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THE UNMASKING OF AMYLOID

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Abstract:
Introduction: Amyloidosis was first described by Virchow. Early detection of amyloidosis is essential as there are effective treatments for the primary causes. The progression and severity of the disease can be halted with newer treatments. The cause of amyloidosis often remains elusive. Amyloidosis is usually incidentally detected in biopsies.
Aim: We aim to evaluate the clinical features and histomorphological findings and document the occurrence of rare presentations of amyloidosis.
Materials and methods: The cases documented as organ amyloidosis were collected from the casefiles and records. The amyloidosis associated with skin and neoplastic lesions are excluded. The histopathological slides and congored stained slides were reviewed with analysis of the microscopic features. Polarizing microscopy was performed to confirm the diagnosis.
Results: These included a total of 14 cases. Three cases of larynx, two each of peripheral nerves and abdominal fat, along with one each of heart, gastric, duodenum, colon, liver, pancreas and spleen are included in the study. The age range was 35 years to 84 years. The median age was 62 years. Male predominance (71%) was noted. Most common organ involved in the present series was larynx.
Conclusion: This is a review of the rare cases of amyloidosis which had unusual presentation and histopathology finally revealed the diagnosis. High suspicion of amyloidosis can help in early diagnosis. Careful histomorphological analysis recognizes the patterns in which this disease entity can present, so that early lesions can also be subjected to special staining with congo red and polarized microscopy for early treatment and to minimize complications and morbidity.

Introduction:

Amyloidosis was first described by Virchow. During his autopsies the lardaceous appearance of spleen or liver was coupled with a very interesting finding. The organs turned mahogany brown after addition of iodine. He felt that this was due to presence of starch like material in the organs and he termed it as amyloidosis. Many years later the detailed structure of amyloid was determined. Amyloid deposits were found to contain misfolded proteins in beta pleated sheets without tertiary structure. These tissues also show positive staining with congo red and apple green birefringence on polarizing microscopy. [1] There are various causes for amyloidosis and early detection is essential as there are effective treatments for the primary causes. The progression and severity of the disease can be halted with newer treatments.

AIM:

We aim to evaluate the clinical features and histomorphological findings and document the occurrence of rare presentations of amyloidosis.

The objective was to review the cases of amyloidosis reported in our department during the time period of 2015 to 2020.

MATERIALS AND METHODS:

Patient records were reviewed for amyloidosis cases in the Department of Pathology, Government Medical College (RIMS), Kadapa for the period of 2015 to 2020.

The cases documented as organ amyloidosis were collected from the casefiles and records. These included a total of 14 cases.

Three cases of larynx, two each of peripheral nerves and abdominal fat, along with one each of heart, gastric, duodenum, colon, liver, pancreas and spleen are included in the study. The amyloidosis associated with skin and neoplastic lesions are excluded. The histopathological slides and congo red stained slides were reviewed with analysis of the microscopic features. Polarising microscopy was performed to confirm the diagnosis.

RESULTS:

The age range of these organ amyloidosis was from 35 years to 84 years. The median age was 62 years. Of these 14 cases, predominance of males and four were females.

The laryngeal cases were all in females. The other case of female gender was amyloidosis of the nerves.

Clinical manifestations were variable with laryngeal amyloidosis presenting with hoarseness of voice. The peripheral nerve involvement produced sensational loss with tingling, numbness and loss of power. The cardiac case was secondary to multiple myeloma with sinus tachycardia. The gastric case presented with low gastric emptying and gastric erosions. The duodenal case presented with constipation. On endoscopy there was dilation of duodenum. The colon amyloidosis presented as bleeding per rectum. The endoscopy showed ulcers and erosions. The liver amyloidosis presented with hepatomegaly with gynecomastia. The pancreatic islet cell amyloidosis was detected at autopsy. The splenic amyloidosis presented as spontaneous splenic rupture in a known case of idiopathic thrombocytopenic purpura who was treated with steroids. The bone marrow examination was within normal limits. He presented with an ecchymosis patch on the abdomen with syncope. Ultrasound and CT scan revealed rupture of spleen with hematoma formation. Splenectomy was performed. Grossly the spleen appeared waxy on cut surface. Microscopically diffuse deposits of pink material were seen throughout with obliteration of splenic parenchyma. These deposits were proven to be amyloid by congo red staining.

