Chromosomal aberrations and chromosomal heteromorphisms among young couples with recurrent spontaneous abortion

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Background
Recurrent spontaneous abortion (RSA) is a common challenging reproductive problem, whereas the frequency of chromosomal abnormalities among couples with RSA varies from 2 to 8%. Chromosomal heteromorphisms that have been considered as normal variants are suspected to have a clinical effect in the form of infertility or RSA.

Objective
The objective of this research is to study the role and incidence of different types of chromosomal findings including chromosomal aberrations and chromosomal heteromorphisms among couples with RSA. The results can add to genetic counseling as well as can serve as a step for collecting national data concerning this issue.

Patients and methods
A total of 73 couples, comprising 146 cases with a history of RSA, have been enrolled in the study. All cases were subjected to full history taking, conventional cytogenetic analysis in addition to fluorescence in-situ hybridization technique whenever required.

Results
Among the studied cases (n = 146), 6.8% showed chromosomal abnormality. They were categorized into three (30%) cases with reciprocal translocation, four (40%) cases with Robertsonian translocation, two (20%) cases showed mosaic aneuploidy, and one (10%) case was chimera. Regarding chromosomal heteromorphisms, nine (6.16%) cases have been detected; the most frequent finding was pericentric inversion in chromosome 9 (*inv (9)*(*p12q13*)).

Conclusion
Before endeavoring any therapeutic intervention, couples who experience two or more abortions of unknown etiology should undergo a cytogenetic analysis, as RSA might be a rescuer mechanism to avoid distressing outcomes. Chromosomal heteromorphisms could be a contributing factor for RSA. Genetic counselors should pay attention to this issue until a conclusive mechanism can be verified.

Keywords:
chimerism, chromosomal aberrations, chromosomal heteromorphism, recurrent spontaneous abortion

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Introduction

Recurrent spontaneous abortion (RSA) or recurrent pregnancy loss is a common clinical complaint. It is a frequent complication of pregnancy to the extent of 15–20% of all identifiable pregnancies (Pal *et al.*, 2018). RSA is usually defined clinically as two or more disrupted pregnancies before the 20th week of gestation (Practice Committee of The American Society for Reproductive Medicine, 2012). Numerous etiological factors can lead to this condition, including genetic, endocrinal, environmental, anatomical, and infectious factors (Gonçalves *et al.*, 2014). Parental chromosomal aberration is an important genetic cause (Fan *et al.*, 2016), as balanced rearrangement is usually a prominent finding within couples complaining of this reproductive complication. The frequency of chromosomal abnormalities among couples with RSA varies from ~2 to 8% (Hyde and Schust, 2015; Nonaka *et al.*, 2015). Chromosomal abnormalities in parents can result in unequal crossing over during meiosis with subsequent gametes characterized by unbalanced chromosomal aberrations, which are lethal and result in spontaneous abortion or unviable outcome in the form of stillbirth or neonatal death (Rao *et al.*, 2005). Moreover, it has been reported that 50% of spontaneously aborted fetuses have chromosomal abnormalities (Ghazaey *et al.*, 2015). Although some of these abnormalities are de novo, most appear to be inherited and could be derived from a balanced carrier parent (Driscoll and Gross, 2009).

Common cytogenetic heteromorphisms, which include heterochromatic regions on chromosomes 1, 9, 16, and Y as well as acrocentric short arms, satellites, and stalks, have been described as normal chromosomal variants (Brothman *et al.*, 2006). As these regions consist of highly repetitive sequences of satellite DNA that does not encode proteins, normal heritable outcome for both genotype and phenotype has been described (Yuce *et al.*, 2007).

However, various recent studies indicate that these chromosomal heteromorphisms may have a clinical effect and can represent its effect in the form of infertility or RSA (An *et al.*, 2016). Most studies have reported that the incidence of heteromorphisms is 3–5-folds higher in cases with reproductive problems compared with the fertile ones (Tempest and Simpson, 2017).

The objective of this research was to study the role and incidence of different types of chromosomal aberrations and chromosomal heteromorphisms among couples with recurrent pregnancy loss, apart from those characterized by consanguineous marriage, advanced age (exceeding the childbearing period), or complaining of any medical illness that may be the cause of this reproductive problem, and to discuss the feasibility of considering chromosomal heteromorphisms during genetic counseling sessions.

