High Frequency of Chromosomal Anomalies and a Novel Chromosomal Insertion Associated with Infertility and Recurrent Miscarriages (Reproductive Failure) in West Turkey

Research Article

Fatma Silan¹, Sinem Yalcintepe¹, Digdem Uysal¹, Mine Urfalı¹, Ahmet Uludag¹, Emine Cosar², Ayse Nur Cakir Gungor², Ozturk Ozdemir¹

¹ Department of Medical Genetics, Canakkale Onsekiz Mart University, Faculty of Medicine, 17100 Çanakkale Turkey
² Department of Obstetric and Gynecology, Canakkale Onsekiz Mart University, Faculty of Medicine, 17100 Çanakkale Turkey

*Correspondence: Prof Dr Fatma Silan, Department of Medical Genetics, Canakkale Onsekiz Mart University, Faculty of Medicine, 17100 Çanakkale Turkey, Email: fsilan@yahoo.com, Tel:+90 286 263 59 50-1080, Fax:+90 286 2635956

Keywords: Chromosome, Cytogenetics, Insertion, Inversion, Translocation

Received: 15 May 2014; Revised: 15 July 2014
Accepted: 17 July 2014; electronically published: 18 July 2014

Summary

Numerical and/or structural chromosomal abnormalities may be a reason of high infertility rates and recurrent pregnancy losses (RPLs) in humans. Karyotype and karyogram profiles of patients with RPLs are presented in current results. A total of 722 patients; 161(44.5%) infertile and 200(55.5%) RPL couples were included in the study. Karyotype and structural chromosome analyses of both patient groups in Canakkale population were made between May 2011-December 2013, using peripheral lymphocyte cell culture and GTG banding technique. High frequency of chromosomal abnormalities(%7.45) were detected in 24 patients of the infertility group(n:322). 10 patients(42%) of this group(n:24) had numerical and 14 patients(58%) had balanced structural chromosomal abnormalities. A novel chromosomal insertion was found in an infertile male, one of the 22th choromosome was totally inserted in 9th choromosome [ins(9;22)(9pter-q12::22q11.1-q13.33::9q12-9qter)]. This is the first report of germline total inserion of a chromosome. Interestingly, this insertion was inherited from father. Balanced structural chromosomal abnormalities was also detected in 17 patients (4.25%) of RPL group without any numerical abnormalities. Current results constitute the first report on the high incidence of structural chromosomal aberrations in RPLs and infertile couples in Canakkale district.
I. Introduction:
Numerical and structural abnormalities may be seen in chromosomes with many reasons. These abnormalities may be the reasons of infertility and RPL. Chromosome abnormalities can be detected with cytogenetic analysis. It has been recommended that the standard investigation of such patients should include karyotyping of both parents for chromosomal aberrations (1). Chromosomal abnormalities, mainly balanced rearrangements, are common in couples with reproductive disorders. The most common complication of pregnancy is recurrent spontaneous abortion, which referred to termination of pregnancy prior to 20 weeks of gestational age. According to this definition, RPL occurs in approximately 1% of all pregnancies. This frequency increases up to 5% when RPL is defined as two or more losses of pregnancy. Approximately 15-20% of all clinically recognized pregnancies are spontaneously aborted at different weeks. Couples with RPL are facing an increased risk of being carriers of a structural balanced chromosome abnormality. The incidence of carrier status is 0.7 % in the general population worldwide and increases to 2.2 % after one miscarriage, 4.8 % after two miscarriages and 5.2 % after three miscarriages (2, 3). The World Health Organization (WHO) defines infertility as the inability to conceive a child after 1 year of regular, unprotected intercourse. In developing countries, there are severe social, psychological and economic consequences for infertile men and women. Infertility currently affects approximately 15% of all couples, with an increase anticipated over the next 20 years. Chromosomal abnormalities are one of the most important causes of infertility (5, 6). The numerical and structural chromosomal abnormalities are seen frequently in infertility etiology (7).

Because a considerable proportion of patients with reproductive dysfunction had various cytogenetic abnormalities, the chromosomal analysis should be considered as a diagnostic tool in the evaluation of reproductive dysfunction. The study of human chromosomes plays a role in the diagnosis, prognosis, treatment and monitoring of fertility problems. Infertility is a condition of failure to conceive after 12 months of unprotected sexual intercourse without the use of contraception. It affects approximately 15% of couples in reproductive age. Infertility may be used synonymously with sterility with only sporadically occurring spontaneous pregnancies. Subfertility generally describes any form of reduced fertility with prolonged time of unwanted non-conception. Subfertility can be primary or secondary: Primary subfertility - a delay for a couple who have had no previous pregnancies; Secondary subfertility - a delay for a couple who have conceived previously, although the pregnancy may not have been successful (for example, miscarriage, ectopic pregnancy). Many factors can cause or contribute to reduced fertility. These concerns may be attributed to an issue with the woman, the man, the couple, or their lifestyle. Causes of infertility can be identified in about half of these cases, many causes are still unclear. The objective of this retrospective study was to report the distribution of structural chromosome rearrangements in couples with a history of RPL or infertility.

