

The Utility Of Congo Red Stain And Cytokeratin Immunostain In The Detection Of Primary Cutaneous Amyloidosis

A , A

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Abstract

Background: Primary cutaneous amyloidosis includes several forms of localized amyloidosis characterized by superficial amyloid deposits occurring at or near the dermal–epidermal junction. This type of amyloidosis is derived from keratin presents in basal keratinocytes.

Aims: To compare the cytokeratin immunohistochemical stain and Congo red stain for the detection of primary cutaneous amyloidosis.

Methods: We examined 19 cases of cutaneous amyloidosis stained with hematoxylin–eosin, Congo red and the cytokeratin immunohistochemical stain.

Results: Most of the cases (16/19) examined were negative for Congo red stain, but all of the cases were positive for the cytokeratin immunohistochemical stain.

Conclusion: Congo red is not useful for the detection of cutaneous amyloidosis. The cytokeratin immunohistochemical stain is superior to Congo red and can detect very small amount of amyloid.

INTRODUCTION

Primary cutaneous amyloidosis can be easily diagnosed by a dermatopathologist on hematoxylin - eosin (H&E) stained slides. General pathologists can detect amyloid deposits as extracellular eosinophilic material, and Congo red staining is usually performed for confirmation. Primary cutaneous amyloidosis is derived from epidermal keratinocytes and is therefore positive for the cytokeratin immunohistochemical stain. In our experience, cases of primary cutaneous amyloidosis generally stain negative when using Congo red. In this study, we compare the cytokeratin immunohistochemical stain and Congo red stain for the detection of primary cutaneous amyloidosis.

MATERIALS AND METHODS

We retrieved the slides and blocks of 19 cases of primary cutaneous amyloidosis from our histopathology lab files at King Khalid University hospital. The cases included macular amyloidosis (16) and lichen amyloidosis (3). The clinical

features of the cases were described. In all cases, we examined hematoxylin-eosin stained slides and slides stained with Congo red and the cytokeratin 5/6 immunohistochemical stain (CK5/6) (Roche, Germany). Congo red stained slides were also examined under polarized light. A positive control of non cutaneous amyloidosis was used for comparison.

RESULTS

A total of 19 cases of primary cutaneous amyloidosis were identified. Of these, 16 were from women (84.2%) and 3 were from men (15.8%). The mean age was 39 years (range: 20-61 years). Among cases of macular amyloidosis (16/19), the back was the most common site of involvement (12/16); other sites included the leg (2 /16), forehead (1 /16) and arm (1 /16). The extensor surface of the leg was the site of involvement in the 3 cases of lichen amyloidosis.

Histopathologically, all cases showed a homogenous

esinophilic deposits within widened dermal papillae (Figure 1). However, the quantity of the deposits varied. In 2 of the cases, the deposits were very minimal and questionable. Congo red staining was positive in the positive control case (Figure 2), but it was negative in (16/19) cases (84.2%) (Figure 3A). Faint positivity was noted in (3/19) cases (15.8%) (Figure 3B). Only one case showed apple green birefringence under polarized light (Figure 3C).

Figure 1 A&B

Deposits of eosinophilic globules in the papillary dermis in cases of macular (A) and lichen amyloidosis (B). (Hematoxylin-eosin (H/E) ×200 magnification)

