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Karyotyping and counseling in bad obstetric history and infertility

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Abstract

Background: Division of Human Genetics (DHG) is a referral center for karyotyping and counseling to the couples as well as to the individuals referred with bad obstetric history and infertility.

Materials and Methods: From 1972 to 2003, overall 1666 couples and 131 female partners with bad obstetric history (BOH) such as; spontaneous abortions, live births with congenital malformations and still born and 73 infertile male partners have been referred for chromosomal analysis.

Results: The chromosomal abnormality was found in 4.4% (83) of the sample studied. Chromosomal abnormality was seen in 56 couples (3.4%), 15 female (11.5%) and 12 male (16.4%) partners. The numerical chromosomal abnormality were seen in 34 (41%) and the structural abnormalities in 49 (59%) cases. The numerical chromosomal abnormalities were associated with sex chromosomes as follows (the number of cases are shown in parenthesis): 47, XXY (9); 46, XY/ 47, XXY (2); 46, XY/ 48, XXXY (1); 46, XY/ 47, XYY (2) and X mosaicism; 45, X/ 46, XX (14); 46, XX/ 47, XXX (6). The structural anomalies were 40 translocations and 9 duplication/ deletion/ marker/ iso chromosome for the X chromosome; Male: 46,XY/ 47,XY+ mar (1); Female: 45,X/ 47,XX+mar (1); 46,XX/ 47,XX+mar (1); 47,XX+frag (1); 46,X,Xq- (2); 46,X,Xp- (1); 46,X,Xp+ (1); 45,X/46,X,i(Xq)(1). The frequently involved chromosomes in the translocations were 4, 11, 15 and X. There were three X; autosomal translocations and a unique combination of translocation 1; 15 in the parents of a female carrier and 13; 14 in a non- consanguineous couple. On the whole, 57.5% of the females (23/ 40) were translocation carriers. Non-significant chromosome polymorphisms were observed in 79 cases (4.2%).

Conclusion: The current study has demonstrated the presence of the chromosomal abnormality and its influence in reproductive failure. On an average, in this study one in 56 couple and one in 12 males with infertility or one in 15 females with BOH has had a chromosomal abnormality as the genetic cause. The identification of chromosomal abnormality as the etiology has facilitated the counseling and appropriate management.

Key words: *Chromosomal abnormality, Bad obstetric history, Infertility, Genetic counseling*

Introduction

In literature, the terms recurrent miscarriage/ habitual abortion/ recurrent spontaneous abortion/ bad obstetric history/ recurrent or repetitive pregnancy loss, reproductive failure and infertility, have been used interchangeably. Even though discrepancies in the work-up parameters in this field exist, the genetic component has achieved near universal acceptance. The estimated chromosomal abnormality (CA) in live births is 9.2 per 1000; out of which the autosomal CA constitutes 75% and sex CA 25%. Among the

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autosomal CA, balanced rearrangements present in 5.2 per 1000 live births; whereas 0.6% have unbalanced autosomal CA (1).

In couples with bad obstetric history (BOH) percentages of CA vary from 1 to 25% for individuals or to 50% for couples. Most frequently occurring CA is balanced chromosomal rearrangement, i.e. translocation; other CAs seen usually are sex chromosomal mosaicism, inversions and ring chromosomes. Reciprocal translocations are found to be 60% and Robertsonian translocation 40% in couples experiencing recurrent pregnancy loss (2). Infertility is reported to affect up to 15% of couples. The infertile males definitely have the increased risk to be the carriers of CA (47, XXY; X mosaicism). The detection of CA is one of the fundamental diagnostic procedures for further management and treatment (3).

In this investigation, the term reproductive failure includes the couples and the female partners who have experienced BOH and the males diagnosed with infertility. In this article, CA was investigated in 1666 couples and 131 female partners who have had BOH and 73 infertile male partners, referred to Division of Human Genetics (DHG) for karyotyping and counseling.

