

Study of karyotypes in Case of Recurrent Abortions in Gujarat

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ABSTRACT:-

Introduction: - The biological definition of miscarriage is the expulsion of the conceptus before viability has been achieved. The definition of recurrent miscarriage is three or more consecutive spontaneous abortions. The risk factors for recurrent miscarriage are epidemiological, genetic, anatomical disorders, endocrinal, reproductive tract infections, thrombophilic disorders, disorders of materno-fetal alloimmune relationships, environmental effects and psychological causes. About 50% to 60% of all first trimester abortions are associated with derangement of one or more chromosomal complements. **Aim:** - The aim of this study was to assess frequency and increasing the awareness of physician about the nature of chromosomal aberration that contribute to the occurrence of repeated abortions. **Material & Methods:** - Patient of recurrent abortion was investigated by history taking, examination and investigations. For present study 20 women having two or more consecutive spontaneous abortions, who attended outdoor & indoor patient department, were selected and karyotyping was done. In 10 of the above cases karyotype study of both partners was done. So in total 30 individuals (20 females & 10 males) were selected for Cytogenetic study. In all cases relevant history and clinical findings and other investigations were noted. Blood samples were obtained and karyotype study was performed at Genetic Laboratory, B. J. Medical College, Ahmedabad. **Results and Conclusions:** - Cytogenetic evaluation by karyotypes revealed robertsonian translocation in one (5%) female; this patient had a history of 2 spontaneous abortions and two times IVF failure, she had history of chocolate cyst of ovary and family history of infertility. No numerical anomaly; mosaicism or inversions were found in this study; 23 cases had normal karyotype and remaining 6 cases came out inconclusive.

Key words: -Karyotype, Cytogenetics, Recurrent abortion

Introduction:-

The biological definition of miscarriage is the expulsion of the conceptus before viability has been achieved. The risk factors for recurrent miscarriage are many for example epidemiological, genetic, anatomical disorders, endocrinal and reproductive tract infections etc.

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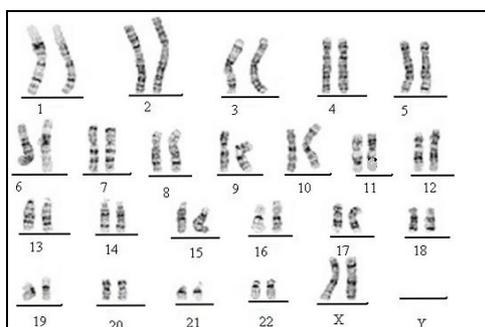
One of the important causes is genetics. The effects of genetic disease may range from spontaneous abortion to chromosomal anomalies in live born children and in adults

with certain types of faulty development. About 50% to 60% of all first trimester abortions are associated with derangement of one or more chromosomal complements.¹ Abnormal chromosomal complement is more likely in first trimester miscarriages. There are about 30 to 35% chances of having another miscarriage in couple having two or three miscarriages.¹ Carriers of balanced translocation were considered likely to produce chromosomally unbalanced offspring which would probably be aborted. Cytogenetics is the study of the structure, function & evolution of chromosomes, the vehicles of inheritance that reside in the cell nucleus. It is interesting and worth mentioning how the concepts in human cytogenetics developed, improved & gave better insight changing the scope of the subject with respect to human disease. The important advances came with the use of metaphase blocking, chromosome spreading device, banding techniques. Greater accessibilities of experimental material & with the recent advances in molecular biology applied to Cytogenetics. Hence study of karyotypic profile of patients with recurrent abortion has been helpful to find out chromosomal constitutions to detect chromosomal abnormalities & genetic cause for recurrent abortion so that proper management and genetic counselling can be done.

