DEVELOPMENTAL DISTURBANCES OF FEMALE INFERTILITY, CYTOGENETIC AND CLINICAL CORRELATIVE STUDIES ON PRIMARY AND SECONDARY AMENORRHEA.

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ABSTRACT

Amenorrhea or absence of menstruation is a symptom and not a disease. Normal menstruation is the end result of coordinated cyclic events in the hypothalamo–pituitary–ovarian axis and appropriate responses by the uterine endometrium, the target organ for ovarian hormones. Two hundred and eighteen women in the age of 16 years to 35 years were referred to our genetic lab due to primary amenorrhea or irregular menstrual cycle and ambiguous genitalia. Some cases were referred to the hospital as a case of mullerian agensis. Some for hormonal therapy. Ultrasound report of pelvis revealed ovarian tissue not made out clearly and small uterus. The present study revealed 45% chromosomal anomalies, subject with primary, secondary amenorrhea and ambiguous genitalia, and whether there exists any correlation between the phenotype and karyotype among Turner patients carrying X-chromosome abnormalities. The outcome of these studies may also be applied in genetic counseling.

KEYWORDS Infertility, Turner’s Syndrome, Karyotyping, Chromosomal abnormality.

MANUSCRIPT

The study has helped in a better understanding of the human sexual disorders, which were loosely classified under the general term, infertility. A classification based on the cytogenetic and clinical features with the gonadal sex in view has been discussed. The estimation of the proportion of the affected cell line in a Turner and its variants reveal the severity of the syndrome and this diagnosis would help the physicians to understand the treatability and non-treatability of the patients of sexual disorders with surgery and contribute hormonal administration.

INTRODUCTION

Amenorrhea or lack of menstrual cycles as the term denotes, is one of the prime causes for female infertility and can be either primary or secondary in nature. PA is a common feature among subjects with Turner syndrome, XX- and XY-gonadal dysgenesis and testicular feminization. In secondary amenorrhea (SA), there are one or more bleeding episodes followed
by a minimum of three months of amenorrhea (Doody and Carr 1990) and it ranged from 6 months to 7 years Safai, (2012). Anomalous sexual development has been attributed to hormonal imbalance, developmental disturbances of unknown etiology (Dougherty, 1972) as well as genetic causes. Among the congenital anomalies contributing to the infertility in the female, ovarian dysgenesis and the unfortunate embryological freak, resulting in the absence of vagina, are the most enigmatic afflictions.

However in an earlier study abnormal chromosomal constitution was reported to be 25.6% in Indian population (Anupam 2004) and 24.5% in Chinese population (Wong and Lam 2005) among PA Patients.

MATERIALS AND METHODS

The study consisted of 184 women with primary amenorrhea and 34 cases of secondary amenorrhea who were referred for cytogenetic investigations from Government and private hospitals. Out of those 218 cases, 165 cases were confirmed of having ovarian dysgenesis and the rest having normal ovaries. Detailed clinical examinations of each patient were done carefully, and recorded with the help of physicians. Clinical pictures were routinely taken. Ultrasonogram pictures and X-ray photographs were taken wherever necessary. The X chromatin test as a cytogenetic screening test and the karyotype for confirmation of the certain diagnosis, in all patients. The early diagnosis is helpful for patient counseling and management ( Popo 2011). A minimum of 50 technically good metaphase plates of each patient was analysed to detect the nature of the karyotype of the patient achronosomal anomalies were designated as per standard guidelines.