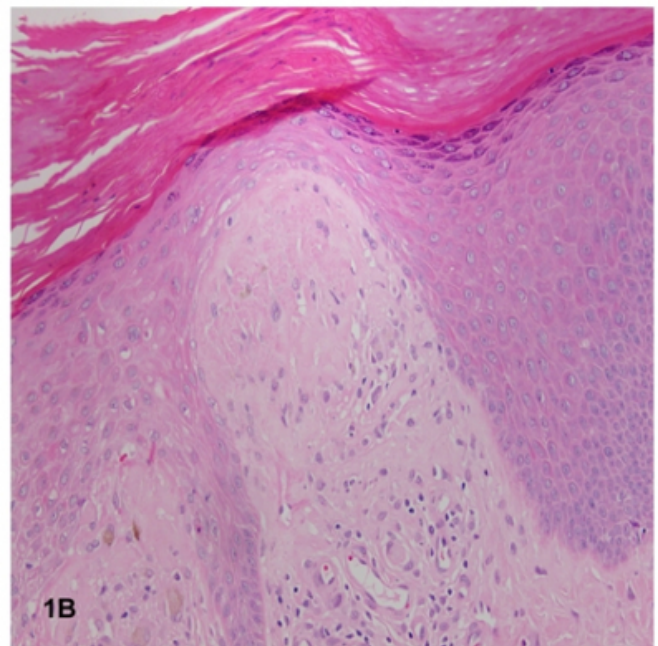
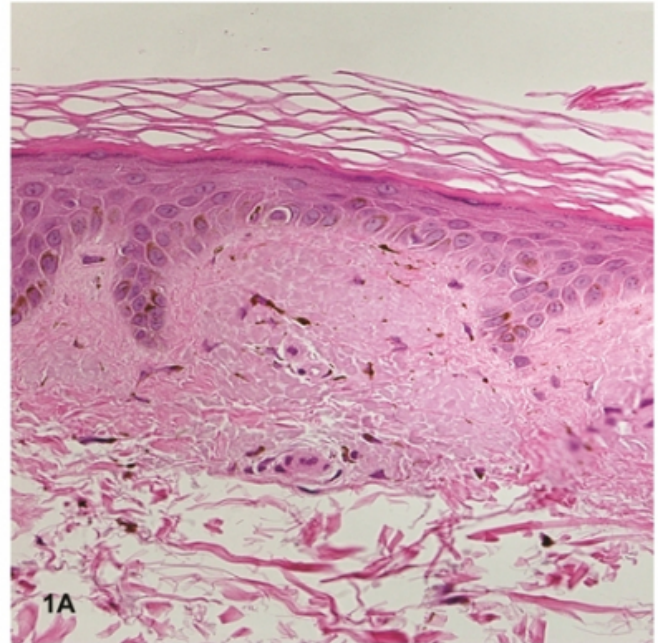


Figure 2 A&B

Congo red staining in the positive control case (A), and apple-green birefringence under polarized light (B). (Congo red stain $\times 200$ magnification)

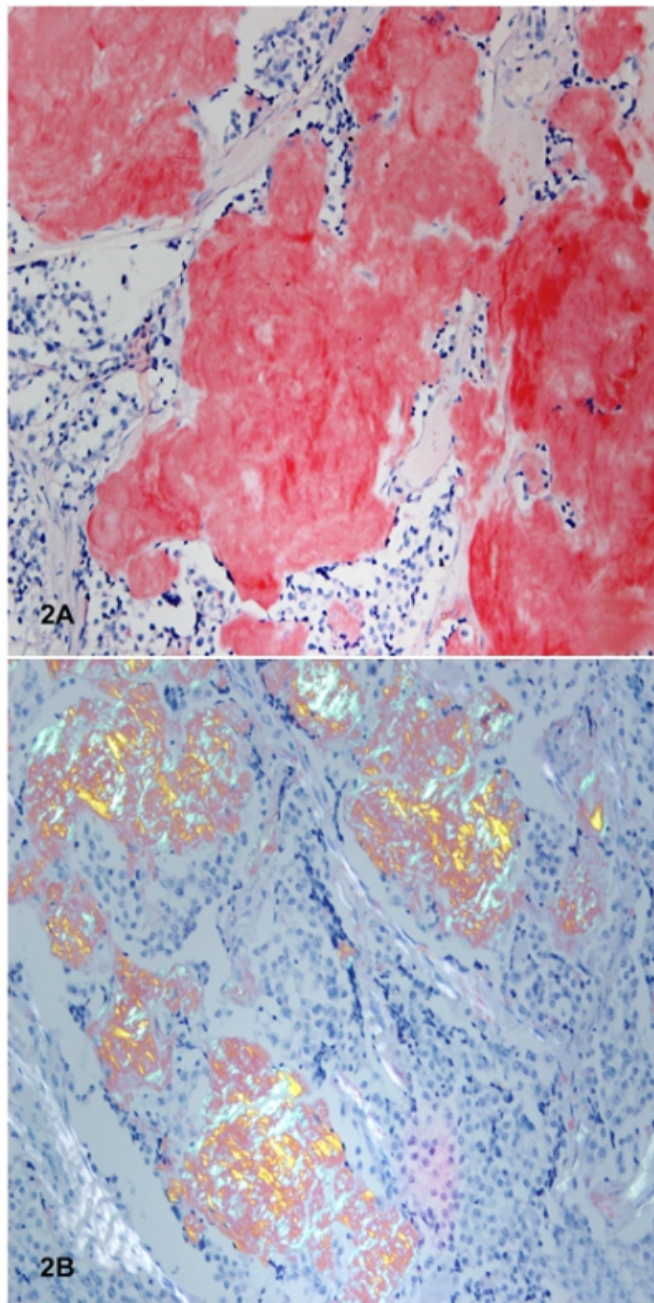


Figure 3 A&B

In most of the evaluated cases, Congo red stain is negative in the papillary dermal deposits (A). Congo red staining is faintly positive in a case of macular amyloidosis (B). Apple-green birefringence under polarized light of the same case (C). (Congo red stain $\times 200$ magnification)

