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Prevalence And Possible Effects of Pericentric Inversion of Chromosome 9 on Reproductive Failure in A Human Population of Northeast Bosnia and Herzegovina: A 10-year Retrospective Study

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ABSTRACT

Introduction: **Since 1956 karyotype analysis becomes an essential part of routine medical diagnostics, and helped medical professionals investigate the origin of genetic abnormalities in many constitutional and cancer diseases. Karyotyping also provided more information in the monitoring of fertility problems. An inversion does not usually have a phenotypic effect, especially if it involves a heterochromatin area, such as 9qh. Chromosome 9 polymorphism, with breakpoints p11q13/p12q13, can be the cause of variant abnormal clinical conditions such as congenital abnormalities, hematological diseasesand also could have a connection with pregnancy loss and fertility failure.**

Methods: **A retrospective study was conducted on 1784 cytogenetics examination results from peripheral blood samples in the period from January, 2012 to December, 2022. The patients, carriers inv(9) in their karyotype were highlighted for detailed analysis.**

Results: **Among the 1784 patients, constitutional pericentric inversion inv(9)(p11q13) was found in 13 females (0,72%), while it was seen in 17 cases of males (0.95%). The total average amount of inv (9) in this study is 1.68%. The inv(9) population consists of 60% cases with infertility problems, 6,66% females who had spontaneous abortus and 33,33% were patientsreferred to our laboratory for other reasons.**

Conclusion: **In this research, the prevalence of inv (9) in the population of patients of Northeast Bosnia and Herzegovina who had the reproductive failure is shown. We believe that these results will help in finding the key to the truth about the association of this chromosome polymorphism with some pathological conditions such as fertility problems.**

Keywords: Chromosome 9, cytogenetics, infertility, polymorphism, spontaneous abortus.

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I. Introduction

For a long time, it has been known in 1956 Joe HinTjio and Albert Levan reported the correct human chromosome number [1]. Since then, karyotype analysis becomes an essential part of routine medical diagnostics. With the development of banding techniques, cytogenetics as a new biological discipline has helped medical professionals investigate the origin of genetic abnormalities in many constitutional and cancer diseases. Karyotyping also provided more information in the monitoring of fertility problems. It is considered, a couple is infertile if they try but fail to get pregnant after 12 months of unprotected sexual intercourse. Infertility does not include miscarrying or being unable to carry a baby to childbirth. Regarding the literature, it affects approximately 15% of couples in reproductive age [2], [3]. Any form of reduced fertility with prolonged time of unwanted non-conception can described as subfertility. It can be primary, in case of a woman who was never pregnant and who fail to get pregnant after one year of not using contraceptives. The secondary type of the subfertility occurs a woman can't get pregnant again after having at least one successful pregnancy [4], [5].

Thereasons for infertilityvary from person to person. The most commoncauses in men with fertility problems may have a low sperm count, low testosterone, testicular cancer or undescended testicles. In women, it can be tubal damage, endometriosis, abnormal menstruation, uterineproblems and many other causes that arise with age [6].

One thing is certain, a common cause of fertility problems for both men and women can be changes detected in the karyotype, found in 2-7% of couples [7].It is still controversial, but uniformly accepted, balanced translocations are one of a cause of recurrent abortion, defective offspring, and lowered fertility capacity. Using new techniques such as fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH),

instead of old fashion banding techniques (Q and C staining), subtle chromosome variants have been detected. Carriers of these balanced chromosome variants seem to have an increased risk of progeny with abnormal karyotype and reproductive failure in the future. Chromosome translocations and inversions are common structural alterations.

Unlike translocations, an inversion does not usually have a phenotypic effect, especially if it involves a heterochromatin area, such as 1qh, 9qh, 16qh and Yqh. According to the last version of International System for Cytogenetic Nomenclature (ISCN) this condition is called polymorphism without clinical significance [8]. But there remains an ongoing debate in the literature [9]. When examining metaphase cells, the most common inversion cytogeneticists see in human karyotype is the pericentric inversion of chromosome 9, with an incidence of about 1% to 3%, depending on the human population [10]. The region of chromosome 9 affected by the inversion, inv(9)(p11q12)/inv(9)(p11q13), contains centromere and centromeric heterochromatin.

A review of the literature showed that this chromosome 9 polymorphism, with breakpoints specified, can be the cause of variant abnormal clinical conditions such as congenital abnormalities, cancer and also could have a connection with pregnancy loss and fertility failure [11].The purpose of this paper is to calculate the frequency of pericentric chromosome 9 inversion in a patients study group and its correlation with possible reproductive failure.

II. Patients and Methods

A. *Patients*

A retrospective study was conducted on 1784 cytogenetics examination results from peripheral blood samples of the patients who were admitted to the Department of Pathology, Polyclinic for laboratory diagnostics, University Clinical Center Tuzla (UCC Tuzla), in the period from January, 2012 to December, 2022. The patients with reproductive failure, carriers inv(9) in their karyotype, were highlighted for detailed analysis.

B. *Karyotyping*

A total volume of 0,5 mL heparinized peripheral blood sample with 5 mL commercially available complete medium, Chromosome medium P (Euroclone, Italy), was cultured at 37 °C for 72 hours. After exposing the culture to the Colcemid, the harvesting procedure started according to the standard laboratory protocol. After fixation treatment, two to three slides were prepared, and chromosomes were GTG banded, using 0,05% solution of the proteolytic enzyme Trypsin and 4% Giemsa solution. At least 10 metaphases per case were completely analyzed, while 10 metaphases were captured using a DP12 camera (Olympus, Japan). A karyogram was done using the image analyzer program. An additional C staining technique was used to confirm the inversion 9 heterochromatin.

We used the 2016 version of the International System for Human Cytogenetic Nomenclature (ISCN 2016) to interpret karyotypes [8].