RESULTS

The clinical features observed in those patients ranged from a true Turner stigmata (fig 1 webbed neck) and to a well developed secondary sexual characters. There were 12 cases with imperforate hymen, 9 cases had congenital absence of vagina, 53 cases had infantile uterus which were felt like nodule and 71 cases had ovarian dysgenesis.(fig:8,) there were 6 cases of XY females (photo), with X- chromatin negative. 2 cases of testicular feminization syndrome normal female body habitus, external genitalia essentially of female type, vagina leading to a blind pouch, no Mullerian duct and Wolfian duct differentiation and were clearly distinguished from incomplete-feminizing testes syndrome. 2 cases of pseudovaginal perineoscrotal hypospadias with eunuchoid body habitus. No Mullerian duct differentiation while normal wolfian duct derivatives were present and male escutschen during puberty. And two cases of XY Gonadal agenesis Syndrome with 46, XY sex chromosome. At laparotomy, no internal genitalia or gonads were found. The pelvic wall on both sides had a small mass of tissue4. Microscopic examination revealed it as a rudimentary fallopian tube. 2 cases of XXX and 2 cases of ring chromosomes with well developed secondary sexual characters, chromatin positive. Chromatin studies from buccal smears of 59 cases bearing a 46, XX karyotype, revealed a range of 32 to 38 percent positive X-chromatin. (fig:3)

50 cases of 46,XX/45,X0 with sex chromatin positive between and, one case with 46,XX/45,X0/47,XX+G, revealed a range between 12 to 15 per cent. Two cases with 46,XX /47,XXY were 18 percent positive for X-Chromatin. All the 32 cases of Turner syndrome having 45,X0 chromosome pattern and 6 cases with 46,XY chromosome complement were sex chromatin negative .One case with 46,XX/46,XY.

As for cytogenetic studies the karyotypic analysis of 77 patients showed 46, XX chromosome complements. This study recorded 34 cases of Turner syndromes, having 45,X0 chromosome pattern. (Fic:4). They were all X-chromatin negative. The mosaic individuals exhibited a phenotypic variation ranging from characteristic features of Turner stigmats to that of normal female phenotype (fig:1,2,5, &6). These clinical variations were analysed in relation to the ratios of affected cell line (X0), in the mosaic and presented in (table 2 ).
Table 1
A CORRELATION BETWEEN THE SEVERITY OF THE SYNDROME AND THE DEGREE OF MOSAICISM IN TURNER

<table>
<thead>
<tr>
<th>KARYOTYPE AND DEGREE OF MOSAICISM</th>
<th>NO. OF CASES</th>
<th>CLINICAL FEATURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>X0</td>
<td>34</td>
<td>Short stature, average height being 132 cms with a range between 129 cms and 135 cms. Cubitus valgus, low posterior hair line. High arched palate increased carrying angle. Broad shield – like chest. Widely spaced nipples, abnormal secondary sex development and ovarian dysgenesis.</td>
</tr>
<tr>
<td>XX/X0 (21.4:78.6)</td>
<td>21</td>
<td>Short stature, average height being 135 cms with a range between 130 cms to 140 cms. Short webbed neck, cubitus valgus, low set ears, low posterior hair line. High arched palate, increased carrying angle. Broad shield – like chest. Widely spaced nipples, abnormal secondary sex development and ovarian dysgenesis.</td>
</tr>
<tr>
<td>XX/X0 (60.5:39.5)</td>
<td>27</td>
<td>Average height being 148 cms with a range between 146 cms to 150 cms. No webbing of neck, absence of cubitus valgus. Moderately developed secondary sex characters with presence of ovarian dysgenesis.</td>
</tr>
<tr>
<td>XX/X0 (71 : 29)</td>
<td>2</td>
<td>Average height being 153.5 cms with a range between 151 cms to 156 cms. Absence of turner features with well developed secondary sex characters.</td>
</tr>
<tr>
<td>47,XXX, X+Ring</td>
<td>2</td>
<td>Abnormal height measures 167.2 and secondary amenorrhea absence of Turner features.</td>
</tr>
<tr>
<td>46,XX/XY</td>
<td>1</td>
<td>Average height, intersexuality, presence of male features.</td>
</tr>
</tbody>
</table>
Table 2

