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Original Research Article

CYTOGENETIC ANALYSIS OF PREMATURE OVARIAN FAILURE PATIENTS

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ABSTRACT

Premature ovarian failure (POF) is defined as amenoirhea for more than 6 months in the presence of raised gonadotrophins, FSH serum level higher than 40 mIU/ml, occulting before the age of 40. In this study, we have done karyotyping of POF patients. The findings which consist of 55% Karyotype abnormality are 17% 46,X0, 4% 46,XX(Xr), 20% 46XX(Xq del), 6% 46,XX(Xinv), 6% 47,XXX and 2% 46,XX(Xqiso).The (mean ± S.D.) height of all the POF patient 142.5±0.18 and the (mean ± S.D.) FSH of all the POF patients 45.21±17.41. As we have correlated the finding especially Xqdel and XO the (mean ± S.D.) height(ft) 4ft 8inch ± 0.39 and 4ft6inch ± 0.21, respectively . The hormonal level FSH especially Xqdel and XO The (mean ± S.D.) FSH (mIU) 93± 31.91 and 92.66 ± 23.75, respectively. The chromosomal abnormality especially turner syndrome, X-chromosomal abnormality associated with POF patients as shown by this study. Hence, the early detection of these cytological abnormalities in individuals of early age group will prevent POF along with their consequences in future.

KEY WORDS: Premature ovarian failure (POF), Amenoirhea, Gonadotrophins, Karyotyping, Chromosomal Abnormality.

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INTRODUCTION

Premature ovarian failure (POF) is defined as amenorrhoea for more than 6 months in the presence of raised gonadotrophins, FSH serum level higher than 40 mIU/ml, occurring before the age of 40 [1,2]. POF accounts for about 10% of all female sterility. POF presents by typical manifestations of climacterium: infertility associated with palpitations, heat intolerance, flushes, anxiety, depression, fatigue. POF is biochemically characterized by low levels of gonadal hormones (estrogens and inhibins) and high levels of gonadotropins (LH and FSH) (hypergonadotropic amenorrhea). Hormone defects in these patients may cause severe neurological, metabolic or cardiovascular defects early onset of osteoporosis etc. The major problems associated with POF are the loss of fertility at an early age and the psychological problems associated with this. In addition there are the physiological effects of reduced estrogen, which include an increased risk of osteoporosis.

For every decade before 40 the prevalence of POF is estimated to decrease by a factor of 10. Thus in presence of a normal karyotype 1: 1,000 of women at 30 have POF, 1: 10,000 at 20 and 1: 100,000 of women will present with gonadal failure and primary amenorrhoea [2]. The prevalence of POF, however, varies by ethnicity, with women of oriental origin having a lower risk and African-Americans a higher risk compared to Caucasian Americans.

In terms of the mode of presentation, POF is the underlying etiology in 10–28% cases with primary amenorrhoea and in 4–18% of those with secondary amenorrhea. POF affects 1% of women and the majority of cases are idiopathic.

It would seem that the more common causes of POF in adolescents include cytogenetic abnormalities involving the X chromosome, ovarian dysfunction occurring in association with other autoimmune endocrine disturbances, and chemotherapy and/or radiation therapy given for any of a number of malignancies**.** This may lead to decreased follicle production or increased follicle artesia resulting in premature loss of germ cells [3].

