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Study Of Fragile Sites In Meos And Sunni Muslims Of Haryana

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Abstract

Fragile sites are heritable points of breaks and gaps in human chromosome. Fragile sites are excellent cytologic markers and can be utilized in gene linkage studies. Fragile site on chromosome 16 was found linked to gene for haptoglobin (Magenis et al., 1970). Sutherland (1981) found tenfold increase in the number of individuals having folate sensitive fragile sites on chromosome 2, 10, 11, 20 in comparison to the normal individuals. Autosomal fragile sites may predispose to mental retardation. Undoubtedly, the fragile X is associated with mental retardation (Lubs, 1969). Hecht and Hecht (1984 a,b) suggested that fragile sites may predispose to heritable chromosome rearrangements. This may result in pregnancy wastage, congenital malformation and mental deficiency (Shabtai et al., 1983). Twenty-eight individuals from each caste, Meos and Sunni Muslims of Haryana, were selected during this investigation to study the effect of consanguinity. After collecting blood samples, cell cultures were set up using culture medium, RPMI-1640 or TC-199 and various inducers or inhibiters 5-fluorodeoxyuridine, Methotrexate and Aphidicolin. Out of fifty-six individuals, one Meo and two Sunni Muslim individuals were found to express the autosomal fragile sites with an expression rate of 1 to 2% of metaphases.

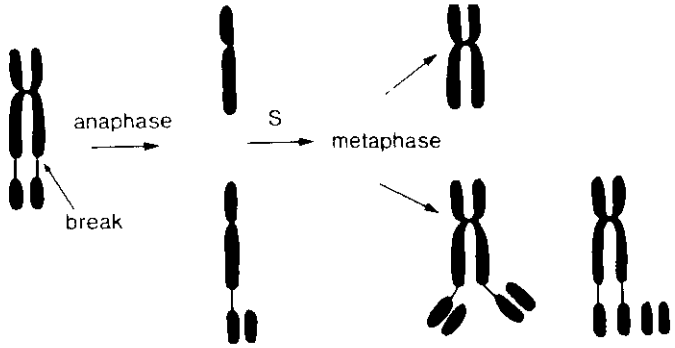
Keywords: Fragile site, 5-fluorodeoxyuridine, Methotrexate, Aphidicolin.

Introduction

The first fragile site on a human chromosome was recorded by Dekaban (1965). The fragile site at q27.3 on the human X chromosome was associated with a form of X-linked mental retardation (Lubs, 1969; Harvey et al; 1977). According to Sutherland (1979a), a heritable fragile site shows some of following features:

1. Fragile site shows a non-staining gap of variable width, usually on both chromatids.
2. The location of site on the chromosome is always same in all cells from an individual.
3. The fragile tendency is inherited in a Mendelian co-dominant fashion.
4. The fragility is evident by appearance of acentric fragments, deleted chromosomes and triradial or multiradial configurations, *in vitro* culture.

The triradial figure is the best observable cytogenetic manifestation of the fragile site and it helps in distinguishing fragile sites from other phenomenon causing chromosomal alteration. The acentric fragments produced by a fragile site get lost from the metaphase spindle or fail to reach the spindle pole at anaphase and become incorporated into micronuclei. According to Sutherland and Hecht (1985) frequency of triradial configuration in autosomal fragile sites is 1% to 4%.



Mechanism of production of triradial figures and deleted chromosome by breakage at fragile site followed by non-disjunction (after Ferguson–Smith, 1973). (reproduced from Sutherland, 1983).

Fragile sites can be divided into two major groups: rare and common. The distinction between these two types is that the most frequent of rare fragile sites is carried by about 1 in 20 individuals in the population of Germany (Schmid et al., 1986), but all individuals are probably homozygous for the common ones.

Materials and Methods

Twenty-eight individuals from each caste, Meos and Sunni Muslims of Haryana, were selected. About 10 ml blood was taken from each individual in heparin coated green top tube by vein puncture in the arm. Then, lymphocyte culture was set up for cytogenetic studies. Short term lymphocyte cultures were setup (Moorhead et al., 1960). About 5ml of culture medium, RPMI-1640 or TC-199 was taken into different culture vials. 0.5-0.8ml of heparinised whole blood was delivered to each culture vial and capped tightly. The contents of the culture bottle were thoroughly mixed by repeatedly tapping the bottom. The cultures were kept in an incubator in a slanting position at 37ºC. Cultures were incubated for 72/96 hours.

