

## Thyroidectomy in severe amiodarone induced thyrotoxicosis

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**Introduction:** Amiodarone can cause thyroid dysfunction in patients with or without previous thyroid disease. Amiodarone induced thyrotoxicosis (AIT) may precipitate life-threatening arrhythmias and may be refractory to medical treatment. Total thyroidectomy constitutes a valid alternative to medical therapy and should be considered in the management of the disease. The aim of this study was to assess perioperative risk of thyroidectomy in patients with AIT.

**Patients/ Methods:** We performed a meta-analysis of trials involving patients with AIT. Ninety-three cases were reported from 1985 to 2007. We included our three patients with refractory to medical therapy AIT treated by total thyroidectomy in 2008. Baseline characteristics, treatment received, laboratory parameters, and events during follow-up were evaluated. The end points of the study were the mortality and morbidity rates.

**Results:** Ninety-six patients were included in the present analysis. There were 76 male and 20 female patients aged 39-85 years, with severe refractory AIT following 3-108 months of amiodarone treatment. All patients had heart disease. Cardiac ejection fractions ranged from 10%-66%. Ten (10.4%) patients were on a heart transplant waiting list. Four (4%) had previously undergone heart transplantation. Type 2 AIT was diagnosed in most patients (86.5%). Sixty-five patients (68%) in the delayed rescue thyroidectomy group - were treated with propylthiouracyl (PTU) 600-1200 mg/d, beta blockers and high dose steroids, for 2 to 4 months. Of these patients, 52 (80%) did not respond to medical therapy and 13 (20%) developed side effects. The rest, 31 (32%) were offered surgery as a first line treatment because of necessity to continue Amiodarone therapy (32%) or the severe condition of the patients (68%). Fourteen patients (45%) – from the early thyroidectomy group - received pre-surgery medical anti-thyroid therapy for 2-3 weeks. Seventeen patients (55%) were treated with urgent thyroidectomy without receiving medical therapy because of debilitated condition. Nine patients underwent plasmapheresis before surgery. Six achieved transient benefit, two did not respond and one patient had a severe adverse reaction. Total thyroidectomy was performed under general anesthesia. The perioperative complication rate was lower in groups of urgent and early thyroidectomy (16%) compared with the delayed rescue thyroidectomy group (26%). Two patients developed severe hypocalcemia, four experienced arrhythmia and eight had respiratory complications. There were no deaths in the early thyroidectomy group. In the urgent thyroidectomy group, two (11.7%) died (two events of arrhythmia). Four (6.2%) died in the delayed rescue group (two events of arrhythmia, one from pneumonia and another one from stroke). The rest of the patients had rapid resolution of their symptoms and were euthyroid on postoperative thyroxin replacement therapy.

**Conclusions:** AIT may be severe and refractory to medical therapy. Despite the potential risks of surgery associated with uncontrolled thyrotoxicosis, thyroidectomy should be considered as the definitive treatment for resistant AIT, with rapid control of the latter.

## **Components of the Minimal Thyroglobulin Enhancer: necessary Element in a future Thyroid Cancer Gene Therapy Paradigm**

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**Introduction:** As a step towards the development of gene therapy vectors targeting human thyroid carcinoma, we set out to define and characterize a minimal thyroid cancer-specific enhancer. It is well known that the promoter region of the thyroglobulin (Tg) gene interacts with at least three thyroid-specific transcription factors: thyroid transcription factor-1 (TTF-1), TTF-2 and PAX-8. Minimalization of the enhancer should potentially allow more flexibility in the subsequent preparation of therapeutic vectors intended to elicit thyroid cancer cell death. Aims: 1) To define a minimal fragment of the Tg gene enhancer required for thyroid-specific transcriptional activity in human thyroid NPA (papillary), MRO (follicular) and ARO (anaplastic) human carcinoma cell lines and from primary human goiter cells. 2) To clarify the mechanism of thyroid specificity for this minimal enhancer through characterization of the transcription factors that interact with this nucleotide sequence from human thyroid carcinoma cell lines by homology mapping and gel shift analysis.

