Original Article

Impact of pericentric inversion of Chromosome 9 [inv (9) (p11q12)] on infertility

Hossein Mozdarani*, Anahita Mohseni Meybodi*†, Hamideh Karimi†
*Department of Medical Genetics, School of Medical Sciences, Tarbiat Modares University, Tehran, †Department of Genetics, Royan Institute, Tehran, Iran

BACKGROUND: One of the frequent occurrences in chromosome rearrangements is pericentric inversion of the Chromosome 9; inv (9) (p11q12), which is consider to be the variant of normal karyotype. Although it seems not to correlate with abnormal phenotypes, there have been many controversial reports indicating that it may lead to abnormal clinical conditions such as infertility. The incidence is found to be about 1.98% in the general population.
MATERIALS AND METHODS: We investigated the karyotypes of 300 infertile couples (600 individuals) being referred to our infertility clinic using standard GTG banding for karyotype preparation.
RESULTS: The chromosomal analysis revealed a total of 15 (2.5%) inversions, among these, 14 male patients were inversion 9 carriers (4.69%) while one female patient was affected (0.33%). The incidence of inversion 9 in male patients is significantly higher than that of normal population and even than that of female patients ($P<0.05$).
CONCLUSIONS: This result suggests that inversion 9 may often cause infertility in men due to spermatogenic disturbances, which are arisen by the loops or acentric fragments formed in meiosis.

Key words: Infertility, inversion 9, Iranian population, metaphase analysis

Introduction

Pericentric inversions result from a two-break event in which there is a break in each arm including the centromere.[1] An inversion does not usually have phenotypic effect in the majority of pericentric inversion heterozygote carriers, when it is a balanced rearrangement. However, infertility, miscarriages and/or chromosomally unbalanced offspring can be observed in carriers of either type of inversions specially pericentric inversions.[2] Carriers of such inversions are at risk of producing abnormal gametes during meiosis that may lead to unbalanced offspring. During meiosis I, a loop will be formed in chromosome with inversion which can lead to produce a percentage of abnormal and unbalanced gametes. These gametes may show duplication of the region outside the inversion segment on one arm of the inverted chromosome and deletion of the terminal segment on the other arm and vice versa, ending up with duplicated/deficient recombinant chromosomes distal to the breakpoints.[3] The most common inversion seen in human chromosomes is a small pericentric inversion of Chromosome 9, with an overall incidence of 1.98%, especially in African-Americans.[4] According to different studies this inversion can also be seen in 1-3% of general population and the exact amount of this phenomenon is still unclear.[5-9] The human Chromosome 9 displays the highest degree of structural variability.[10] Review of literature showed that inversions of Chromosome 9 with different breakpoints could be the cause of different disturbances in carriers. Pericentric inversions of Chromosome 9, inv (9) (p11q12)/ inv (9) (p11q13), are such common occurrences that cytogeneticist would consider it as normal variants. Despite being categorized as minor chromosomal rearrangements, which does not correlate with abnormal phenotypes, many reports raised the association of these inversions with sub fertility, recurrent abortions and abnormal clinical phenotypes.[9] The present study aimed to evaluate the association between inversion 9 (p11q12) and infertility.

Materials and Methods

The patient population consisted of those Iranian couples who attended the infertility clinic. A total of

Address for Correspondence: Hossein Mozdarani, Department of Medical Genetics, School of Medical Sciences, Tarbiat Modares University, Tehran, P.O. Box 14115 - 111, Iran. E-mail: mozdarah@modares.ac.ir

601 patients with at least three years of infertility were examined. The age of the referred wives ranged from 21-38 years, while the age of the husbands ranged from 25-42 years.

Cytogenetic analysis was performed with GTG banding technique for their lymphocytes.

G-banded chromosomes from peripheral blood were prepared according to the modifications of Verma and Babu.[11] Whole complete blood cultures were set up in 10 ml culture tubes. The medium had the following composition: 100 ml RPMI medium (Sigma) supplemented with 20 ml fetal calf serum (Gibco BRL), 1.2 ml Glutamine (Sigma), 1 ml phytohaemagglutinine (Sigma) and penicillin streptomycin (Sigma) mix was added. Five to ten 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 4 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 ethanol/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 in an oven at 55-60°C then treating them with trypsin solution and stained in 4% Giemsa solution (Merck), dried and examined microscopically using the image analyzer programmed (Cytovision, version 2000, Applied Imaging). Metaphases were karyotyped and interpreted according to the international system for human cytogenetic nomenclature (ISCN).[12]

Results

A total of 601 patients with at least three years of infertility were examined. The age of the referred wives ranged from 21-38 years, with a mean of 27.81 years (SD ± 5.276), while the age of the husbands ranged from 25-42 years, with a mean of 32.09 years (SD ± 5.665). There was no significant correlation between the age and pericentric inversion of Chromosome 9. Using the G-banded technique among the 601 patients, 15 (2.5%) individual was found to have inversion 9 (p11q12). Inversion 9 was observed for just 1(0.33%) female, while it was seen in 14 cases of males (4.69%).