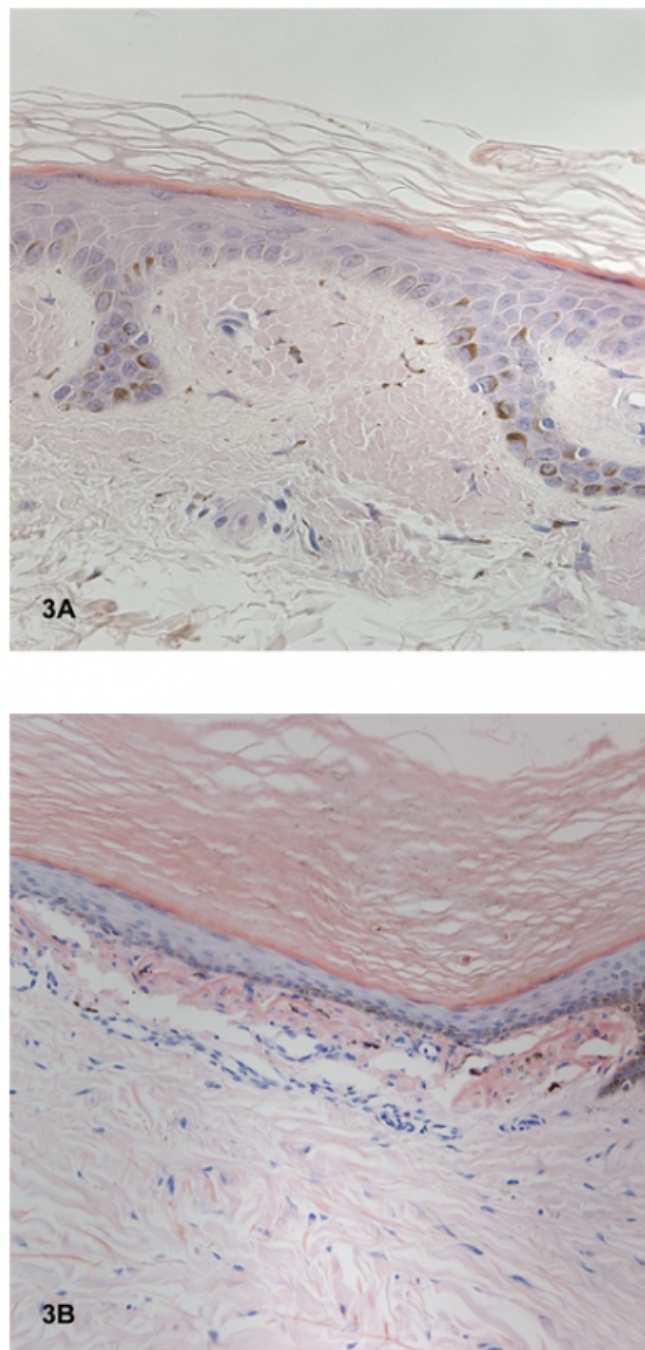
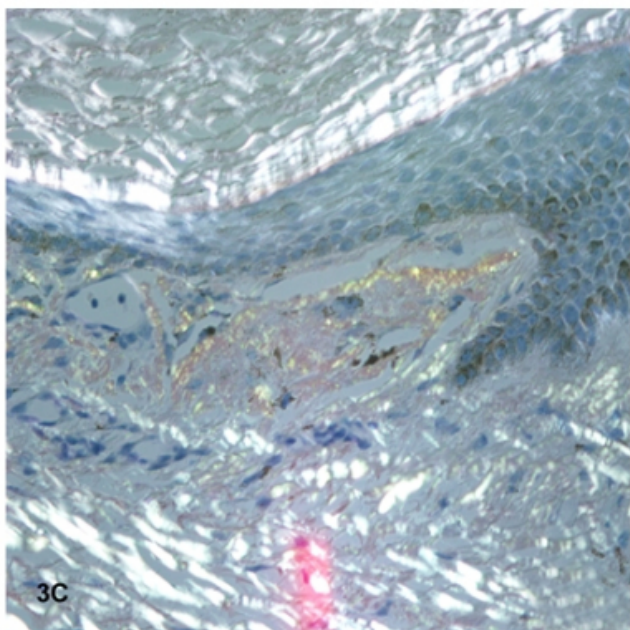


Figure 3 C



Immunohistochemical staining for CK5/6 was positive in all of the reviewed cases (19/19) (100%). This positivity was characteristically fainter than the overlying keratinocytes (Figure 4). A very minimal amount of amyloidosis was detected using the CK5/6 immunohistochemical stain (Figure 5).