Patients and methods

Patients

This study was conducted on 146 patients. They were grouped into 73 couples. Each couple included a male and a female in the childbearing period (which lies between 17 and 35 years) with a history and clinical diagnosis of RSA. All selected cases were confined between 20 and 35 years of age, with a mean age of 26 years for females (20–31 years) and 28 years for males (23–34 years). Cases with age above 35 years, positive consanguinity, or history of any medical illness that explains their current complaint have been excluded from the study, in an attempt to localize the causal factors in the studied sample.

Cases were selected from the Human Genetics Department Clinics – The National Research Centre. An informed consent was taken from all cases according to the guidelines, and approval of the Medical Research Ethics Committee of the National Research Centre was obtained.

Methods

All cases were subjected to the following:

(1) Full history taking, laying stress on pregnancy history, including exposure to environmental agents, for example, infections, pesticides, cigarette smoking, or any chronic medical condition that can be the cause of recurrent abortion; occupational history for each couple to exclude environmental risk factors; as well as history of any previous child with birth defects or dysmorphic features. A family pedigree was constructed for each couple. Thorough clinical examination and investigations have already been done previously for the studied cases to exclude various common causes of abortion
(2) Cytogenetic studies: A sample of venous blood (3–3.5 ml) was taken from both partners under aseptic conditions into a sterile heparin-coated vacutainer.

Conventional cytogenetic analysis (CCA) was performed to detect any numerical or structural chromosomal aberrations among the selected cases, as well as chromosomal heteromorphism using, GTG banding technique (Verma and Babu, 1995). Approximately 30 metaphases have been analyzed and karyotyped for each patient, and cytogenetic nomenclature was written following the International System for Human Cytogenomic Nomenclature (2016) recommendations. Fluorescence in-situ hybridization (FISH) technique was done for selected cases to detect the chromosomal breakpoints in cases with reciprocal translocation or to detect the percentage of mosaicism in cases that showed two cell lines by CCA, to allow for proper genetic counseling and recommendations for subsequent pregnancies. The technique was carried out according to modification of Pinkle *et al.* (1986). Specific DNA probes in addition to DAPI II counter stain (4,6-diamidino-2-phenylindole dihydrochloride) have been used.

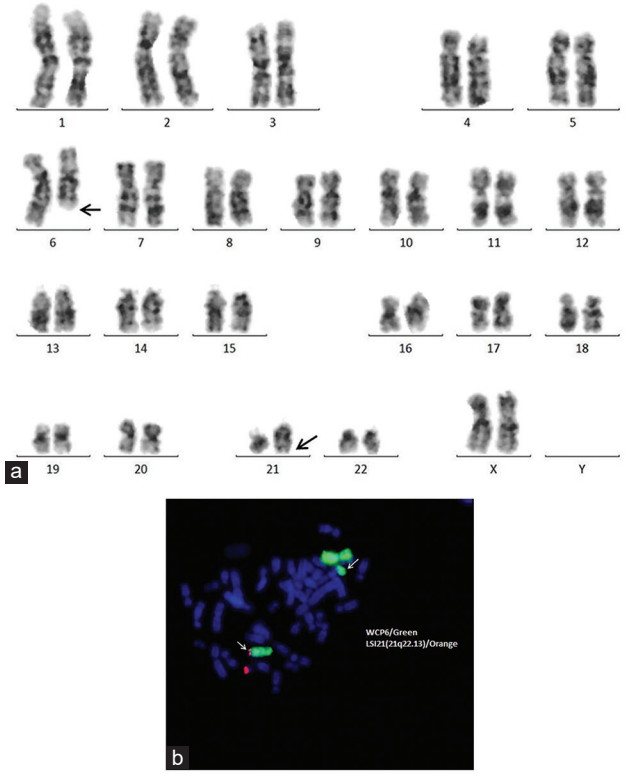
According to each case, specific DNA probes, including centromere-specific probes such as CEP X (DXZ1) (spectrum green)/CEP Y (DYZ3) (spectrum orange; Abott Molecular, Lake Bluff, Illinois, USA) as well as locus-specific and whole chromosome painting probes (Cytocell FISH probes; Oxford Gene Technology-The Molecular Genetics Company, Cambridge, UK), have been used. At least 100 cells were scanned in every case using image analysis system (computer-assisted camera system and fluorescence microscope with suitable filter combination; Applied Imaging, San Jose, California, USA). Only metaphases and interphases with nonoverlapping and clear signals were analyzed.