**Methods:** We prepared three different fragments covering the minimal Tg enhancer region, some of which were labeled (DIG): EcoRV-BglII (624bp: -2.82 to -2.2 kbp), EcoRV-HindIII (146bp: -2.82 to -2.68 kbp) and Hind III-BglII (478 bp: -2.68 to -2.2 kbp). We also prepared cytoplasmic and nuclear extracts from the cells above. Supershift analysis was applied to define the presence, specificity and amount of TTF-1 in the binding activity for each fragment.

**Results:** We defined a new minimal thyroid cancer-specific enhancer (size 624bp, localized between -2.8 and -2.2 kbp from the Tg transcriptional start site), based on the thyroglobulin (Tg) upstream enhancer. We then analyzed the Tg minimal enhancer sequence for thyroid-specific transcription factors and found five putative TTF-1 recognition sites, but none for TTF-2 or PAX-8. We confirmed this region to be a thyroid-specific enhancer by transfection studies. The presence of TTF-1 and PAX-8 in all three thyroid carcinoma lines by Western blot analysis confirmed their thyroid origin. Using nuclear extracts for the three thyroid carcinoma lines, competitive gel shifts were found for each fragment. A supershift compatible anti-TTF-1 antibody (Ab) was only able to supershift bands for the EcoRV-BglII as well as the HindIII-BglII fragments. The gel shift band(s) visible for the EcoRV-HindIII fragment, while competable with cold DNA, was not supershifted by the TTF-1 Ab. No other known transcription factor has yet been described to bind the sequence in this region. This is particularly important as we found this region to be essential for enhancer activity in the papillary carcinoma cell line (NPA). Furthermore, the single putative TTF-1 binding site in this fragment did not bind TTF-1.

**Conclusions:** The new minimal upstream Tg enhancer fragment we constructed was characterized as thyroid-specific and to contain five TTF-1 binding site homologies, at least one of which does not bind TTF-1. Additionally, we demonstrated the presence of a new thyroid enhancer transcription factor essential for enhancer activity in the papillary carcinoma cell line (NPA). The new minimal enhancer element may serve to increase the activity of a Tg promoter-driven suicide gene as a future tool in thyroid cancer gene therapy.

# THE CONTRIBUTION OF GC-MS URINARY STEROID METABOLOME IN THE STUDY OF A CONSANGUINEOUS FAMILY WITH 6 MEMBERS WITH APPARENT MINERALOCORTICOID EXCESS (AME).

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**Introduction:** Apparent mineralocorticoid excess (AME) was first defined in 1977 in a patient with hypertension, hypokalemia, low renin and low aldosterone. Subsequently, it was demonstrated that AME is caused by impaired activity of 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (11 $\beta$ -HSD2), which converts biologically active cortisol into inactive cortisone. The enzymatic action of 11 $\beta$ -HSD2 guarantees the selectivity of the mineralocorticoid Receptor (MR) for aldosterone (A) by converting cortisol (F) to its inactive metabolite cortisone (E). In the case of impaired 11 $\beta$ -HSD2 activity, cortisol binds inappropriately to the MR, thus acting as potent mineralocorticoid, leading to sodium resorption, potassium excretion, and severe hypertension.

**Patients/ Methods:** The first patient in this study (A, 13y.o) was accidentally diagnosed with hypertension (190/125) after a bone fracture. He was an offspring of consanguineous parents from Arab descent. His family history was highlighted by hypertension, and premature death from "kidney problems" in three of his siblings. A 23y.o. sister (B) suffered from hypertension and hypokalemia since the age of two. After kidney transplantation, her blood pressure and kalemia normalized. An additional brother, (C), had only occasional slightly elevated blood pressure.

**Results:** Blood test results from patient A exhibited hypokalemic metabolic alkalosis (K=2.3 mEq/L), low plasma rennin activity PRA (1.08 ng/ml/h, Normal: 1.33-3.95), and low aldosterone (41pg/ml, Normal: 70-350). The GC-MS urinary profiles of all the family members were performed (parents and 5 children). The results are expressed as ratios of cortisone metabolites (THE,  $\alpha$ CL,  $\beta$ CL) to cortisol metabolites (THF,  $\alpha$ THF,  $\alpha$ C,  $\beta$ C). Two ratios were calculated: ratio1=THE/THF+ $\alpha$ THF, ratio2= $\alpha$ CL+ $\beta$ CL/ $\alpha$ C+ $\beta$ C. The ratios for the affected members were: A: ratio1=0.01, ratio2=0.06, B: ratio1=0.02, ratio2=0.18, C: ratio1=0.26, ratio2=1.04 (Reference values: ratio1= 0.4-1.8, ratio2=1.5-5.0. The other members of the family had normal ratios. Genetic studies confirmed the diagnosis of AME in these three patients (A, B,C).

