

CHROMOSOMAL ABNORMALITIES IN RECURRENT PREGNANCY LOSS – A STUDY IN NORTH-EASTERN PART OF INDIA.

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Accepted Date: 07-07-2025

ABSTRACT

Recurrent Pregnancy Loss (RPL) is a cause of childlessness affecting 2-5% women across the globe. Among the varied known and unknown causative factors of RPL, chromosomal abnormalities are often detectable ones with cytogenetic tests.

This study aims to explore the incidence of chromosomal abnormalities observed in RPL cases, by conventional karyotyping technique, in north-eastern part of India.

It was observed that chromosomal abnormalities are a cause of RPL in 2.6% of the cases.

KEYWORDS- recurrent pregnancy loss, karyotyping, chromosomal abnormality

INTRODUCTION

Recurrent Pregnancy Loss (RPL) also referred to as recurrent spontaneous abortion or recurrent miscarriage, is classically defined as the loss of three or more consecutive pregnancies before 20th gestational weeks according to the Practice committee of the American Society for Reproductive Medicine, whereas for clinical purposes, two or more losses constitute RPL and deserve evaluation.^[1] RPL has been estimated as the cause of childlessness in 2-5% of reproducing couples.^[2] Chromosomal abnormalities contribute about 6% of all causes of RPL.^[3] Structural and numerical defects in the chromosome of both parents and the fetus can lead to abortion. Genetic causes also include genetic mutations besides the chromosomal abnormalities. Other causes that can lead to RPL are uterine structural abnormalities, infection from TORCH, endocrine, immunologic, environmental factors, and unknown causes. RPL is a negative reproductive outcome faced by women across the world and poses as a great challenge to maternal health. It is officially recommended by both the American^[4] and Royal^[5] Colleges of Obstetricians and Gynecologists to karyotype both parents in RPL cases. This study aims to explore the association between RPL and chromosomal abnormalities observed by karyotyping in the women of different ethnic origins of Assam, in north-eastern part of India.

The chances a woman carries a pregnancy to term is greatly reduced after two or more abortions.^[6] There may be a specific and repeated reason of pregnancy loss in such cases. Knowledge about this repeated cause can help the women overcome the emotional grief associated with RPL. It is an indication as well as justification of a diagnostic workup in women suffering from RPL. Therefore, a study was conducted using conventional cytogenetic techniques to look for any chromosomal abnormality in the women attending Gauhati Medical College Hospital clinics with recurrent pregnancy loss

- Objectives-

- To document the prevalence of chromosomal abnormalities as a probable cause of recurrent miscarriage, among women visiting the clinics of Obstetrics and Gynaecology Department of Gauhati Medical College.

MATERIALS AND METHODS

- Design of the study was both retrospective and prospective analysis.
- Sample size- 269
- Inclusion criteria - History of 2 to 10 number of spontaneous abortions
- Exclusion criteria-
 - Obesity (BMI more than 30)
 - Factor v laden mutation/ thrombophilias
 - Thyroid Disorders/ hormonal disorders
 - Diabetes Mellitus
 - Antiphospholipid IgG antibodies/ lupus anticoagulants
 - Uterine anatomic defects

In the present study 269 women with not less than two spontaneous abortions participated, during the years 2017 to 2019 from the out-patient-departments of Obstetrics and Gynaecology Department of Gauhati Medical College and Hospital. These women were handed a Questionnaire and an Informed Consent form.

The women were categorized according to age groups (18-25yrs, 26-30years, 31-35years & 36-40years, 41years and above), according as number of previous abortions (2, 3, 4, more than 5), primary/secondary/ tertiary aborter state, and according to their ethno-linguistic origins (Hindi, Assamese, Bengali-Muslim & Hindu, and Bodo/other tribes) and compared.

Primary aborters were- women who never had a previous viable infant. ^[8]

Secondary aborters were-women who had previously delivered a pregnancy beyond 20 weeks gestational weeks and then suffered pregnancy losses. ^[8]

Tertiary aborters were-women with multiple miscarriages interspersed with normal pregnancies. ^[8]

Institutional ethics committee clearance was taken for the study.

Consent was taken from each participant while informing them about the purpose of study and counselled accordingly. Out of these 269 patients, 77 patients' peripheral blood was collected for karyotypic analysis.

