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Research Article

Ambiguous genitalia: a clinical and chromosomal study

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ABSTRACT

Background: To correlate the findings of physical examination, ultrasonography and chromosomal analysis, so that to give proper advice to the concerned parents as early as possible in rearing up of the child as male or female according to the situation.
Methods: The study is undertaken with forty cases with ages ranging from new borne to 20 yrs. Out of these 40 cases eight cases are below one year. In these cases physical examination is correlated with ultrasonography and chromosomal analysis.
Results: In chromosomal analysis three persons out of forty cases were mosaics with 45, XO/46, twenty one cases who showed the chromosomal pattern as 46, XY mostly showed with no mullarian reminents. On examination palpable gonads were found in labio-scrotal sacs in seventeen cases. One of these cases was reared as girl found cytogenetically as 46, XY with the ultrasonographic impression as small uterus with no ovaries. Nineteen cases who with ambiguous genitalia showed the chromosomal pattern as 46, XX one out of these cases showed enlargement of the breast, and on examination of external genitalia found enlarged clitoris with labiamajora and minora. The child was brought up as male. Genitogram showed the absence of uterus.
Conclusions: Chromosomal studies with ultrasonography can help in rearing a child male or female in young generation by surgical and Hormonal therapy. This prevents many problems in later life. This fact should be advertised openly in the public so that illiterate people should be alert.

Keywords: Ultrasonography, Chromosomal analysis, Mullarian reminents

INTRODUCTION

In recent years there in an increasing awareness among general public regarding ambiguous genitalia in new born babies. The confused parents often post pone the problem thinking that the problem solves on itself as the child grows. However, now-a-days with greater input from the media with newspaper articles and medical talks and chart shows on small screens, there is an increase in the number of parents consulting the gynaecologists, genetists for assistance, Urologists are also consulted for the treatment of hypospadias. Pediatricians and endocrinologists are consulted with symptomology of adrenogenital syndrome. Early involvement of a clinical psychologist with experience in this field should be mandatory. Other professionals including geneticists and gynaecologists may also become involved. There must be access to specialist laboratory facilities and experienced radiologists. The incidence of genital ambiguity that results in the child's sex being uncertain is 1 per 4500,[1] although some degree of male undervirilisation, or female virilisation may be present in as many as 2% of live births.[2]

However the outcome of some of the investigations may take some weeks and registration of the child's birth should be deferred until gender has been assigned. This may require communication with the Registrar of Births, and a skilled clinical psychologist will help the parents in deciding what to tell family and friends in the interim. It

is also helpful (if appropriate) to reassure the parents that their child is otherwise healthy.[3,4]

Ambiguous genitalia come under a group of congenital anamolies known as Hermaphrodism. The word hermophrodism is derived from a bisexual Greek God Hermophroditos, the offspring of Hermes and Aphrodite. Hermaphrodism is of two types. Pseudohermaphrodism and True hermaphrodites.[5] True hermaphrodites are defined as having gonads of both sexes separately or as ovotestis but chromosomal complement can be 46, XX or 46,XX/46,XY. Pseudohermaphrodite is defined as a state when a person is having ambiguous genetalia, so that sex cannot be determined and having gonads of any sex, usually with chromosomal complement showing 46 XX or 46 XY or mosaic. The pseudohermophrodism can cause so many social and psychological problem.[6]

These cases are referred for chromosomal analysis. Due to ambiguity a male child would have been brought up as a female and vice versa. When the signs of masculinity or feminity do not appear at the appropriate age, then alarm bell rings for the parents.[7] If the child is brought up as a girl, then the question of marriage looms large in their minds. The psychological and physical trauma to the child and parents is immense. As mentioned earlier parents are driven from speciality to speciality. It is this aspect of the problem that provoked us to undertake a study for Karyotyping to find out chromosomal sex, comparing the chromosomal study with the ultra sound scanning for internal sex organs and Education of public for early detection along with establishing a help line for confused parents.

It is this aspect of the problem that provoked the author to undertake a study to know Incidence so far reported in India/and other countries or whether the condition found at birth, before puberty or after puberty, Karyotyping done to find out chromosomal sex, Comparing the chromosomal study with the ultra sound scanning for internal sex organs and also Education of public for early detection along with establishing a help line for confused parents.

METHODS

Present study includes 40 cases of ambiguous genitalia, referred to the Cytogenetic Laboratory of Department of Anatomy of Osmania Medical College, Hyderabad. All the cases were referred from Niloufer Hospital, Govt. Maternity Hospital, Urology and Endocrinology Department of Osmania Hospital – Hyderabad.

