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Cytogenetic abnormalities in 1162 couples with recurrent miscarriages in Southern region of India: report and review

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Abstract
Purpose The purpose of the present study was to investigate the contribution of chromosomal anomalies and the frequency of a particular type of aberration in couples with recurrent miscarriages.
Methods A total of 1,162 couples with recurrent miscarriages were analyzed using G-banding and Fluorescence in situ hybridization where ever necessary.
Results Chromosomal anomalies were detected in 78 cases. This study describes majority of the cases with balanced reciprocal translocations. Among the abnormal karyotypes we also report for the first time three unique translocations involving (3;14), (18;22) and (X;22) chromosomes which were confirmed by molecular cytogenetic methods.
Conclusions The review of literature and the overall incidence of the abnormalities suggest that chromosomal analysis in couples with recurrent miscarriages should be taken up by all the practioners at all levels. This not only helps to check the cytological abnormalities but also helps to correlate the recurrent abnormalities in a given population. Thus establishing and correlating the environmental and genetic condition of that particular phenotype and genotype.

Capsule This is the first report of 2,324 cases from Southern part of India with a table showing the review of literature and also summarizing the overall incidence.

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Introduction

Recurrent miscarriage (RM) is defined as a condition of three or more consecutive pregnancy losses before 24 weeks of gestation [1]. In almost 50% of cases the etiology is unknown. The causes of RM are heterogeneous and include endocrine dysfunction, auto immune disorders, genetic abnormalities, maternal and paternal age, infectious diseases, environmental toxins and congenital or structural uterine anomalies etc [2].

Chromosomal abnormalities, mainly balanced rearrangements, are common in couples with reproductive disorders including recurrent abortions [3]. Almost 15–20% of all pregnancies end up as spontaneous miscarriages, out of which the contribution of chromosomal abnormalities is as high as 70%. Parental chromosomal abnormalities represent an important etiology of recurrent miscarriage; studies published elsewhere have shown a prevalence of chromosomal anomalies that varies from 2% to 8% of couples who are affected by RM [4]. The presence of chromosomal rearrangements can lead to unequal crossing over during meiosis which can result in gametes with unbalanced chromosomes like duplications or deletions. The clinical consequences of such imbalances usually are lethal to the developing embryo leading to spontaneous miscarriages or early neonatal deaths [5].

The contribution of cytogenetic studies in reproductive disorders has been reported from many countries including India, but to our knowledge no such study has been done with a sample size of 2,324 cases in South India. The aim of the present study was to identify the types of

chromosomal abnormalities in couples with RM who were referred to our genetic clinic and to review the literature.

Materials and methods

A retrospective study was done in couples with RM from the period February 1998 to June 2010. A total of 1,162 couples with RM were offered chromosomal analysis. In all the cases the detailed reproductive case histories were taken and karyotypes were generated from the peripheral blood lymphocyte cultures and the cytogenetic analysis was performed.

Metaphase chromosome preparations from the peripheral blood cultures were made according to standard cytogenetic protocols. Cytogenetic analysis was performed by GTG (G-banding using Trypsin and Giemsa) banding at approximately 400–450 band level. NOR (Nuclear organizing regions) staining was also done to confirm the satellites on acrocentric chromosomes wherever necessary. FISH (Fluorescence in situ hybridization) was performed using commercially available (WCP) whole chromosome-painting probes for chromosomes 3, 14, 18 and 22 (Kreatech). Chromosome denaturation, hybridization and signal detection were done according to the published protocols [6, 7].

Twenty metaphases were analyzed in all the patients but in cases of abnormalities and mosaicism the study was extended to 50 metaphases. Chromosomal abnormalities were reported according to the current international standard nomenclature (ISCN). Along with the structural rearrangements and aneuploidies the common chromosomal variants were also studied.

