

## ORIGINAL ARTICLE

# Thyroid cysts: a new extra-adrenal site of aldosterone synthase expression and increased aldosterone content

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## Abstract

**Background** The rapid re-accumulation of fluid following aspiration of thyroid cystic lesions suggests that active transport of sodium and water may be involved in volume regulation of these lesions. In this study we address the possibility that aldosterone may take part in this process.

**Subjects and methods** Thirty-one patients (29 women and two men), with a mean age of  $52.7 \pm 13.2$  years (range: 27–77 years) underwent evaluation for thyroid nodules that had a sonographic cystic component. Cystic fluid obtained by FNA biopsy was sent for cytological examination and biochemical measurements. In 10 patients, material was collected for RNA extraction and determination of aldosterone synthase expression by RT-PCR amplification.

**Results** All lesions were benign, cystic, colloid nodules. Cyst fluid aldosterone levels as measured by routine radioimmunoassay (RIA) were elevated above the normal plasma levels in all but five patients. Mean aldosterone levels were  $27.1 \pm 22.9$  ng/dl (SD) (range: 5.9–117.5 ng/dl). In contrast, cyst cortisol values were in the low, low normal serum range ( $6.2 \pm 2.9$  µg/dl, range: 0.2–10.2 µg/dl). Sodium, chloride and potassium levels were  $137 \pm 4.7$  mEq/l,  $98 \pm 5$  mEq/l and  $4.9 \pm 1.4$  mEq/l, respectively. Plasma aldosterone levels were normal in all patients tested. To confirm these results, 12 samples were assayed after extraction and chromatography using a highly specific antibody. Cyst aldosterone levels in this group were elevated above the normal serum range in all but one patient (mean concentration:  $24.5 \pm 14.6$  ng/dl, range: 8.72–40.1 ng/dl). In this group, 18(OH)B levels were within the normal plasma range (12–55 ng/dl) in all but one patient ( $34.9 \pm 17$  ng/dl). Furthermore, aldosterone synthase mRNA expression was found in aspirates of four of 10 patients.

**Conclusions** The increased aldosterone concentration and the presence of aldosterone synthase expression suggest that aldosterone may be locally produced and secreted in thyroid tissue. The pathological implications of this finding remain to be established.

‡In memoriam.

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Although clinically significant simple thyroid cysts are rather uncommon, small cystic lesions can be detected in up to 20% of individuals referred for thyroid ultrasonography.<sup>1</sup> Further, many thyroid lesions have a large cystic component.

Such mixed cystic lesions are presumed to represent degenerated nodules. However, the rapid re-accumulation of fluid following aspiration of thyroid cystic lesions suggests that active transport of sodium and water may be involved in volume regulation of these lesions.<sup>2</sup> The potential role of aldosterone in this putative process has not been studied previously.

Whether or not aldosterone can be synthesized in extra-adrenal tissues remains controversial. Several reports indicated that aldosterone synthase, the enzyme which converts deoxycorticosterone to aldosterone through a unique triple effect, is expressed in cell types other than the adrenal-cortical glomerulosa cells.<sup>3,4</sup> Further, aldosterone synthase expression in endothelial cells and vascular smooth muscle was reportedly up regulated by angiotensin II or low salt intake,<sup>5–7</sup> whereas its expression in cardiomyocytes was shown to increase in hypertensive rats, following myocardial infarction or in the failing heart.<sup>8–10</sup> Nevertheless, although the possibility that aldosterone is generated in cardiomyocytes has been explored more extensively than in any other extra-adrenal cell type, recent evidence suggests that the expression of aldosterone synthase in the heart is unlikely to result in significant local production of aldosterone.<sup>11</sup> On the other hand, evidence for local and functionally significant production of aldosterone at other sites, such as some areas in the brain, is thus far undisputed.<sup>12</sup>

Based on these observations we reasoned that thyroid cysts, known for their common tendency to recur despite repeat aspiration of fluid, may require a local machinery to sustain the local accumulation of salt and water. Aldosterone seemed a reasonable candidate to control active local re-absorption of salt and water, which has led us to measure its concentrations in thyroid cyst aspirates.

