CYTOGENETIC STUDY OF REFERRAL CASES WITH GENITAL DISORDERS FROM JAMMU REGION OF J&K STATE

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Abstract

Background: Chromosome abnormalities account significantly for the genesis of aberrant genital and sexual development. Aims: In present study suspected cases with clinical symptoms of abnormal genital malformation were referred to Human Genetic Research cum Counselling Centre (HGRCC) Govt. Medical College GMC/JU to elucidate the presence of different type of genital malformations, cytogenetic confirmation and determining real genetic sex of referral. Methods and materials: Cytogenetic analysis was done by short term lymphocyte culture, GTG banding and Karyotype preparation of metaphase chromosomes by using automated karyotyping workstation. Results: A total of 25 Cases were enrolled, out of them 18 were of Hypospadias, 3 cases of cryptorchidism, 2 of ambiguous genitilia and 1 each of bifid scrotum and hypogonadism associated with gynecomastia. Among 18 cases of hypospadias 13 of distal penile Hypospadias(12 normal male & 1 normal female), 4 of penoscrotal Hypospadias associated with bifid scrotum and split scrotum(46,XY), 2 of ambiguous genitilia (normal female),3 of cryptorchidism possessing bilateral or unilateral undescended testis(46,XY),1case each of bifid scrotum and hypogonadism with gynecomastia(46,XY). Conclusion: All patients were found to be having normal chromosome constitution after karyotyping (22 males and 3 females). Among studied cases Hypospadias is commonest followed by cryptorchidism and ambiguous genitilia and others. Genetic sex was determined in ambiguous genitilia patients was 46,XX. Overall genital malformations were common in male than in female.

Keywords: Genital malformations, Hypospadias, Cryptorchidism, Ambiguous genitilia.

Introduction

Genital malformations are the human congenital anomalies in which developing genitalia is abnormal, that may arises due to the gene mutation or chromosomal aberrations or
environmental and / endocrinological. Although most of them are not lethal but effect may seen as infertility.

The relationship of external genital malformations with chromosomal manipulation is still a matter of debate as several malformations like Hypospadias show negligible association with any chromosomal anomalies.\[1\] Though in some other malformations like intersex sometimes show presence of abnormal Karyotype.\[2\] External genitalia in both male and female are meant for sexual reproduction but in males it’s a common organ for urination and reproduction so any malformation not only affect the fertility but physiological activities also. Chromosomal study aids in correlating the abnormal Karyotype with external genital malformation. The different genital malformations are classified as follows:

Hypospadias: It is the most prevalent urinogenital malformation in which urethral opening is anywhere from anus to nearest position to the tip of penis. The reason behind the occurrence of the disease is incomplete fusion of urethral folds in early embryonic stages.\[6\] The incidence of hypospadias in general population is from 1 in 300 to 1 in 1000.\[7,21\]

Cryptorchidism: In Cryptorchidism one or both the testes have not descended into the scrotum. In some cases it develops later in life, as late as young adulthood. Cryptorchidism is the most common anomaly of external genitilia in males.\[8\] About 4% of full term and 20% of premature infant boys are born with at least one undescended testis. However, about 70% of cryptorchidism testis descends by first year of life, making incidence around 2%. It is different from monarchism, the condition of having only one testicle.\[9\]

Ambiguous genitalia: In this condition only the genital organ is confusing as it does not completely look like either male or female genital organ hence no phenotypic sex can be assigned at birth. This may be sometimes accompanied with bifurcated scrotum which looks like female vulva.\[3\] Also Proximal Hypospadias sometimes associated with penoscrotal transposition and or bifid scrotum. The prevalence has been found increasing in the recent decade, 50 live births now a day’s include 1 patient of sexual ambiguity.\[4\]

The present work was undertaken to locate the chromosome pattern in different individuals with diverse genital malformation phenotypes which will help in determining and assigning their real genetic sex and to rule out any possibility of chromosomal aberration in the referred cases.
MATERIAL AND METHOD:

Study design: Hospital based study.

Ethical approval & consent: Study plan and questionnaire was duly approved by the Animal and Human Experimentation Ethical committee (AHEEC), University of Jammu. Before enrolment, written consent was taken from each case and it was explained that the information revealed by them would be kept strictly confidential.

Inclusion criteria: Patients suspected for genetic disorders were referred by different Department of paediatrics, medicine, neurology, gynaecology and surgery of Govt. Medical College, Jammu and associated hospitals.