**Conclusions:** Analysis of urines metabolites of cortisone and cortisol can be used in the diagnosis of AME. Moreover, there is a good correlation between the ratios of the different metabolites and the severity of the disease. Although hypertension and hypokalemia normalized after renal transplant in B, and the minimal symptomatology in C, the ratios still exhibited pathologic values, showing the sensitivity of this diagnostic tool.

# A Novel Mutation Causing 3 $\beta$ Hydroxysteroid Dehydrogenase Deficiency

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**Introduction:** 3 $\beta$  hydroxysteroid dehydrogenase 2 deficiency is a rare cause of defective glucocorticoid and mineralocorticoid synthesis that may be fatal if not diagnosed in infancy. In its classic form, genetic females have clitoromegaly and mild virilization due to DHEA overproduction and its conversion to testosterone via extra-adrenal 3  $\beta$ HSD1. Here we describe a novel mutation in 3  $\beta$ HSD2 gene causing glucocorticoid and mineralocorticoid deficiency in a girl with no virilization.

**Patients/ Methods:** Patient: A 9.3 years old girl, born to non relative Palestinian Christian parents, was diagnosed with severe salt-losing crisis shortly after birth, (Na= 99 meq/l). No clitoromegaly or fusion of the labia was observed. ACTH test at 0 and 60 minutes showed: ACTH 18.6 pmol/, cortisol <27.6 -> < 27.6 nmol/l, 17OH progesterone 0.1 ->0.1 nmol/l, 17OH pregnenolone <1-> 13.9 nmol/l, DHEAS 6.49 -> 6.13 micromol/l, D4A <0.35 -> <0.35 micromol/l, Testosterone <0.3 -> 0.3 nmol/l. She has been successfully treated with Hydrocortisone and Flurinef. Currently she is pre pubertal with height and weight in the 25th and 75th percentiles respectively. Her physical examination does not reveal acne or hirsutism. Genetic studies for the family were done.

**Results:** Genetic analysis of DNA from the proband and her parents using micro satellite markers on chromosome 1 flanking the  $\beta$ HSD2 gene found our patient to be homozygous and the parents heterozygous in the gene locus.  $\beta$ HSD2 gene sequencing revealed the patient to carry a novel homozygous mutation in exon 3 (439), C->A corresponding to the A231D amino acid substitution in the  $\beta$ HSD2 protein.

**Conclusions:** We present a unique case of 3 $\beta$ HSD2 deficiency with severe hyponatremia and adrenocortical insufficiency, and no signs of virilization, caused by a novel mutation in exon 3 in a location that was described in the past to be responsible for enzyme activity. Functional studies may explain the absence of virilization and the severe hyponatremia that are special in this case.

## **Papillary Carcinoma of the Thyroid gland – A Subclinical Disease**

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**Introduction:** The incidence of thyroid cancer more than doubled over the past 3 decades. The increase in incidence reflects increased detection of subclinical disease rather than a true occurrence of thyroid cancer. There is sizable data implicating that thyroid “incidentalomas” are a growing phenomenon, albeit there is missing information considering the rate of “incidentaloma” cases out of the overall treated cases of papillary carcinoma. The purpose of this study is to explore the manner in which patients with papillary cancer were initially discovered since the year 2008.

**Patients:** Retrospective epidemiologic investigational study, reviewing the charts of 109 cases with the pathologic diagnosis of papillary carcinoma of the thyroid, operated on in our department between the years 2000 and 2007.

**Results:** 55% of patients were discovered incidentally. The most common imaging study leading to the incidental finding of thyroid cancer was thyroid sonography, used in the follow-up of multi nodular thyroid goiter. 78.3% of the cases were cancers that were smaller than 2 cm. Average size of the tumor became smaller as the years progressed.