As the patients were received in the department, a detailed family history and clinical history were taken, physical examination was done and the findings were correlated with the clinical findings. The chromosomal analysis was done in all the cases. Family history was taken as Parental age, other affected children in the family, History of consanguinity and Rearing of child (as male or as female), Clinical history was taken to rule out other systamic abnormalities like C.V.S, Urinary and phychological disturbances. Physical examination was done to examine External genitalia as size of phallus, labioscrotal folds, and gonads. Secondary sexual characters were noted as development of breast, axillary and pubic hair and change in voice and also any other abnormalities were observed. Clinical investigations are done as ultrasonography, hormonal assay and genitogram.

Chromosomal analysis for all the persons was done by making a lymphocyte culture from peripheral blood. A karyotype is prepared from the cells and was studied.

Procedure for lymphocyte culture

Procedure for lymphocytic culture in micromethod for karyotyping is discussed under the following headings: As a pre-requisite to lymphocyte culture all the glassware to be used should be cleaned thoroughly and sterilized properly.

Following steps are followed:

A. Collection of serum

- Calf blood: About 500 ml is drawn from jugular veins.
- The blood is set aside for 24 hrs in a refrigerator.
- The serum is collected or pippetted out into other sterilized alloquets and kept in the water bath (55°C) for 2 hrs for detoxification, (optimum temperature is selected to avoid coagulation of plasma proteins).

B. Preparation of media

RPMI 1640 Media (HI Media - 1640 media with glutamine without $NaHCO_3$ with antibiotic 10.3 gm/litre). RMPI 1640 media powder 100 gms is weighted under strict asceptic precautions and is transferred into a sterilized media bottle. To this 100 cc of sterilized glass distilled water is (triple distilled) added.

- To the media thus prepared, the first ingredient added is the antibiotic gentamycin 10 drops (0.5 cc)
- Then Heparin – 1 ml is added
- Human serum 20 ml is added which is the nutrient part
- Phytoheomoagglutinin M 1 ml is added.
- pH of medium is maintained by adding $NAHCO_3$ 0.4 to 0.5 ml at 7.1
- Once 100 ml of medium is prepared it is divided in alloquets of 10 ml each culture tubes.

C. Culture technique

- About 1 ml of blood is drawn with heparinised syringe. 10-12 drops are added to the media in the strerilized culture tubes.

- The culture tubes were incubated for 70 hrs at 37.5° C. At the end of 70 hrs four drops of 0.4 micrograms of colchicin is added to arrest the cell division at metaphase stage. Culture is incubated in the incubator for a further period of 2 hrs.
- At the end of 72 hrs the culture is taken out, centrifuged at 1000 RPM for 10 mts. The supernatant fluid is discarded; the pellet at the bottom of the tube is taken.
- To the pellet, 5 ml of hypotonic saline (0.075 KCl) is added. The culture tubes are kept at room temperature for 25 mts.
- After 25 mts, centrifuged at the rate of 500 RPM for 5 minutes. The supernatant is pippetted off and recidue dispersed by shaking.
- The pellet is fixed in 3:1 methonol and glacial acetic acid (Conroy fixative) for 8 min, the centrifuged at the speed of 1000 RPM for 10 mts.
- Supernatant fluid is discarded and the same procedure is repeated twice or thrice until the sediment is clear.
- After the 3rd fixation the pellet with the little fixative is left over and thoroughly mixed.
- The pellet is then drawn with the micro pipette and droped over a clean chilled slide kept at 45◦ angle and delivered 2 to 3 drops of the fixed cell suspension from a height of 10 to 20 cm above the slide.

D. Staining of slides

- Slides thus prepared and dried are stained by Giemsa stain as per standard procedures (Rooney & Czepulkowski)

E. Karyotyping

- Presently microphotographs of metaphase spreads are taken in oil immersion and the characters of chromosomes studied. Most of the study is based on eye karyotyping.

RESULTS

In this study 40 cases of ambiguous genitalia analysed the chromosomes compared it with ultrasonography and physical examination. It was very interesting to know out of these 40 cases half of them were reared as males and half were reared as females. Age groups varied from 1 month to 20 years. Below one year there were 8 cases in which 4 cases were reared as females and 4 as males. Most of the cases i.e., about 45% were in the age group of 1 – 5 years. (18 cases) In this large group 25% were reared as male and 20% as females.

