URINARY STEROID PROFILING IN THE EVALUATION OF PATIENTS WITH ADRENOCORTICAL NEOPLASMS.

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ABSTRACT

Background: Adrenocortical tumours are relatively common in childhood. Clinical presentation with an abdominal mass often signifies metastatic disease with poor prognosis. On the other hand, modern imaging techniques have significantly increased the pick up rate of adrenal masses (dubbed 'incidentalomas'). Endocrine hypersecretion may result in varying degrees of vague symptomatology that may be clinically challenging to adequately evaluate.

Objectives: To determine the urinary steroid profiles (by capillary gas chromatography) in a series of patients presenting with adrenocortical masses).

Methods: The fifty patients included in this study had an adrenocortical tumour diagnosed on the basis of abdominal ultrasound, CT or at laparatomy. An aliquot of a 24 hours urine collection was analysed by capillary column gas chromatography after enzymatic hydrolysis and derivatization of constituent steroid metabolites. Twenty steroid derivative compounds were identified and quantified.

Results: All 23 children (less than 16 years) in this series presented with endocrine symptoms with only 3 additionally presenting with a clinically evident abdominal mass. 11 out of 20 adult patients presented with Cushing's syndrome, including 5 presenting with a clinically evident abdominal mass. Only one of the 50 patients studied here had a normal urinary steroid profile. The pattern or the amount of overall steroid metabolites excreted was abnormal in all the other patients. There was heterogeneity of urinary steroid profiles in both children (=<16 years) and adults. There were no profiles clearly distinguishing carcinoma from adenoma. In particular, 11-deoxy cortisol and its metabolites were not sufficiently discriminatory to distinguish malignant from benign adrenocortical neoplasms: 16 of the 22 patients with carcinoma and 8 of the 15 patients with adenoma had raised excretion of 11-deoxycortisol metabolites.

Conclusion: The pattern and quantities of urinary steroid metabolites are often a significantly deranged in both benign and malignant adrenocortical neoplasms. Such profiling should form part of the routine evaluation of adrenal masses (including 'incidentalomas') and Cushing's syndrome in adults and precocity and virilism in childhood. The search for specific steroid metabolite patterns exclusively associated with malignant adrenocortical tumours remains elusive.

INTRODUCTION

Adrenocortical tumours are rare with an incidence rate of about 2 per million persons and accounting for only 0.02% of all cancers (Vankatesh et al 1989). The symptoms are often diffuse. About half the cases have no clinical evidence of endocrine hypersecretion. Presentation with an abdominal mass often signifies advanced (metastatic) disease since the

glands have a deep retroperitoneal location (Brodie et al 1989). The diagnosis is consequently often difficult and delayed, largely accounting for the depressingly poor prognosis (Demeure & Somberg 1998), although prognosis seems a bit better in childhood (Teinturier et al 1999). Aggressive surgical resection continues to be the mainstay of treatment as the use of adjuvant therapy is generally of dubious value (Brodie et al 1989, Vankatesh et al 1989). The detection of recurrent disease is similarly often delayed.

While localisation has been much improved by non-invasive scanning techniques, biochemical methods based on urinary steroid profiling are useful to: (1) assist tumour detection, (2) define the activity of the tumour, and to (3) provide markers of tomour recurrence post resection. Histological differentiation of benign and malignant adrenocortical tumours is difficult and controversial (Cagle et al 1986, Weiss et al 1989,). Progress has been made through the use of flow cytometric analysis of adrenocortical tumour DNA to detect aneuploidy which seems to predict tumour aggressiveness and poor patient prognosis (Bowlby et al 1986). The patterns of urinary steroid metabolite excretion have been studied in small series of patients with adrenocortical tumours (Honour et al 1984, Grondal et al 1990). Steroid profiling has been used in these studies to confirm suspected endocrinopathies, to detect unusual metabolite excretion patterns and in the assessment of 'incidentalomas' – asymptomatic adrenocortical tumours detected by abdominal CT or ultrasound done for other reasons (Chang et al 1989). Incidentalomas often exhibit subtle hormonal abnormalities (Kasperlik-Zeluska et al 1997, Mantero et al 1997).

We have evaluated the use of urinary steroid profiling (by capillary gas chromatography) in a series of 50 patients ultimately found to have an adrenocortical tumour either at laparatomy or by imaging techniques.

METHODS

Patients

Patients were referred to us during the operation of a UK national steroid profiling referral service over a number of years. All patients included in this study had an adrenocortical tumour diagnosed on the basis of abdominal ultrasound, CT or at laparatomy. Urine was collected over 24 hours, the total volume recorded and an aliquot (10ml) was sent to our laboratory by the physician who requested the steroid profile.

