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RESEARCH ARTICLE

CYTOGENETIC ANALYSIS OF CASES REFERRED FOR SUSPECTED AMBIGUOUS GENITALIA IN WESTERN INDIA: METROPOLIS HEALTHCARE EXPERIENCE

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ABSTRACT

Ambiguous Genitalia (AG) is the birth defect where the gender of an individual is ambiguous. The retrospective study aimed at finding the frequency of sex chromosomal abnormalities and its various patterns in the samples with history of Ambiguous Genitalia. This retrospective study was carried out for two years on 487 cases referred in view of clinical suspicion of AG. The samples were received at the department of cytogenetics, Metropolis Healthcare Ltd, Mumbai, India. Peripheral blood (2-3 ml) from all study subjects was collected in sodium heparin green top vacutainer tube. Both, 48 hours and 72 hours' cultures were set & analysed by GTG–banding at 450-550 band level. Out of 487 study samples, 243 cases and 220 cases were registered as male and female, respectively. For 24 cases, gender was not mentioned or identified. Out of the total 487 cases, 73cases showed cytogenetic abnormality and normal polymorphic variation was seen in 15 cases. Out of 32 abnormal cases registered as male, mosaic pattern for sex chromosome was seen in 11 cases. Out of 41 abnormal cases registered as females, 4 cases showed mosaic pattern. Since Metropolis Healthcare Ltd is a referral laboratory and because of the selection bias, there could be slight variations in percentage of abnormality as compared to the published data in scientific literature. This study concludes that for all cases of genital ambiguity, a cytogenetic investigation as a basic approach could be very informative and should be suggested.

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INTRODUCTION

According to several literature studies carried out by Aaronson (2010), Al-Mutair (2004), Choi (2008) and Cohen-Kettenis (2005) Ambiguous Genitalia (AG) is defined as the birth defect in which the gender of the individual is not clear whether male or female. Cox (2014) has reported that the presentation may vary from case to case and the causes for these also can be different. According to Fausto-Sterling (2000), cytogenetic abnormality or chromosomal abnormality as a cause is one of the major contributory factor for AG. As per research studies of Grumbach (2005) and Guaragna-Filho (2012), in neonatal cases and in cytogenetic practice, AG is one of the cases of medical emergency and such cases should be taken on priority and reported as soon as possible not only for conditions like congenital adrenal hyperplasia and certain malformation syndromes which have potential risk of life to the neonate but its associated with lot of social and psychological issues. It is important to rule out the genetic aspect as the cause of AG. This retrospective study aimed to determine the frequency of chromosomal abnormalities and its various cytogenetic types in the samples received by Metropolis Healthcare laboratory, Mumbai. This study was conducted on the samples referred for chromosomal

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karyotyping with a history of AG. A total of 487 cases were referred within the term of two years which include January-2015 to December-2016. Out of the total cases, 243 were registered as males and 220 were registered as females. While, in 24 cases, the gender was not mentioned or was not identifiable.The cytogenetic abnormality was found in total 73 (14.98%) cases and polymorphic variation in a total of 15(3.08%) cases. Wherever applicable, in addition to karyotyping, FISH studies for confirmation and for presence or absence of SRY gene were done.

MATERIALS AND METHODS

The study was carried out on 487 cases referred in view of clinical suspicion of AG.This retrospective study was conducted between January-2015 to December-2016. Peripheral blood (2-3 ml) was collected in sodium heparin green top vacutainer tube from the patients between the age group of one day to one year.

Both, 48 hours and 72 hours' cultures were set & analysed by GTG–banding at 450-550 band level and reported as per the guidelines of The International System for Human Cytogenetic Nomenclature (ISCN), College of American Pathologists (CAP) and The National Accreditation Board for Testing and Calibration Laboratories (NABL). The reason for a 48-hour old culture was additionally performed to report the cases on urgent basis and to have a back-up if no growth or in possibility of low mitotic index. For each case, 20-30 metaphases were studied and for mosaic cases 50-100 metaphases were studied. Wherever necessary, FISH was performed for confirmation of low-grade sex chromosomal mosaicism and for SRY gene depending upon clinical findings in in-house cases and history provided for referred cases after discussing with the referring clinician.