III. Results

Among the 1784 patients involved in this study, constitutional pericentric inversion inv(9)(p11q13) was found in 13 females (0,72%), while it was seen in 17 cases of males (0.95%) (Fig. 1). We find no cases that had the breakpoint of p12q13. The total average amount of inv(9), in both males and females, in this study is 1.68% (n=30). The age of female patients ranged from 2-50 years, with a mean of 35 years, while the age of male patients ranged from 1-58 years, with a median age of 34 years. Out of 1784 patients, 614 (34,41%) had spontaneous abortions in women and reproductive failure in both genders.

We found that 60% (18/30) of pericentric inv(9) carriers were persons who had fertility problems for at least two years. The rest of them, 6.66 % (2/30) were female patients who had spontaneous abortus. Furthermore, 33.33% (10/30) were patients referred to our laboratory for other reasons (Central nervous system disease, hematological reasons, gynecomastia...) (Table I).

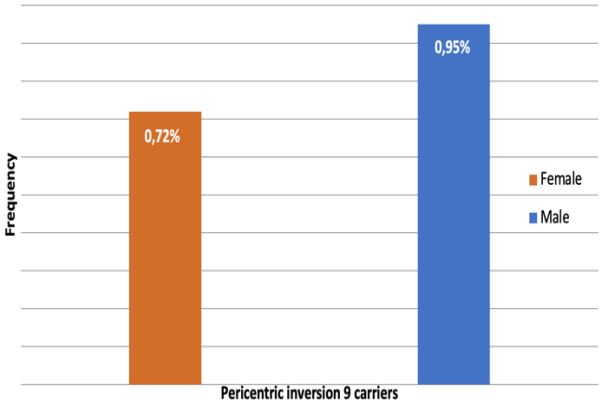

Fig.1. Incidence of pericentric inversion 9 in different sexes.

TABLE I: The Patients with Inversion 9 Referred to Our Lab for Other Reasons

Patients (%)	Diagnose
3 (10)	Hematological disease
1 (3,3)	Congenital malformation
2 (6,7)	Psyhomotor retardation
1 (3,3)	Child with neurorisks
1 (3,3)	Central nervous system disease
2 (6,7)	Gynecomastia

IV. Discussion

Today, even though we use modern cytogenetic techniques, many cytogeneticists still believe that the inversion of chromosome 9 (p11q13/p12q13) is a variant of constitutive heterochromatin, and natural polymorphism in the population [12]. But, many of them still have controversial opinions, believing that this chromosomal change is associated with certain clinical conditions[13]-[15]. However, one thing is certain, pericentric inversion 9 is the most common pericentric inversion cytogeneticists found in 1% to 3.57% of the general population [16]-[18]. According to the literature data, it was found that inv(9) prevalence is the highest among the African American population (3.57%), then they follow by 2.42% Hispanic population, and 0.26% of the Asian population [19]. In Europe, inv(9) is detected in 2,32% Romanian population [17], while in Türkiye the percentage is 1,71% [20].The

prevalence of pericentric inversion of chromosome 9, in the present study (1.68%), correlates with data observed from the Turkish population. Many studies have reported no difference in frequency among the male and female carriers of inv (9), but some of them noted that the difference is statistically significant (P<0.05) [21]-[23]. Reference [24], in their research on 500 couples from India with reproductive failure, discovered 18 couples where females had a normal karyotype and only one male patient had inversion 9. They also noticed 7 couples where males had normal chromosomes 9, while their wives carried inversion 9 [24].

Comparing the gender and age-specific incidence of chromosome 9 inversion in our study, we found no significant correlation. Since this structural change is also encountered in prenatal cytogenetics, some authors observed that most of the clinical phenotypes associated with inv 9, during the fetal period, were anatomical malformations [25], [26].

Data published from 1996 to 2011 showed that chromosome 9 polymorphism in adult carriers affected outcomes by decreasing fertilization rate and idiopathic reproductive failure[3], [27].In a study by Romanian authors, conducted on 1800 infertile patients, inv (9) was found in 2.27% of cases, while in the healthy control group, it was found to be 3.76% [17].In another research group from Türkiye, pericentric inversion 9 was associated with subfertility and sterility, detected in 1,42% of cases, 18 males and 23 females[28]. The association between inv(9) and infertility was detected also in Chinese infertile couples [29].

Reference [30] reported a case of a middle-aged couple with a fertility problems. The woman had a previous marriage in which she had children. In the new marriage, she tries to have a child with a new father, but it fails. Results from the infertility workup of both parents revealed the presence of the inv(9)(p12q13) in the father's karyotype, as a sole cytogenetic abnormality. It seems that these findings may support unexplained infertility in this couple and all the cases mentioned above. We detected 4,88 % (30/614) inversion 9 in cases with fertility failure which is higher, but not statistically significant, compared to the results conducted by Romanian authors [17]. On the other side, according to the data, conducted by researchers from another part of Europe, the incidence ofinv(9) in persons with fertility problems is much higher [20]. Anyway, the results we obtained can be very helpful for medical professionals in the field of marital infertility and for patients who intend to have In Vitro Fertilisation (IVF) or Intracytoplasmic Sperm Injection (ICSI) treatment.

V. Conclusion

Since the pathogenesis and clinical manifestations associated with the pericentric inversion of chromosome 9 are still unclear, it is necessary to investigate all structural changes on this chromosome. For this reason, we recommend genetic counselling for all patients with unexplained infertility followed by karyotype with pericentric inversion of chromosome 9.

We believe that these results will help in finding the key to the truth about the association of this chromosome polymorphism with some pathological conditions such as fertility problems.

CONFLICT OF INTEREST

Authors declare that they don't have any conflict of interest.

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