Between 6 – 10 years of age there were 5 cases about (12.5%). Out of which 7.5% reared as females and rest as 5.5% as males. The author observed only 3 cases i.e., 7.5% is coming to this institution between 11 – 15 years of age group. Out of which 1 case reared as female and rest i.e., 2 cases as male. In 16 – 20 years of age group there were 6 cases i.e., about 15% of total cases of which 2 of them were reared as female and 4 of them reared as males.

Table 1: Age groups analysed for ambiguous genitalia.

Age group	No. of cases
0 – 1 yrs	8
1 – 5 yrs	18
6– 10yrs	5
11 – 15 yrs	3
16 – 20 yrs	6

Examining the external genetalia in detail, author has described the ext. genitalia into following type of classification (followed from the text book of Disorders of Sexual differentiation etiology and clinical delineation. By Joe Leigh Simpson as examination for phallus, labio-scrotal folds and position of gonads.

Most of the cases (about 23) were having the Microphallus (57%), Penile Hypospadiasis was observed in 22 cases to (55%). In 10 cases the author has observed that labio-scrotal folds were not fused (25%). The cases which showed non-union of labio-scrotal folds and had showed vaginal opening are only 5 cases (about 12.5%).

In these cases the position, size and consistency of the gonads were observed. Out of 40 cases, 27 cases showed the gonads in various positions-some in inguinal region and some in Labio-scotal region. Out of these 27 cases, gonads observed in labio-scrotal sacs were 17 (42.5%). Gonads observed in the inguinal region were 6 i.e., (17.5%). Out of these, 3 cases showed one gonad in Labio-scrotal sac and one gonad in inguinal region.

It is observed inguinal gonads with unfused scrotal sac in 5 cases (12.5%). Scrotal gonads in unfused scrotal sacs are 4 cases (i.e., 10%), about 4 cases partially (10%). 9 cases were observed with fused scrotal sacs with scrotal gonads (i.e. 22.5%). Fused scrotal sacs with inguinal gonads in 6 cases (15%). One case of these fused scrotal sacs showed one gonad in inguinal region one gonad in scrotal region. Most of the gonads showed firm consistency.

Ultrasonography is taken into consideration. Of the 75% of cases which showed fusion of labioscrotal folds ultrasonography revealed uterus in 5 cases (12.5%). Out of these 5 cases, only one case showed presence of ovaries. Then with labioscrotal sacs unfused (i.e., out of 25%), only 2 cases showed uterus without ovaries. The author made an effort to get the biopsy for these cases to correlate between chromosomal analysis and external genitalia. Because of lack of awareness in the patients she could not succeed in getting the biopsy done as the patients have not turned up.

Lastly the author is interested to correlate the chromosomal analysis with the above mentioned classification. In 40 cases the author has observed 21 cases as 46 XY, 13 cases as 46, XX and 2 cases showed true hermorphroditism i.e 46, XX/46 XY and 4 cases showed mosaic pattern 46, XO/46, XY. Therefore out of 46, XY chromosomal components, 21 numbers of cases showed variable forms of external genitalia. 14 cases were with hypospadiasis 15 cases were having fused labioscrotal swellings. 6 were having unfused labioscrotal swellings. In 13 cases gonads were palpable in fused labio scrotal swellings.

In 3 cases uterus was like tissue found on ultrasonography. Ovarian tissue could not be seen on ultrasonography in all those cases. In 3 cases, testes in the scrotal sacs were found in ultrasonogram. With 46, XX component 13 cases showed the following variations in external genetalia. 7 cases presented with fused labioscrotal swellings. 6 cases presented with unfused labioscrotal folds.