Results

A total of 1,162 couples i.e., 2,324 cases with RM were evaluated. Chromosomal rearrangements were found in 78 cases (3.35%) (Table 1), among those 33 cases showed structural aberrations (1.41%) (Table 2), 44 cases showed normal polymorphic variants (1.89%) (Table 3) and one case of numerical anomaly was found. Majority of the abnormalities were BRT (Balanced Reciprocal Translocations) (21 cases). Robertsonian translocation was found in 6 cases involving chromosome 13, 14 and 15; and inversion Y chromosome in two cases. Two cases of deletions Xq and 17q were also identified and a case of derived 15 and also an additional 15p were observed, one marker chromosome and one aneuploidy with a mosaic XY/XXY were also identified.

In the present study three novel BRTs were described. Case 1: In a 30 year old male referred for chromosomal analysis due to RM in the spouse, cytogenetic analysis had revealed a karyotype of 46,XY,t(3;14)(p12;q12~13). Molecular cytogenetic techniques like FISH using WCP of chromosome 3 and 14 confirmed the translocation. In silico analysis of the gene content of the break point regions showed that the 14q12~13 is mapped to a locus for PAX9 gene, a novel member of the paired box-containing gene family. The history showed that his wife had two neonatal deaths one, an anencephalic child and the second child showed absence of stomach bubble and oesophageal atresia. Thus, hypothesized that two neonatal deaths could be due to the deletion of the PAX9 gene which might have occurred due to the unbalanced rearrangements from the proband.

Table 1 Total chromosomal abnormalities among 1,162 couples

Abnormalities	Number
Reciprocal translocation	21
Robertsonian translocation	6
Inversion	2
Deletion	2
Duplication	1
Marker	1
Aneuploidy	1
Polymorphic variants	44
Total	78

Case 2: A 26-year old woman was referred for chromosomal analysis due to RM. Cytogenetic analysis had revealed a BRT with a karyotype of 46,XX t(X;22)(p11.21;q13.3). Initially, WCP probe for chromosome 22 was used to confirm the translocation. With the availability of the Di George's probe in the laboratory, FISH was performed using this probe. The Di George's probe on chromosome 22 has two regions-the critical region (red) is on the 22q11.2 band and the control region (green) is on 22qter band. Interestingly, in this case the critical region was intact on derived chromosome 22 but the control region was found on the derived X confirming the breakpoints in this translocation.

Case 3: A 33 year-old was referred for chromosomal analysis due to RM. The karyotype showed 46, XY,t(18;22)(q21.2;qter). WCP FISH probes on 18 and 22 confirmed the translocation. In all these cases the advanced molecular cytogenetic techniques like FISH helped in the precise identification of the breakpoint regions. Thus genetics of RM also helps especially in assisted reproductive procedures.

Discussion

The most likely pathogenic mechanism behind RM is a multifactorial mode of inheritance. Several causes such as

Table 2 Structural chromosomal abnormalities identified in this study

S No	Reciprocal translocation	Sex		S No	Reciprocal translocation	Sex
1	46,XX,t(6;11)(q14;q14)	F		11	46,XX,t(5;9)(p13.1;pter)	F
2	46,XX,t(6;13)(p25;q22)	F		12	46,XX,t(7;14)(pter;q21)	F
3	46,XX,t(1;14)(q32;q32)	F		13	46,XX,t(X;22)(p11.21;q13.3)	F
4	46,XY,t(4;5)(q35;p15)	M		14	46,XY,t(5;16)(q33.3;p13.3)	M
5	46,XX,t(5;7)(p32.1;q31)	F		15	46,XX,t(1;18)(pter;p11.2)	F
6	46,XX,t(4;5)(q25;q35)	F		16	46,XX,t(7;16)(q31.1;q23)	F
7	46,XY,t(3;14)(p12;q12~13)	M		17	46,XX,t(16;20)(p13.3;p12)	F
8	46,XY,t(7;14)(q33;q32.3)	M		18	46,XX,t(9;12)(p24;q24.1)	F
9	46,XY,t(1;9)(q23;q22.3)	M		19	46,XX,t(7;9)(p13;p22)	F
10	46,XY,t(18;22)(q21.2;qter)	M		20	46,XX,t(1;13)(q42.1;q14.3)	F
				21	46,XX,t(8;10)(q24.2;q25.2)	F
S No	Robertsonian translocation	Sex	No	S No	Robertsonian translocation	Sex
1	45,XX,rob(13;14)	F	3	3.	45,XY,t(13;15)	M
2	45,XY,rob(13;14)	M	2			
S No	Inversion	Sex	No			
1	46,X,inv(Y)	M	2			
S No	Deletion	Sex		S No	Deletion	Sex
1	46,XX,del(Xq)	F		3	46,XX/46,XXdel(17)(q)	F
2	46,XX,der(15)add(15)(p)	F				
S No	Marker	Sex				
1	47,XY+marker	M				
S No	Aneuploidy	Sex				
1	46,XY/47,XYY	M				

