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Chromosomal Instability in Recurrent Spontaneous Aborters

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KEYWORDS Acrocentric chromosome associations; premature centromere division; spontaneous abortions; gametogenesis; aneuploidy; nondisjunction; chromosomal instability

ABSTRACT Association between the acrocentric chromosomes are very obvious in human metaphases. The incidence of acrocentric chromosome association is high in both partners experienced two or more recurrent miscarriages. Acrocentric chromosome associations are highly relevant because most aneuploidic conceptuses results from meiotic nondisjunction during gametogenesis. The altered centromere functions may have an increased risk for chromosomal instability and this leads to spontaneous abortion due to cell division errors. To find out this anomaly blood culture was performed in a series of 50 women with repeated spontaneous abortions and also in their husbands. Out of 100 individuals, a 36 years old man whose wife has experienced two spontaneous abortions was having Acrocentic chromosome association. His wife was normal with 46, XX chromosomal complement. Another 30 years old female experienced three spontaneous abortions was also having Acrocentric Chromosome association. Her husband was normal with 46, XY chromosomal complement. Four probands i.e, one female and three males showed premature centromeric division. The female was 26 years old with two recurrent spontaneous abortions. Her husband was normal with 46, XY chromosomal complement. Other three males are with 31, 33 and 33 years of age respectively. Their wives have experienced 2, 2 and 4 recurrent spontaneous abortions respectively with normal 46, XX chromosomal complement.

INTRODUCTION

It is considered that about 15% of all recognized pregnancies terminate in spontaneous abortions (Reid et al. 1972). The hypothesis advanced by Streeter (1931), Hertig and Rock (1949) that the principal aetiological factor in spontaneous abortion is an intrinsic anomaly in the fertilized ovum, a so called germplasm defect has confirmed by the observation that a high proportion of such abortuses are chromosomally abnormal (review by Carr 1971). It is generally accepted that between 25 to 35% of all recognizable spontaneous abortions are caused by chromosomal anomalies (Carr 1969; Larson and Titus 1970; Pawlowitzki 1972). The frequency of chromosomal abnormalities in embryos 3.8 to 7.5%, in fetuses 1.5 to 2% in still births 6.0 to 7.0%, in newborns 0.6%, in children (upto 7-8 years) 0.5% and in adults 0.4% (Diwald and Michels 1986; Boue and Boue 1978). In adults chromosomal abnormalities are found 4 in 1000, of these 50% occur in genetically balanced form. Of all spontaneous abortions 50 to 80% show a chromosome anomaly 94% of all detectable chromosomal abnormalities are associated with clinically recognizable fetal wastage. Chromosomal abnormality is a major cause of fetal loss and extensive surveys have shown that some 20-30% abortuses are aneuploid (Carr 1971; Boue at al. 1975; Creasy et al. 1976). The mean incidence of cytogenetic anomalies in 6639 couples investigated for recurrent abortions (Chandley 1983; Schwertz and Planner 1983; Fryns et al. 1984; Travers, personal communications) is 6.65%. Bhasin et al. (1972) reported among 16 couples and 11 women one of the partners in two cases shows balanced translocation of 13q/15q and a pericentic inversion of a chromosome A1.

MATERIAL AND METHODS

In the present study fifty couples were selected from Visakhapatnam district and included in the study ascertained with recurrent spontaneous abortions, majority of individuals were couples who had two or more spontaneous abortions. A complete case history was taken from the patient, this include personal particulars, family history, detailed pedigree chart and the result of laboratory or other investigations that had been conducted.

The mitotic chromosomes of the subjects investigated were prepared for cytogenetic evaluation by standard techniques. G banding in order to facilitate identification of individual chro-

mosomes was done for all cases. A minimum of 25 banded metaphase plates were analysed for routine Karytyping and for observing chromosomal aberrations.

RESULTS

The cytogenetic abnormalities among recurrent spontaneous aborters of 28 separate surveys have been presented in table 1. There are altogether 17047 patients from all the 28 separate series and out of which 486 patients exhibited chromosomal abnormalities. Increased percentage of abnormalities were recorded by Nordenson (1980) and Gupta et al. (1995) where the percentage of abnormalities were 45.00 and 28.57 respectively. One of the causes of this increased figures may be attributed to the small size studied. The mean percentage of abnormalities can be recorded as 2.86. The present study records abnormality in six individual (6.00%) out of 100 cases studied.