Figure 4 A&B

Positive CK5/6 staining in the papillary dermal deposits of macular (A) and lichen amyloidosis (B). (Immunohistochemistry ×200 magnification)

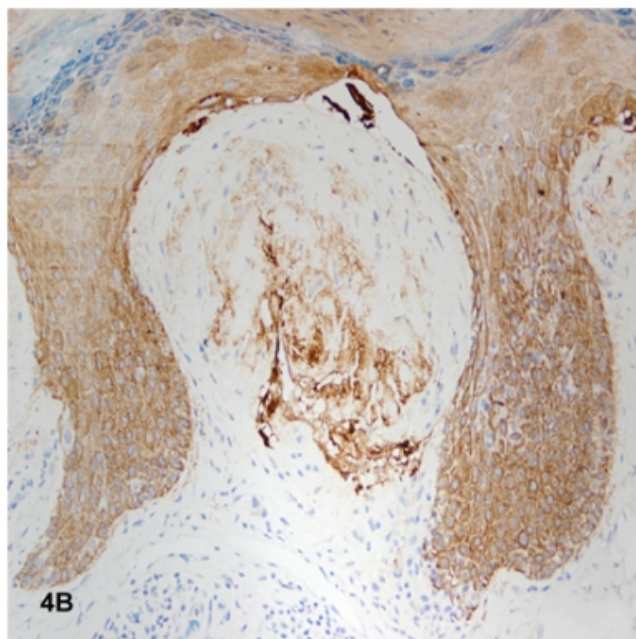
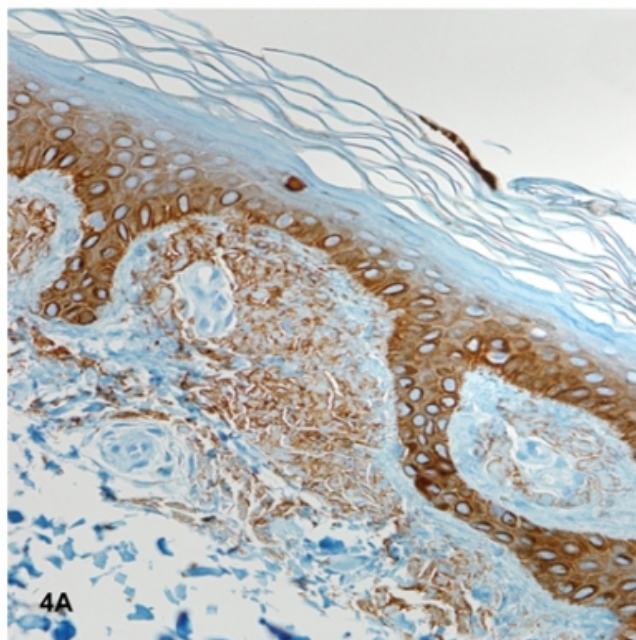
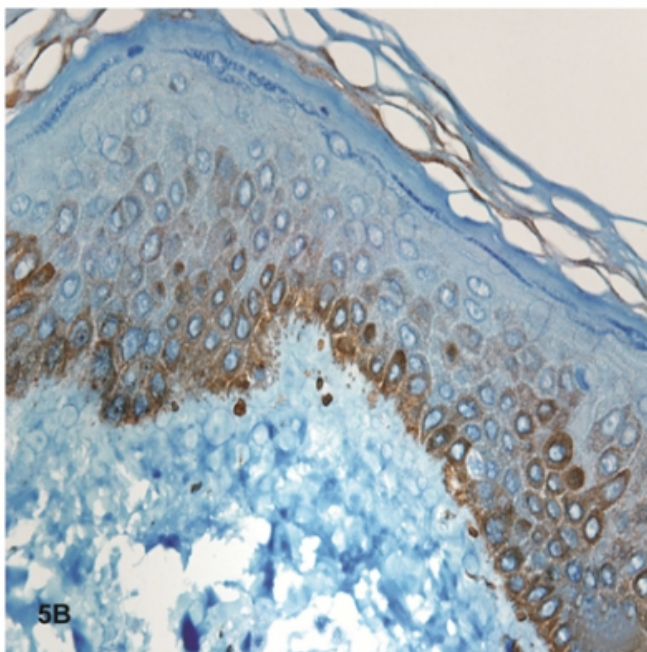
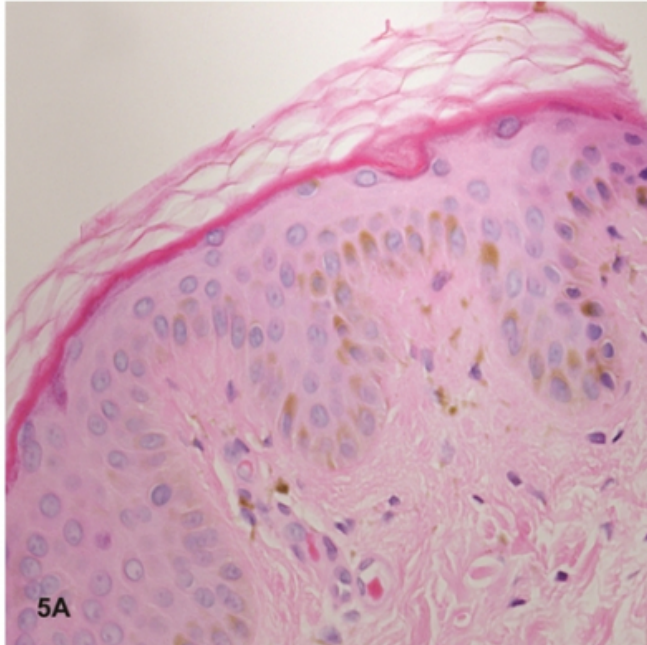