A genetic basis is well established and was definitively demonstrated by the report of numerous familial cases. Identification of genes responsible for autosomal recessive [4], X-linked dominant [5] or autosomal dominant syndromic forms of the disease demonstrated a monogenic component, but the genes identified account for a very small percentage of the POF cases. There may be autosome or X-chromosome associated with the premature ovarian failure. It may be in the form of translocation, ring formation, iso-chromosome, inversion, deletion of regions located on the p and q arm especially X-chromosome. Cytogenetically visible rearrangements of the X chromosome are associated with POF. Many of those rearrangements occur in specific Xq regions. Two main critical regions have been located on the long arm of the X chromosome, at Xq13–q21 [6] and at Xq26–q27 [7]. Thus, the two functioning X chromosomes appear necessary for normal ovarian function. The most obvious genetic cause of POF is Turner Syndrome, in which a complete or near-complete loss of the second X chromosome occurs. Turner Syndrome typically results in the most severe and irreversible cause of POF, often clinically evident prior to menarche. Typically, in Turner Syndrome, menopause precedes menarche, and there is no evidence of ovarian function. Lesser degrees of ovarian failure have also been attributed to partial X chromosome deletions and milder degrees of X chromosome mosaicism. Fragile X syndrome is another cause of mild POF. Fragile X syndrome is another example of mild POF that can be linked to disorders of the X chromosome. Other genetic defects are believed to cause POF, yet their prevalence has been difficult to determine. The localization of the gene for the blepharophimosis/ptosis/POF Syndrome has been reported, yet this finding has not been seen commonly in POF. Other genetic syndromes including POF await elucidation. Many transgenic “knock-out” animals have been created with deficient ovarian function. Most interesting along these lines is the heterozygous FSH receptor knock-out, which exhibits a reduced follicle reserve and early menopause [8]. Bentov et al., 2010 [9] reported that aging and age-related pathogenesis are associated with loss of mitochondrial function, mainly due to accumulation of mtDNA mutations and deletions. These may also lead to POF. Seda Ateº *et al*., (2016) [10] concluded in his study on the retrospective karyotype analysis of 65 women with idiopathic POI referred to the Medical Genetics Department at the Bezmialem Vakif University Hos- pital. Shows Chromosomal abnormalities were present in 12 of 65 cases (18.4%). The most frequently detected abnormalities were X chromosome mosaicisms. Two cases had fragile X premutation carriers. Eight (12.3%) women were considered as familial POI, as per their results the underline the essential role of the X chromosome is there in the etiology of POI. In the future, a better knowledge of the cellular and biochemical components involved in folliculogenesis and apoptosis should elucidate the mechanisms of POF (Christin-Maitre et.al 1998)[11].

Pedigree data indicate that early menopause and premature menopause sort similarly within families. The only difference between women with true POF and those with early menopause may be in the timing of the expression of the syndrome, and not in the genetics in individuals without POF who are seeking extension of their reproductive life spans or fertility enhancement by other means. The aim of the study to find out chromosomal abnormalities in POF patients along with the raised FSH level and both variables would be correlated.

MATERIALS AND METHODS

Patients: 50 cases of idiopathic were enrolled. This study was done only by informed consent and this study was approved by institutional review board of All India Institute of Medical Sciences, and all participants gave their written informed consent for this study .The critical age of these patients d"40years along with a FSH concentration of >40mIU/ml. The complete physical, clinical including gynecological and family history was taken for every patient.

Conventional Cytogenetic Analysis: Chromosome preparation [12]: In POF patients, chromosome analysis was done to identify for any numerical or structural chromosomal aberrations. For this lymphocyte cultures were setup and chromosome were analyzed by G banding. 5 ml. of heparinized blood was drawn and kept in an upright position at 37°C for 30 minutes. This helps in the separation of plasma from red blood cells. Then, the plasma and the settled lymphocyte (PLS, plasma lymphocyte suspension) in buffy coat was tapped gently and mixed together. The needle was bent and 0.5 ml of PLS was transferred into a sterile culture vial containing 5ml of media RPMI-1640 and 0.2 ml Phytohaemagglutinin (PHA). The cultures were incubated for 72 hours at 37°C. After 70 hours of incubation, 0.1 ml (0.2%) of colcemid was added to the cultures. At 72 hours the samples were washed for removing colcemid. Then, they were centrifuged at a speed of 1000 rpm for 10 minutes, the supernatants was discarded and freshly prepared pre-warmed hypotonic solution (0.56% KCl) was added and incubated for 20-25 minutes at 37°C. The cell suspension were centrifuged again and after discarding the supernatant, freshly prepared chilled carnoy's fixative (methanol: acetic acid/3:1) was added to the cell pellet slowly. At least three changes of fixative were given till the pellet became pale. Two drops of cell suspension were dropped from a height on a clean wet slide.