The purpose of the present study was to examine aldosterone concentration in fluid aspirates of thyroid cystic lesions and assess the

possible expression of aldosterone synthase, an enzyme involved in aldosterone production in thyroid cysts.

## Patients and methods

All patients included in this study underwent evaluation for nodular goitre at the Institute of Endocrinology and Metabolism, Tel Aviv – Sourasky Medical Center. They presented with palpable thyroid nodules that contained a dominant cystic component as imaged by ultrasound, and had decreased  $^{99}$ technetium uptake in the thyroid scan. Fine needle aspiration biopsy (FNA) was clinically indicated in all patients, and informed consent was obtained for its performance. None of the patients suffered from uncontrolled hypertension requiring endocrine evaluation. Nonetheless, plasma samples from nine patients were available for aldosterone measurement.

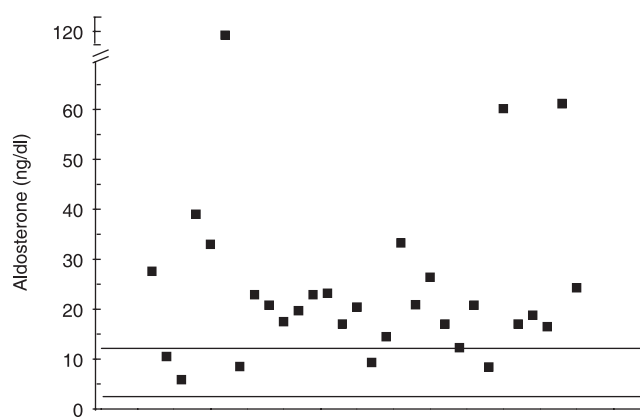
Cystic fluid obtained by FNA biopsy was sent for cytological examination and biochemical measurements of aldosterone, cortisol, sodium, potassium and chloride. The needle biopsy was then washed in 1 ml of Trizol reagent (Gibco-BRL, Gaithersburg, MD) for subsequent RNA extraction as recommended by the manufacturer. The RNA pellet was resuspended in 20  $\mu$ l of DEPC-treated water and frozen at  $-80^{\circ}\text{C}$ .

Aldosterone was measured in cystic fluid by a solid-phase  $^{125}\text{I}$  radioimmunoassay (RIA) (Coat-A-Count Aldosterone, Diagnostic Products Corporation, Los Angeles, CA, USA). To confirm results obtained by this method, 12 samples were assayed by an 'in house' RIA using a highly specific anti-aldosterone antibody, at the Institute of Pharmacology at the Ruprecht-Karls-University at Heidelberg, Germany.<sup>15</sup> Antibody cross-reactivity with other steroids was as follows: 17  $\alpha$ -isoaldosterone, 0.4%; 21-deoxyaldosterone, 2.5%; corticosterone, 0.06%; 18-OH-corticosterone, 0.04%; DOC, 0.01%; 18OH-DOC, 0.02%; cortisol, 0.04%; cortisone, 0.01%; progesterone, 0.01%; testosterone, 0.05%. Samples were assayed after extraction and chromatography conducted on celite mini-columns. 18-OH-corticosterone was assayed in the same samples using specific antibodies. All values are presented as mean  $\pm$  standard deviation (SD).

## RT-PCR

Twelve  $\mu$ l of the RNA suspension was reverse transcribed in a 20- $\mu$ l reaction, using oligo-dt primers, 10 mM of each dNTP, and MMLV reverse transcriptase (Clontech, Palo Alto, CA, USA). The suspension volume was brought up to 100  $\mu$ l by adding DEPC-treated water. Five  $\mu$ l of this cDNA suspension together with specific aldosterone synthase primers in a final concentration of 0.5  $\mu\text{M}$  each were used in the PCR reaction, which was performed using the Advantage cDNA PCR Kit (Clontech). Cycling parameters consisted of an initial denaturation step of 94  $^{\circ}\text{C}$  for 4 min, followed by 35 cycles of 94  $^{\circ}\text{C}$  for 1 min, 55  $^{\circ}\text{C}$  for 1 min and 72  $^{\circ}\text{C}$  for 2 min and a final extension cycle of 72  $^{\circ}\text{C}$  for 7 min.