Analysis

Hydrolysis

Two ml of urine was applied to Sep Pak C18 cartridges (Waters Associates, USA) and eluted with 4 ml methanol. The eluate was dried on a rotary evaporator immersed in a water bath at 37^{o}C. The residue was resuspended in 5 ml 0.5M sodium acetate (pH 4.6). 200mg sodium acetate and 5 drops of *Helix pomatia* digestive juice (containing glucuronidase and sulphatase) were added to each tube and incubated for 48 hours at 37^{o}C. Free steroids were subsequently extracted with 4 ml methanol after application on Sep Pak C18 cartridges, dried and resuspended in 2 ml ethanol.

Preparation of methyl oxime trimethylsilyl ether (MO-TSIM) derivatives

To fractions of extract were added three internal standards (androstanetriol (A), stigmasterol (S), and cholesterol butyrate (CB). The solvent (ethanol) was evaporated under a stream of nitrogen at 60^{o}C. Seven drops of 2% methoxyamine hydrochloride (MO) in pyridine were added under a hood and incubated for 2 hours at 60^{o}C. Following the oxime formation, the hydroxyl groups of the steroids were derivatised by addition of seven drops of trimethylsilylimidazole (TMSI), overnight incubation at 100^{o}C and evaporation of volatile solvent. Lipidex gel permeation was used , with cyclohexane:pyridine:hexamethydisilane (HMDS) (98:1:1) as solvent, to recover the volatile MO-TSIM derivatives. These were stored in 2ml cyclohexane in screw-capped vials.

Capillary cloumn gas chromatography

Steroid derivatives were analysed using a 25m fused silica capillary column coated with OV-1 in a Packard Model 437A gas chromatograph fitted with a flame ionisation detector and an automatic solid sample injector. The operating programme was:

Initial temperature	180°C
Final temperature	270°C
Rise in temperature	2.5°C
Injector temperature	250°C
Detector temperature	250°C
Recorder speed	300mm/hr

The compounds were provisionally identified by comparison with GC retention times obtained from a chromatogram displaying a standard profile of all major urinary steroid metabolites. The concentration of individual steroid metabolites was calculated by relating their peak heights to that of the internal standards A (androstenedione) and CB (cholesterol butyrate). 24 hours excretion rates were computed by relating the aliquot used in the laboratory analysis to the total 24 hours urinary volume collected. The reference ranges used are those established in our laboratory for the relevant age and sex.

Results

Clinical data

Of the 50 patients, 36 (72%) were female. The median age was 16 years (range 5 months to 74 years). There were 8 patients whose age was not recorded. This included one case with adenoma and one with carcinoma. In the remaining 5, the tumour type was not specified. Among patients aged 16 years or less, the commonest clinical presentation was precocious puberty, followed by virilism and Cushing's syndrome (Table 1). The majority of those aged over 16 years presented with Cushing's syndrome (55%). In 8 of the patients, the initial presentation was an abdominal mass. Overall, 93% of the patients had surgical resection. There were only 4 patients who had chemotherapy (mitotane), in three cases this was as adjuvant therapy post resection, while in the remaining patient this was as sole therapy for inoperable tumour.

Table 1. Clinical features of patients according to age and type of tumour

Age group	Nature of mass (number of cases)	Precocious puberty(n)	Virilism (n)	Cushing's (n)	Abdominal mass(n)
16 yr	adenoma (10)	4	4	2	1
Or	carcinoma (8)	5	2	2	1
Less	unspecified (5)	2	5	3	1
Above	adenoma (4)	0	0	1	2
16yr	carcinoma 15)	0	1	9	3
Old	unspecified (1)	0	0	1	0

Steroid profiles

Approximately 20 of the major steroid metabolites were quantified using high-resolution gas chromatography. The patterns of excretion were classified according to which steroid(s) predominated (Table 2). Androgen metabolites were the most frequently elevated. Both adenoma and carcinoma were identified histologically in each of the categories of metabolite excretion patterns. One belonging to the miscellaneous category had a normal profile. 16 of the 22 patients with proven carcinoma and 8 of the 15 patients with adenoma had raised excretion of 11-deoxycortisol metabolites.

Table 2. Classification of 50 patients with adrenocortical neoplasms according to major urinary steroid metabolite(s) excreted.

Major metabolite(s)	No of patients (%)
DHA	13 (26)
16 alpha-hydroxy DHA	7 (15)
11-hydroxyandrosterone	6 (12)
3 beta-OH-ene C21 metabolites	2 (4)
DHA & pregnenetriol	2 (4)
11-deoxycortisol metabolites	6 (12)
cortisol metabolites	5 (10)
Miscellaneous	9 (18)

DISCUSSION

Only one of the 50 patients studied here had a normal urinary steroid profile. The pattern or the amount of overall steroid metabolites excreted was abnormal in all the other patients with adrenocortical neoplasm. Thus urinary steroid profiling is an important means of identifying adrenocortical neoplasms. However, there was heterogeneity of urinary steroid profiles in both children (=<16 years) and adults. There were no profiles clearly distinguishing carcinoma from adenoma. This is in agreement with the findings of Malunowicz et al (1995). Grondal et al (1990, 1991), on the other hand, found that 11-deoxy cortisol and its metabolites were commonly elevated in carcinoma and therefore could be used to discriminate malignant and benign adrenocortical neoplasms. In our series, 16 of the 22 patients with carcinoma and 8 of the 15 patients with adenoma had raised excretion of 11-deoxycortisol metabolites. Thus we are not able to use this marker in our series to distinguish malignant and benign adrenocortical neoplasms. One interesting steroid profile pattern showed acquired enzyme deficiency. For example, a 3 beta-hydroxysteroid dehydrogenase deficiciency was noted in 4 patients in our series, all of whom had carcinoma. Sakai et al (1994) showed that the mRNA concentrations for this enzyme (thus its activity) were markedly lower in carcinomas compared to normal or adenomatous adrenal glands.