skewed X-chromosome inactivation, genomic imprinting, single gene mutations, chromosomal instability, and sperm chromosome abnormalities have been suggested to explain the so called idiopathic reproductive loses. This has an important implication for future research as it may lead to the identification of some new genes involved in RM. Apparently; cytogenetic studies give considerable information about the genetic makeup leading to RM and still remain an important tool. For example when a history of repetitive abortions, malformative syndrome, or mental retardation is found in the family of one of the two parents, the risk of finding a structural chromosomal anomaly is significantly higher. If such rearrangements are present the chromosomes have difficulty in pairing up and dividing evenly during meiosis. Especially this is due to the fact that carriers of BRT have a risk of partial trisomy or partial monosomy for chromosomal regions involved in the translocation due to meiotic segregation.

Table 3 The identified Polymorphic chromosomal variants

S No	Variants	No of cases
1	9qh+	10
2	Pericentric inversion 9	14
3	22p+	2
4	15p+	2
5	9qh−	1
6	1qh+	2
7	13s+	3
8	15s+	7
9	22s+	2
10	22p−	1
		44

Most of the spontaneous miscarriages are caused due to chromosomal abnormalities in the embryo or foetus [8]. The genetic etiology for multiple spontaneous miscarriages includes an unbalanced chromosomal rearrangement which may be the result of one parent being carrier for BRT [9]. In 4–8% of couples with RM, at least one of the partners has chromosomal abnormalities that probably contain balanced chromosomal anomalies [10]. Usually one in 500 people carries a BRT. When one member of a couple carries a BRT; the risk of having a miscarriage is approximately doubled [11]. Further, BRT is ascertained in 68% of phenotypically normal couples because of their reproductive problems [12]. Also, Boue et al. [13] identified in prenatal diagnosis around 3.4% unbalanced foetal karyotypes, in couples in which a parent had a BRT.

According to the literature review the prevalence of chromosomal aberrations among the couples with repeated spontaneous miscarriages varied in different studies from none [14] to as high as 21.4% [15]. These differences may be related to sample size and to different criteria (Table 4). The overall chromosomal anomalies found in our study are 3.35%. Similar to other studies [23] translocations were the common abnormalities in our study too with 79.41%. The affected translocation partner was female in 18 cases (66.66%) and male in 9 cases (33.33%). The incidence of translocation is more in females than in males in the literature too [19]. As it is known that BRT involve meiotic blocking of the spermatogenesis, but ovogenesis usually is conserved and produces gametes with a high risk of presenting unbalanced forms of chromosomal anomaly [24].

In literature, there have been reports of reciprocal translocation carriers with varying combination of the involved chromosomes, resulting in RM and reproductive failure [19]. For example, though a few chromosomes are frequently involved in translocations in RM there seems to be still a variation in the breakpoint regions which enter into translocation. Translocations involving 2q, 5q, 7p, 7q, 12q, 13q, 17q, 18q & 22q are frequent. The size of the chromosomal segment involved the frequency of the breakpoints and their positions have a vital role in reproduction. In translocations, breakpoints are non-random, especially in couples with Bad Obstetric History [25].The frequently involved chromosomes in the translocations in the present study were 1, 5, 7, 9, 14 & 16. The molecular characterization of the three novel translocation cases mentioned in this study was not reported in the literature involving those breakpoint regions. In addition introduction of such molecular characterizations to clinical practise helps in identifying the precise breakpoint regions which could be helpful in assisted reproduction where the zygote could be checked or sperm chromosomes could be considered for further evaluation.