RESULTS

Out of 487 samples referred with history of AG, 243 (49.89%) cases were registered as male and 220 (45.17%) cases were registered as females. Only in 24 (4.92%) cases, gender was not mentioned or identified. Out of the total 487 cases, around 73 (14.98%) cases showed cytogenetic abnormality and normal polymorphic variation was seen in 15 (3.08%) cases. Of the 73 cases, 32 cases were registered as male and 41 cases were registered as female. Gender of 24 (4.98%) cases was not mentioned but the report of these 24 cases were discussed with the referring doctor. Out of 24 cases, 19 (79.16%) were matching (more clinical deviation towards same sex) and 5 (20.83%) cases were not matching (more clinical deviation towards opposite sex). Out of 73 cases with abnormalities,1 (1.36%) was having translocation between chromosome Y and chromosome 4 and 1 (1.36%) case was found to have trisomy of 21. Polymorphic variations in the form of inversion of 9 was seen in 3 (0.61%) cases, out of which 2 (0.41%) were females and 1 (0.2%) were males. Inversion of Y chromosome was seen in 3 (0.61%) cases and other polymorphic variations in the form of increase in the length of satellite on acrocentric chromosome was also recorded.

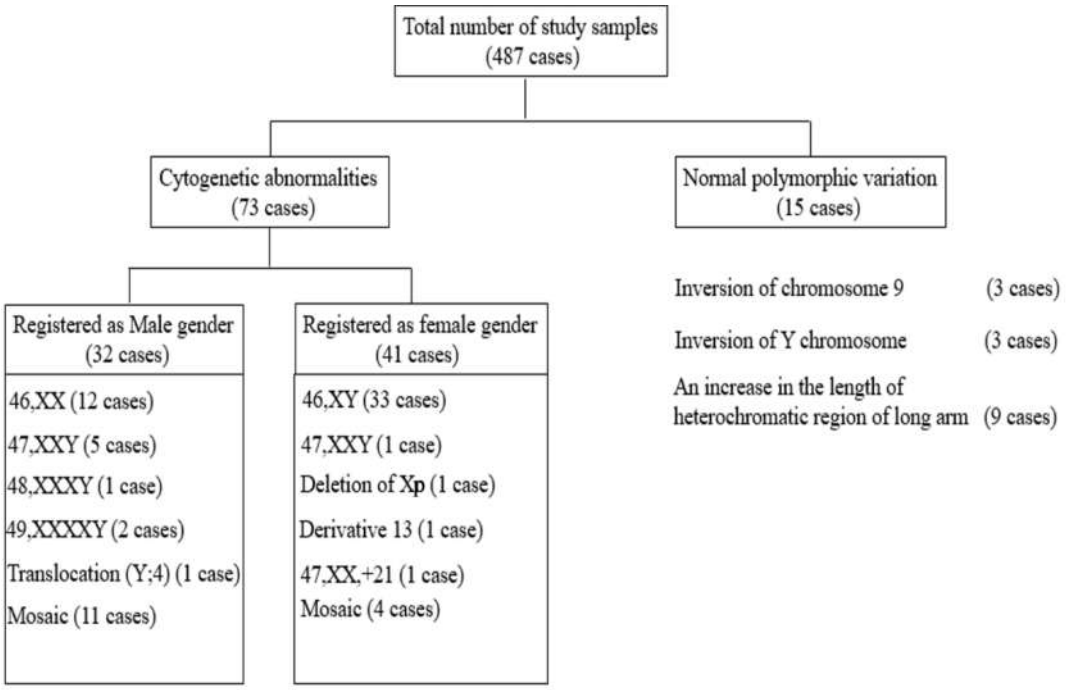

Figure 1. Cytogenetic evaluation of the study samples

An increase in the length of heterochromatic region of long arm in 9 (1.84%) cases. Out of 32 abnormal cases registered as male, 5 (15.62%) cases showed 47, XXY pattern, 12 (37.5%) cases showed 46,XX pattern, 2 (6.25%) cases showed 49, XXXXY, one case (3.12%) showed 48, XXXY pattern. Mosaic pattern for sex chromosome was seen in 11 (34.37%) cases and translocation between chromosome Y and chromosome 4 in one (3.12%) case. Out of 15 (3.08%) cases, polymorphic variations were recorded in 9 (60%) male cases. Out of which inversion of chromosome Y in male was seen in 3 (20%) cases, inversion of chromosome 9 in 1 case (6.66%) and increase in the length of satellite on acrocentric chromosome and increase in the length of heterochromatic region of long arm in 5 (33.33%) cases. Out of 41 abnormal cases registered as females, 33 (80.48%) cases showed 46, XY pattern,one (2.43%) case showed 47, XXY pattern and 4 (9.75%) cases showed mosaic pattern, one (2.43%) case each of trisomy 21, derivative chromosome 13 and deletion of short arm of chromosome X and other polymorphic variations in the form of inversion of chromosome 9 in two cases (4.86%)

increase in the length of satellite on acrocentric chromosome and increase in the length of heterochromatic region of long arm in 3 (20%) cases also one (2.43%) case had inversion of chromosome 4.