Advances in imaging (Bardet et al 1996, Dominguez-Gadea et al 1994) and immunohistology (Marx et al 1996) have increased the diagnostic ability to distinguish benign from malignant adrenocortical neoplasms. These methods are helpful in the evaluation of incidentalomas as well as in patients presenting with endocrinopathies possibly related to the adrenal glands. Steroid profiling would complement these. Furthermore, we would recommend that profiling always be carried out before tumour excision in order to identify suitable steroids as markers of recurrence. Preliminary results suggest that this is feasible and useful (Grondal et al 1990; Khorram-Manesh et al 1998).

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REFERENCES

1. Bardet S, Rohmer V, Murat A et al. 131I-6 beta-iodomethylnorvholesterol scintigraphy: an assessment of its role in the investigation of adrenocortical incidentalomas. *Clin Endocrinol* 1996;44(5):587-96.
2. Bowlby LS, DeBault LE, Abraham SR. Flow cytrometric analysis of adrenal cortical tumour DNA. Relationship between cellular DNA and histopathologic classification. *Cancer* 1986;58(7):1499-505.
3. Brodie B, Novick AC, Pontes JE et al. The Cleveland Clinic experience with adrenal cortical carcinoma. *J Urol* 1989;141(2):257-60.
4. Cagle PT, Hough AJ, Pysher T et al. Comparison of adrenocortical tumours in children and adults. *Cancer* 1986;57(11):2235-37.

5. Chang SY, Lee SS, Ma CP & Lee SK. Non-functioning tumours of the adrenal cortex. *Br J Urol* 1989;63(5):462-4.
6. Dominguez-Gadea L, Diez L, Bas C, Crespo A. Differential diagnosis of solid adrenal mass using adrenocortical scintigraphy. Clin Radiol 1994;49(11):796-9.
7. Grondal S, Cedermark B, Erikkson B et al. Adrenocortical carcinoma: A retrospective study of a rare tumour with a poor prognosis. *E J Surg Oncology* 1990; 16(6):500-6.
8. Grondal S, Curstedt T. Steroid profile in serum: increased levels of sulphated pregnenolone and preg-5-ene-3 beta,20 alpha-diol in patients with adrenocortical carcinoma. *Acta Endocrinol* 1991;124(4):381-5.
9. Grondal S, Eriksson B, Hagenas L et al. Steroid profile in urine: a useful tool in the diagnosis and follow up of adrenocortical carcinoma. *Acta Endocrinol* 1990;122:656-663.
10. Honour JW, Price DA, Taylor NF et al. Steroid biochemistry of virilising adrenal tumours in childhood. *E J Pediatr* 1984;142:165-169.
11. Kasperlik-Zeluska AA, Roslonowska E, Slowinska-Srzednicka J et al. Incidentally discovered adrenal mass (incidentaloma): investigation and management of 208 patients. *Clin Endocrinol* 1997;46(1):29-37.
12. Khorram-Manesh A, Ahlman H, Jansson S et al. Adrenocortical carcinoma: surgery and mitotane for treatment and steroid profile for follow-up. *World J Surg* 1998;22(6):605-12.
13. Malunowicz EM, Ginalska-Malinowska M, Romer TE et al. Heterogeneity of urinary steroid profiles in children with adrenocortical tumors. *Horm Res* 1995;44(4):182-8.
14. Marx C, Wolkersdorfer GW, Brown JW et al. MHC class II expression—a new tool to assess dignity in adrenocortical tumours. *J Clin Endocrinol Metab* 1996;81(12):4488-91.
15. Sakai Y, Yanase T, hara T et al. Mechanism of abnormal production of adrenal androgens in patients with adrenocortical adenomas and carcinomas. *J Clin Endocrinol Metab* 1994;78(1):36-40.
16. Teinturier C, Pauchard MS, Brugieres L et al. Clinical and prognostic aspects of adrenocortical neoplasms in childhood. *Med Pediatr Oncol* 1999;32(2):106-11.
17. Vankatesh S, Hickey RC, Sellin RV et al. Adrenal cortical carcinoma. *Cancer* 1989;64(3):765-769.
18. Weiss LM, Medeiros LJ, Vickey Al Jr. Pathologic features of prognostic significance in adrenocortical carcinoma. Am J Surg Pathology 1989;13(3):202-6.