MOLECULAR ANALYSIS IN GENDER ASSIGNMENT AND MANAGEMENT OF AMBIGUOUS GENITALIA

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ABSTRACT

Patients presenting with ambiguous genitalia (AG) often pose a dilemma to the attending clinicians as to definite sex assignment. To avoid any doubt about gender of rearing it is necessary that correct diagnosis is made within a short period of birth. Current hormonal and imaging studies for sex determination could be supported by the application of chromosomal and molecular genetic studies using the SRY gene. Delay in diagnosis and treatment of congenital adrenal hyperplasia (CAH) lead to adrenal crisis. Moreover inappropriate gender assignment has tragic consequences. Clinicians must be aware as well that pseudohypoaldosteronism can masquerade as CAH and causes inappropriate hormonal therapy. The SRY gene complements the investigations and has aided us in patient management and counseling of parents. In the near future we are proceeding to the analysis of the CYP21 gene to confirm diagnosis of CAH due to 21- hydroxylase deficiency.

INTRODUCTION

The issue of correct gender assignment causes some amount of anxiety and the problem of acceptance on the part of the parents. Patients with ambiguous genitalia should be investigated for definite sex assignment. Diagnosis will be aided by hormonal, imaging studies and genetics information. Current clinical and hormonal investigation protocols for sex determination could be supported by the application of chromosomal and molecular genetic studies using SRY gene as one of the tools for gender assignment.

AIM

To apply molecular studies to complement investigations for the genetic assignment of patient with ambiguous genitalia.

METHODS

Three patients were referred to Hospital Kota Bharu (HKB) and Hospital Universiti Sains Malaysia (HUSM) for further management of ambiguous genitalia. 3 - 5 ml blood specimen from the patients with ambiguous genitalia was collected in sodium EDTA or lithium heparin container. Blood was cultured for metaphase cells for cytogenetic and FISH (Fluorescence in situ hybridization) according to the standard protocol. Genomic DNA was extracted and subjected to the PCR analysis of SRY gene using modified method described earlier [1,2].

RESULTS

Table 1

Tests/Patient	I	II	III
Sodium 135-150 mmol/L	92	118	138**
Potassium 3.5 – 5.0 mmol/L	7.4	8.2	5.5**
17-OHP	>20 3 mths-5 yrs : up to 1.1 ng/ml	33.2 (<0.7-2.5)	2.8 nmol/L* (normal for age)
Cortisol	484 am :139-501	NA	67 nmol/L (am: 221-690)
ACTH	ND	50.7 pg/ml (N: <37)	ND
Testosterone	NA	<20 (ng/dl)	3.0 nmol/L adult female: 0.9-2.8
Aldosterone	>3,300 (111-860 pmol/L)	NA	1,400 pmol/L* erect: 110-860 supine : 30-440
PRA	NA	NA	72.0 ng/ml/h erect: 1.3-4.0 supine:0.1-2.4
Karyotype	46,XX	46,XX	46,XX
SRY gene	Not present	Not present	Not present
US pelvis/abdomen	Uterus and ovaries	Uterus	Uterus. Bilateral pelvi-calyceal system dilatation. Mildly distended bladder.
Genitogram	Figure 2	Figure 3	Not done