II. Materials and Methods:
Couples with a history of at least two documented miscarriages before 20 weeks of gestation or to not have any child after 1 year of regular, unprotected intercourse, included. A total of 722 patients including 200 couples with RPL and 161 couples with infertility were examined. In the RPL group the mean age was
31 for wives and 33 for husbands. In the infertility group the mean age was 35 for wives and 37 for husbands. Cytogenetic analysis was performed with GTG banding technique for their lymphocytes. Whole complete blood cultures were set up in 10 ml culture tubes. The medium had the following composition: 100 ml RPMI 1640 medium (Sigma) supplemented with 20 ml fetal calf serum (Gibco BRL), 2 ml L-Glutamine (Sigma), 1 ml phytohaemagglutinine (Sigma) and 2 ml penicillin streptomycin (Sigma) mix was added. Eight drops of heparinized blood were added to 5 ml of the complete media. Culture tubes were incubated for 72h in a slanting position at 37°C. Colcemid (Sigma) was added at 100 µg/ml to the cultures 2h before harvesting. Slides were prepared after hypotonic treatment of the cells with KCl (0.075 M) followed by fixation in methanol/glacial acetic acid (3/1 vol/vol). A concentrated suspension of the cells was dropped on slides, which were dried on a slide warmer at 60°C for a few seconds. G-banded chromosomes were obtained by keeping the slides overnight in an oven at 55-60°C then treating them with trypsin solution and stained in 6% Giemsa solution (Merck), dried and examined microscopically using the image analyzer programmed (Cytovision, version 2000, Applied Imaging). Minimum 20 metaphases counted and analysed for acrocentric chromosomes and gonosomes (DU, MU, SA) and minimum three metaphases were analysed for all numeric and structural chromosomal abnormalities (FS, OO). Metaphases were karyotyped and interpreted according to the International System for Human Cytogenetic Nomenclature (ISCN).

III. Results
In infertility group total chromosomal anomaly was 7.45%. 10 patients(42%) of this group(n:24) had numerical and 14 patients(58%) had balanced structural chromosomal abnormalities: 45,X in 3 patients, 47,XXY in 3 patients, 47XXX in 1 patient, 46,X,i(X)(qter-q10:q10-qter), mos46,X,i(X)(qter-q10:q10-qter)[14]/45,X[7] in 1 patient (Figure 2), mos46,XX[4]46,XY[21] in 1 patient, mos47,XXY/46,XY in 2 patients, 45,XX,rob(13;14) in 3 patients, 45,XY,rob(13;14) in 1 patient, 46,XY,t(1;2)(p36;p14-16) in 1 patient, 46,XY,t(3;8)(q21;q24) in 1 patient, 46,XY,inv(9)(p11q13) in 1 patient, 46,XX,22p+ in 2 patients, 46,XY,inv(15)(p11,2;q14) in 1 patient, 46,XY,del(22)(q13) in 1 patient, 46,XY,9qh(+) in 1 patient and 46,XY,Yq(+) in 1 patient. We detected rob(13;14) in a couple who has first degree cousin marriage and three unsuccessful IVF cycles (UIVF). One of the male patient with inv 9 has two UIVF. From this group, 4 couples has 9 UIVF cycles.

In RPL group, we detected 4.25% balanced structural chromosomal abnormality, there was no numerical chromosomal anomaly (Table 1). There were structural abnormalities in 17 patients: 46,XY,inv(11)(p13q11), in 1 patient (Figure 1), 46,XX,inv(11)(p13q11)(no consanguineous marriage) in 1 patient, 46,XX,ins(9;22)(9pter-q12::22q11.1-q13.33::9q12-9qter) in 1 patient, 46,XX,9qh+ in 4 patients, 46,XX,inv(9)(p11q13) in 2 patient, 46,XY,inv(9)(p11q13) in 2 patient, 46,XY,Yq(+) in 2 patients, 46,XY,t(4;6)(q13.1;pter)(4pter-4q13;6pter-6qter::4q13-4qter) in 1 patient, 46,XX,t(8;12)(p22;p12.3) in 1 patient, 46,XX,t(X;2)(q27;p25) in 1 patient and 46,XY,t(5;11)(p13;q24) in one patient.
Figure 1. Shows Karyogram analysis of a case with 46,XY,inv(11)(p^{13};q^{11}). Arrow indicates the inverted chromosome 11 in a case with 2 RPLs.