The fragile sites were detected according to Sutherland (1991) method. About 5ml of medium (TC-199 or RPMI-1640) was taken into four different culture vials. TC199 was taken into 1st culture vial while the remaining three culture vials were having RPMI 1640. TC 199 medium was used for detecting folate sensitive fragile sites in routine culture technique. In 2nd, 3rd and 4th culture vials FUdR (0.01 mg/L), Methotrexate (0.01 mg/ml) and Aphidicolin (0.07 mg/ml) were added respectively 24 hours before harvesting the cultures.

The type of medium used, pH, duration of culture, inhibitor/inducer along with its concentration and duration for routine cultures and for fragile sites is tabulated below:

Medium	pH	Duration (hrs)	Inhibitor/ Inducer	Conc. of Inhibitor/ inducer	Duration of Inhibiting/ Inducing	Remarks
RPMI-1640	7.0-7.2	72	–	–	–	Normal Karyotyping
TC-199	7.2-7.4	72	–	–	–	Fragile sites
RPMI-1640	7.0-7.2	96	FUdR	.01mg/l	final 24 hrs	Fragile sites
RPMI-1640	7.0-7.2	96	MTX	.01mg/ml	final 24 hrs	Fragile sites
RPMI-1640	7.0-7.2	96	Aphidicolin	.07mg/l	final 24 hrs	Fragile sites

Two hours prior to harvesting 0.02ml (10µg/ml) colchicine solution was added to each culture vial. The culture vials were gently shaken and incubated for additional 50-60 minutes at 37°C. After this the tubes were centrifuged at 800 rpm for 8 minutes. The supernatant was discarded leaving the cell button in the tube. The cell button was resuspended in 5ml of hypotonic solution (0.075M KCl) already warmed at 37°C. These tubes were incubated for 10-15 minutes at 37°C followed by centrifugation at 800 rpm for 8 minutes. After the removal of supernatant freshly prepared fixative (3:1, Methanol: glacial acetic acid) was added slowly to the cell button. The supernatant was again discarded, pellet was resuspended in fixative and tubes were again centrifuged. This process was repeated 3-4 times until the pellet turned white and the fixative appeared clear. After the final centrifugation, the cells were suspended in 0.5 to 1ml of fresh fixative to form a slightly milky suspension. Then 2-3 drops of cell suspension were dropped evenly from a height of about 2 feet on a wet, cold, grease free glass slide. The slides were kept in a slanting position and allowed to dry at room temperature. Then the slides were coded and studied under Trinocular Research Microscope. Photo micrographs of some selected metaphases were also taken for record to study various fragile sites

Results

Out of fifty-six individuals, one Meo and two Sunni Muslim individuals were found to express the autosomal fragile sites with an expression rate of 1 to 2% of metaphases. The fragile sites noted during the present investigation in Meos were – 2q13, 3p14 and 6q26 and in Sunni Muslims these were – 2q13, 2q31, 5q31, 6q26 and 10q23 (Table 1 Figs. 1, 2) Fragile site 2p13 was observed by using TC-199 as culture medium, without any inducer/inhibitor whereas fragile sites 2q31, 3p14, 5q31, 6q26 were noticed by using RPMI-1640 as culture medium and aphidicolin which act as an inducer. Fragile site 10q23 was detected by using RPMI-1640 as culture medium and methotrexate as an inhibitor of folate metabolism. Since the fragile sites are expressed at a very low level i.e. 1-2% they are considered to be less significant in present populations.

Table 1: Various Autosomal Fragile Sites seen among Meos and Sunni Muslims of Haryana

Fragile site	Inducer/ Inhibitor	No. of Ascertainment	% of Expression	Nature of Fragile site.
2q13[ab]	–	2	1%	Folate sensitive
2q31[b]	Aphidicolin	1	2%	Common
3p14[a]	Aphidicolin	1	2%	Common
5q31[b]	Aphidicolin	1	1%	Common
6q26[ab]	Aphidicolin	2	1%	Common
10q23[b]	Methotrexate	1	1%	Folate sensitive

a = Observed in Meos
b = Observed in Sunni Muslims
ab = Observed in Moes and Sunni Muslims

