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AMYLOID GOITER: A DIAGNOSTIC AND THERAPEUTIC CHALLENGE

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Amyloid goiter is a very rare clinical entity that has to be distinguished from the more common types of goiter. In the present study, we report seven cases of amyloid goiter, admitted to the Head and Neck Surgery Unit, Alexandria Main University Hospital, during a three-year period (1998-2000). Six patients were female and one male. Their ages ranged between 26 and 55 years with a mean age of 38.1 years. Four patients had dialysis-related amyloid goiter (DRAG), and three had secondary (reactionary) amyloid goiter. Rapid and progressive enlargement of the gland was a striking feature. Clinically, all patients were euthyroid, except one was hyperthyroid. Serum levels of T3, T4 and TSH were not characteristic. Anti-microsomal and anti-thyroglobulin antibodies were negative in all patients. Fine needle aspiration cytology (FNAC), done for six patients, aided in the pre-operative diagnosis in three (50%). Final diagnosis rested on a positive Congo red staining with characteristic apple-green birefringence under polarized light, and demonstration of the characteristic amyloid fibrils by transmission electron microscopic examination. Immunohistochemical staining showed the amyloid material to be of the beta-2 microglobulin (β-2 M) type in the four patients with DRAG, and of the AA type in the remaining three patients with reactionary amyloidosis. Total thyroidectomy was performed for all patients due to the diffuse nature of the gland, to alleviate pressure symptoms, and to exclude malignancy. Early evaluation of patients with rapidly progressive enlargement of the thyroid gland, with the possibility of amyloid goiter in mind, would allow proper diagnosis, and prompt and adequate surgical treatment, thus decreasing the duration of morbidity.

INTRODUCTION

Dialysis-related amyloidosis (DRA) is a disorder which commonly develops in long-term dialysis patients. The clinical manifestations include carpal tunnel syndrome, spondylarthropathy, pathologic fractures, and swollen painful joints, especially in the form of scapulohumeral periarthritis[1,3]. These symptoms are a reflection of the preferential amyloid deposition in the bones, joints and synovium[4]. However, DRA is a systemic disease since deposition also occurs in subcutaneous tissues and skin, and less frequently in rectal mucosa, liver, spleen and blood vessels[5,7].

In the present communication, we report four patients with dialysis-related amyloid goiter (DRAG), their clinical features, diagnosis and management. In addition, we report three more cases of reactionary amyloid goiter secondary to chronic inflammatory disease.

PATIENTS AND METHODS

Population: The present study included seven patients with amyloid goiter admitted to the Head and Neck Surgery Unit, Main Alexandria University Hospital, during the years 1998-2000. Six patients were female and one male. Their ages ranged between 26 and 55 years, with a mean age of 38.1 years. All patients were subjected to careful history-taking, and thorough clinical examination that was geared to thyroid dysfunction manifestations.

Biochemical Studies: Laboratory tests included routine thyroid function tests (T3, T4 and TSH serum levels), as well as anti-thyroglobulin[8] and anti-microsomal antibodies[9].

Pathological Studies: Six patients (all except the first) were subjected to fine needle aspiration cytology pre-operatively, and all patients to histological examination, post-operatively .Biopsies of the thyroidectomy specimens submitted were fixed in 10% formalin, embedded in paraffin, and cut at 5 μ-thick sections, and stained with the following techniques:

1. Hematoxylin and eosin (H&E) for histologic diagnosis.
2. Congo red for characterization of suspected hyaline material, whether amyloid or not[10].
3. Visualization by fluorescence microscopy for further proof of the amyloid nature of the Congo red positive material by demonstration of the positive autofluorescence[11].
4. Immunohistochemical staining for identification of the amyloid type[12]. Sections from the goiterous specimens were stained immunohistochemically with the anti-β2 microglobulin polyclonal antibody (Zymed , CA) using a peroxidase-streptavidin kit (Zymed, CA) for DRAG, and anti-SAA polyclonal antibody for type AA amyloid goiter. Briefly, representative sections from the specimen were deparaffinized, rehydrated in ascending grades of alcohol and equilibrated in buffer. Subsequently, blocking of endogenous peroxidase and non-specific staining with 3% hydrogen peroxide in methanol and a protein-blocking agent were performed with intervening washes. Following protein blocking and without washing, the primary antibody was added (prediluted) with overnight incubation at 4°C. On day 2, the primary antibody was washed off and the secondary antibody added for 30 minutes. After washing, the label was added for another 30 minutes. Detection of positivity was performed using a visual detection system with DAB as the chromogen. All steps were performed in a humidity chamber at room temperature unless otherwise indicated. Positive cells exhibited diffuse brownish coloration in the interstitium.
5. Electron microscopic examination for demonstration of the characteristic amyloid fibrils: Freshly obtained representative specimens of goiterous tissue were immediately fixed in 3% glutaraldehyde in PBS overnight, postfixed in 1% Osmium tetraoxide and K Ferricyanide for one hour at 4°C, washed in PBS and dehydrated through a graded series of ethanol and embedded in araldite by inverting ampoules filled with the embedding substance over the tissues and incubated at 64°C for 48 hours. After polymerization of the resin, the ampoules containing the specimens were sectioned to obtain semithin sections and stained with toluidine blue. Thin sections were cut and mounted on unsupported 300 mesh grids. Sections were stained with uranyl acetate and lead citrate before being examined by the electron microscope (Joel 100X).

Surgical Procedure: All patients were subjected to total thyroidectomy due to widespread infiltration of the gland, to exclude malignancy, and to alleviate pressure symptoms.

RESULTS

Characteristic Features of the Study Population:

Characteristic features of the studied patients are summarized in Table 1. As may be seen, the underlying disease was chronic renal failure (CRF) in four patients who were on dialysis for a period that ranged between 4 and 7 years. One patient was transplanted 3 months before being enrolled in the study. The underlying disease in the remaining three patients was chronic osteomyelitis, tuberculosis, and subacute thyroiditis respectively.

Clinical Findings:

The clinical presentation in six of the seven patients included a non-tender, rapidly enlarging thyroid gland, firm or elastic in consistency and diffuse in appearance, associated with dysphagia, dyspnea, or hoarseness of voice. Clinical evaluation of these patients failed to detect any evidence of thyroid dysfunction. In the last patient (number 7), the gland was tender, nodular, and firm in consistency. In addition to local compression manifestations, the patient suffered from palpitation, hyperhiderosis, and loss of weight.

Biochemical Results:

Serum T3, T4 and TSH levels were within the normal range except in two patients. One patient with CRF (number 5) showed low T3 and T4 levels despite being clinically euthyroid, and another patient with subacute thyroiditis (number 7) showed slightly elevated T3 and T4 levels. Anti-thyroglobulin and anti-microsomal antibodies were negative in all patients.

Histopathologic Findings:

Fine needle aspiration cytology was able to aid in the diagnosis preoperatively in three patients out of six (50%). Final diagnosis was based, however, on post-surgical histology.

On gross examination of the submitted specimens, the thyroid gland was enlarged, diffusely or focally nodular, with a smooth external surface, and firm in consistency. Cut sections showed a characteristic grayish yellow coloration of the affected areas.

On microscopic examination, the main feature was the deposition of hyaline material extra-cellularly which

appeared on H&E-sections in the form of structureless homogenous eosinophilic material (Fig. 1a). Under high power examination, the surrounding acini of the thyroid gland showed effects of pressure atrophy and degenerative changes in the form of nuclear abnormalities with cytoplasmic vacuolization (Fig. 1b). The hyaline material stained bright orange on Congo red stain (Fig. 2). On examination of these sections under the fluorescent microscope, the characteristic apple-green birefringence was demonstrated. Immuno-histochemical staining showed that the amyloid was of the β2-microglobulin (β2-M) type in the four patients on dialysis (Fig. 3), and of the AA type in the remaining three patients with secondary amyloidosis.