Results

By CCA, 10 cases (6.8% of all the studied cases) showed chromosomal abnormality (Table 1), comprising six (4.1%) females and four (2.7%) males.

Among these 10 cases, seven cases revealed structural chromosomal aberrations and were categorized into three (30%) cases with reciprocal translocation (Fig. 1a), four (40%) cases with Robertsonian translocation (Fig. 2), two (20%) cases showed mosaic aneuploidy, and one (10%) case was chimera.

Figure 1



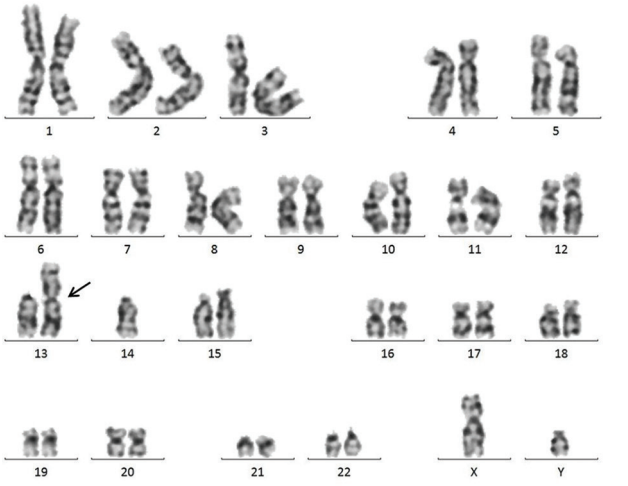
(a) Karyotype of the female partner of couple no. 7, showing reciprocal translocation, 46,XX,t(6;21)(q25;q22). (b) Metaphase fluorescence in-situ hybridization of the same female, revealed ish t(6;21) using Cytocell probes, WCP6 spectrum green, and LSI 21 (21q22.13) spectrum orange.

Regarding chromosomal heteromorphisms, nine (6.16%) cases have been detected (Table 2 and Fig. 3), comprising seven (4.79%) males and two (1.36%) females.

Using FISH technique, reciprocal translocation has been confirmed for male partner of couple no. 6 and female partners of couples no. 5 and 7 (Fig. 1b). Percentage of different cell lines by FISH technique was compatible with that by CCA (Table 1) for both female partners of couples no. 8 and 9 (Fig. 4) and male partner of couple no. 10.

Remarkably, CCA of female partner of couple no. 9, revealed a rare finding of 46,XX[16]/XY[11]. This case was presented with history of two previous abortions during the first trimester; she was 27 years of age with irrelevant medical history. Radiological and pathological investigations were not available, and the patient was not available again for requesting further investigations. Results of her CCA were confirmed by FISH and revealed chi

Figure 2



Karyotype of the male partner of couple no. 1 showing Robertsonian translocation, 45,XY, rob(13;14)(q10;q10).

Table 1 Detailed numerical and structural aberrations among the studied cases

Couple numbers	Karyotype of male partner by CCA	Karyotype of female partner by CCA	FISH results of the affected partner
1	45,XY,rob(13;14)(q10;q10)	46,XX	ND
2	46,XY	45,XX,rob(14;14)(q10;q10)	ND
3	46,XY	45,XX,rob(14;15)(q10;q10)	ND
4	45,XY,rob(21;22)(q10;q10)	46,XX	ND
5	46,XY	46,XX,t(3;4)(q12;p14)	ish t (3;4)
6	46,XY,t(5;8)(q22;p22)	46,XX	ish t (5;8)
7	46,XY	46,XX,t(6;21)(q25;q22)	ish t (6;21)
8	46,XY	mos 45,X[5]/46,XX[20]	nuc ish (DXZ1×1)[25/100]/(DXZ1×2)[75/100]
9	46,XY	chi 46,XX[16]/XY[11]	nuc ish (DXZ1×2)[60/100]/(DXZ1, DYZ3) x1[40/100]
10	mos 47,XYY[4]/46,XY[26]	46,XX	nuc ish (DXZ1×1, DYZ3×2)/[16/100]/(DXZ1, DYZ3) x1[84/100]

CCA, conventional cytogenetic analysis; chi, chimerism; FISH, fluorescence in-situ hybridization technique; ish, FISH; mos, mosaicism; ND, has not been done; rob, Robertsonian translocation; t, translocation.