Primers were 5'TACAGGTTTTCCTCTACTCG3' and 5'AGAT-GCAAGACTAGTTAATC3', corresponding to nucleotides 1208–1227 and 1503–1522 of the aldosterone synthase cDNA, amplifying a fragment of 315 base pairs. PCR products were resolved in an ethidium bromide-stained 2% agarose gel. PCR bands were cut and products were extracted from the gel for subsequent sequencing



**Fig. 1** Aldosterone levels in thyroid cyst fluid of 31 patients as measured by routine RIA. The normal serum range is denoted by the horizontal lines.

**Table 1.** Results of thyroid cyst fluid measurements

	Mean (SD)	Range
'Routine' aldosterone RIA ( $n = 2-12.5$ ng/dl)	27.1 $\pm$ 22.9	5.9–117.5
'In house' aldosterone RIA ( $n = 2-10$ ng/dl)	24.5 $\pm$ 14.6	8.7 $\pm$ 40.1
Cortisol ( $\mu\text{g/dl}$ )	6.2 $\pm$ 2.9	0.2–10.2
18(OH)B ( $n = 12-55$ ng/dl)*	34.9 $\pm$ 17	21–69.4
Na <sup>+</sup> (mEq/l)	137 $\pm$ 4.7	126–145
K <sup>+</sup> (mEq/l)	4.9 $\pm$ 1.4	3.8–10.2
Cl <sup>-</sup> (mEq/l)	98 $\pm$ 5	93–107

\*18(OH)B = 18-OH-corticosterone

verification. RNA extracted from the human NCI-H295 adrenocortical carcinoma cell line was used as a positive control for the RT-PCR.<sup>14</sup>

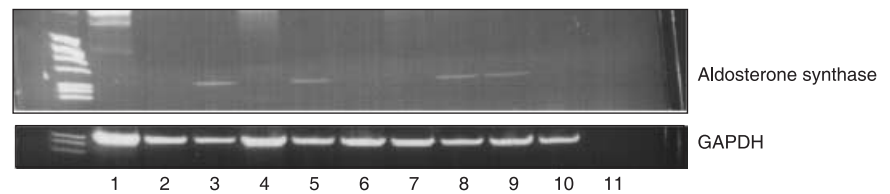
RNA and cDNA integrity was verified through parallel amplification of all samples with specific primers for GAPDH.

## Results

Thirty-one patients (29 women and two men), with a mean age of 52.7  $\pm$  13.2 years (range: 27–77 years) underwent evaluation for thyroid nodules. All lesions were cytologically defined as benign cystic colloid goitres.

Results of cyst fluid measurements are summarized in Table 1. Aldosterone levels as measured by routine RIA were elevated above normal plasma levels (2–12.5 ng/dl) in all but five patients (Fig. 1). Mean cyst aldosterone levels were 27.1  $\pm$  22.9 ng/dl (SD), range: 5.9–117.5 ng/dl. In contrast, cyst cortisol levels were in the low, low normal serum range (6.2  $\pm$  2.9  $\mu\text{g/dl}$ ; range: 0.2–10.2  $\mu\text{g/dl}$ ). Plasma aldosterone was normal (8.1  $\pm$  2.7 ng/dl; range: 4.8–12.8 ng/dl) in the nine patients in whom it was measured. In these nine patients mean cystic fluid aldosterone levels was 25.9  $\pm$  35 ng/dl (range: 2.8–117.5 ng/dl), with a mean cyst/plasma ratio of 3.5  $\pm$  4.4 and a median cyst/plasma ratio of 2.2 (range 0.2–14.8,  $n = 9$ ). Cyst aldosterone levels were higher than plasma levels in seven out of nine patients.