* results from overseas

** patient was on glucocorticoids and mineralocorticoids

NA: not available; ND : not done

RESULTS

PT	AGE	SEX	CLINICAL FEATURES	DIAGNOSIS	THERAPY	COMPLICATION / PROGRESS
I	10 w	F	Failure to thrive. Adrenal crisis. AG: phallus 2 cm, no gonads palpable, no rugae, no hyperpigmentation, single orifice at base of the phallus.	CAH, salt loser	G and M	**Undertreatment and poor compliance caused virilisation, Patient was put on high dose of glucocorticoids to suppress virilisation. At 6 years old, she had fits and treated as meningitis. Had hypertension and left ventricular hypertrophy.**
II	6 d 9 m	M F	Severe neonatal jaundice. AG: prominent phallus, hyperpigmented labio-scrotal folds, single orifice at the base of phallus, no gonads palpable. Reassigned as female. Clitoris measures 2.5 cm	CAH, salt loser	G and M	**Sex reassignment at 6 months old.**
III	13 d	F	AG: clitoris 0.9 cm Slight hyperpigmentation and scrotalisation of the medial aspect of the labia majora. No palpable gonads, no fusion of labia minora, 2 slit–like orifices seen. Mild enlargement of adrenal glands.	Pseudohypoal-dosteronism	G and M	**Bilateral pelvi-calyceal system dilatation. Mildly distended urinary bladder. Both abnormalities resolved after one month. Normal electrolytes after discontinuation of hormonal treatment. Normal repeat US of genitourinary system. Normal MCU.**

PT: patient, d: day, w: week, m: months, F: female, M: male, G: Glucocorticoids, M: mineralocorticoids, US: ultrasound, MCU : micturating cystourethrogram

RESULTS

Fig. 1 : External genitalia of patient enlarged clitoris of 3.2 cm.

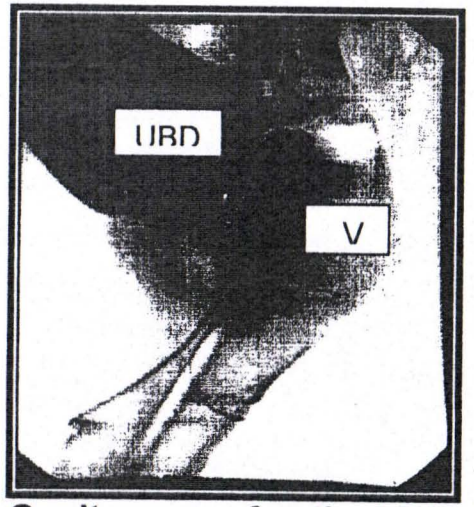

Fig. 2 : Genitogram of patient I, both the bladder and vagina (V) are opacified. Single perineal opening with short segment urogenital sinus.

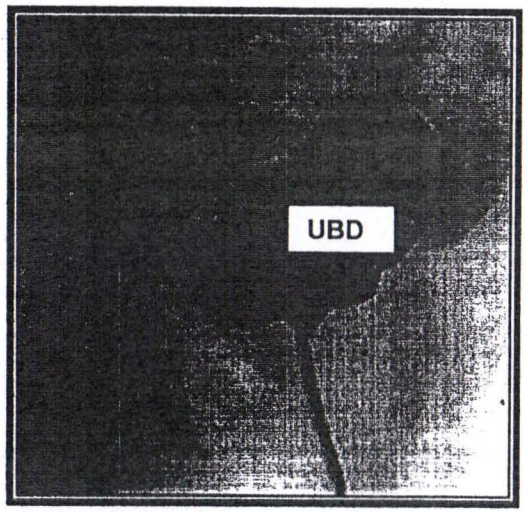

Fig. 3 : Genitogram of patient II, the urinary bladder (UBD) was filled with contrast and normal in outline

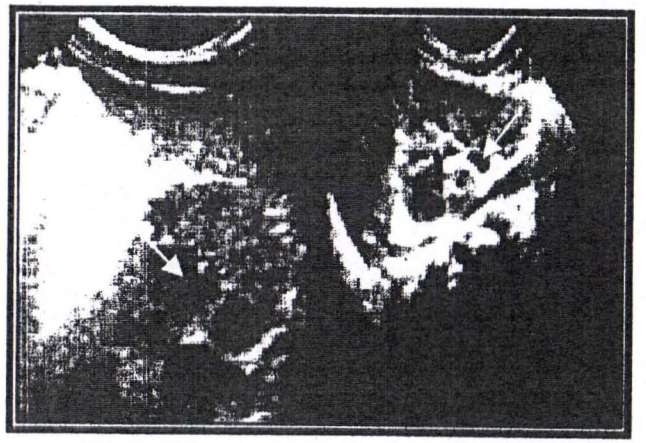

Fig. 4 : Ultrasound of the kidneys of patient III : Dilated pelvicalyceal system of both kidneys (arrow). Mild dilatation of urinary bladder. No adrenal mass seen.