46,XX[16]/XY[11]. nuc ish (DXZ1×2)[60/100]/(DXZ1, DYZ3) x1[40/100]. Cytogenetic study of her couple revealed a normal male karyotype of 46,XY.

Discussion

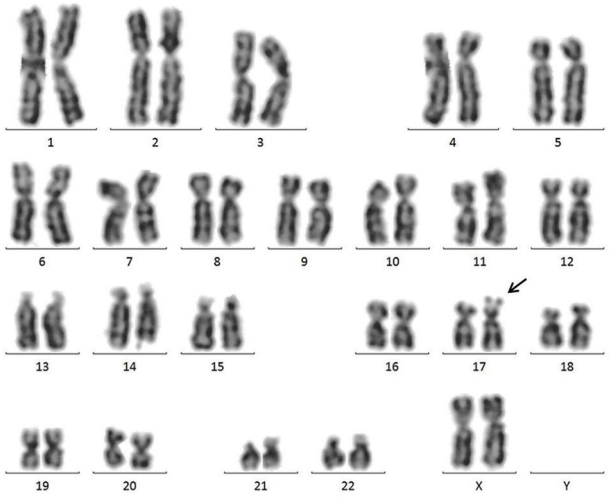
RSA is a common reproductive problem. Various causal factors can be responsible, including genetic, hormonal imbalance, environmental, uterine abnormalities as well as infectious agents. Parental chromosomal abnormality is a cardinal genetic factor (Flynn *et al.*, 2014), particularly for balanced translocations, which are responsible for ~2–5% of cases (Ozawa *et al.*, 2008). Parents with balanced translocations are liable for meiotic nondisjunction (Marqui, 2018). The mispairing of the abnormal chromosomes during the first meiotic division may result in aneuploidy of the aberrant chromosome, partial trisomy, or partial monosomy, with subsequent outcome in the form of abortion or affected viable offspring (Gonçalves *et al.*, 2014).

Table 2 Chromosomal heteromorphisms detected in the studied cases

Couple numbers	Karyotype of male partner by CCA	Karyotype of female partner by CCA
11	46,XY,inv(9)(p12q13)	46,XX
12	46,XY,inv(9)(p12q13)	46,XX
13	46,XY	46,XX,inv(9)(p12q13)
14	46,XY,15ps+	46,XX
15	46,XY,22ps+	46,XX
16	46,XY,9phqh	46,XX
17	46,XY,13ps+,15ps+,16qh+	46,XX
18	46,XY,16qh+	46,XX
19	46,XY	46,XX,17ps

CCA, conventional cytogenetic analysis; FISH, fluorescence in-situ hybridization technique; inv, inversion.

Figure 3



Karyotype of the female partner of couple no. 19, showing chromosomal heteromorphism in the form of satellite on the short arm of chromosome 17; 46,XX,17ps.

The incidence of heteromorphisms among couples with RSA has been studied by diverse researches (Xu *et al.*, 2016). Many studies reported an elevated incidence, and different theories have been postulated to depict the contribution of these variants in the reproductive outcome (Tempest and Simpson, 2017).

This research was aimed to study the role and incidence of various chromosomal aberrations and chromosomal heteromorphisms among couples with recurrent pregnancy loss and discuss whether chromosomal heteromorphisms can be considered during genetic counseling sessions.