Material & Methods:-

For present study 20 women having two or more consecutive spontaneous abortions, who attended outdoor & indoor patient department in obstetrics & gynaecology at civil hospital, and some private infertility clinics, Ahmedabad were selected. In 10 of the above cases karyotype study of both partners was done. So, karyotype study included total 30 individuals, out of which 20 were females & 10 were males. For present study, clinically diagnosed patient of recurrent abortion was selected. Patient's personal data including name, age, sex, registration number as well as onset and type of illness, relevant personal history, past history and family history was noted. In addition, menstrual history, obstetric history, vital statistics & brief clinical assessment were also noted. All routine and specific investigations, regarding recurrent abortion were also noted. Blood samples of the patients were obtained in a heparinized container. Cultivation was done on the same day of the aspiration. After an incubation period of 69 hours at 37⁰ C, the harvesting was done and finally the metaphases on the slides were obtained. All the slides were scanned for metaphase detection. The readings were then taken from each slide and noted. Thereafter, those slides showing metaphase with good morphology were selected and kept under non-humid dry wooden boxes for aging process. Approximately after 7 days of harvesting, banding procedure was done using freshly prepared EDTA-Trypsin solution and giemsa stain. A photograph was obtained from a good quality metaphase slide with the help of a digital camera attached with a photo microscope with an exposure time of 4-8 seconds. The chromosomal findings were described according to the international system of human Cytogenetic Nomenclature and finally, karyotype, were prepared using conventional cut and paste technique as shown in Image.1. Correlation of chromosomal finding was done with other parameters.

Image-1 Normal chromosomal Karyotyping



Observations & Results:-

In total 30 individuals 20 females & 10 males were selected for Cytogenetic study.

Out of 20 females studied; 15(75%) females were in the age group of 21-35years, 2(10%) females were 20 years of age and 3(15%) females were more than 35years of age.

Out of 20 females studied; 14(70%) females had two or three abortions, 6(30%) females had more than three abortions.

Out of 20 female studied; 14(70%) had primary, and 6(30%) had secondary recurrent abortion. Out of 20 females studied; 13(65%) females had recurrent abortion in first trimester, Only 2(10%) females had recurrent abortion in second trimester and 5(25%) females had recurrent abortion in both first and second trimester.

Out of 30 individuals studied; 3(10%) were having positive family history. One patient gave history of male baby born with congenital anomaly in his family, one female had family history of recurrent abortion and one female had family history of infertility.

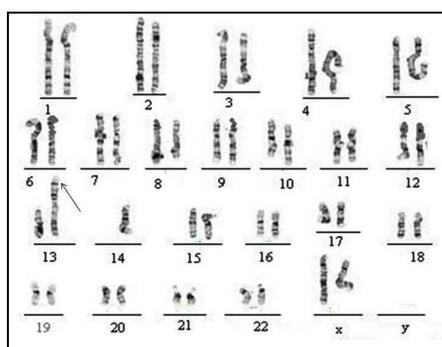
Out of Ten male partners, only 1 (10%) had decreased motility of sperms; rest of the males had normal sperm count & morphology.

Table-1 Cytogenetic analysis in patients studied:

Metaphase findings	F	M	Total
Numerical abnormality	0	0	0
Structural abnormality(Translocation)	1	0	1
Normal	15	8	23
Metaphase not found	4	2	6
Total	20	10	30

In present study, 30 individuals; 20 females and 10 males were studied for cytogenetic assessment. Out of 30, one female (3.3%) had robertsonian type of translocation as shown in **Image.2**. No one (0%) had numerical abnormality and 23 (76.6%) showed normal chromosomal constitution. In 6 cases metaphase was not found.

Image.2 Robertsonian type of translocation



Discussion:-

In present study 20 clinically diagnosed patients of recurrent abortion were selected. In 10 of the above cases karyotype study of both partners was done. Relevant and significant clinical parameters were noted and karyotypes of all 30 cases were studied, out of which 20 were females and 10 were males. An attempt was made to find out chromosomal abnormalities in cases of recurrent abortion and to determine the types of chromosomal abnormalities that play a major role in the causation of recurrent abortion. The data of present study were compared with others reported in literature.

Virtually, several surveys have reported the frequency and types of chromosomal abnormalities in patients of recurrent abortion. The frequencies of chromosomal abnormalities in these surveys varied from 4% to 14% .Some of the concerned studies are discussed here with.