Figure 2. Shows Karyogram analysis of a case with 46,X,i(X)(q^{ter}-q^{10};q^{10}-q^{ter}). Arrow indicates the isochromosome X in an infertile case.
Chromosomal Aberrations | Habituel Abortion (n=400) | Infertility (n=322) | Total Patients (n=722)
---|---|---|---
**Numerical Abnormalities (n%)** | - | 10 (3.10%) | 10 (1.38%)
45,X | - | 3 | 
47,XXY | - | 3 | 
47,XXX | - | 1 | 
mos47,XXY/46,XY | - | 2 | 
mos46,XX/46,XY | - | 1 | 
**Structural Otosomal Abnormalities (n%)** | 15 (3.75%) | 12 (3.72%) | 27 (3.73%)
Robertsonian Translocation | - | 4 | 
Resiprocal Translocation | 4 | 2 | 
Inversion | 6 | 4 | 
Insertion | 1 | - | 
9qh(+) | 4 | 1 | 
Others | - | 1 | 
**Structural Gonosomal Abnormalities (n%)** | 2 (0.50%) | 2 (0.62%) | 4 (0.55%)
isochromosome | - | 1 | 
Yq(+) | 2 | 1 | 
**TOTAL** | 17 (4.25%) | 24 (7.45%) | 41 (5.67%) |

Table 1. Some clinical characteristics (main age, gender etc) karyotypes and chromosomal abnormalities in the current cohort of reproductive failure couples from Canakkale population.
IV. Discussion

Chromosomal abnormalities are the cause for pregnancy loss in 50% to 80% of cases, depending on maternal age and gestational age at time of the loss. Karyotyping of spontaneous losses in the first trimester beginning with the patient's second loss provides clinically important etiologic information and decreases the number of evaluations necessary for RPL. Chromosome analyses are an important and necessary part of the etiological research in couples with RPL. Although reciprocal translocations are balanced rearrangements, they are important for the offspring of carriers that have increased risk of chromosomal imbalance during gametogenesis due to unequal meiotic segregation. When one of the parents is a carrier of a balanced reciprocal translocation, a pregnancy can result in three types of offspring: a child with a normal karyotype, a child with a balanced reciprocal translocation, or a conceptus with an unbalanced karyotype that may lead spontaneous miscarriage or live-born child with malformations and mental retardation. Since cytogenetic findings do not only lead to RPL, but also increase frequency of bearing malformed child, genetic counseling for subsequent pregnancies of couples who have balanced translocation is important. Carriers of balanced structural aberrations appear to have an increased risk of progeny with unbalanced karyotype resulting spontaneous abortion in first and second trimester (17). According to the literature review the prevalence of chromosomal aberrations among the couples with repeated spontaneous miscarriages varied in different studies from none (23, 24) to as high as 21.4% (24). These differences may be related to sample size and to different criteria.

The overall chromosomal anomalies found in our study are 5.67%. Similar to other studies (26) translocations were the common abnormalities in our study too with 1.4%.

In literature, there have been reports of reciprocal translocation carriers with varying combination of the involved chromosomes, resulting in RPL. For example, though some are frequently involved in translocations in RPL there seems to be still a variation in the breakpoint regions which enter into translocation (25). Chromosome 2 and chromosome 8 at our patients had similar breakpoints to the literature.

Inversions are another group of chromosomal abnormalities which have been frequently reported in couples with RPL. It is known that paracentric inversion carriers have risk to produce unbalanced gametes with a dicentric chromosome and an acentric fragment due to homolog pairing in meiosis occurring through the formation of an inversion loop between the normally structured and inverted loop. Because of these chromosomally unbalanced gametes, the incidence of fetal loss increases in paracentric inversion carriers. Pericentric inversion carriers also have increased risk for pregnancy loss. The consequences of crossing over in a pericentric inversion loop may be deletion or duplication of a chromosome segment. The size of loss or gain of genetic material depends on the length of the inversion segment (8, 9). Inversions were found in four cases in our study, four of them were pericentric 2 of them at 11th and 4 of them are at 9th chromosome. One of the our pericentric inversion 11 patient has a pregnancy loss with triploidy, and one of the 9qh+ male patient has an abortion with 45,X with paternal loss. These patients show us to Interchromosomal effect of balanced structural chromosomal abnormalities.
Most of the cytogeneticists says 9qh+ is a polymorphism (10), but our results support this abnormality's significance similar to Mozdarani’s report (11). The presence of balanced chromosomal translocations was evident in 4.25% of 200 RPL couples who studied. These results were comparable with the results of Makino et al. who observed that among the 639 couples, 8.6% of partners showed chromosomal balanced carrier (12). Similar work by Mameli and colleagues indicated an incidence of 8% for balanced translocation in 100 examined individuals, which is near to the mode (about 9%) observed in previous studies (13). Tsui et al. claimed that the overall incidence of chromosomal anomaly was 51 (9.92%) out of 514 individuals (14).