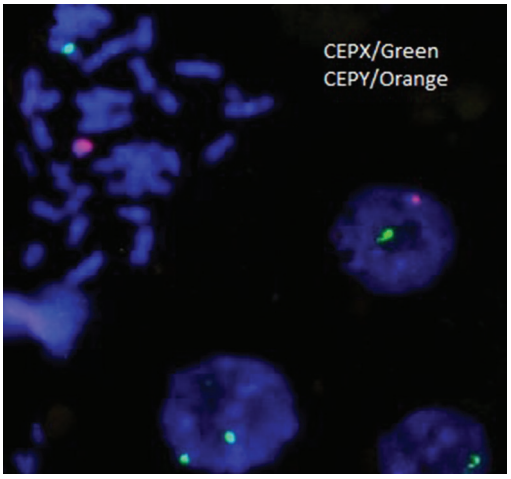
Chromosomal findings have been detected in 19 cases, they were 10 case with chromosomal aberration and nine cases with chromosomal heteromorphisms.

Regarding cases with chromosomal aberration, this study revealed a percentage of 6.8% of all the studied cases. This result was comparable to the study done by Gaboon *et al.* (2015) who reported a percentage of 6.4, whereas Ghazaey *et al.* (2015) and Pal *et al.* (2018) reported a percentages of 5.8 and 4.94%, respectively.

The slight discrepancy in the previous results can be attributed to the differences in size and criteria of the selected sample (AlGhamdi and Makhashen, 2016).

Couples with balanced reciprocal translocation have a 50% chance of having RSA and a 20% risk of having affected viable offspring (De *et al.*, 2015).

Figure 4



Metaphase and interphase fluorescence in-situ hybridization of the female partner of couple no. 9 showing chimerism in the form of nuc ishX/Ycen (DXZ1, DYZ3) x1, using Vysis probes CEP X (DXZ1) spectrum green/CEP Y (DYZ3) spectrum orange.

The formation of normal or unbalanced gametes is dependent on both the breakpoints and the involved chromosomes (Practice Committee of The American Society for Reproductive Medicine, 2012).

This study revealed balanced reciprocal translocation in 30% of all cases with chromosomal abnormalities, whereas Pal *et al.* (2018) reported a percentage of 47.05. The divergence between these results can be attributed to the criteria of the selected sample as our research was confined only to young couples (<35 years of age).

Regarding Robertsonian translocation, there is a risk of ~75% of producing gametes with chromosomal imbalances (Coco, 2018), whereas the risk of RSA is ~25% (Ghazaey *et al.,* 2015).

Four cases of Robertsonian translocation were detected in our study which represent 40% of the affected cases. These findings were in reverse to the studies done by both Gaboon *et al.* (2015) and Pal *et al.* (2018), which verified that reciprocal translocation is the most frequent followed by Robertsonian translocation. Both studies found approximately analogous percentages of 12.5 and 11.76%, respectively.

This study revealed numerical chromosomal abnormality in two cases, which represent 20% of all cases with chromosomal aberrations; both cases were mosaic sex chromosomal aneuploidy. Correspondingly, An *et al.* (2016) and Pal *et al.* (2018) reported only sex chromosomal aneuploidy in all cases with numerical aberration. Indeed this was expected, as phenotypic features associated with sex chromosomal aneuploidy are generally tolerable or may be actually abolished in cases of mosaicism. This can be attributed to X chromosome inactivation as well as low gene dosage of Y chromosome, which can minimize or obscure symptoms associated with sex chromosomal aneuploidy in the affected partners apart from that associated with reproductive abnormalities (Ostrer, 2014).

Despite the variability in the percentage, the pattern of distribution of structural and numerical chromosomal aberrations in our research study is nearly similar to that of previous research studies, as numerical chromosomal aberrations are less frequent than structural aberrations among cases with RSA.

We have reported one case (10% of affected cases) that revealed chimerism in the form of 46,XX/XY. This case was the female partner of couple no. 9. She was a phenotypically normal female of 27 years of age, presented with two times previous abortions; her couple was 32 years of age with normal male karyotype of 46,XY. Unfortunately, this case did not show up in the subsequent visit for requesting further investigations.

The cytogenetic result of 46,XX/46,XY is a rare finding that is considered to be chimerism (Chen *et al.,* 2005). It is defined as chimerism rather than mosaicism, whether molecular studies have been done or not. Mosaicism results from different cells originating from a single zygote as a consequence of mitotic defect during the first blastomeric division or at a later stage. However, chimerism is the presence of two or more cell lines derived from different zygotes (Malan *et al*., 2007).