Sample size: A total of 25 Cases were referred to Human Genetic Research Cum Counselling Centre (HGRCC) Govt. Medical College (GMC/JU), University of Jammu for Chromosomal study.

Methodology: Short term lymphocyte cultures were set up with slight modification in the protocol given.\(^{[10]}\) In each case 0.3ml of blood incubated in culture at 37° C for 72 hr. After 70 hr Colchicine was added to arrest culture growth, followed by slide preparation and GTG banding to analyse metaphase chromosome.\(^{[11]}\)

Results:

Table 1 depicts 18 cases of Hypospadias, were having distal penile Hypospadias, 12 were found normal male having 46,XY chromosome constitution and one found to have 46,XX normal female Karyotype, 4 were clinically suspected for penoscrotal Hypospadias having 46,XY Karyotype associated with bifid scrotum or split scrotum. In Table 2, three cases of cryptorchidism possessing bilateral or left undescended testis were karyotypically normal with male sex (46, XY).Table 3 shows two cases of ambiguous genitilia detected with normal female chromosome complement. In Table 4, normal male complement was observed in each case of bifid scrotum and hypogonadism associated with gynecomastia. No structural as well as numerical aberration was observed in the present cases.
### Table 1: Showing patients with Hypospadias

<table>
<thead>
<tr>
<th>CLINICAL DIAGNOSIS</th>
<th>Type</th>
<th>SEX</th>
<th>AGE</th>
<th>CONSANGUINITY</th>
<th>CASES ENROLLED</th>
<th>KARYOTYPE ANALYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distal penile hypospadias</td>
<td>12-male 1-female</td>
<td>8 month-30yrs</td>
<td>NC</td>
<td>13</td>
<td>46,XY 46,XX</td>
</tr>
<tr>
<td></td>
<td>Penoscrotal hypospadias with bifid scrotum</td>
<td>Male</td>
<td>5 months</td>
<td>NC</td>
<td>1</td>
<td>46,XY</td>
</tr>
<tr>
<td></td>
<td>Penoscrotal hypospadias with split scrotum</td>
<td>Male</td>
<td>9 months</td>
<td>NC</td>
<td>1</td>
<td>46,XY</td>
</tr>
<tr>
<td></td>
<td>Penoscrotal hypospadias</td>
<td>Male</td>
<td>6-24yrs</td>
<td>NC</td>
<td>2</td>
<td>46,XY</td>
</tr>
<tr>
<td></td>
<td>Mid penile hypospadias</td>
<td>Male</td>
<td>1.2yrs</td>
<td>NC</td>
<td>1</td>
<td>46,XY</td>
</tr>
<tr>
<td>Total cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18</td>
<td>17-male 1-female</td>
</tr>
</tbody>
</table>

### Table 2: Showing patients with Cryptorchidism

<table>
<thead>
<tr>
<th>CLINICAL DIAGNOSIS</th>
<th>TYPE</th>
<th>SEX</th>
<th>AGE</th>
<th>CONSANGUINITY</th>
<th>CASES ENROLLED</th>
<th>KARYOTYPE ANALYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Undescended testis</td>
<td>Male</td>
<td>9 months</td>
<td>NC</td>
<td>1</td>
<td>46,XY</td>
</tr>
<tr>
<td></td>
<td>Bilateral undescended testis</td>
<td>Male</td>
<td>4yrs</td>
<td>NC</td>
<td>1</td>
<td>46,XY</td>
</tr>
<tr>
<td></td>
<td>Left undescended testis</td>
<td>Male</td>
<td>8yrs</td>
<td>NC</td>
<td>1</td>
<td>46,XY</td>
</tr>
<tr>
<td>Total cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>3-male</td>
</tr>
</tbody>
</table>

### Table 3: Showing patients with Ambiguous Genitalia

<table>
<thead>
<tr>
<th>CLINICAL DIAGNOSIS</th>
<th>SEX</th>
<th>AGE</th>
<th>CONSANGUINITY</th>
<th>CASES ENROLLED</th>
<th>KARYOTYPE ANALYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambiguous genitilia</td>
<td>2 female</td>
<td>8 months-6yr</td>
<td>NC</td>
<td>2</td>
<td>46,XX</td>
</tr>
<tr>
<td>Total cases</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>2- female</td>
</tr>
</tbody>
</table>
Table 4: Showing patients with Bifid Scrotal and Hypogonadism