On transmission electron microscopic (TEM) examination of DRAG, the characteristic appearance of amyloid fibrils was seen in the form of curved, haphazardly arranged fibrils of 10-12 mm width (Fig. 4).

Table 1.: ***Characteristic Features of the Studied Population (N=7).***

No.	*Sex*	*Age (y)*	*Underlying Disease*	*Thyroid Function*	*T3 & T4 Levels*	*Thyroid Antibodies*	*FNAC*	*Amyloid Type*
1	F	31	CRF*	Euthyroid	Normal	Negative	Not Done	β2-M
2	F	26	Chronic Osteomyelitis	Euthyroid	Normal	Negative	Positive	AA
3	M	41	CRF	Euthyroid	Normal	Negative	Positive	β2-M
4	F	38	T.B.	Euthyroid	Normal	Negative	Negative	AA
5	F	55	CRF	Euthyroid	Low	Negative	Positive	β2-M
6	M	37	CRF	Euthyroid	Normal	Negative	Negative	β2-M
7	F	39	Subacute Thyroiditis	Hyper-thyroid	High	Negative	Negative	AA

CRF: Chronic renal failure, CRF*: Transplanted patient, TB: Tuberculosis
β2-M: Beta-2 microglobulin, AA: Amyloid A.

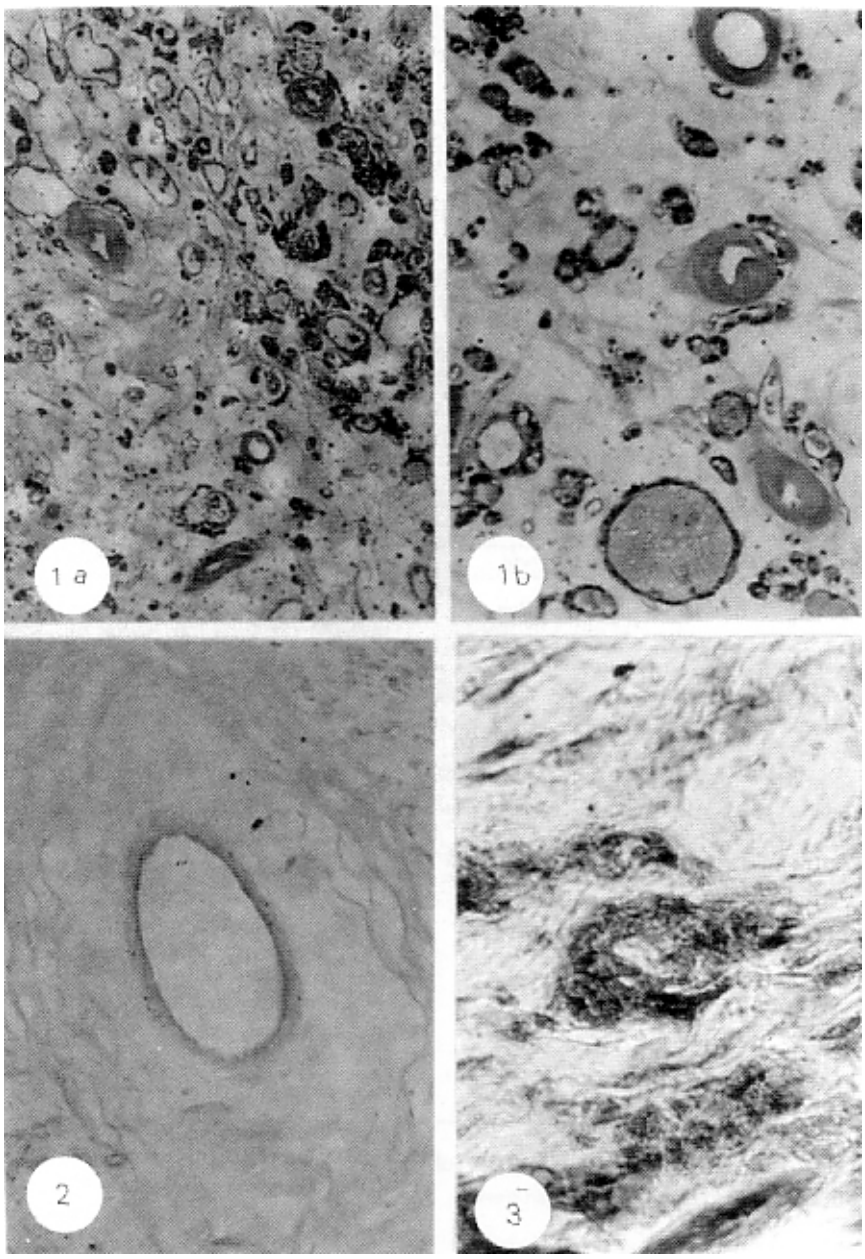

Fig. 1 :

A- ***Section of a case of amyloidosis of the thyroid gland featuring widely dispersed thyroid follicles with a lining of cuboidal acinar cells showing different grades of degenerative features. The glands are separated by a vascular stroma with thickened blood vessels exhibiting heavy deposits of structureless, homogenous eosinophilic appearance (H&E x 100).***

B- ***High power view of the previous section featuring the thickened blood vessel walls with the structureless homogeneous eosinophilic material (H&E x 400).***