The patients with amyloid deposits in abdominal fat presented with chronic diarrhea. In two male patients aged 48 and 62 years amyloid was detected on abdominal fat biopsy. The younger patient presented with chronic diarrhea and hypertrophic cardiomyopathy. Sections of abdominal and arm fat biopsy revealed lobules of mature adipose tissue, intervening collagenous septae and congested blood vessels. The congo red stain was positive for amyloid in the subendothelium of the blood vessels.

Amyloid neuropathy cases occurred in a 63 year old male and a 37 year old female. They both had paresthesia, weakness of limbs and areflexia. The 63 year old patient was suspected to have vasculitis with ischemic polyneuropathy. On histopathological examination asymmetric moderate to severe degree loss of small more than large myelinated nerve fibre loss. Bands of Bungner endoneurial fibrosis noted. Amorphous substance is seen deposited around the endoneurial capillaries. Perineurium is unremarkable. Epineurium shows sparse perivascular lymphocytic infiltrate. Kulchitsky Pal stain showed asymmetric moderate to severe degree loss of small more than large myelinated nerve fibre loss, and few thinly myelinated nerve fibres. Congo red stain was positive for amyloid. The 37 year old patient’s biopsy also showed loss of large and small myelinated fibres with amyloid deposits. The amyloid was recognized on deeper sections.

The results are tabulated below:

Table 1: Clinicopathological features of amyloid cases

Number of patients	Diagnosis	Age and sex	Presenting symptoms
3	Laryngeal amyloidosis	53,45,35/F	Hoarseness of voice with a nodule on the vocal cords
2	Amyloid neuropathy	63 /M and 37 /F	Paresthesia, weakness and areflexia.
2	Diagnostic abdominal fat biopsy	48,62/M	Chronic diarrhea.
1	Myocardial amyloid deposition(secondary to multiple myeloma)	70 /M	Sinus tachycardia
3	GIT: stomach,duodenum and colon	61,65 66/ M	Constipation, bleeding per rectum, ulcers and erosions.
1	Liver	35/M	Hepatomegaly, gynaecomastia
1	Pancreas(islet amyloidosis)	84/M	Detected at autopsy
1	Spleen	45/M	Spontaneous splenic rupture

DISCUSSION:

First description of amyloidosis comes from Rokitansky who described it in 1842. [2] The term 'amyloid' was coined by Virchow, in 1854, which is a greek word for starch. This was named so after violet staining with iodine and sulphuric acid. Amyloid refers to diverse extracellular protein deposits with common morphological properties, affinities for certain dyes and apple green birefringence under polarized light. Amyloidosis comprises the clinical disorders caused by amyloid deposits ranging from minor deposits which are incidentally detected to florid forms where end organ damage and dysfunction manifests.

Clinical amyloidosis is classified by the nature of the fibril protein. More than 25 such proteins have been described which are different and unrelated. It can be acquired or hereditary. They are deposited in vivo in localized or systemic fashion. In systemic amyloidosis, any tissue is involved and the disease is often fatal. Advances in the clinical therapy for the underlying cause has improved the prognosis dramatically. On the other hand, localized deposits of amyloid are confined to particular organ or tissue. Occasionally, localized disease can also be life threatening if it involves vital organs like heart. Other important disorders with localized amyloidosis include Alzheimer disease and type 2 diabetes mellitus.

Amyloid deposition occurs under various circumstances. It can be deposition of abnormally high levels of proteins which are normally present at low levels or presence of acquired or inherited variant protein with abnormal amyloidogenic structure. Some of the amyloid proteins commonly implicated include AL (amyloid light chain) type, AA (amyloid associated) type, ATTR (amyloid transthyretin), beta-2 microglobulin and SAP (serum amyloid P component). [3]

In the present review, the median age of patients was 62 years (35 to 84 years). Male predominance (71%) was noted among the studied patients. About 60% of the patients affected by this disease are men and 70% are between 50 to 70 years old. Among all the hospital admissions, less than 1% manifest with severe amyloidosis. [4]

Most common organ involved in the present series was larynx. The median age of presentation was 45 years in only female sex. The presenting complaints were hoarseness of voice. The laryngoscopy showed masses in the false cord and true cord in two cases. The other case had a subglottic growth mimicking neoplasm. The mucosa of the vocal cords showed smooth surface with nodular bulge.