Table 1. Frequency of abnormal cytogenetic pattern observed in males

Cytogenetic pattern	No. of cases (32)
46,XX	12(37.5%)
47,XXY	5(15.62%)
48,XXXY	1(3.12%)
49,XXXXY	2(6.25%)
Mosaic	11(34.37%)
Translocation (Y;4)	1(3.12%)
Total	32

Table 2. Frequency of abnormal cytogenetic pattern observed in females

Cytogenetic pattern	No. of cases (41)
46,XY	33(80.48%)
47,XXY	1(2.43%)
Mosaic	4(9.75%)
Deletion of Xp	1(2.43%)
Derivative 13	1(2.43%)
47,XX,+21	1(2.43%)
Total	41

Table 3. Patterns of mosaicism in males

Type of Mosaicism	No. of cases (11)
45,X/46,XY	6 (54.54%)
46,XX/46,XY	2 (18.18%)
45,X/46,X,idic(Y)	1 (9.09%)
46,X,iso(Yq)	1 (9.09%)
48,XYYY/45,X	1 (9.09%)
Total	11

Table 4. Patterns of mosaicism in females

Type of Mosaicism	No. of cases 4
47,XXY/46,XX	1 (25%)
45,X/46,XY	1(25%)
45,X/46,X,i(Xq)	1(25%)
46,XX/46,XY	1(25%)
Total	4

Since Metropolis Healthcare Ltd is a referral laboratory and because of the selection bias, there could be slight variations in percentage of abnormality as compared to the published data in scientific literature.

DISCUSSION

This is a retrospective study undertaken in a referral laboratoryper formed on the peripheral blood samples. All the samples were sent for chromosomal karyotyping in view of clinical findings of Ambiguous Genitalia (AG). In our laboratory, every case with history provided of AG is taken on the priority and findings are discussed with the referring doctor before release of report. Also, after discussing the case many a times, some additional tests may be required to be done as per the need of the situation. The sex mentioned/registered are based on the more deviation to the respective gender as per the clinical findings observed by the clinician.

All the cases registered for ambiguous genitalia are taken on priority and the reports are discussed usually with the clinician and then released. Considering the urgency of report in addition to 72 hours' cultures, a 48 hours' culture is also set and every effort is undertaken to inform the report to the referring clinician at the earliest. Early detection of cytogenetic abnormality can sometimes be life-saving. Moreover, understanding the cytogenetic status assists the clinician to decide the mode of treatment modalities. In certain cases, where the females have a cytogenetic pattern of 46, XY (presence of Y chromosome), the possibility of developing gonadoblastoma remains persistent.Therefore, understanding the cytogenetic status along with other investigation in such females helps the clinician to decide the surgical treatment. A careful cytogenetic, endocrine and molecular evaluation is critical for genetic counselling and the management. Our results are in agreement with the studies carried out by Hackel (2005), Houk (2012), Hutson (2014) and Moreno Morcillo (2005). For this condition, the XX males are usually sporadic. This condition is due to Y chromosome material translocation on X chromosome and this can be detected by FISH or by other molecular methods. In XY females, usually the presentation is with androgen insensitivity syndrome where a normal female usually has testis. In typical ambiguous genitalia case, the reasons for referral could be congenital adrenal hyperplasia or sex chromosome disorder. Therefore, this study was conducted to observe the cytogenetically sex chromosomal abnormalities. The findings observed in our study are quite significant and some variation may be because of slight selection bias.

Conclusion

We wish to highlight from our study of two years on 487 cases that Ambiguous Genitalia (AG) cases should be taken on priority. Proper Cytogenetic diagnosis and early detection helps the clinician to undertake an informed approach to make the family/patient understand the condition for rearing the child with proper gender. It also helps the clinician to choose appropriate treatment modalities. As management of these cases requires multidisciplinary approach along with the clinicians, genetic counselling to the family/patient is also equally important. Timely detection of cytogenetic abnormality can be life-saving. Therefore, for all cases of genital ambiguity, a cytogenetic investigation as a basic approach could be very informative and should be suggested.

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Conflicts of interest

There is no conflict of interest whatsoever among the authors of this study.

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