In 1981, Sahsook Hahn and Dong Sik Kim² have done, Cytogenetic Studies of Leucocytes of 18 Couples with Habitual Abortions. The abnormal karyotypes seen were one case with 20% of 45,XX,-14,-15, t(14/15),one case of 46,XY/45,XY,-21 mosaicism, one case of 45,XY,-14,-21t(14/21), one case of 46,.XY/45,XO mosaicism and one case of 46,XYq+.

In 1982, Kostaraki, Z. Retzepopoulou Z. Kosmaidou-Aravidou³; examined G-banded chromosome complements from both partners of 150 couples who had two or more spontaneous abortions. Two women and four men were found to be balanced translocation carriers, as follows: 46, XX, t(2;10), 46, XX, t(6;II), 46, XY, t(6;10), 45, XY, t(13;14), 45, XY, t(13;14), 45, XY, t(14;21). Another woman had an abnormal karyotype 46, XX/47, XXX and one man had a pericentric inversion of chromosome 1; six other men and two women had pericentric inversions of chromosome 9.

In 1990, Makino T, Tabuchi T, Nakada K, Iwasaki K, Tamura S, Iizuka R⁴ have done, chromosomal analysis of peripheral blood cells of 639 Japanese couples. Among the 639

couples, 32 major chromosomal anomalies (5.0%) and 23 minor chromosomal variants (3.6%) were found. Both partners of one couple had an abnormal karyotype. The 32 major anomalies consisted of 19 reciprocal translocations, 9 Robertsonian translocations, one large inversion, two triple-X females, and one Turner mosaicism. The 23 minor variants included 15 cases of pericentric inversion of chromosome 9.

In 2005, Dubey S, Chowdhury MR, Prahlad B, Kumar V, Mathur⁵ have done Cytogenetic study to know the causes for recurrent spontaneous abortions on 742 couples (1484 cases). Chromosomal abnormalities reported were, 22 (2.9%) structural aberrations, 9 (1.2%) numerical anomalies. In addition to these abnormalities, 21 (3.2%) chromosomal variants were also found.

In May 2006, Razieh sehghani Firoozabadi, M, D., Seyed sehdi Klantar, Syed mohammad Seyed-Hasani and co- workers⁶ have done a Cytogenetic analysis of 88 couples and karyotypes of 12.5% of them were abnormal. The majority of them had monosomy X (6.82%), followed by balanced translocation (2.27%).

In 2008, Mozdarani H, Meybodi AM, Zari-Moradi S⁷ did a cytogenetic study of couples with recurrent spontaneous abortions and infertile patients with recurrent IVF/ICSI failure. Out of 221 individuals; 79 had three or more recurrent spontaneous abortions and 142 had at least three IVF/ICSI failures. Abnormal karyotype was found in 21 (9.50%) individuals. Of these 21 subjects, 4 (19.04%) exhibited sex chromosomal abnormalities and 17 (80.96%) had autosomal abnormalities. Male partners had significantly higher chromosomal abnormalities (5.88%) than of females (3.61%). These abnormalities were also higher in patients with recurrent spontaneous abortions than with IVF/ICSI failure ($P < 0.05$).

In present study as shown in table-I, out of 30, one (3.3%) had robertsonian type of translocation, 45 XX, D-D-t (13q, 14q). No one (0%) had numerical abnormality and 23 (76.6%) showed normal chromosomal constitution. In 6 cases metaphase was not found. Parents who are carriers of abnormal chromosomes are at higher risk of producing child with chromosomal abnormalities, which have been recognized as major cause of early spontaneous abortion.

Conclusions:-

Cytogenetic evaluation by karyotypes revealed robertsonian translocation in one (5%) female; this patient had a history of 2 spontaneous abortions and two times IVF failure, she had history of chocolate cyst of ovary and family history of infertility.

No numerical anomaly; mosaicism or inversions were found in this study; 23 cases had normal karyotype and remaining 6 cases came out inconclusive.

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