The most frequent site of involvement by amyloidosis in the upper airway is the larynx. In a study by McAlpine etal of upper airway amyloidosis they have found 75% occurrence in the larynx. [5] Amyloidomas in the region of the head and neck is rare, but most commonly involves the

supraglottic and subglottic larynx. Localized amyloidosis restricted to the larynx is rare, however, it should prompt a search for other sites of involvement or circulating light chains, in which case a diagnosis of systemic amyloidosis can be made. In the present study the larynx manifested with amyloidoma in two cases. Systemic disease was not recorded. Amyloidosis is characterized by extracellular deposition of homogeneous eosinophilic material, often with a "cracked" appearance. Similar histologial features were documented in our case. Lymphoplasmacytic inflammation is occasionally present, but usually sparse. The amyloid classically stains positively with Congo red and displays apple-green birefringence under polarized light. [6]

Yoitakis et al reported a case of young 23 year old female presenting with hoarseness of voice and dysphagia due to localized laryngeal amyloidosis. [7] Daudia reported a case of 47 year old female with change in voice who was diagnosed as subglottic amyloidosis. She progressed rapidly with further change in voice and stridor even after the first resection. Tracheostomy was needed in this case. [8]

We had case of 45 year old male who was a known case of ITP on steroid treatment. In a study by Aubrey-Bassler etal, out of 276 cases of spontaneous splenic rupture 24 were because of amyloidosis. Amyloidosis of the spleen, though rare is a known cause for spontaneous splenic rupture. Renzulli etal studied causes of atraumatic splenic rupture. They have found an incidence of 3.8% of amyloidosis out of 928 causes. Oran et al reported a high incidence of splenic involvement in AL amyloidosis along with liver involvement. They found that splenic involvement could lead to spontaneous splenic rupture. [9,10,11]

Gastric amyloid was seen in a 66yr old male. He had delayed gastric emptying with erosions. Histological study of the antral biopsy revealed homogenous, acellular, eosinophilic deposits in the lamina propria and perivascular areas. This was confirmed by polarized microscopy of congo red stain. The endoscopy of stomach showed mucosal erosions, ulcers or tumoral mass. The histology often has extracellular homogenous deposits in the lamina propria, submucosal layer, muscularis propria and around nerves and blood vessels. The chemical type of amyloid determines the dominant clinical features. Two mechanisms are responsible for intestinal motor dysfunction which is amyloid induced neural damage and the other is amyloid deposits in the gastrointestinal smooth muscle. The tissue processing artefact produces slit like spaces due to cracking. The AA deposits are often in the lamina propria while AL deposits are often in the full thickness of the wall. Connective tissue disorders like scleroderma often result in fibrosis of the deeper layers which may be mistaken for amyloid. Atherosclerosis of vessels with hyalinization may be mistaken for amyloid which is often stained blue with Masson trichrome stain. [12]

A 65 year male presented with constipation, anemia and gastric discomfort. Endoscopy revealed nodular mucosa of the duodenum with erosions. Histology showed atrophic duodenal mucosa with homogenous extra-cellular deposits in the lamina propria. These were confirmed as amyloid deposits. Gastrointestinal tract is a frequent site for amyloid in patients with systemic or isolated disease. Malabsorption, diarrhea, weight loss, pain abdomen, protein losing enteropathy, ischemia, perforation or motility disorders are the common manifestations. AA deposits has fine granular elevations in the mucosa which reflect expansion of the lamina propria due to amyloid deposits. Stomach and rectum are better sites for biopsy than the small intestine since they are more likely to be involved. The type of amyloid chain could be distinguished by histologic appearance as documented by Ichimata et al. The deposition of AH type was fine and focal. AL type showed heavy diffuse deposits. AH type was deposited in the villi and AL type was mainly seen in the muscularis mucosae and submucosa in their study. [13]

