CYTOGENETICAL AND CLINICAL INVESTIGATIONS
OF A CASE OF
FEMALE PSEUDOHERMAFRODITISM

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SUMMARY (online version)
A case of a female pseudohermaphroditism is presented. The aetiologic frame and
genetic counseling indications are based on cytogenetic, biochemical and clinical
investigations of the patient and on familial inquiry concerning sibship and pregnancy
evolution.

KEY WORDS: female pseudohermaphroditism, karyotype, genetic counseling.

INTRODUCTION
After the fertilization of the secondary oocyte by the spermatozoa
result a zygote and genetic sex of future organism is established. During
embryonic and fetal development sex differentiation occurs in a cascade like
manner with temporally successive steps according to genetic programme.
From initially indifferent stage develop the gonads, the efferent ducts and
the external genitalia. (Checiu)

If a Y chromosome is present, early embryonic testes develop under
the influence of testis – determining factor. Due to a testis – derived
hormone (the müllerian inhibition factor) the further development of the
müllerian ducts is suppressed and the embryo develops as a male.

If a zygote there are two X chromosomes and it is not a Y
chromosome, testes are not formed and from undifferentiated gonads
develop ovaries, the müllerian ducts differentiate into the uterus, uterine
tubes and upper vagina. (Max, Vogel)

A lot of situations of genetically determined disorders of sex
determination and differentiation were described. In these disorders
mutation or chromosomal aberrations are involved. The abnormal sexual
development is expressed by some chromosomal syndromes and by three
types of intersex: true hermaphroditism, male pseudohermaphroditism and
female pseudohermaphroditism. Each of these disorders can be
characterized after clinical observations and laboratory investigations.
MATERIAL AND METHODS

Patient was carefully examined for morpho-anatomical features and family inquiry concerning state of pregnancy and sibship were undertaken.

For cyrogenetic diagnosis periferic blood cells were obtained by venous punction in heparinised vacuum tainer. Culture medium consisted from RPMI 1640 Gibco supplemented with 15% fetal calf serum, PHA Gibco and antibiotics. Cell culture was incubated for 72 hours at 37°C in a CO₂ incubator. Two hours before the end of culture time a colcemid solution was added to have a 0,2 µg/ml concentration in culture medium. Cells were hypotonised in 0,075 M KCl 10 min and then fixed in absolute methanol/ acetic acid 3:1. Slides obtained from cells suspension were treated with trypsin solution and after their drying were stained with Giemsa. Good metaphases were observed and photographed finally obtaining the karyotype.

X chromatin was pointed out on smears obtained by drawing cells from lower lip of the patient.

Biochemical investigations and anatomico-pathological examinations were practiced.

RESULTS AND DISCUSSIONS

The new-born N.R., nine days of age presented at the entrance in the pediatric hospital sexual ambiguity, pronounced weight diminution and extracelular type of dehydratation state. Familial inquiry indicated young healthy parents, without relationship. Child’s parents denied any malformation among near or distant kindred excepting a congenital deaf and dumb father’s nephew. The patient has a brother of two years and six months affirmatively in good state of health. A sister of the patient deceased at five months age having Debre - Fiebiger syndrome and presenting hypospadias was declared after her birth that being a boy. Mother declared that the aspect of external genitalia of this deceased child was similar to the patient. The evolution of health state was favorable and the patient died at five months and two weeks of age.

Anatomico – pathological examination revealed: a bilateral suprarenal glands hyperplasia (both glands have 8 g weight); internal genitalia of female type; uterus, fallopian tubes and ovaries normal constituted; widened
vagina without perineal opening, hypertrophy of the clitoris, perineal urethra, scrotum - aliased vulva.

The newborn come after a pregnancy with disturbances in the first trimester and uterine contractions since the sixth month. The birth was spontaneously at 36 weeks of gestation, eutocious. The placenta was senescent with infarctions. The newborn presented the stigma of placentary insufficiency syndrome: fetal hypotrophy, weight 2700 g, body size, very dry teguments having tendency to desquamate, absence of cellular subcutaneous tissue.

Striking are the alterations of external genitalia: hypertrophy clitoris – shaped, the big labia tumefied and coalescent without feeling of gonads. A single orifice is situated at the basis of the clitoris and a ditch on the dorsal clitoris face; hyper pigmentation of the big labia, of white line and of mammary nipples.

As first step of the diagnosis this status of sexual ambiguity must be integrate in one of the three great groups proposed by Job. For this integration some investigations were necessary: genetic sex of the newborn is female revealed by 25% positive X chromatin and 46, XX karyotype. The genitography indicated the presence of vagina, uterus, fallopian tubes and urogenital sinus.

It is possible to consider that this case is one of female pseudohermaphroditism (FPH) and can be classified in the four type of five anatomic types described by Prader, having the following features: hypertrophy of the clitoris giving the aspect of a hypospadias with dorsal prepuce and the existence of a ditch starting from glands till the basis, labia majora partially fused.

In order to define more accurately the etiological frame of this kind of FPH it is necessary to compare clinical and paraclinical data of the affections known to be include in FPH with data about our case.

Three main groups of FPH are recognized. The most frequent is group with suprarenal virility inducing hyperplasia which is due to an enzyme blocking: 21 hydroxylase or 11 hydroxylase or 3-beta-01-dehydrogenase. In all cases common deficiency is cortisol biosynthesis defect leading to a growth of ACTH secretion. The consequence of this growth is suprarenal hyperplasia and the growth of androgens secretions continuing during all the life and indicating an evolutive virilization. The diagnosis is based on
hormonal dosing. For other cases of FPH there is neither evolutive virilization nor height or bones growth. Hormonal dosing gives normal results. The puberty is realized following a normal female pattern without treatment.

More types of disturbances are found by paraclinical data analysis:
- hydroelectrolytical disturbances generally characterized by high blood levels of sodium and potassium and high level of sodium and lower level of potassium in urine, in fact these disturbances belongs to salt loss syndrome.
- acid and basic disturbances characterized by uncompensatory metabolic acidosis.
- grown elimination of 17 – cetosteroids.
- blood pressure values constantly grown.

The results of these data analysis are that our case is one of FPH (IV Prader type) with salt loss syndrome and arterial hypertension. Subsequent etiological considerations could not be based on paraclinical arguments because of the absence of the urinary steroids gas chromatography but such considerations could come from theoretical arguments about pathogenesis. Analyzing the table of suprarenal hormones synthesis we find that only blocking of 11 – hydroxylase comprise symptomatic criteria of presented case: in this care appear a deficit in synthesis of gluco and mineralocorticoids consecutively existing ACTH and MSH hypersecretion DOC accumulation explaining arterial hypertension DHEA accumulation accounting for virilization and growth elimination of 17 cetosteroids.

Therapeutic problem of this case is solved, as in all cases of FPH with suprarenal hyperplasia. The treatment consist in administration of cortisone a mineralocorticoids stopping in this manner in excess formation of ACTH.

Regarding genetic counseling line on conduct we take into account that FPH is a genetic autosomal recessive disease. Being heterozygotes parents could have theoretically a homozygote ill child in a ratio of \( \frac{1}{4} \). Practically, around this value, many or all children of a couple could be normal or the number of abnormal children can be higher than \( \frac{1}{4} \). This is the situation of our case which comes from a couple having one normal child and two affected children. So for future pregnancies the risk of recurrence is important taking into account also the advanced form of pseudohermaphroditism (IV Prader type) for two children. This value of risk
was communicated to the parents and only they can decide about their determinations to have another child for the future.

REFERENCES


FIG.1 Cariotipul unei femei cu pseudohermafroditism