Table 1: Percentage of chromosomal abnormalities reported in recurrent spontaneous aborters

Reference	*No. of cases investigated*	*No. abnormal*	*% abnormal*
Stenchever et al. (1976)	56	5	8.92
Mennuti et al. (1978)	74	5	6.57
Kajii et al. (1978)	808	7	0.86
Nordenson (1980)	40	18	45.00
Subrt (1980)	230	9	3.91
Simpson et al. (1981)	224	2	0.89
Virginia et al. (1982)	440	8	1.8
Diedrich et al. (1983)	272	15	5.5
Wolstenholme et al. (1983)	156	6	3.84
Lippman- Hand (1983)	347	10	2.88
Fryns et al. (1984)	2136	59	2.76
Frederick Hecht (1984)	290	6	2.06
Sachs et al. (1985)	1000	50	5.00
Mouro Campana (1986)	792	20	2.52
Albert Fortuny et al. (1988)	890	27	3.03
David Castle & Renee Bernstein (1988)	1376	16	1.16
Gadow et al. (1991)	1364	28	2.03
Junge et al. (1991)	482	17	3.52
Tsui et al. (1991)	1028	51	4.96
Midro et al. (1992)	1000	10	1.00
Wu et al. (1993)	210	6	2.85
Orozco et al. (1993)	1347	30	2.22
Tulppala et al. (1993)	126	6	4.76
Gupta et al. (1995)	56	16	28.57
Celbellos - Quintal et al. (1996)	246	5	2.03
Imai et al. (1996)	74	7	9.45
Sasiadek et al. (1997)	258	8	3.1
Thomas et al. (1998)	1725	39	2.26
Total	17047	486	2.86
Present Study (2002)	100	6	6.00

From table 1 it may be observed that the frequency of chromosomal abnormalities that are recorded range from 0.86% - 45% The upper limit of 45% was reported by Nordenson (1980) and the lower limit of 0.86% was reported by Kajii et at. (1978). The high variation in percentage of abnormal cases in these surveys may be attributed to the lack of uniformity in selecting the couples i.e those with different number of spontaneous abortions (one or more spontaneous abortions) and with different gestational ages.

From the table 2 it is evident that a male aged 35, whose wife has experienced 2 spontaneous abortions and a female aged 30 experienced 3 spontaneous abortions showed Acrocentric Chromosome Association. Three males (31,33 and 33 years of age, respectively) whose wives have experienced 2,4 and 2 spontaneous abortions respectively exhibited premature centromeric division of cells. A female with 26 years of age experienced 2 spontaneous abortions also shows premature centromeric division

In the present study out of 100 cases, a male aged 35, with 2 repeated spontaneous abortions to her wife showed acrocentric chromosome association. Out of 2 pregnancies, 1of which ended at about 2 months of gestation and 1at about 3 months of gestation. The proband was born when her father and mother were 38 years and 31 years of age respectively and no abnormalities were noticed at birth or thereafter. He was normal phenotypically and mentally. The wife of the proband aged 29 is a healthy and well developed female with normal 46, XX chromosomal complement. Another female aged 30 years with 3 repeated spontaneous abortions exhibited acro-

Fig. 1. Metaphase showing acrocentric association

Table 2: Chromosomal abnormalities observed in 50 couples with spontaneous abortions

S.No.	*Sex*	*Age (in years)*	*No. of abortions*	*Chromosomal anomaly*
1.	Male	35	02	Acrocentric Chromosome Association
2.	Female	30	03	Acrocentric Chromosome Association
3.	Male	31	02	Premature Centromeric division
4.	Male	33	02	Premature Centromeric division
5.	Male	33	04	Premature Centromeric division
6.	Female	26	02	Premature Centromeric division

centric chromosome association (Fig.1). Out of 3 pregnancies 2 of which ended at about 2 months of gestation and one at about 3 months of gestation. The proband was born when her father and mother were 34 and 28 years of age respectively. She was normal phenotypically and mentally. The husband of the proband aged 34 is a healthy and well developed male with normal 46, XY chromosomal complement.

From the table 3 it is evident that three males whose wives have experienced 2, 2 and 4 spontaneous abortions showed 9%, 17% and 21% of premature centromeric division cells respectively (Fig. 2). A female with 2 spontaneous abortions shows 47% of premature centromeric division cells.

Table 3: Percentage of premature centromeric division cells in lymphocyte cultures observed in the present study.

S.No.	*Sex*	*% of PCD cells*	*No. of abortions*	*Karyotype*
1	Male	09	2	46, XY
2	Male	17	2	46, XY
3	Male	21	4	46, XY
4	Female	47	2	46, XX

Fig. 2. Metaphase showing premature centromere division

In the present study it is observed that premature centromere division cells are observed in 4 individuals out of 50 spontaneous abortion couples (4.00%). Here only few cells are showing premature centromere division. But they showed normal 46, XX (female) and 46, XY (male) chromosomal complement in other cells.