Also polymorphisms were more associated to chromosome 9 and Y. Pericentric inversion is one type of chromosomal rearrangement, which has been categorized as a minor chromosomal rearrangement not expected to correlate with abnormal phenotype. Most clinicians have considered inversion 9 to be a benign chromosomal polymorphism [26]. The significance of the variants is a subject of debate. The role played by the variants is controversial as large number was also found in normal populations [27]. In this study the variants were mostly involved with chromosome 9.

The first specific characteristic of BRT is the absence of phenotypic manifestations and the second very important characteristic is the high risk to give birth to children with unbalanced chromosomal rearrangements. The overall incidence of chromosomal abnormalities indicates that chromosomal analysis of the couples with RM should be essentially considered. A chromosomal anomaly finding in either of the parent can make it possible to evaluate the prognosis of future pregnancies because the risk of miscarriages in couples with BRT is approx 25%–50%, and with Robertsonian translocation it is approx 25%. Therefore all the couples with BRT should be strongly advised to monitor their future pregnancies by prenatal diagnosis to exclude the possibility of a chromosomally unbalanced zygote. Cytogenetic studies gives the important genetic information thus acts as a good genetic tool. This study has emphasized the importance of proper work up of the RM cases, considering different etiological factors including karyotyping in order to find the cause for such

Table 4 Review of literature in reproductive disorders

Author	Total cases	Total abn[a].	%	Structural	%	Numerical	%	Variants	%
Dubey et al. [16]	1484	31	2	22	2.9	9	1.2	21	3.2
L Rao et al. [5]	320	18	11.25	–	–	–	–	–	–
Akgul et al. [17]	179	21	11.74	3	1.68	15	8.38	3	1.68
Tavokina et al. [18]	420	46	10.95	41	89.13	5	10.87	–	–
Mozdarani et al. [11]	221	21	9.50	–	–	–	–	–	–
Bourrouillou [19]	2136	–	–	–	–	–	–	–	–
Rajangam et al. [20]	3332	83	4.4	49	59	34	41	79	4.2
Amudha et al. [21]	1465	–	–	65	21.3	240	78.7	–	–
Elghezal et al. [4]	2800	97	6.93	57	–	32	–	–	–
Fryns et al. [3]	2136	59	5.5	33	3.09	6	0.56	–	–
Espino et al. [22]	1832	52	2.76	43	2.28	9	0.47	17	0.9
Our cases	2324	78	3.35	33	1.41	1	0.04	44	1.89

[a] abnormalities

a problem, thereby the affected couples may be effectively counselled and also decide about further reproductive options.

Conclusions

The results of this study about the prevalence of chromosomal abnormalities found in our sample are consistent with figures described in several populations around the world. The overall incidence of chromosomal abnormalities indicates that chromosomal analysis of the couples with RM should be essentially considered. Among the novel karyotypes, we report for the first time three unique cases of chromosomal translocation associated with RM. Further precise molecular characterization of these chromosomal breakpoint regions could pave way for identification of new genes or genes involved in RM and also help in elucidating the molecular mechanism underlying the aberrations. It also establishes the cause of the miscarriages and helps in genetic counselling.