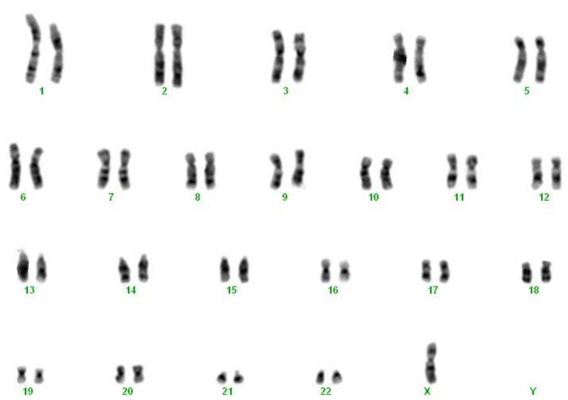
G-banding [13] Giemsa staining of chromosome preparation after proteolytic enzyme treatment revealed G-banding. The 3 days old matured unstained chromosome preparations were flooded with 0.25% Trypsin for 10-15 seconds, then the slides were rinsed in phosphate buffer saline. The slides were stained in 2% Gimesa stain for 5-7 minutes, thereafter, they were washed in distilled water. Metaphases were analyzed using cytovision software (zeiss microscope) classified according to ISCN 1995. At least 50 metaphases in each patient were analyzed and karyotype.

RESULTS

50 patients of POF included in this study. 45% patients having the normal karyotype and increase hormonal levels with normal Average Wt. and Ht. but remaining 55% patients found abnormality in their karyotype as well as their hormonal level. Among the patients, the mean age and height was found to be 21.35±6.32 years (mean ± SD) and 142.5±0.18 (mean ± SD) respectively.

Fig. 1: GTG Banded Mataphase spread of POF patient.

Fig. 2: Karyotype shown 45, XO (Turner's Syndrome).



The findings which consist of 55% Karyotype abnormality are 17% 46,XO; 4% 46,XX(Xr), 20% 46XX(Xq del) , 6% 46,XX(Xinv) , 6% 47,XXX , 2% 46,XX(Xqiso) .The (mean ± S.D.) **Height** of all the POF patient 142.5±0.18 and the (mean ± S.D.) **FSH** of all the POF patients 45.21±17.41. As we have correlated the finding especially Xqdel and XO the (mean ± S.D.) height(ft) 4ft8inch ± 0.39 and 4ft6inch ± 0.21 respectively . The hormonal level FSH especially Xqdel and XO The (mean ± S.D.) FSH (mIU) 93± 31.91 and 92.66 ± 23.75 respectively.

Table 1: Showing the findings of the various chromosomal abnormalities against the Body weiught (kg), Body height (cm) and FSH level (mIU/ml).

S.No.	Chromosomal Abnormalities	Body weight (Kg.)	Body height (cm)	FSH level (mIU/ml)
1	45,XO	36.3	135	92.66
2	46,XX(Xr)	37	130	56
3	46,XX(Xqdel 11-27)	44.4	140	93
4	46,XX(Xinv9)	50	157.5	62.32
5	47,XXX	57.2	155	62.28
6	46XX(Xqiso)	52	155	52.6

DISCUSSION

Worldwide POF affects 1% of all women and occurs in 0.1% before the age of 30 years. The major problems associated with POF are the loss of fertility at an early age and the psychological problems associated with this. In addition there are the physiological effects of reduced oestrogen, which include an increased risk of osteoporosis. POF is a heterogeneous disorder and the cause of most cases is unknown. A significant proportion (20-30%) POF women is having genetic predisposition._In our study we included 50 patients of premature ovarian failure out of which 45% of normal karyotype and 55% of abnormal karyotype