**Conclusions:** Over half the cases are diagnosed incidentally and this trend seems to be accelerating over the years. 80% of these cases are small (<2cm) tumors that are mostly non palpable and thus represent a subclinical disease. While we are expanding our use of imaging studies we are only scratching the surface of a huge subclinical reservoir

## **Incorporation of BRAF mutational analysis in clinical decision making- initial experience in papillary thyroid carcinoma**

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**Introduction:** Thyroid cancer is the most rapidly growing cancer in incidence, increasing by 2.4 in the last decades. Virtually all increase results from improved detection of papillary thyroid carcinomas (PTC's) less than 10 mm's termed microcarcinomas. The BRAF activating mutation is a T1799A transversion mutation in exon 15 of the gene, which causes a V600E amino acid substitution in the protein and consequent constitutive activation of the kinase. This mutation occurs in 29-83% of PTC's and has been shown to correlate with a more aggressive phenotype. Here we present initial experience with the incorporation of BRAF mutation analysis in both diagnostic and therapeutic clinical decision making in cases with low risk PTC.

**Methods:** BRAF mutation analysis was performed on 3 cytological specimens obtained by US-guided FNAB of subcentimeter thyroid nodules and on 3 post surgical paraffin blocks of patients with PTC smaller than 1.5 cm using the Mutector assay.

**Results:** DNA was isolated from 3 histological specimens of patients which had undergone total thyroidectomy. The first was primarily diagnosed as harboring a 0.4 cm PTC with several additional indeterminate lesions in the surgical specimen. Mutational analysis was positive for BRAF in the primary lesion and in one histologically suspicious lesion (figure 1A) and negative in an adjacent seemingly benign lesion (figure 1B). The patient was therefore diagnosed with multifocal PTC and subsequently underwent radioiodine treatment. Two other patients presenting post surgically with BRAF positive PTC's 0.7cm and 1.3 cm's in size were treated with radioiodine post analysis. DNA was also isolated from washout of three FNAB samples and analyzed for the BRAF T1799A mutation. One sample was highly diagnostic of the BRAF T1799A mutation and in accordance with a positive cytological diagnosis of papillary carcinoma of the thyroid. The patient subsequently underwent total thyroidectomy with a diagnosis of thyroid papillary carcinoma. The other two FNAB's were negative for BRAF T1799A mutation with benign cytology and are currently followed without surgery.

**Conclusions:** Papillary thyroid carcinoma, a seemingly indolent tumor, raises several dilemmas regarding diagnostic and therapeutic strategies. We suggest that compilation of BRAF mutational analysis with clinicopathologic criteria may aid in clinical decision making in controversial cases.

## **Familial and Sporadic Non-Medullary Thyroid Cancer Share a Similar Clinical Behavior and Prognosis**

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**Introduction:** Familial non-medullary thyroid cancer (FNMTC) accounts for 3-5% of all differentiated thyroid cancer, yet, very little is known about its genetic background and clinical behavior. We report on the clinical characteristics and long-term outcome of a cohort of 67 patients with FNMTC.

**Patients/ Methods:** The study group was composed of 54 cases from the Rabin Medical Center and 13 from collaborating physicians, representing a total of 46 families. Data was obtained through review of charts and compared with 375 patients (SNMTC) from the RMC registry with no family history.

**Results:** Patients with FNMTC were slightly younger at diagnosis (42.8 vs 47.2 yrs,  $p=0.06$ ), had a similar F:M ratio (59:7 vs 300:75,  $p=0.1$ ), and were similarly affected by PTC type (88.8% vs 91%,  $p$  smaller than NP). The disease was multicentric in 44.1% vs 55.8% ( $p=0.12$ ) and extrathyroidal extension was present in 27.6% vs 21.8% ( $p=NS$ ), at initial treatment, lymph nodes were involved in 29.3% vs 24.6% ( $p=NS$ ) and distant metastases were found in 4.9% vs 5% ( $p=NS$ ) in FNMTC and SNMTC, respectively. Persistent/recurrent disease was seen in 32% vs 26.2% ( $p=NS$ ) and new distant metastases in 5.2% vs 6.2% ( $p=NS$ ) (FNMTC and SNMTC, respectively). With a mean follow up of  $8.6 \pm 10$  and  $8.4 \pm 9.1$  years for each group, 95.4% in the FNMTC group were disease-free, compared to 85.8% in the SNMTC group ( $p=0.12$ ).

**Conclusions:** The small differences seen in patients with familial and sporadic non-medullary thyroid cancer do not justify a different therapeutic approach.