Only one case had the break point of (p11q13) which has not considered in statistical analysis.

The incidence of inversion 9 in male patients was found significantly higher than that in female partners (P<0.05). The total average amount of inversion in both male and females in this study (2.5%) is not significantly higher than normal population [Table 1, Figure 1].

Discussion

A summary of reports for various abnormalities associated with pericentric inversion 9 (p11q13) and (p11q12) is shown in Table 2. The finding of homozygosity for a pericentric inversion of Chromosome 9 is rare[5] and none of our cases was homozygote. We have observed 2.5% inversion 9 in cases with infertility which is similar to the earlier report by Sasiadek *et al*[13] in the cases of recurrent spontaneous abortions. However, our results cannot be compared to normal or general population

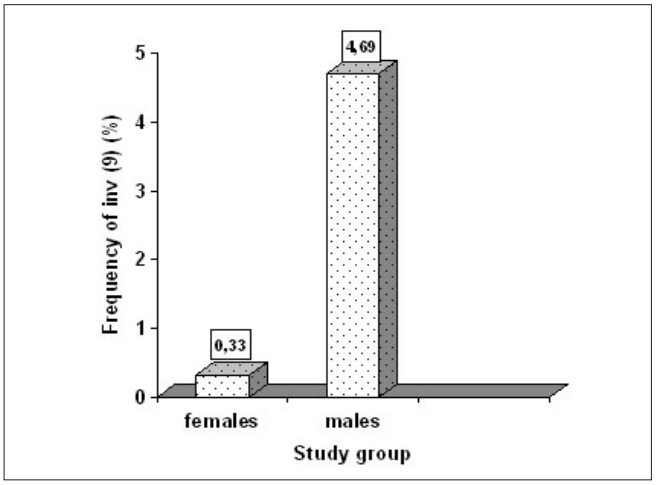

Figure 1: Comparison of the frequency of inv (9) in patients referred for infertility reasons

Table 1: Frequency of inv (9) in male and female infertility cases

Sex	Mean age ± SD	Total	Normal	inv (9) carriers	Mean frequency
Female	27.81 ± 5.276	302	301	1 ($p^{11}q^{12}$)	0.0033
				0 ($p^{11}q^{13}$)	
Male	32.09 ± 5.665	299	284	14 ($p^{11}q^{12}$)	0.0469
				1 ($p^{11}q^{13}$)	

Table 2: Associations between the different disturbances and inversion 9 with (p11q12) or (p11q13) breakpoints as well as infertility

Karyotype	Disturbance	Reference
Inv (9) (p11q12)	Infertility/recurrent abortion	Sasiadek *et al*, 1997[13]
inv(9)	Male infertility	Sasagawa *et al*, 1998[23]
inv(9)(p11q13)	Asperger syndrome	Pia Verri and Cimbro, 2002[24]
inv(9)(p11q13)	Goldenhar syndrome or oculo-auriculo-vertebral spectrum	Stanojevic *et al*, 2000[25]
inv(9)	Immotile/ultrastructural sperm defect	Baccetti *et al*,1997[26]
inv(9)(p11q13)	Schizophrenia-like psychosis	Miyaoka *et al*, 1999[27]
inv(9)	Recurrent spontaneous first trimester abortions	Parmar and Sira,2003[28]
Inv (9) (p11q12)	Infertility/recurrent abortion	The present study

because the rate of inv (9) is reported in a wide range from 1-3% by other investigators.[5-9]

The major observations in this study was the difference of the rate of inv (9)(p11q12) between the male and female patients as shown in Table 1 and Figure 1. In contrast to some other studies on inversions of other chromosomes,[14,15] there is considerable difference in frequency of inv (9) among two sexes (P<0.05).

These results of inv (9) may explain some idiopathic male infertility. In fact pairing in spermatogenesis constitutes the main problem in inversion heterozygosis. This phenomenon can be more effective in male partners because of their higher rate of meiotic division. An odd number of crossover events (during the pachytene stage of meiosis I) within the inversion segment can lead to two monocentric recombinants with reciprocal duplications/ deficiencies in the sperms, ending up in a risk of inheriting such an imbalance when conception occurs. However, it has been shown that the classical assumption that loops are invariably present at meiotic prophase to realize a homologous pairing in inversion heterozygotes[16-21] however, the other reports do not agree.[22] These results indicate that inv (9) may often cause clinical problems in offspring of the carriers and infertility with sex related unknown certain mechanism.

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