DISCUSSION

Human acrocentric chromosomes are frequently found in association. These associations have been subject of a number of investigations, with some workers reporting random and others nonrandom involvement of the acrocentrics and contradictory results when their dependence on sex has been examined (Warburton et al. 1973; Galperin 1969; Galperin-Lemaitre et al. 1977). This phenomenon might be related to frequencies of nondisjunction (Hansson 1979). While it is recognized that all chromosomes are predisposed to nondisjunction it has been suggested that certain chromosomes display an increased frequency of nondisjunction. As one third of trisomies observed in spontaneous abortions and live borns involve acrocentric chromosomes (Hassold and Jacobs 1984), it has been proposed that the presence of nucleoli organizing regions on the short arms of all five acrocentric chromosomes predispose them to nondisjunction (Polani et al. 1960; Mirre et al. 1980; Schmickel et al. 1985; Garcia et al. 1989) with the identification of DNA polymorphism, it is now possible to determine with confidence the parental origin of the additional chromosomes in most trisomies (Hassold and Sherman 1993).

Association between acrocentric chromosomes are very obvious in human metaphases. The phenomenon called satellite association was first reported by Ferguson Smith and Handmaker (1961). Harnaden (1961) Ohno et al. (1961) and observed in mitotic metaphases. The phenomenon of satellite association was also reported in meiosis by Ferguson-Smith (1964). The question

Table 4: Premature centromeric division reported in various studies

References	*Number of cases investigated*	*Number abnormal*	*%of PCD cells*	*Type of study*	*% of PCD*
Rudd et al. (1983)	03	03	14	Habitual Abortions	100%
			61.5	Cancer	
			6	Infertility	
Gabarron et al. (1986)	01	01	36	Habitual Abortions	100
Murthy and Prabhakaran (1990)	01	01	20.5	Habitual Abortions	100%
Katalin Bajnoczky (1993)	02	02	29.7(W)	Habitual Abortions	100%
			37.7(H)	Habitual Abortions	
Anuradha (1999)	140	06	16	Habitual Abortions	
			09	Habitual Abortions	
			47	Habitual Abortions	4.28%
			23	Habitual Abortions	
			21	Habitual Abortions	
			17	Habitual Abortions	
Present Study (2002)	100	04	09	Habitual Abortions	
			17	Habitual Abortions	4.00%
			21	Habitual Abortions	
			47	Habitual Abortions	

of non-random participation of the acrocentic chromosomes in satellite association was discussed by many authors even before the banding techniques made it possible to identify the individual chromosome types within the D and G groups. A higher tendency of the G group chromosomes to be involved in satellite association has been reported by e.g Zang and Back (1966) and Cohen and Shaw (1967). Other authors reported a random association pattern of the D group Chromosomes (Shaw et al. 1969; Cuevas Sosa 1970).

Thus the observation in the present study shows the acrocentic Chromosome association is higher in both partners experiences two or more recurrent miscarriages. Acrocentric Chromosome associations are highly relevant because most aneuploidic conceptuses result from meiotic nondisjuction during gametogenesis.

Premature centromeric division has been described by Fitzgerland (1975), Fitzgerland and McEwan (1977), Galloway and Buckton (1978) in aged women in association with aneuploidy of X-chromosome. Vig (1984) proposed a hypothesis that premature centromeric division may result in non-disjuction by impairing the attachment of prematurely separated centromeres to spindle fibres. Miller et al. (1990) reported association of Premature centromeric division with various aneuploidies in a high percentage supporting a functional relationship between disturbances in the mechanism of centromere separation and chromatid separation at cell division. An increased frequency of mitoses with centromere separation affecting all chromosomes was found in lymphocyte cultures from a couple with recurrent spontaneous abortions. Katalin Bajnoczky et al. (1993) concludes that patients with altered centromere functions may have an increased risk for chromosome instability and that the abnormal behaviour of centromeres may predispose the individual to cell division errors, the consequences of which may be a spontaneous abortion.

The increased frequency of mitosis with centromere separation affecting all chromosomes was found in Lymphocyte culture from a couple with recurrent spontaneous abortions reported by Bajnoczky et al. (1993) who concluded that patients with changed centromeric functions may have an increased risk for chromosomal instability and that the abnormal behaviour of centromere may predispose the individual to cell division errors, the consequence of which may be a spontaneous abortion. Increased frequency of mitosis showing premature centromere division was also reported by Gabarron et al. (1986).

Murthy and Prabhakaran (1990) reported a female with a history of spontaneous abortions and subsequent birth of Downs Syndrome Child. She was normal female with 46, XX chromosome complement with 20.5% cells with premature centromere division. Her husband was normal with 46, XY chromosome complement. He concluded that higher incidence of mitotic disturbances finally resulting in aneuploidy. This predisposi-

tion is evident by spontaneous abortions and Downs Syndrome child.

Thus the observation in the present study shows the patients with altered centromere functions may predispose to cell division errors due to chromosome instability and thus may lead to spontaneous abortion.

Investigation using molecular techniques on large samples will help to understand the exact reason for the increase in the Acrocentric chromosome association and premature centromere division in aborted couples and its role in nondisjuction.

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