**Fig. 2** Aldosterone synthase and GAPDH mRNA expression in thyroid cysts. Lanes 1–10: samples. Lane 11: negative control.



'In house' aldosterone and 18-OH-corticosterone assays were performed in 12 cyst samples as described above. Cyst aldosterone levels were elevated above the normal serum range (2–10 ng/dl) in all but one patient in this group. Mean cystic aldosterone levels were  $24.5 \pm 14.6$  ng/dl (range: 8.72–40.1 ng/dl). Cyst 18(OH)B levels were within the normal plasma range (12–55 ng/dl) in all but one patient, with a mean 18(OH)B levels of  $34.9 \pm 17$  ng/dl.

Cyst sodium levels were  $137 \pm 4.7$  mEq/l, with chloride  $98 \pm 5$  mEq/l and potassium levels of  $4.9 \pm 1.4$  mEq/l in the whole group (Table 1).

Aldosterone synthase mRNA expression was found in four of the 10 FNA samples examined (Fig. 2). Aldosterone synthase was also expressed in one sample of a papillary thyroid carcinoma and its normal adjacent thyroid tissue (not shown).

RT-PCR products were sequenced and found to be 100% homologous to the corresponding aldosterone synthase gene fragment.

## Discussion

Several aspects of our findings merit consideration. First, the electrolyte environment within the cyst was reminiscent of the extracellular/intravascular distribution of sodium, potassium and chloride. Hence, this would be inconsistent with fluid accumulating as a result of ongoing cell degeneration, which would have reflected more closely intracellular electrolyte distribution. Such original composition, though, could have undergone secondary re-equilibration with neighbouring extracellular pools adjacent to the cystic formation. Second, based on two independent assays, one of which has been developed and used for several decades in a laboratory specializing in the measurement of various corticosteroids, aldosterone concentrations within the cyst were higher than the normal range for serum aldosterone whereas the cystic cortisol levels were relatively low, and indeed, grossly low in some cases. This would yield an aldosterone/cortisol ratio that is considerably higher within the cystic fluid than in the serum. Finally, the thyroid cystic levels of 18-OH-corticosterone, another product of aldosterone synthase, were quite similar to normal circulating 18-OH-corticosterone.

The possibility that high cyst aldosterone levels reflect elevated plasma aldosterone is unlikely. Although a high prevalence of ultrasonographic abnormalities has been reported in patients with primary hyperaldosteronism,<sup>15</sup> the probability that 25 of 31 consecutive patients examined for clinical thyroid nodular disease also suffer from primary hyperaldosteronism is very low. Additionally, none of the patients had uncontrolled hypertension, which might have been suggestive of primary hyperaldosteronism. Finally, plasma aldosterone levels were found to be normal in all patients tested.

We are unable to determine, based on these results, whether aldosterone is generated intrathyroidally, by some aberrant

(over)expression of the aldosterone synthase gene induced by local mechanisms, or is simply extracted by the trapped fluid populating the cyst, where it assists in the maintenance of sodium and water. The increased aldosterone concentration and the presence of aldosterone synthase expression suggest that aldosterone may be locally produced and secreted in thyroid tissue. However, based on the present findings alone, an equally valid explanation is that aldosterone is somehow preferentially trafficked to thyroid cysts. Neither the mechanism, nor the purpose of such a putative process can be presently addressed. We are also unaware of reports of increased aldosterone concentrations in other cyst types at other organs.

In summary, thyroid aldosterone cystic fluid contains aldosterone concentrations, which exceed that of plasma aldosterone, in association with low cystic cortisol and an electrolyte composition that is essentially similar to that found in the extracellular compartment. Additionally, aldosterone synthase mRNA expression is found in thyroid specimens extracted during thyroid FNA. The pathophysiological significance of this finding and, specifically, the possible contribution of thyroid aldosterone to the formation or maintenance of thyroid cysts require examination.

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