<table>
<thead>
<tr>
<th>No</th>
<th>Genotype</th>
<th>Ratio of the cell lines</th>
<th>No. of cases studied</th>
<th>No. of metaphases studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45, XO</td>
<td>-</td>
<td>34</td>
<td>2581</td>
</tr>
<tr>
<td>2</td>
<td>46, XY/45, XO</td>
<td>21.5:78.5</td>
<td>21</td>
<td>1694</td>
</tr>
<tr>
<td>3</td>
<td>46, XX/45, XO</td>
<td>60.5 : 39.5</td>
<td>27</td>
<td>1969</td>
</tr>
<tr>
<td>4</td>
<td>46, XX/45, XO</td>
<td>71 : 29</td>
<td>2</td>
<td>154</td>
</tr>
<tr>
<td>5</td>
<td>46, XX/45, XO/47, XX+G</td>
<td>68:20:12</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>46, XX/47, XXY</td>
<td>34:28</td>
<td>2</td>
<td>170</td>
</tr>
<tr>
<td>7</td>
<td>46, Xi(Xq)</td>
<td>-</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>8</td>
<td>XY(Females)</td>
<td>-</td>
<td>6</td>
<td>469</td>
</tr>
<tr>
<td>9</td>
<td>46, XX(amenorrhea)</td>
<td>-</td>
<td>77</td>
<td>5366</td>
</tr>
<tr>
<td>10</td>
<td>47, XXX (secondary amenorrhea)</td>
<td>-</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>45, X+(ring)</td>
<td>50:50</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>46, XX/XY</td>
<td>50:50</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>13</td>
<td>XX (control females)</td>
<td>-</td>
<td>20</td>
<td>1432</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>196</td>
<td>14,265</td>
</tr>
</tbody>
</table>
DISCUSSION

The cytogenetic analysis of cases of primary and secondary amenorrhea revealed 93 cases (45%) had chromosomal anomalies (Table 1). From the study of the previous workers (Jacobs et al., 1961; Makino et al.; Okada et al., 1968) a range between 31% - 43% of chromosomal anomalies. The frequency of sex chromosomal anomalies in patients present with primary or secondary amenorrhea have shown a wide variation (Van Niekerk 1978; Joseph and Thomas 1982; Ko et al. 1982; Mulye et al. 1983; Optiz et al. 1983; Chuang et al. 1985; Ghalib et al. 1988; Ten et al. 1990; Park and Kang 1999). The most common abnormal karyotypes observed were 45,X; 45,X/46,XX; 45,X/46,X,i(Xq) and 46,XY in amenorrhea patients.

The karyotype was abnormal in 269 (54.56%) patients; the most frequent abnormality detected was X chromosome monosomy, Franks (1987); Hunter (2006) Lăcrămioara Butnariu et al (2011). The percentage of chromosomal abnormalities varies from 15.9% - 63.3% (Joseph and Thomas 1982); (Wong and Lam 2005), Cortes - Gutierrez et al, 2007 Vijayalakshmi et al, 2010, Kalavathi et al, 2010 Ramireza et al, 2000 Safaei et al 2010. In several metaphases chromatid breaks were in frequently observed karyotyping is one of the fundamental investigations in the evaluation of amenorrhea. It has highlighted chromosomal abnormality, one of the genetic etiology as the causal factor in amenorrhea, Sayee Rajangam,(2007). Autosomal translocations are relatively common; gonosomal-autosome translocations with rearrangement of genes are rather unique. In the mix of both balanced and unbalanced translocation between the X- and 18 chromosomes has led to various degrees of fertility. Attila Szvetko (2012). A translocation between X and an autosome (chromosome # 20) J.Vijayalakshmi (2010), and it also has been reported by (Wong and Lam 2005). Turner syndrome manifests itself differently in each female affected by the condition, and no two individuals were found to Share the same features in the present study Kalavathy et al,( 2010).

Attempts were made on the basis of qualitative changes in a genome either (i) at the genic or (ii) at chromosomal level or (iii) due to the presence of more than one cell line (mosaicism) in a patient. For instance, the developmental abnormality of Mullerian ducts leading to abnormalities of the vagina and / or uterus was found to be one of the most common causes of primary amenorrhea in an otherwise normally developed woman. In the present study the patients suffering from such disorder were not always associated with chromosomal aberrations, showing only a normal 46,XX chromosome constitution. This abnormal development of Mullerian duct was explained on the basis of gene mutation.