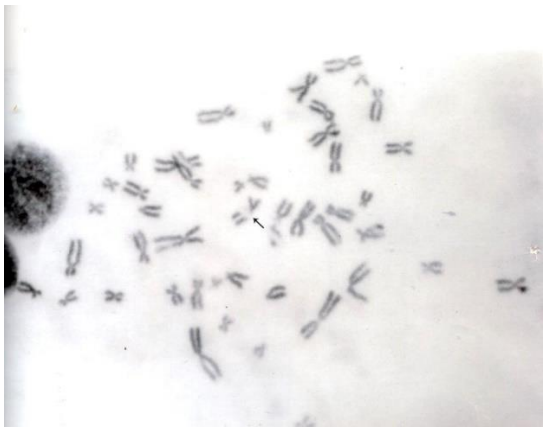


Fig 1: Mitotic metaphase showing Fragile site 6q26

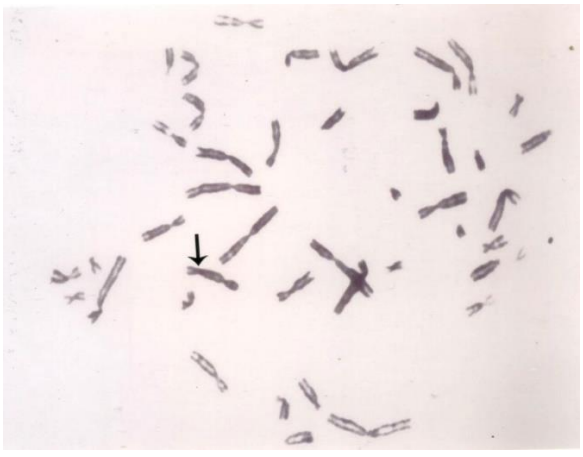


Fig 2: Mitotic metaphase showing Fragile site 5q31

Discussion

The fragile sites exhibit fragility under appropriate conditions of induction as evidenced by acentric fragments, deleted chromosomes, triradial figures etc. Fragile sites are not reported in the 100% of the metaphases observed. They are usually seen in a lower proportion of metaphases, which in turn is influenced by the culture medium in which the cells are cultured (Sutherland, 1979b). According to Craig-Holmes et al. (1987), the common fragile sites are almost certainly present in homozygous form, some of them like 3p14, 16q23 are easy to detect as they are seen at reasonable level of expression and in some metaphases, both homologues will be seen to express fragile sites while other common fragile sites are seen in fewer than 5% of metaphases and their detection may not be reproducible even within same laboratory. Sutherland (1991) reported that most of rare autosomal fragile sites are expressed in 10-40% of metaphases. In the present study, 100 metaphases were studied from each person and fragile site expression was reported only in 1-2% of metaphases (Table 1).

There are different reports about the expression rate of fragile sites. Quack et al. (1978) studied 1000 individuals and frequency of autosomal fragile sites expression was 0.14%. Soudek and McGeorge (1981) reported that frequency of expression of fragile sites is 1.2%. Guichaoua et al. (1982) studied 7786 individuals and frequency of autosomal fragile sites expression was found to be 0.22%. Sutherland (1983) studied 3187 individuals and frequency of fragile sites expression was found to be 0.60%. Sutherland (1985) reported only 0.16% frequency of autosomal fragile sites in 2439 randomly selected neonates. Schmid et al. (1986) suggested that rare fragile site is carried by 1 in 20 individuals, but all individuals are probably homozygous for common fragile sites. The frequency of rare fragile sites was detected about 0.5-1% in different populations (Takahashi et al., 1988; Kahkonen et al., 1989). In present investigation out of 56 individuals, only 3 individuals were found to carry fragile sites. The highest frequency of fragile X expression in the normal population was estimated by Herbst and Miller (1980) at 0.09%. About 0.04% frequency of fragile X was noted in 10000 individuals (Blomquist et al. 1982; Turner and Jacobs, 1983; Sherman et al., 1984). Fishburn et al. (1983) showed the incidence of fragile X to be 0.019% in 10000 normal males. In the present study, no fragile X was reported.