As many studies are already done on Turner syndrome but this study is quite different. In this, presence of only one X chromosome in Turner's syndrome, ovarian follicles undergo accelerated apoptosis. This may be the result of a lack of diploid dosage of one or more vital genes, both alleles of which are active in oogenesis [14]. In this study, we find XO (turner syndrome) in 16% patients having (mean ± S.D.) FSH 92.66±23.75 and ht. 135±0.21 respectively. It shows that these patients have short stature because they are below 140cm as described (W.H.O.).We also found the mosaic Turner syndrome in 4% cases. These have FSH and ht 45.66±16 and 143.75±13.75 respectively. Thus we can compare and distinguish mosaic and nonmosaic Turner syndrome. The non-mosaic turner having very high values for FSH hormone level and short stature as compared to mosaic Turner syndrome ,which is having FSH hormone level >40mIU but not short stature. lack of diploid dosage of one or more vital genes, both alleles of which are active in oogenesis [14]. The molecular mechanism based on the POF phenotype could be due to haploinsufficiency of genes located within the deleted region. Maraschio *et al*., 1996 [15] reported that the same deletion can have different clinical consequences on menses and fertility. However, the majority of patients with Xq deletions have oligomenorrhoea, followed by secondary amenorrhoea or premature menopause, irrespective of the size of the deletion [16]. An association between POF and abnormalities of the X chromosome has been extensively confirmed in the literature [17]. A Dutch study has recently suggested that the involvement of the X chromosome may not be limited to POF but may influence the broader spectrum of menopausal age [18]. In POF patients deletions of the X chromosome have suggested two main critical regions located in Xq13.3–q22 (Powell *et al*., 1994)[6] and Xq26–q28 [18]. Few deletions in distal Xq have been reported. Marozzi *et al*. (2000) [19] described in POF patients with rearranged Xq chromosomes and confirmed that the second region extends from Xq26.2 to Xq28. Rossetti *et al*. (2004) [20] have reported an interstitial and distal deletion of the X chromosome in two affected women and their fertile mother In our study we find 20% cases 46,XX(Xq del12-27) .Our study also proved that deletion X-chromosome may lead to POF. These POF patients having, ht:140±0.397and FSH:93.0±31.91 and when we compare with Turner syndrome, both have almost same height, very high FSH levels as compared to other patients of POF. This may helpful to distinguish these deleted Xq region and Turner syndrome patients from other POF patient.

CONCLUSION

In conclusion, this study is beneficial for the premature ovarian failure patients. We identified mosaic 46, XX(Xq del) (20%) in region 46,XX(Xqdel12 – 27) that leads to chromosome abnormalities in a large population which result into POF in women. This study also including patients with clinical stigmata of Turner's syndrome (16%). It implies that karyotyping is helpful in the evaluation of POF patients. These both Turner's syndrome and mosaic del region of X-chromosome can detect biochemical i.e. high level FSH and their average height POF patients. These X- chromosome deletions associated with POF are more common than translocations. These may lead to the deletion or disruption of genes on the X-chromosome that are critical to ovarian function; however, it may also affect the correct alignment of chromosomes during meiosis, eventually leading to follicular atresia. The deleterious effect on ovarian function results from X chromosome breakpoints that fall on the long arm between Xq13 and Xq26. A 'critical region' for normal ovarian function has therefore been proposed for Xq13–q26 [16,21]. Recently, Eggermann *et al*. (2005) [23] have narrowed the distal region after a case report of a woman with a small deletion spanning from Xq27.2/Xq27.3 to Xqter in a familial case of POF with secondary amenorrhea. In a more recent study, Demirhan et al. (2014) [24] analysed chromosome abnormalities in 393 women presenting with primary and second- ary amenorrhea and 46,XX,15p+ was found in 1 case. As the p arm of acrocentric chromo- somes has no gene, it is expected that the increasing heterochromatin material in the p arm of acrocentric chromosomes considered as a polymorphic feature does not inûuence phenotype. Our study also gave distinguishing features of mosaic and nonmosaic of Turner syndrome.

Further studies using FISH and DNA microarrays allowing a high chromosomal resolution analysis will help delineate the Xq critical region in POF patients.

Conflicts of Interests: None

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