Materials and methods

This study was designed as a retrospective study. The data from 1666 couples and 131 female partners with BOH and 73 male partners with infertility referred to DHG has been considered for the study. Couples and the female partners were considered for the study only if they have had two or more than two abortions, neonatal deaths or offspring with multiple congenital anomalies. The reasons for the referral of the female and male partners alone were as follows; their spouses had the testing elsewhere, spouses genuinely could not come because their career was outside India, referral was only for the suspected partner and in-spite of the repeated requests the other partner did not turn up for the karyotyping.

The protocol for the preparation of the chromosomes for karyotyping was as follows: About 2ml of heparinized blood was collected from peripheral veins of the referred patients. Lymphocytes were grown in RPMI 1640 culture media containing antibiotics (penicillin/ streptomycin) and 15 % serum supplemention. Phytohemaglutinin (PHA) was added as the mitotic stimulant (0.5 ml of the innoculum) and the samples were incubated for 72 hours at 37^0C incubator. The cells were arrested at metaphase with 0.1% colchicine; chromosome elongation was accomplished by adding 1% ethydium bromide. Hypotonic treatment was done with Ohnuki solution (KCl / NaCOOH/ NaNo3) and cells were fixed with 3 changes of fixative (3:1, methanol: acetic acid). The prepared slides were stained with GTG (G-bands using Trypsin and Geimsa stain). Chromosomal analysis was done under 100x, magnification. Overall 15 metaphase spreads were screened and 5 metaphases were captured using a CCD camera. The captured picture was further enhanced by adjusting the sharpness, brightness and contrast and the printout was taken. According to ISCN standards, the karyotype was prepared**.**

Additional investigations for the complete work up of the referred patients include anatomical (scanning), endocrinological (hormonal assay), immunological (anti phospholipid profile–APL, anti cardio lipin-ACL) and environmental (infections, occupational hazards, cigarette smoking, alcohol, nutritional deficiency) factors.

Results

As it is shown in table I**,** major CA was found in 83 cases of the 1870 total samples (4.4%). This include; 56 out of 1666 couples (3.4%), 15 out of 131 females (11.5%) and 12 out of 73 males (16.4%).

The noted chromosomal polymorphism/ heteromorphism/ variants are presented in table II. The chromosomal variants were present in 79 out of the 1870 (4.2%). Polymorphisms were more frequently associated to chromosomes 9 and Y. Numerical CA (34/83, 41%) were either Klinefelter or Turner syndrome which had the typical as well as the variant karyotypes or mosaicism for the sex chromosomes. Mosaicism involved mostly the X chromosome, except one instance of 47, XYY in a male partner along with normal cell line of 46, XY (Table III).

The structural CA (49/83, 59%) was translocation in 40 (81.6%) and the deletion/ duplication/ marker/ isochromosome associated with X in 9 patients (18.4%). They were:(Male: 46,XY/ 47,XY+ mar 01, Female: 45,X/ 47,XX+mar 01; 46,XX/ 47,XX+mar 01; 47,XX+frag 01; 46,X,Xq- 02; 46,X,Xp- 01; 46,X,Xp+01;45,X/46,X,iX(q)01) (Table IV). In total 28 cases had reciprocal (70%) and 9 had Robertsonian (30%) translocation. The frequently involved chromosomes in reciprocal translocation were 1, 11 and 15 followed by 2, 4, 13, 20, 22 and X. A unique case of translocation t(1; 15) was detected in a female partner wherein both parents of her also were the carriers. Among Robertsonian translocations t(13; 14) was prevalent. In one case, both partners had robt(13; 14). In total, 23 females were found to be translocation carriers (82.14%) (Tables V and VI).

Table I. Chromosomal Abnormality

	Couples (1666)	Male (73)	Female (131)
Normal karyotype	1610 (96.6%)	61 (83.6%)	116 (88.5%)
CA (83) (4.4%)	56 (3.4%)	12 (16.4%)	15 (11.5%)
Numerical CA (34) (41%)	17	10	07
Structural CA (49) (59%)	39	02	08

Table II. Chromosomal Polymorphism (number) (%)

Couples (74/ 1666) (4.1%)	Male (2/ 73) (2.7%) Female (3/131) (2.3%)
1qh+ - 01	21p+ - 02
9 qh+ - 13	22p+ - 05
Inv9 - 11	Long Y - 19
13p+ - 03	Short Y - 04
14p+ - 06	Inv Y - 02
15p+ - 08	Total 79 (4.2%)