RESULTS

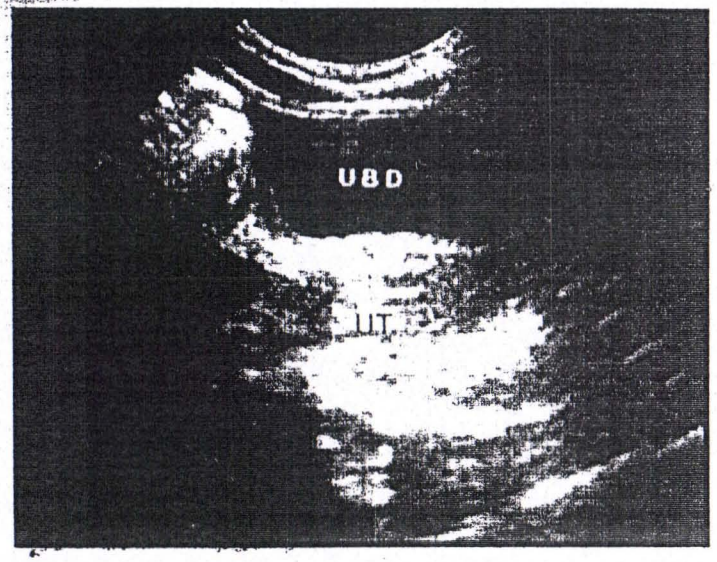

Fig. 5 : US pelvis showing the uterus (UT) & urinary bladder (UBD).

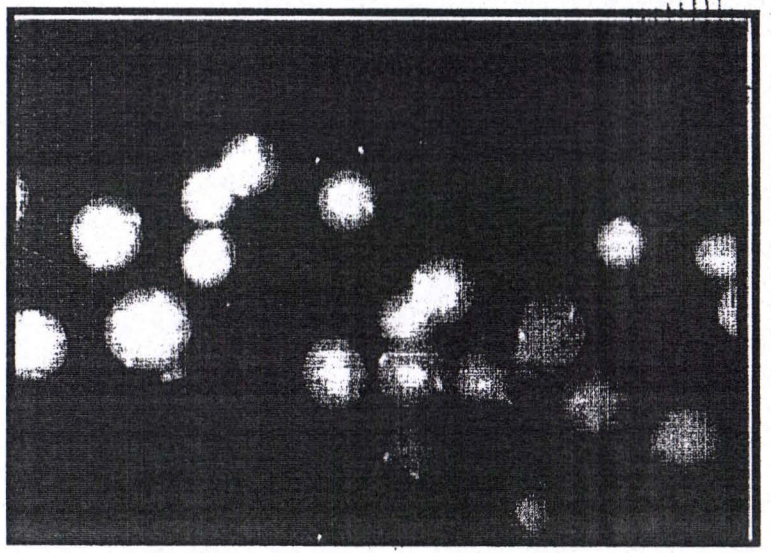

Fig. 6:FISH with X chromosome.