The prevalence of parental chromosomal aberrations among RPL couples in this study was 4.25% (17 couples), which is greater than that reported by other authors. However, the pattern of chromosomal abnormalities is similar to that seen in previous studies (31, 32, 33) except whole chromosome 22th insertion of the 9th. The high prevalence may be because our cases were selected from among subjects who had had two or more spontaneous abortions, whereas most of the other studies included subjects with three or more repeated miscarriages. We determined that eight women and nine men had chromosomal aberrations in RPL group. This male-to-female ratio (1.1/1) was not different from that found in most other studies (31, 34). A likely explanation is that chromosomal aberrations in male carriers may cause severe meiotic disturbances and spermatogenic arrest. Some chromosomal abnormalities (such as Robertsonian translocations) that are compatible with fertility in women may be associated with sterility in men (29, 35). In the present study, the frequency of chromosomal aberrations (7.45%) among infertile men was found to lie within the previously reported range (1.9–16.9%) (15, 16). The majority of infertile men were diagnosed as having Klinefelter syndrome (3.1%). Total chromosomal anomaly in infertile men was 4.5%. This is in accordance with previously published studies, showing that both numerical sex chromosome aberrations and translocations are the most common chromosomal aberrations associated with male infertility (17). Infertility in such patients may result from the interference of chromosomal aberrations with normal spermatogenesis (18). The most common type of karyotype abnormality in infertile cases is represented by Klinefelter Syndrome (KS). KS is the most common abnormality of sexual differentiation, and occurs in approximately 1 in 500 live births. KS is a form of primary testicular failure and elevated gonadotropin plasma levels, and it represents the most common form of male hypogonadism (20). Ferlin et al. reported that the prevalence of KS among infertile men is very high, up to 5% in severe oligozoospermia and 10% in azoospermia (21). It has always been assumed that more than 90% of non-mosaic 47, XXY males are azoospermic. In their series, 74.4% of mosaic 47, XXY/46,XY patients were azoospermic, whereas the remaining had severe oligospermia (22). KS was the most frequent chromosome-related cause of infertility in our study group. In our study, we detected that two cases have mosaic KS (47,XXY/46,XY) and three patients had 47,XXY. There are some other cytogenetic studies conducted on infertile patients from different part of Turkey (22,23, 24). Akgül et al. have found that chromosomal abnormalities were detected in 17.4% of 86 azoospermic cases and 6.8% of 73 oligozoospermic cases revealed 11.7% of all cases in a regional study from Turkey.
Samli et al. have detected that chromosomal abnormality was detected in 47 (12%) of 383 non-obstructive azoospermia cases and in 20 (4%) of 436 oligospermia cases. Balkan M et al. have found that the total prevalence of chromosomal abnormalities was found to be 11.2% (9/80; 52 were azoospermic, 25 oligozoospermic and 3 asthenozoospermic), including seven patients with KS. All of the patients with KS had azoospermia. Our results also shows high incidence of structural chromosome abnormalities at normospermic infertile patients. Our data confirm the importance of karyotype analysis in infertile men with or without sperm anomalies. The current results are the first reports on cytogenetics and numerical-structural chromosomal profiles in RPL and infertile couples from Canakkale population. This analysis can provide useful information also for patients with long-term infertility and considering assisted reproduction techniques. Early diagnose of parental chromosome abnormalities will provide Preimplantation Genetic Diagnosis for these couples and lower unsuccessful IVF cycles. 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 RPL and infertility should be essentially considered.

Competing interests
Authors declare that they have no competing interests.

Authors' contributions
F.S.; A.U.; E.C.; and A.N.C.G.; performed the bioinformatical work, analyzed the clinical data and designed the clinical experiments, S.A.Y.; M.U.; and D.U; performed the lymphocyte cell culture, harvest, and karyotyping F.S.; O.O.; karyotype analysis and S.A.Y., M.U., F.S., wrote the manuscript. O.O.; supervised the study.

All authors read and approved the final manuscript.

References:
Celep F, Karagüzel A, Ozeren M, Bozkaya H: The frequency of chromosomal abnormalities in patients with reproductive
de Braekeleer M, Dao TN: Cytogenetic studies in couples experiencing repeated pregnancy losses. Hum Reprod 5:519-528(1990)
Rowley PT, Marshall R, Ellis JR: Genetic and