The Phenotypic features of Turner syndrome, such as short stature, shield – like chest, sexual infantilism and ovarian dysgenesis are without doubt due to the loss of an X-chromosome in a small proportion of cells. The SHOX gene mapped to the pseudoautosomal region of the X and Y chromosomes has been causally responsible as reported earlier (Abir and Fisch 2001).

Almost all the patients in first two groups with short stature have a detectable sex chromosome abnormality either numerically or structurally. In patients with abnormal chromosome constitutents, 74% exhibit numerical aberrations (n=29) and26% with structural aberrations (n=10) (Vijayalakshmi 2010). In the third group, a few patients have a chromosome anomaly while the majority of cases are chromosomally normal (46,XX) Polani (1970), German (1970,1971) regarded Turner syndrome and its variations including mixed gonadal dysgenesis as members of a single group of disorders, the “gonadal dysgenesis”. According to his views, Turner syndrome is the prototype of the disorders and characterized by the 45,X0 complement. The X-gonadal dysgenesis is characterized by a mosaicism of 45,X0/46,XX; and y – gonadal dysgenesis is represented by a mosaicism of 45,X0/46,XY.

This theory holds good in all the 34 cases of Turner with 45, X0 chromosome constitution observed in our study. The occurrence of similar short stature in a patient with 46,Xi(X q) recorded in the present study strengthens further the view of Ferguson –Smith (1965) that the short arm of the X-Chromosome exercises a stronger influence on stature than the long arm. Polani (1970) in his excellent review on patients with dysgenetic gonads has made a similar
mention that in the cases of pure gonadal dysgenesis with average height, there was only a very minute proportion of non mosaic 45,X0 individuals, but there was by contrast, a good proportion of mosaics, either only with X-chromosome or with Y-chromosomes. A female karyotype can occur in XY embryo when testis determining factor in the testes determining pathway are lost, mutilated or compromised (Oster et al 1989). The expression of the Turner’s Phenotype being modified under the effect of the presence of an XX cell line. Here 20% of cases with Y chromosome constitution referred for PA (Anupam 2004).

Fraccare et al. (1960), described that mosaic cases with 45,X0 /46,XX had short stature and normal female genitalia except poor pubic hair and rudimentary sexual organ, but neither cubitus valgus nor webbed neck was noted. The Clinical features of 45, X0 /46,XX mosaics generally being variable and show phenotypes ranging from those of Turner’s syndrome to those of normal females. Presumably the expression of the Turner’s Phenotype is modified under the effect of the presence of an XX cell – line. Such a wide range of clinical features is well portrayed in the 48 cases of primary amenorrhea and two cases of secondary amenorrhea with 45, X0 / 46,XX which are recorded in the present study. Marker X karyotypes may have usually severe phenotypes, which include mental retardation or abnormal facial features (Wolff 1996). In our study same effect with patient (fig 2).

Ferguson – Smith (1965) Observed in the 45,X0/46,XX mosaic cases, the clinical pictures ranged from an apparently normal female of average height to the complete Turner syndrome. Likewise, patient with 45,X / 46,XY mosaicism also vary in phenotypic expression from an apparently normal male to a complete Turner stigmata. A boy with normal clinical features in the 46,XX/46,XY cells forming about 50% XX and 50% XY evidence of intersexuality observed..

In our study we not only took note of the mosaic cell lines but also estimated the ratio between the affected cell population and normal cells population in the Turners and mosaic Turner. This explains the variations in the clinical features of the mosics, For instance in a mosaic turner in whom the ratio between the normal (46,XX) cell population reveal the degree of severity of the syndrome. This diagnosis may be of great help in treating the patient.

**SUMMARY AND CONCLUSIONS**

In the majority of the cases, sex chromosomal abnormalities were associated with the reproductive disorders leading to infertility. It is predictable from our studies that more of the structural abnormalities involving minute translocations and deletions will be detected in near future by the use of ‘C’ and ‘G’ banding techniques; thereby more depth in the understanding of the cytogenetic of sexual disorders in Infertility will be achieved.

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