021