Colonic amyloidosis was seen in a 61 year male who presented with melena and weight loss. Colonic mucosa was edematous with erosions and ulcerated at endoscopy. Gastrointestinal amyloidosis generally presents as a motility disorder, ulcers, and areas of hemorrhage or pseudotumors. The most common form of large intestinal amyloid is secondary to AL followed by AA. Occasionally colorectum has deposits of alpha-2 microglobulin presenting with amyloid deposits in the wall of the intestine along with perivascular deposits. Rectal biopsies are commonly

obtained in patients usually suspected to have amyloidosis. However it is necessary to have the presence of submucosal vasculature in these sections. [14]

Gastrointestinal amyloidosis was extensively analysed by Said et al in 79 patients. The median age of presentation was 62 years, similar to our study. The other gastrointestinal sites apart from stomach were small intestine (89%), colon(81%) and esophagus(31%) were also affected. The histopathological study was further supplemented by ancillary tests. Source of amyloid was light chain immunoglobulin in 67%, transthyretin in 18%, SAA in 9% and apolipoprotein in 3%.[15]

In a study of islet amyloidosis of type 2 diabetes mellitus patients 10% aged 50-60 years, 30% of individuals aged 60-70 years and above 50% of individuals aged above 70 years were found to have amyloid deposits in the islets. He has also documented that in 4-23% of non-diabetic patients also show islet amyloidosis. [16]

In a study examination of minimum three abdominal. Fat aspirate slides stained by congored was shown to give up to 100% specificity and up to 90% sensitivity for detection of amyloid. A grading of deposits can also be done from 0(negative) to 4+ according as proposed by the authors. [17]

Amyloid neuropathy can be a consequence of familial or acquired diseases. The transthyretin gene mutations are the most common form of inherited amyloid neuropathy. AL amyloidosis is the most common form of acquired form. They present as sensorimotor polyneuropathy or autonomic neuropathy. The peripheral neuropathy manifests late because of delay in diagnosis due to considering diabetic or autoimmune disorders first. [18] Amyloidosis of liver may present with hepatosplenomegaly, portal hypertension and jaundice. Primary amyloidosis is associated with the deposition of amyloidogenic light chain protein in various lymphoproliferative diseases such as plasma cell dyscrasia, myeloma, B-cell lymphomas and Waldenstrom's macroglbulinemia. The amyloid deposits are preferentially within vessel walls, along the sinusoids, in the space of Disse or in portal tracts. Primary amyloidosis involving the liver consists of the deposition in the portal areas, sometimes restricted only to the vessel. In contrast, secondary amyloidosis consists of the deposition in the space of Disse with the hepatocytes shrunken and embedded in the thick amorphous material. [19]

The availability of new technologies has improved diagnosis and enabled accurate fibril typing and better risk stratification. Outcomes have improved, at least in AL amyloidosis, and several novel therapies are on the horizon for various types of amyloidosis, including antibody-based therapy and RNA inhibition strategies. However, management of patients with advanced organ involvement at diagnosis remains a major challenge, with nearly a third of all patients with AL amyloidosis still dying within a few months of diagnosis. Early diagnosis of amyloidosis remains an elusive goal that requires education of both physicians and patients. [20]

Cardiac amyloidosis is a manifestation of amyloidosis which is a multisystem disorder. This is difficult to diagnose, rare disease which eventually leads to the mortality. Diagnosis requires a high index of clinical suspicion along with echocardiographic clues like, diastolic dysfunction, bi-atrial enlargement and ventricular thickening. Treatment is mainly supportive with disappointing outcomes. [21]

Amyloid typing can also be performed by mass spectrometry or laser microdissection, immunohistochemistry, immunofluorescence, and/or genetic testing

CONCLUSION:

This is a review of the rare cases of amyloidosis which had unusual presentation and histopathology finally revealed the diagnosis. Minute deposits of amyloid can be easily missed on routine histology. This review enables to recognize the unique nature of the disease. High suspicion of amyloidosis can help in early diagnosis. Careful histomorphological analysis recognizes the patterns in which this disease entity can present, so that early lesions can also be subjected to special staining with congo red and polarized microscopy for early treatment and to minimize complications and morbidity associated with this rare disease.

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