Although different mechanisms have been proposed to describe chimerism, Malan *et al*. (2007) emphasized that tetragametic chimera is the most verified mechanism that has been confirmed by molecular studies through which two oocytes are fertilized by two sperms with subsequent fusion of two zygotes into single embryo. Pelvic ultrasound and gonadal biopsy followed by histopathological examination is required to exclude or confirm the presence of testicular tissue.

Bone marrow transplantation is another explanation for this cytogenetic finding as the transplanted marrow cell progeny will attain genotype of the donor while the patient initially still acquires his own cells. At a transient state, the patient will acquire two cell lines derived from different genotypic origin (Rasche *et al.*, 2016).

There are increasing research study hypotheses which integrate heteromorphisms with both infertility and RSA (Tempest and Simpson, 2017). Most of these studies reported an increase in the incidence of heteromorphisms in cases with reproductive problems to the extent of 3–5-folds compared with normal population (Xu *et al.*, 2016).

In this study, chromosomal heteromorphisms have been detected in nine cases (6.16% among the studied cases); they comprised inv (9)(p12q13), 17ps, 22ps+,13ps+,15ps+,16qh+, and 9phqh. The most frequent heteromorphism detected was pericentric inversion in chromosome 9 (inv (9)(p12q13)).

In accordance with this study, An *et al.* (2016) reported that inv (9)(p12q13) *is* the most frequent heteromorphism detected in their research. However, Dana and Stoian (2012) in their study declared that the most frequent chromosomal polymorphism in the general population is inv (9)(p12q13); furthermore, they revealed a nonsignificant difference for the incidence of inv (9)(p12q13) between an infertile and a control group.

Tempest and Simpson (2017) postulated that chromosomal heteromorphisms may have a crucial role in genome regulation and modulation during reproduction, as reproduction is a complicated biological process that requires genome regulation and expression at different levels. This could, to some extent, clarify the absence of clinical phenotype in heteromorphic variant carriers apart from infertility or RSA.

Besides, they hypothesized that the heterochromatic regions around the centromeres of the acrocentric chromosomes have a role in spindle attachment, chromosome pairing, and cell division. Thus disruption in these heterochromatic regions may in fact have its consequences on gene expression, affecting gamete formation, fertilization, and embryogenesis. Furthermore, this disruption could lead to defect in chromosome and chromatid cohesion. This defective cohesion usually induces malsegregation which increases the risk of chromosomal aneuploidy (Hussen *et al.*, 2018).

A recent study has declared a significant increase in chromosome aneuploidy in both sperm and embryos from males with chromosomal heteromorphism (Morales *et al.*, 2016).

Boronova *et al.* (2015) assumed that hidden functional genes are located in the heterochromatin of the (q) arm of chromosomes 1, 9, and 16 and Y. They anticipated that these hidden genes regulate the cellular function in the reproductive process, and thus, heteromorphic variants related to these regions could be associated with reproductive failure.

Despite increasing theories to correlate heteromorphisms with RSA and infertility, additional research studies are required to determine a compact mechanism through which these heteromorphisms can exert their effect. Nevertheless, genetic counselors should pay attention to these heteromorphisms until the exact mechanism can be clarified.

Generally, the probability of a subsequent normal offspring in cases of RSA depends on the involved chromosome(s) and the type of aberration. Whenever either of the partners has particularly structural chromosomal rearrangement, preimplantation genetic diagnosis, amniocentesis, or chorionic villus sampling is a recommended option to detect the genetic abnormality in the upcoming offspring (Practice Committee of The American Society for Reproductive Medicine, 2012).

Conclusion

This study showed that the incidence and distribution of chromosomal abnormalities among couples of RSA are comparable to that reported worldwide.

It is worth to emphasize that before endeavoring any therapeutic intervention, couples who experience abortion of unknown etiology more than two times should undergo a cytogenetic analysis, as RSA might be a rescuer mechanism formulated to prevent distressing outcomes. Cases that experienced chromosomal findings, either chromosomal aberrations or chromosomal heteromorphisms, should be referred for genetic counseling.

Chromosome heteromorphisms could be a contributing factor for RSA. Genetic counselors should pay attention to this issue until a conclusive mechanism can be verified.

Studies on the genetic basis of RSA should be taken up to create an informative database from different regions.

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Conflicts of interest

There are no conflicts of interest.

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