Table III. Numerical Chromosomal Abnormality (NCA)

34/83(41%)	Couples		Male	Female
	H*	W**		
Aneuploidy	04	13	10	07
47,XXY	01	-	08	
Mosaicism	03	13	02	07
Male				
46,XY/47,XXY	01	-	01	-
46,XY/48,XXXY	01	-	-	-
46,XY/47,XYY	01	-	01	-
Female				
45,X/46,XX	-	07	-	07
46,XX/ 47,XXX	-	06	-	

* *Husband* ** *Wife*

Table IV. Structural Chromosomal Abnormality (SCA)

49/83(59%)	Couples		Male	Female
	H*	W**		
Structural CA (49)	16	23	02	08
Translocation (40)(81.6%)	15	19	02	04
Reciprocal (28, 70%)	11(73.5%)	14(73.7)	02	01
Robertsonian (12,30%)	04(26.5%)	05(26.3)	-	03
Sex chromosomal (09)(18.4%)	01	04	-	04

* *Husband* ** *Wife*

Table V. Reciprocal translocation: Chromosomes involved

H* (11)		W** (14)	Male (02)	Female (01)
1;3	1;4	1;15 (mat and pat)	1;7 (mat)	-
2;4	2;13	2;11	4;21	-
3;11		-	-	-
4;6;15	5;6	5;13		X;12
-		6;8, 7;8, 7;11, 10;14, 11;16		
13;17	14;22	-		
		15;20; 15;22		
19;20		20;22		
Y;Y		X;14, X;22		

* *Husband* ** *Wife*

Table VI. Robertsonian translocation: Chromosomes involved

H*	W**	Male	Female
45,XY,t(13;14)(4)	45,XX,t(13;13)(2)	-	45,XX,t(13;14)(2)
	45,XX,t(13;14)(3)		45,XX,t(14;15)(1)

* *Husband* ** *Wife*

Discussion

For any given pregnancy, the reported risk of pregnancy loss is 15% and the likelihood of consecutive three losses, which is the classic definition of repetitive pregnancy loss (RPL), would be 0.34%. However, 1 to 2 % of couples experience three or more consecutive losses. Hence, medical help is sought in order to identify the causal factor as well as the strategy to alleviate the problem (4).

CA is included in the factors influencing the recurrence risk of RPL. Numerous studies have demonstrated that in around 5.5% of the couples, who have had RPL, one of the partners is the carrier for a balanced chromosomal rearrangement, in contrast to its incidence of less than 0.55% in the general population (5). It has been observed that the balanced chromosomal rearrangements have been detected to be present twice often in the female partners (6). In male the rearrangements are often associated with infertility (7).

Translocation (reciprocal and Robertsonian) is the most commonly observed CA (8). Overall, 75% are autosomal balanced translocation in the couples with pregnancy loss and this incidence is supposed to be 30 times higher than the report in the general population (5). The presence of the low - level sex chromosomal mosaic aneuploidy in the couples with the history of abortions has also been recorded. It may be due to age related phenomenon or an indicator of Turner or Klinefelter syndrome (9). It was thought that the frequency of CAs might be higher in couples with mixed problems in obstetric history than in couples with only abortions. However, it was not found to be significant, as various studies have quoted variable incidence from 2.9 to 23% (10-15). In a combined study done by collecting computerized database on 22,299 couples (44,398 individuals), 2.35% had CAs. Even from the pooled data, statistically significant differences have not been observed between the major CAs and the types of reproductive wastage and / or the presence or absence of normal live births (16). The incidence of CA in male infertility is reflected to the conditions such as hypogonadism, azoospermia or oligospermia. The CA frequency is found to be 4.6% and 13.7% in men with oligospermia and azoospermia respectively (17). The sex CA has the highest rate in azoospermic males, while autosomal CA predominates in the oligospermic males. Klinefelter syndrome with the classic karyotype of 47, XXY or mosaics with 46, XY / 47, XXY karyotypes are the frequently observed sex CA in infertile males. Robertsonian translocation is the frequently seen autosomal CA in infertile men (0.7%). A correlation has been observed between the XY bivalent and the chromosomes involved in Robertsonian trivalent in the pachytene stage of meiosis leading to the impairment of the germ cells (18). Pooling the data from the different series of infertile males, 0.5% reciprocal translocations were observed as compared to 0.1% in the newborn children. The correlation between reciprocal translocations with involvement of chromosomes 3, 4, 5, 6, 7, 9, 11, 13, 14, 15, 16, 17, 19, 20, 21, 22 and the impairment of sperm production is well known (19).

