Incidence of Isochromosome Xq at Division of Human Genetics, Bangalore Medical College and Research Institute, Bangalore

SUDHA J[1] & PRIYA RANGANATH[2]

Department of Anatomy, Bangalore Medical College and Research Institute, Bangalore 560002, Karnataka
E-mail: priya_ranganath@rediffmail.com

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ABSTRACT: Isochromosome is a structural chromosomal abnormality where there is an abnormal transverse division of the centromere, so that there are either two short or long arms. The present study aimed at finding out the incidence of isochromosome. Karyotyping was done on 460 patients with different clinical diagnosis, who were of all ages, male and female, attending the laboratory of Division of Human Genetics, Bangalore Medical College and Research Institute, Bangalore. Cytogenetic analysis of peripheral blood lymphocyte by GTG banding was done. The karyotyping of 459 patients was normal with 46 XX or 46 XY karyotype. One patient (0.2%) had 3 different cell lines - 45, X (60%)/ 46,X,i(X)(q10) (35%)/47,XX,i(X)(q10)(5%).

INTRODUCTION

Humans have two types of chromosomes, autosomes and allosomes or sex chromosomes. In case of female individual there are 22 pairs of autosomes and 1 pair of sex chromosomes while in male along with 22 pair's autosomes, single X and Single Y chromosome are present. Sex chromosomal abnormality like turner syndrome is very common but less attentive syndrome because sex chromosomal abnormality may not be as severe or fetal as those from autosomal abnormality (Ford *et al.,* '59). But, this kind of sex chromosomal abnormality have various kind of phenotypic expression because genes present on X chromosomes are partially responsible for the development of body stature, puberty, primary and secondary sexual characters. So, mutation and complete or partial deletion of these genes may cause developmental failure of body stature and puberty. Generally, Turners are phenotypically female and suffering with ovarian dysgenesis and many other characteristics collectively called Turner stigmata (Rao *et al*., '97). Cytogenetically turners are generally X chromosome aneuploids or 45X, but other chromosomal numerical variation also occurs. This may be presence of cell line like 46XX or 46XY along with 45X, are call mosaic individuals. Presence of structural abnormality like deletion, duplication and isochromosome formation in both arms of X chromosome also occurs in significant manner (Jacobs *et al*., '97). Several possible mechanisms have been postulated for the formation of i(Xq), including mis-division of the centromere either during meiosis or mitosis, X;X translocations, crossing-over within an inversion loop, and chromatid breakage and reunion involving either sister chromatids or different homologues (Harbison *et al*., '88).

Isochromosome is a structural chromosomal abnormality where there is an abnormal transverse division of the centromere, so that there are either two short or long arms. This causes monosomy for the missing and trisomy for the duplicated arms (Young, 2005). The formation may also be because of the more complex U-type exchange resulting in acentric or dicentric products. The process may occur

† Research Assistant cum Tutor
‡ Professor (Corresponding author)

in pre- meiotic gamete, during meiotic cell divisions or in post-zygotic cell divisions of a normal or trisomic conceptus (Gardner and Sutherland, 2004). The symbol 'i' is in use to indicate the isochromosomal status (ISCN, 2005).

Turner syndrome was first described in 1938 and the chromosomal cause was established in 1959. It is X chromosomal aneuploidy, that is monosomy status compatible with relatively normal growth and development (Turner, '38; Ford *et al.*, '59). The incidence of Turner syndrome is about 1 in 5000 newborn females, even though 97% of these conceptions are spontaneously aborted.

Mosaicism is the presence of two or more cell lines with different chromosomal constitutions in the affected individuals. The cell lines mostly are derived due to post zygotic mitotic non disjunction (45,X/47,XXX/46,XX). The number of cell lines or the percentage may be given in brackets and the normal diploid karyotype is written last (Turnpenny and Ellard, 2005; ISCN, 2005).

On chromosomal analysis, the percentage occurrence of the various karyotypes observed in Turner syndrome are: 45,X (50%), 45,X/46,XX (20%), 46,X,i(Xq)(15%),46,X,r(X) or 46,X,del(X)(10%), and others (5%) (Graham *et al.*, 2007).

Isochromosomes of the long arm of the human X chromosome occur in approximately 0.002% of all newborns and is greater than or equal to 15% of individuals with Turner syndrome (Hook and Warburton, '83).

Hence the present study was done to know the incidence of formation of isochromosome in long arm of X chromosome.