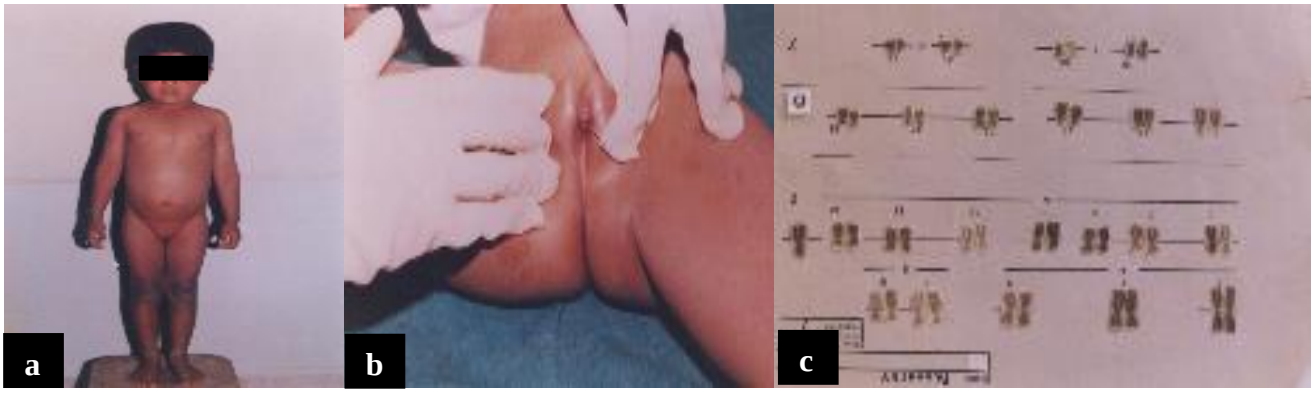

Figure 1: Case 1.

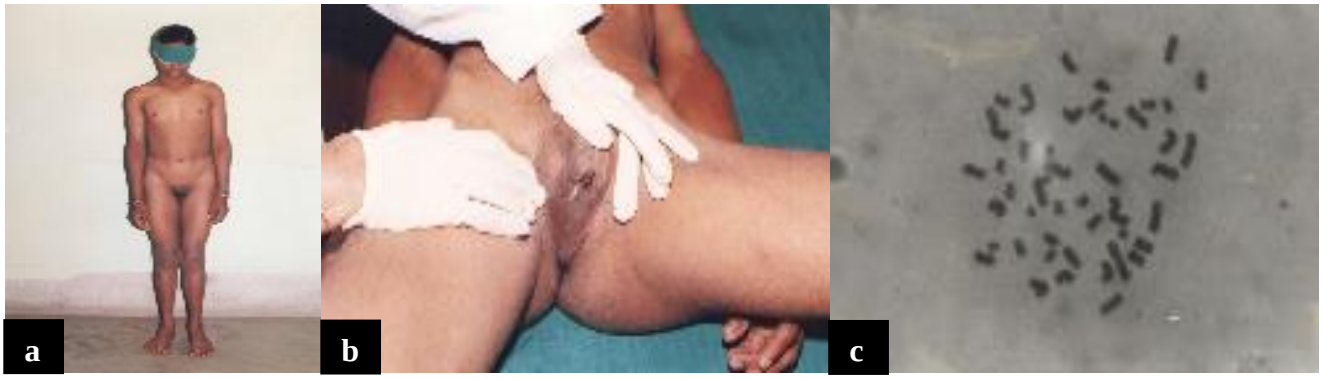

Figure 2: Case 2.

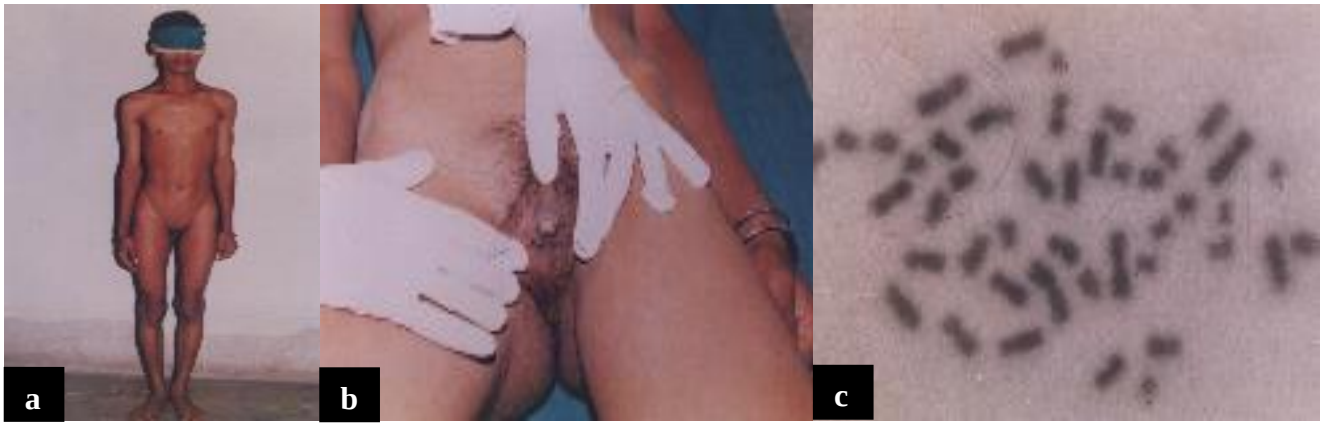

Figure 3: Case 3.