Fig. 2: ***Positive staining reaction for Congo red stain in the walls of blood vessels (Congo red x 400).***

Fig . 3: ***Section of a thyroid gland showing positive staining of the interstitium with β2-microglobulin (Streptavidin-peroxidase x 200).***

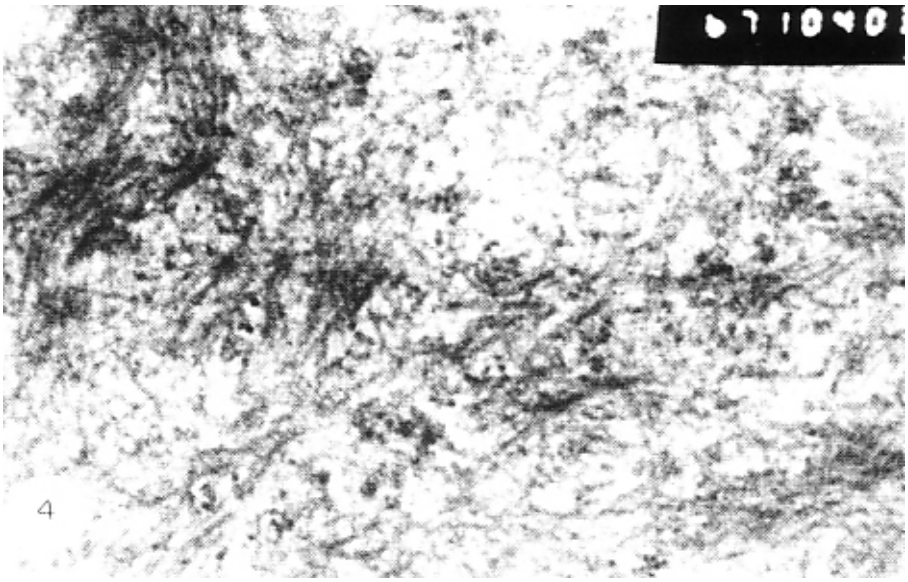

Fig. 4: TEM of a case of dialysis-related amyloidosis of the thyroid gland. Note the characteristic curved elongated filamentous appearance of the constituent fibrils (Uranyl acetate and lead citrate x 67000).

DISCUSSION

Amyloid goiter is a very rare clinical entity. It may be due to either primary or secondary systemic or local amyloidosis[13]. In the present study we presented four patients with dialysis-related amyloid goiter (DRAG) and three with reactionary (secondary or AA) amyloid goiter. The amyloid protein in DRA is composed primarily of β2-microglobulin (β2-M)(2,14), a trace protein that is normally catabolized in the kidney. Beta-2 microglobulin is retained in renal failure[2]. Hemodialysis only partially alleviates the problem, and peritoneal dialysis is even less efficient[15,16]. Secondary amyloidosis, also called reactive amyloidosis, is generally associated with chronic inflammatory disease, but has also been noted with non-suppurative diseases such as rheumatoid arthritis and various tumors. The liver, spleen, kidney and adrenal glands are the organs most commonly involved, the thyroid gland is rarely affected[17].