In the present study, cultures were first set up in medium TC-199, a folate deficient medium and RPMI-1640, to study the expression of fragile sites. This was done so because all the folate sensitive fragile sites would be expressed in TC-199 and also other fragile sites which are thought to be expressed independently of tissue culture conditions would expressed in RPMI-1640. Later cultures were set up by using inducers/inhibitors like Aphidicolin, Methotrexate and FUdR to increase the frequency of fragile sties. Folic acid by itself is not biologically active. It is first reduced to dihydrofolic acid (DHF), which in turn is reduced to biologically active co-enzyme tetrahydrofolic acid. Both these reactions are catalyzed by the enzyme dihydrofolate reductase. Inhibitors of this reaction are an effective means of inducing expression of folate sensitive fragile sites by preventing reduction of supplemented folic acid and of DHF generated by thymidylate synthetase catalyzed methylation of dUMP. Thus, inhibitors of dihydrofolate reductase inhibit essentially all folate pathway reactions.

Methotrexate (MTX) is a potent competitive inhibitor of dihydrofolate reductase and is effective in inducing expression of folate sensitive fragile sites (Sutherland, 1979a, Mattei et al., 1981). Folate sensitive fragile sites are also expressed by inhibition of thymidylate synthetase catalyzed reaction producing dTMP for DNA synthesis. FUdR inhibits thymidylate synthetase and can induce expression of folate sensitive fragile sites (Glover, 1981; Jacky and Sutherland, 1983).

Induction of Chromosome breakage in human cells by aphidicolin is also reported (Van Zeeland et al., 1982), but no site-specific breakage was described. Glover et al. (1984) clearly showed that chromosome lesions induced by aphidicolin are non-random and that greatest breakage occurred at "hot-spots" induced by thymidylate stress. Aphidicolin is competitive with dCTP for binding sites on DNA polymerase molecule (Oguro et al., 1979). Aphidicolin has been shown both to block progression of replication fork (Lonn and Lonn, 1983). Aphidicolin is known to partially inhibit DNA polymerase α affecting the progression of DNA replication at those sites (Glover et al., 1984). An indirect effect of polymerase α inhibition on other enzyme involved in DNA synthesis such as thymidylate synthetase is also possibly the direct cause of fragile site expression, however, this seems unlikely (Glover et al., 1984).

Considerable variations in both the frequency and location of aphidicolin-induced fragile sites have been noted primarily due to sampling differences (Craig-Homes et al., 1987). Ten fragile sites (1p31, 2q32, 3p14, 4p16, 4q31, 6q25, 7q21, 14q24, 16q23, 20p12) were observed in at least 40% of individuals among control population by inducing with aphidicolin (Tedeschi et al., 1987). Fragile site 3p14 was observed in every examined individual in a group of 70 normal healthy subjects. It was found to be most common fragile site (Smeets et al., 1986). Furuya et al. (1989) also reported that most frequent fragile site was 3p14 and second most frequent fragile site was 16q23. Methotrexate and fluorodeoxyuridine (FUdR) markedly enhanced the expression of 3p14 and other fragile sites (Smeets et al., 1986). Kuwano et al., (1988) reported 13 folate sensitive, 20 BrdU induced and 8 aphidicolin induced sites in one or more of the 15 normal healthy individuals studied in Japan. There is no systematic data on the population cytogenetics of autosomal fragile sties from the Indian population, except for the reports on for 5q31 by Shoba Rani and Ahuja (1984), and some other autosomal fragile sites by Manjunatha et al. (1989) among mentally retarded subjects.

The fragile site observed during the present investigation in 2q13, 2q31, 3p14, 5q31, 6q26 and 10q23. In Meos fra 2q13, 3p14 and 6q26 were observed whereas in Sunni Muslims fra 2q13, 2q31, sq31, 6q26 and 10q23 were observed. Fra 2q13 is a folate sensitive and reported by Conen and Erkman (1966), Williams and Howell (1976) and Sutherland (1977). Fra 2q31 is a common fragile site which was first reported by Sutherland et al. (1985). Fra 3p14 is a common fragile site reported by Markkanen et al. (1982) in normal individuals. The other fragile site reported was 5q31. It was reported by Hecht and Hecht (1984a) and Shoba Rani and Ahuja (1984). Fra 6q26 is a common fragile site. It was also reported by Leversha (1981). Fra 10q23 is a folate sensitive fragile

site. It was identified as 10q24.2 using R-banding (Giraud, 1976) but by using G-banding it was found to be at position 10q23 (Sutherland, 1979b). More data on Indian populations are required to draw any conclusion.

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