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References

1. Royal College of Obstetricians and Gynecologists. The management of recurrent miscarriage. London: Royal College of Obstetricians and Gynecologists; 1998.
2. Stephenson MD. Frequency of factors associated with habitual abortion in 197 couples. Fertil Steril. 1996;66:24–7.
3. Fryns JP, Van Buggenhout G. Structural chromosome rearrangements in couples with recurrent fetal wastage. Eur J Obstet Gynecol Reprod Biol. 1998;81:171–6.
4. Elghezal H, Hidar S, Mougou S, Khairi H, Saad A. Prevalence of chromosomal abnormalities in couples with recurrent miscarriage. Fertil Steril. 2007;88:721–3.
5. Rao L, Murthy K, Babu A, Venkata P, Deenadayal M, Singh L. Chromosome inversions and a novel chromosome insertion associated with recurrent miscarriages in South India. Arch Gynecol Obstet. 2005;272:273–7.
6. Licher P, Cremer T, Borden J, Manuelidis L, Ward DC. Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. Hum Genet. 1988;80:224–34.
7. Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas J, et al. Fluorescence in situ hybridization with human chromosome specific libraries: detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci USA. 1988;85:9138–42.
8. Rubio C, Pehlivan T, Rodrigo L, Simon C, Remohi J, Pellicer A. Embryo aneuploidy screening for unexplained recurrent miscarriages. Am J Reprod Immunol. 2005;53:159–65.
9. Gadow EC, Lippold S, Otano L, Srafin E, Scarpati R, Matayoshi T. Chromosome rearrangements among couples with pregnancy losses and other adverse reproductive outcomes. Am J Med Genet. 1991;41:279–81.
10. Kavalier F. Investigation of recurrent miscarriages. BMJ. 2005;331:121–2.
11. Mozdarani H, Meybodi AM, Zari-Moradi S. A cytogenetic study of couples with recurrent spontaneous abortions and infertile patients with recurrent IVF/ICSI failure. Indian J Hum Genet. 2008;14:1–5.
12. Schwartz S, Palmer CG. Chromosomal findings in 164 couples with repeated spontaneous abortions: with special considerations to prior reproductive history. Hum Genet. 1983;63:28–34.
13. Boue A, Gallano P. A collaborative study of the segregation of inherited chromosomal structural rearrangements in 1356 prenatal diagnosis. Prenat Diagn. 1984;4:45–67.
14. Rowley PT, Marshall R, Ellis JR. Genetic and cytological study of repeated spontaneous abortion. Ann Hum Genet. 1963;27:78–100.
15. Khudr G. Cytogenetics of habitual abortion: a review. Obstet Gynecol Surv. 1974;29:299–310.
16. Dubey S, Chowdary MR, Prahlad B, Kumar V, Mathur R, Hamilton S, et al. Cytogenetic causes for recurrent spontaneous abortions-An experience of 742 couples (1484 cases). Indian J Hum Genet. 2005;11:94–8.
17. Akgul M, Ozkinay D, Ercal D, Cogulu O, Dogan O, Altay B, et al. Cytogenetic abnormalities in 179 cases with male infertility in Western Region of Turkey: report and review. J Assist Reprod Genet. 2009;26:119–22.
18. Tavokina LV, Baronova EV, Sopko NI. The most frequent chromosomal abnormalities in karyotypes of patients with reproductive disorders. Cytol Genet. 2007;41:237–42.
19. Bourrouillou G, Colombies P, Dastugue N. Chomosome studies in 2136 couples with spontaneous abortions. Hum Genet. 1986;74:399–401.
20. Rajangam S, Tilak P, Aruna N. Rema devi: Karyotyping and counseling in bad obstetric history and infertility. Iranian J Reprod Med. 2007;5:7–12.
21. Amudha S, Aruna N, Rajangam S. Consanguinity and chromosomal abnormality. Indian J Hum Genet. 2005;11:108–10.
22. Espinoza JP, Anguiano LO, Rivera H. Chromosomal abnormalities in couples with reproductive disorders. Gynecol Obstet Investig. 2008;66:237–40.
23. Neri G, Serra A, Campana M, Tedeschi B. Reproductive risks for translocation carriers: cytogenetic study and analysis of pregnancy outcome in 58 families. Am J Med Genet. 1983;16:535–61.
24. De Braekeleer M, Dao TN. Cytogenetic studies in couples experiencing repeated pregnancy losses. Hum Reprod. 1990;5:519–28.
25. Campana M, Serra A, Neri G. Role of chromosome aberrations in recurrent abortions: a study of 269 balanced translocations. Am J Med Genet. 1986;24:341–56.
26. Tibiletti MG, Simoni G, Terzoli GL, Romitti L, Fedele L, Candiani GB. Pericentric inversion of chromosome 9 in couples with repeated spontaneous abortion. Acta Eur Fertil. 1981;12:245–8.
27. Portnoi MF, van den Akker J, Le Porrier N, Youssef S, Taillemite JL. A new case of ring chromosome 9. Sem Hôp. 1983;59:185–8.