The clinical features of patients who present with amyloid goiter indicate a progressive, rapid enlargement of the thyroid. The enlargement may be asymmetrical at first but soon involves the isthmus and the other lobe, or it may affect both lobes simultaneously. If the swelling attains large proportions it may induce pressure symptoms, and may even cause vocal cord paralysis due to involvement of the recurrent laryngeal nerve by amyloidosis and fibrosis, or amyloid involvement of the intrinsic laryngeal muscles[18]. Because of rarity of the condition and its resemblance to Hashimoto's disease and thyroid cancer, the diagnosis of amyloid goiter is seldom made pre-operatively. However, long-term dialysis or chronic suppurative disease accompanied by a rapidly enlarging thyroid gland should raise the suspicion of amyloid goiter. In addition, Hashimoto's disease may be excluded on clinical and immunological grounds. Clinically, patients with amyloid goiter are more commonly euthyroid, but may be hyperthyroid more frequently than hypothyroid, for unknown reasons, despite the widespread infiltration of the gland[19].

In the present study, FNAC could aid in the pre-operative diagnosis in three patients out of six (50%). Reports from the literature showed conflicting results; where as it was non-diagnostic in the single cases reported by Sinha et al[20], and Samuel et al[21], it established the diagnosis pre-operatively in the four cases reported by Nijhawan and colleagues[22], and the two cases reported by Lucas et al[23]. Fontan and associates[24] suggested that FNAC should be done under CT-guidance and directed to solid areas of the mass because amyloid material may not be present in the colloidal cysts. Nevertheless, adequate tissue specimens and proficient histological technique are necessary to maintain high diagnostic sensitivity and specificity. Congo red histology should always be followed by immunohistochemical staining of tissue to determine the amyloid type[25].

Since dialysis related amyloidosis (DRA) is a progressive disease, early surgical intervention is warranted in cases of DRAG for relief of the existing pressure

symptoms and prevention of the consequences of an expanding mass. Successful renal transplantation reduces plasma β2-M levels to normal and joint pains reduce quickly[26]. Over time, there may also be some regression in the amyloid deposits. One study, for example, found regression of amyloid in eight of nine patients with DRA as detected with radiolabled serum amyloid P component approximately 5 years after successful transplantation[7]. In comparison, bone cysts reduce much more slowly[25]. There are no reports, to our knowledge, on resolution of amyloid deposits in the thyroid gland after renal transplantation. In this study, only one patient was a transplant recipient and underwent total thyroidectomy three months after renal transplantation, because of rapid and progressive enlargement of the gland.

If untreated, secondary (reactionary) AA amyloidosis is a serious disease with high mortality rate due to end-stage renal disease, infection, congestive heart failure, bowel perforation, and gastrointestinal bleeding[27,28]. The preferred therapy of AA amyloidosis is control of the underlying inflammatory disease. This can lead to stabilization of renal function, reduction in protein excretion, and partial resolution of amyloid deposits[29]. Long-term treatment with colchicine controls the underlying inflammatory disorder, and inhibits secretion of SAA from hepatocytes[30]. However, involvement of the thyroid gland in AA amyloidosis requires prompt exploration and thyroidectomy, usually total, to alleviate pressure symptoms of the rapidly growing gland, and to exclude malignancy.

Based on the data presented, it may be concluded that [1] amyloid goiter is a rare clinical entity that has to be distinguished from the more common types of goiter, particularly Hashimoto's disease and thyroid cancer, [2] the former can be excluded on clinical and immunological grounds, and the latter by exploration and histology, [3] although FNAC may aid in the diagnosis pre-operatively, post-surgical special tissue stains and electron microscopy are necessary for diagnosis and identification of amyloid type, and [4] with the possibility of this diagnosis in mind, prompt adequate treatment, in the form of total thyroidectomy, would decrease the duration of morbidity

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