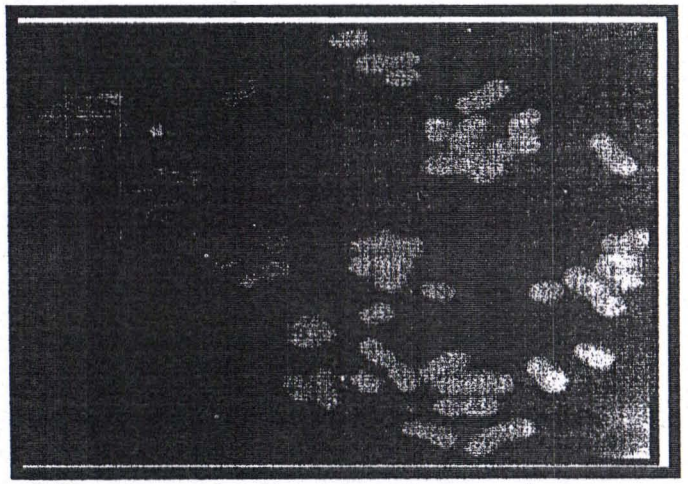

Fig. 7:FISH with Y chromosome

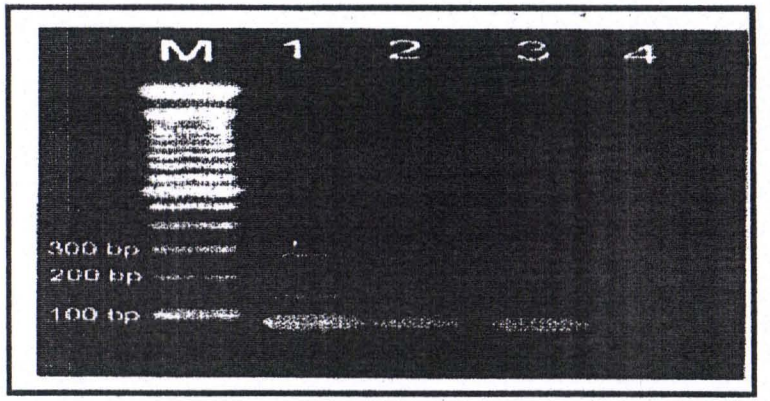

Fig. 8 : Multiplex PCR of X,Y chromosome and SRY gene specific regions.
M-100 bp DNA ladder
1-DNA sample from 46 XY with the presence of SRY gene
2- DNA sample from 46 XX
3- DNA sample from 46 XY with the absence of SRY gene
4-negative control (without DNA)

DISCUSSION

The presentation of the 3 patients illustrated the problem encountered in managing AG/CAH. The diagnosis of CAH has to be suspected and confirmed through investigations. Delay in diagnosis and treatment of CAH lead to development of adrenal crisis which can be life threatening **(pt.1 & 2)**.

Inappropriate gender assignment has tragic consequences. In a newborn baby with AG, sex assignment should not be attempted until the results of appropriate investigations are available. Patient 2 was assigned as male at birth but after the AG was noted and investigated, patient was reassigned as female. Sex confirmation through genetic studies includes karyotyping and SRY gene.

Patient 3 was treated as CAH and relevant hormonal investigations performed. From the results of the investigations **(table 1)**, pseudohypoaldosteronism was suspected. Urosepsis was ruled out from the urine examination. However, the full blood picture showed reactive lymphocytes, thrombocytosis and an umbilical swab showed a mixed growth of Gram negative bacilli suggestive of a possible infection. Subsequent ultrasound of the genitourinary system and electrolytes was normal after cessation of hormonal medications. Pseudohypoaldosteronism can masquarade as the salt losing type of CAH.

Imaging studies can aid in identifying the uterus **(fig.5)** and gonads. Genitogram will identify the anatomy of the genitourinary system **(fig. 2&3)**, which is important for future surgical correction. Clitoroplasty was not performed in patient I due to parents' poor understanding of the importance of surgical correction.

Close monitoring of the patient is needed to ensure that virilisation or hypertension did not occur due to under or overdosage of hormonal therapy respectively.

CONCLUSION

Molecular studies complement hormonal and imaging studies for sex confirmation and appropriate sex assignment or reassignment. Regular medical assessment and monitoring of treatment is the key to the successful management of CAH. Pseudohypoaldosteronism is another differential diagnosis to be thought of in cases of electrolyte imbalances that causes inappropriate hormonal therapy. Counseling of the parents is important to avoid mismanagement, complication and psychosocial issues. We intend to study the CYP21 gene rearrangement to confirm diagnosis of CAH in those patients presenting with AG.

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