MATERIAL AND METHOD

Karyotyping was done on 460 patients with different clinical diagnosis, who were of all ages, male and female, attending the laboratory of Division of Human Genetics, Bangalore Medical College and Research Institute, Bangalore. Cytogenetic analysis of peripheral blood lymphocyte by GTG banding was done as follows:

About 0.5 ml of heparinised whole blood was inoculated into 5 ml sterile RPMI – 1640 medium with 20% fetal bovine serum, antibiotics and phytohemagglutinin (PHA). The culture was incubated at 37 degree Celsius for 72 hours, after which add 50 µl of colcemide solution. The culture was incubated for an additional of 45 minutes, then centrifuged for 5 minutes at 1000 rpm. The supernatant was removed and the cells resuspended in 5 ml of pre-warmed KCl hypotonic solution. The solution was subsequently incubated at 37 degree Celsius for 15 minutes. It was spinned at 1000 rpm for 5 minutes and the supernatant removed. The cellular sediment was agitated in vortex and 5 ml of freshly prepared ice-cold fixative (1:3 parts of acetic acid and methanol) added. The cells were refrigerated overnight. The suspension was centrifuged, supernatant discarded and the pellets mixed. Freshly prepared fixative was added. The step was repeated for 3 changes in fixative until the pellet becomes clear. After the last change in fixative about 0.5 ml of fresh fixative was added to the pellet and mixed. 1-2 drops of cells suspension dropped onto the slide surface and dried. 4-5 slides were prepared for each case. Slides were aged at room temperature for 4-5 days. Then slides were stained by GTG banding method as follows:

The following solutions were added to 4 couplin jars –

1. 30 ml of phosphate buffer solution, 0.0125 % of trypsin
2. 30 ml of phosphate buffer solution
3. 30 ml of Sorensen's phosphate buffer and 3 ml of Giemsa stain
4. Running tap water

A slide was placed in 1st jar for 15-60 seconds. The slide was removed and rinsed by sequential dipping into 2nd jar. The slide was then placed in the 3rd jar for 5 minutes. The slide was removed and rinsed by sequential dipping in 4th jar. The slide was air dried and mounted with DPX after which it was ready for metaphase scanning under the microscope. Chromosomes were analysed under the automatic karyotyping microscope.

RESULTS

The karyotyping of 459 patients was normal with 46 XX or 46 XY karyotype. One patient (0.2%) had 3 different cell lines - 45, X (60%) / 46,X,i(X)(q10) (35%) / 47,XX,i(X)(q10)(5%). This 20-year old female had

short stature with a height of 4.1 feet and normal built. Her secondary sexual characteristics were poorly developed, that is, scanty axillary hair, absent pubic hair, hypoplasia of breasts and child-like voice. Primary amenorrhea was the chief complaints at the time of referral. Her ultrasound scanning of pelvis showed hypoplastic uterus.

DISCUSSION

In X chromosome, the mechanism of the formation is the U-type exchange and reunion between the sister chromatids of the X and frequently associated with 45, X cell line. The absence of the normal cell line in mosaic state indicates that i (Xq) may be predominantly of meiotic origin. The absence of normal cell line 46, XX in the present case could suggest that a post zygotic mechanism is not likely, unless the error occurred during the post zygotic mitotic cell division.

In 45, X Turner Syndrome, the haplo-sufficiency for SHOX gene (short stature homeo-box containing gene) in Xp may account for short stature. Likewise, from the critical region in Xq, functional monosomy may result in primary or secondary ovarian failure and affect ovarian and uterine cycles (Gardner and Sutherland, 2004). The same has been elicited in the present study.

It is important not to confuse the 46,X,i(Xq) syndrome with the 45,X classical Turner's syndrome. There are profound cytogenetic and clinical differences between the two syndromes, which must be borne in mind in the differential diagnosis of amenorrhea and of infertility (Santana *et al*., '77). The i(Xq) appearing as the metacentric chromosome consists of the isologous arms, which are structurally identical and contain the same genes. Almost all 46, X, i(Xq) individuals manifest streak gonads. Complete ovarian failure and partial ovarian failure have been reported in 91% and 9% of cases with i(Xq) individuals. Short stature and TS stigmata are also found to be frequent. The almost complete lack of gonadal development in 46,X,i(Xq) contrasts with 46X,del (Xq11), about 50% of whom menstruate or develop breasts (Simpson and Elias, 2003).

In our study a single case of 46,X,i(Xq) was observed who had primary amenorrhea with short stature and hypoplastic uterus.

Hook and Warburton ('83) observed 0.002% of newborns to have isochromosomes in long arm of X chromosome. In the present study, the incidence of isochromosome of the long arm of chromosome X was seen in one case (0.2%. The difference in the incidence is because the cases analysed in our lab are only the ones with clinical symptoms, and not all newborns in our hospital.

SUMMARY

Karyotyping of 460 cases with different clinical diagnosis referred to the Division of Human Genetics, Bangalore Medical College and Research Institute, Bangalore was done. 99.8% of the cases had normal karyotype; one patient (0.2%) had 3 different cell lines - 45, X (60%) / 46,X,i(X)(q10) (35%) / 47,XX,i(X)(q10)(5%). The present study may be useful in delineating the phenotype associated with abnormal X chromosome, reasons for primary amenorrhea, secondary amenorrhea, infertility, dysgenesis of female reproductive organs which may be due to Mullerian agenesis, short stature and for understanding the formation of isochromosome.

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