Out of these 13 cases vaginal opening was found in 6 cases. No gonads were palpable either in labioscrotal swellings or in the inguinal region. On ultrasonography, 6 cases showed variable development of Mullerian system and ovaries. In 4 cases with chromosomal component 46, XO/ 46 XY (mosaic) showed the following external genitalia variations. Fused labioscrotal swelling one case and Unfused scrotal swelling one case other Two cases which showed the true hermaphoditism (46, XY/46 XX) showed the external genitialia as Fused labio-sacrotal swelling one case and Unfused labio-scrotal swelling one case.

DISCUSSION

An extensive review of available literature has revealed that Ambiguous genitalia are known by 300 B.C. the term hermaphrodite was used by Theoprost and Alkiphran. The literature shows that there was a lot of confusion regarding the acceptance of hermaphrodite.

Identifying this condition at an early age is far better for the patient, was in concurrence with the opinion of peadiatrician Moloshik et al[8] who said that identifying hermaphrodite is equally important as identifying them at an early age. Barbara M. Lippee[9] has mentioned that ambiguous genetalia is a medical emergency and the gender of the child must be assigned as rapidly as possible to avoid later complications.

In reported case reared as girl but her chromosomal study revealed 46, XY with USG impresson as small rudimentary uterus without any ovaries. This was coinciding with the studies of Kajam. M. Khatoous[10] reported a case reared as girl with chromosomal compliment 46, XY. Ultrasound findings revealed only streaks of testicular tissue with no uterus or fallopian tubes.

Presently it is studied as 3 cases were siblings reared as girls but the chromosomal study revealed for all three cases as 45, XO. The mothers of the 3 affected patients are sisters married to same person. The reason for ambiguity of sex in these is due to a weakness of 'Y' which was not enough to produce testosterone. Author Frimberger et al[11] reported sex reversal individual, reversal of sexing 4 cases, of which 2 were siblings, one having 46, XX male and 46, XX/46 XXXY true hermaphrodite. The two were 46, XX/46, XY true hermaphrodite. The other two were 46, XX male.

It is found that 9 out of her 40 cases of ambiguous genetalia with masculinization of the external genitalia with the cytogenetic analysis as 46, XX, which was very much concurrent with the study of Seaver Grimes[12] in his study of female hermaphroditism. He had given that 46, XX female pseudohermophrodites described with nearly complete masculinization of the external genitalia and no apparent source of testosterone. Out of his 4 cases had apparently normal chromosomes 46, XX, one had a 46, XX del(10) chromosome constitution. The chromosome break point is in the region of Px2. A developmentally important paired boxgene which is expressed in urogenital tissue using the polymerase chain reaction, screened for the presence of multiple Y-Specific sequences, including SRY, could explain masculinization of the external genitalia. All were negative for 'Y' centromere sequence. Furthermore, there was no evidence for adrenal or other source of Testosterone. So he suggested that in these cases, it is the result of abnormal expression of genes which would normally be regulated by testosterone.

Kulleh et al[13] in their study with infants born with ambiguous genitalia investigated with ultrasonography and concluded that those who have a uterus will almost always be assigned the female sex. Infants with ambiguous genitalia but without a uterus will have male pseudohermaphroditism. In present study, there was a case of 46, XY individual reared as female but USG revealed a small rudimentary uterus. Ovaries not visualized. This finding is not coinciding with the study of Kulleh et al.[13]

In 40 cases it is observed 21 cases as 46 XY, 13 cases ass 46, XX and 2 Cases showed true hermaphroditism and 4 cases showed mosasic pattern which differed to some extent with the study of Shipet et al.[14] In his study of intersex children (59 cases studied) and found 18 female pseudohermaphrodites with congenital adrenal hyperplasia (CAH) 20 male pseudohermaphrodites 2 true hermaphrodites born with ambiguous external genitalia assigned the female sex (ambiguous girls).

Present study is centered around only on the numerical aspect of chromosomes in ambiguity in genital organ and the percentage are coinciding with foreign statistics.

CONCLUSIONS

From the above study, conclusion can be drawn that chromosomal studies with ultrasonography can help in rearing a child male or female in young generation by surgical and Hormonal therapy. This prevents many problems in later life. This fact should be advertised openly in the public, so that illiterate people should be alert.

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