Figure 5 A&B

A very small amount of amyloidosis cannot be observed using H/E (A) but can be detected by the CK5/6 immunohistochemical stain (B). (Immunohistochemistry ×200 magnification)



DISCUSSION

Amyloidosis is a condition associated with a number of inherited and inflammatory disorders in which extracellular deposits of fibrillar proteins are responsible for tissue damage and functional compromise. These abnormal fibrils are produced by the aggregation of misfolded proteins

(which are soluble in their normal folded configuration). More than 20 (at last count, 23) different proteins can aggregate and form fibrils with the appearance of amyloid. Regardless of their derivation, all amyloid deposits are composed of nonbranching fibrils that are, 7.5 to 10 nm in diameter, and each is formed of β -sheet polypeptide chains that are wound together. The dye Congo red binds to these fibrils and produces a red-green dichroism (birefringence), which is commonly used to identify amyloid deposits in tissues¹.

Amyloidosis involving the skin is either systemic or cutaneous derived. In its systemic form, it results from plasma cell dyscrasia with the production of monoclonal light chains². Histologic examination reveals faintly eosinophilic, amorphous, often fissured masses of amyloid deposited in the dermis and the subcutaneous tissues. Quite frequently, accumulations of amyloid are deposited close to the epidermis. The involvement of blood vessels is responsible for the frequent presence of extravasated erythrocytes³. Primary localized cutaneous amyloidosis presents in either a lichen or a macular form. They are best considered as different manifestations of the same disease process. Lichen amyloidosis is characterized by closely set, discrete, brown-red papules that often show some scaling and are most commonly located on the legs, especially the shins. Macular amyloidosis is characterized by pruritic macules showing pigmentation with a reticulated or rippled pattern⁴.

By light microscopy, amyloid appears amorphous, eosinophilic, and hyaline. Characteristic staining qualities distinguish it from other glassy, pink substances. The Congo-red stain results in a brick-red staining reaction and apple-green birefringence, and amyloid stains metachromatically with crystal violet and methyl violet stains⁵. A study performed by Wenson et al revealed that nine out of nine cases of amyloidosis of the external ear stained positively with Congo red with birefringence (9/9)⁶. However, we believe that their Congo red image is negative not positive, and that the polarizing structures noted are the collagen bundles, not amyloid deposits. Another study by A. Fernandez-Flores concluded that eight out of ten cases of cutaneous amyloidosis were positive for Congo red but no images were shown⁷. In both studies, the cytokeratin immunohistochemical stain was positive in all cases examined.

We and other dermatopathologists have observed that Congo

red is negative in cutaneous derived amyloidosis, but no studies have been performed to confirm this observation. In our study, we examined 19 cases of cutaneous amyloidosis, and the majority stained negative for Congo red with the positive control stain on the same slide. Cytokeratin 5/6, a high molecular weight cytokeratin, was positive in all cases examined.

In conclusion, cutaneous amyloidosis (macular and lichen types) stains negative for Congo red, and positive for high molecular weight cytokeratin. This finding will be valuable for general pathologist as negative Congo red staining does not exclude macular or lichen amyloidosis. High molecular weight cytokeratin immunohistochemical staining must be performed to confirm this diagnosis.

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