<table>
<thead>
<tr>
<th>CLINICAL DIAGNOSIS</th>
<th>SEX</th>
<th>AGE</th>
<th>CONSANGUINITY</th>
<th>CASES ENROLLED</th>
<th>KARYOTYPE ANALYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifid scrotum</td>
<td>Male</td>
<td>5yrs</td>
<td>NC</td>
<td>1</td>
<td>46,XY</td>
</tr>
<tr>
<td>Hypogonadism and gynecomastia</td>
<td>Male</td>
<td>19yrs</td>
<td>NC</td>
<td>1</td>
<td>46,XY</td>
</tr>
<tr>
<td>Total cases</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>2-male</td>
</tr>
</tbody>
</table>

**DISCUSSION:**

Chromosomal study helps in correlating the development of external genital malformation with abnormal Karyotype; this increases the awareness of the occurrence of cytogenetic abnormalities in the patients reporting for infertility, mental retardation, dysmorphism and sex chromosomal abnormalities.[12] Patients having external genital abnormalities are unable to produce offspring and the reason behind this can be physical or physiological. By analysing individual at cytogenetic level their true genetic sex can be determined. The genetic sex of the child is advocated by chromosome study and genitilia corrected accordingly through surgery.[13]

In the present study 25 cases having deformed genitilia were taken up for their chromosomal analysis. The number of hypospadias cases was higher one followed by cryptorchidism and ambiguous genitilia. In addition to this two cases of bifid scrotum and hypogonadism were also reported.

In a study, it was projected that the number of hypospadiasis was 2.89 per 1000 male newborns; 15.3% of all infants with hypospadias also had other malformations.[21] The present study enrolled a total of 18 cases of Hypospadias, out of which 17 cases were male and 1 was female, all from non consanguineous couples, ranging 8 months to 30 years age. Female hypospadias is rare congenital anomaly of urethra due to the defect in differentiation of wolffian duct or urinogenital sinus- leading to more severe proximal Hypospadias or less severe distal Hypospadias respectively [22,23] and found to have 46, XX Karyotype, while other male cases have normal 46, XY chromosome constitution. We have identified female patient with distal penile hypospadiasis and was showing normal chromosomal karyotype(46,XX). No chromosomal abnormality was observed in the Karyotype analysis of enrolled cases in the present study. The current study is in accord with previous findings.[7,24]
However another study reported, 2 male cases with hypogonadism and gynecomastia on Karyotype analysis found to have 2 cell lines 45,X/46, XY in the 1:1 ratio.\[25\]

Cryptorchidism is a common anomaly of external genitilia. Total 3 cases were referred for chromosome study, all were male, related to non-consanguinous couples with their age ranging from 9 months to 8 years and all were found to have normal male Karyotype. Our study is justified with previous study.\[26\] Sex chromosome anomalies were analysed karyotypically, in which 4 patients of cryptorchidism showed normal male Karyotype 46, XY.\[16\]

The number of ambiguous genitilia has increased in the recent decade, 50 live births now days include 1 patient of sexual ambiguity.\[4\] Although 60% of affected children are diagnosed prenatally many parents faced it at birth.\[14\] In another chromosomal analysis of an 2 ½yr old ambiguous genitilia child, researcher reported sex- chromosome constitution 45,X/46,XY mosaicism as well as structural anomalies like ring chromosome and telomeric association that may be held responsible for death at infancy.\[15\] Another study on 21 patients revealed normal genetic composition and also 80% were genetically male.\[16\] In another study of a children with ambiguous genitilia 2 types of cell lines in a child (46,XY/46,XX) both male and female cell line were found.\[17\] Similarly a report of earlier research workers on genital ambiguity, obtained genetic and karyotypic normal cases, except in one case inversion of Y chromosome noticed.\[18\] In present study only two cases with ambiguous genitilia, were referred for chromosome study. On Karyotype analysis, both cases were found normal females.

Men with normal external genitilia and associated hypogonadism rarely express any chromosomal abnormality and most commonly klinfelter syndrome association observed in hypogonadism cases. Among klinfelter and its variants and males with mosaicism of sex chromosome, posses hypogonadism along with gynecomastia.\[19, 20,5\] The present investigation reported, a 19 years male individual suspected with hypogonadism and gynecomastia showed normal Karyotype(46,XY).

Thus the present work is in concordant with previous literature showing that Hypospadias cases have normal Karyotype and is common in male than female.
Conclusion:

The present cytogenetic study of referred cases with genital malformation did not reveal any chromosomal abnormality with hypospadias, ambiguous genitalia, cryptorchidism & hypogonadism. The number of genital malformations was higher in males than females.

References

Undescended testis—46,XY

Ambiguous genitilia—46,XX