The incidence of CA in the present study has been found to be 3.4% in couples, 16.4% in the female partners with BOH and 11.5% in the males with infertility. The translocation and the sex chromosomes abnormalities (43/83, 51.8%) were the major observed CAs in the present study. In this study, the probands with X mosaicism have been considered as Turner or Klinefelter mosaic. The percentage of normal cell lines indicated that the probands might have started as normal zygote. In female patients with X mosaicism, there may be reduced amount of the genetic products transcribed from the critical region of the X chromosome, leading to BOH.

Mixed obstetric history in a couple may increase the probability of identifying the balanced translocation carrier status in one of the partners (20). The mechanism by which a balanced chromosomal translocation exerts a negative effect on the reproductive performance of the carrier is through production of unbalanced gametes during meiotic segregation. These carriers have an increased risk of abortions compared to the general population (15 to 50%). This risk is also increased, although to a lesser extent in the carriers of Robertsonian translocation.

In the present study, the incidence of the reciprocal and Robertsonian translocations in BOH were 65% (26/40) and 30% (12/40) respectively. The higher percentage of these two types of translocation in this study compared to the literature (50% and 2 to 25%) may be due to the sampling bias. As stated in the literature, the carrier status seemed to be prevalent in the females (57.5%).

Carriers of Balanced Complex Translocation (BCT) have a high risk of having spontaneous abortions or children with an unbalanced karyotype. BCT is ascertained in 68% of phenotypically normal couples because of their reproductive problems, around 23% in those born with multiple congenital anomalies and 8% through prenatal diagnosis (21). Infertility in a male BCT carrier is reflected as pre and post implantation losses rather than as disturbed spermatogenesis. Although the number of complex rearrangements seemed to be low, it is very important

to look at the family history and to karyotype all the siblings before predicting the risk.

In literature, there have been reports of reciprocal translocation carriers with varying combination of the involved chromosomes, resulting in BOH and reproductive failure (22, 23). As far as the Robertsonian translocation is concerned, the frequencies of the chromosomes involved are as follows: 13; 14 (57.5 - 63 %), 14; 21 (8%), 13; 13 (19%) and other chromosomes (29%).

Even though frequently involved chromosomes in translocation in couples with BOH have been reported, there seems to be still a variation in the breakpoints as well as in the chromosomes, which enter into translocation. Statistical analysis has shown that although all the chromosome arms have been involved in translocations some seemed to be preferentially involved such as: 2q, 5q, 7p, 7q, 12q, 13q, 17q, 18q and 22q. The size of the chromosomal segments involved, the frequency of the break points and their positions have a vital role in reproduction. In translocations, break points are non-random, especially in couples with mixed obstetric history (24). The frequently involved chromosomes in the translocations in the present study were 4, 11, 15 and X. There were three X; autosomal translocations and a unique combination of translocation 1; 15 in the parent of a female carrier and translocation 13; 14 in a non- consanguineous couple.

The significance of the variants is the frequent subject of debate (23, 25). It has been suggested that chromosomal heteromorphisms does not induce miscarriage (26). The role played by the variants is still controversial, because of the large number of variants found in the normal population (26). In this study, the chromosomal polymorphisms were considered to be non- significant. The disparity observed in the present study with that of the literature is because of the differences in the sample size. Previous reported studies have included only couples with repeated spontaneous abortions/ recurrent miscarriages/ repeated pregnancy losses or couples with mixed obstetric history. Hence, the frequencies of the CAs vary from series to series, but definitely it has a greater frequency than in the general population. This study has emphasized the importance of a proper work up of BOH and infertility, considering the different etiological factors including the karyotyping in order to find the cause for such a problem, thereby the affected couples may be effectively counseled.

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