Peripheral blood (2ml) was collected in heparinized vials and cultured for 72 hours according to conventional cytogenetic protocol and slides are prepared of metaphase spreads that are stained with standard G-banding techniques. These were analyzed using the Cytovision software for chromosomal abnormalities. In each individual, a minimum of 20 metaphase plates were prepared and at least five cells were counted.

The peripheral whole blood sample collected in heparinized vials was brought to the tissue culture lab. The heparinized whole blood was inoculated in 5 ml of culture media in Bio-Safety Cabinet. The tubes were kept in a 5% CO₂ incubator at 37 degrees centigrade for 68-72 hrs. For arresting of the culture, 20µl of colchicine was added after 67.5 hrs. incubation. Culture tubes were then incubated at 37 degrees centigrade for about two hours. The tubes were centrifuged at 1000 rpm for 10 minutes and the supernatant drained. For harvesting, 6ml of hypotonic KCl was added drop wise to the culture tubes while vibrating on a vortex. A 6ml fixative was added dropwise while vortexing and centrifuged at 1000 rpm for 10 minutes. Slide staining was performed in the Giemsa stain.

Results-

Among the 77 women participants in the cytogenetic study, 2 major chromosomal abnormalities were found.

A 46, XX, t (13;14) with two spontaneous abortions

A 46, XX, inv.9 with three spontaneous abortions

◦ Table 1- The chromosomal abnormalities and the prevalence rate (%).

SL NO.	CHROMOSOMAL ABNORMALITY	AGE OF PATIENT	ETHNO-LINGUISTIC ORIGIN	NO OF PREVIOUS ABORTIONS	PERCENTAGE (%)
1	ROBERTSONIAN TRANSLOCATION	27	Assamese	2	2.597%
2	INVERSION 9	32	Hindi	3	

Table2-The Primary/Secondary/Tertiary aborters and their mean age \pm SD, prevalence (in %)

	No of women	Minimum age	Maximum age	Mean age \pm SD	percentage (%)
Primary aborters	160	21	38	29.5 \pm 12.02	59
Secondary aborters	71	22	45	33.5 \pm 16.26	26
Tertiary aborters	38	25	36	30.5 \pm 07.77	14

Table3- The Ethno-Linguistic Origin and prevalence (%) and mean age \pm SD.

Ethno-linguistic groups	No of women	Minimum Age	Maximum Age	Mean age \pm SD	percentage (%)
Hindi	9	22	33	27.5 \pm 7.77	3.34
Assamese	147	22	45	33.5 \pm 16.26	53.90
Bengali Muslim (Sylheti)	61	20	42	31 \pm 15.55	22.64
Bengali Hindu	29	25	43	34 \pm 12.72	10.78
Bodo/other tribes	23	26	35	30.5 \pm 6.36	8.55

Table4- The age groups and the number of women and percentages.

Age Group	Number of women	Percentage (%)
18-25 years	39	14.49
26-30 years	98	36.43
31-35 years	59	21.93
36-40 years	49	18.21
41 years and above	24	8.92

Table5- The Number of Previous abortions and association with age.

No. of previous abortions	No. of women	Minimum age	Maximum age	Mean age \pm SD
2	163	20	43	31.5 \pm 16.26
3	59	25	38	31.5 \pm 9.12
4	29	33	45	39 \pm 8.48
More than 4	18	27	37	32 \pm 7.07

DISCUSSION-

Rearrangements involving chromosomes 13 and 14 (der (13;14) (q10; q10)) account for 75% of Robertsonian translocations.^[9]

Pregnancies from carriers of this translocation can produce viable gestations with Patau syndrome (trisomy 13).

However, most conceptions in der (13; 14) carriers result in early pregnancy loss,^[10] with only 0.4% of second trimester prenatal diagnostic tests demonstrating an unbalanced result. However, this risk is dependent on the parental origin of the translocation. Pregnancies with a maternal origin of the translocation demonstrate a significantly increased chance of a positive second trimester screen than those with a paternal origin (15% vs. <0.5%).^[11]

In this present study we found an overall incidence of chromosomal abnormalities as 2.6%.

An inversion occurs when a piece of a chromosome breaks at two points and reinserts within the same chromosome. Pericentric inversions involve the short and long arms (p-arm and q-arm, respectively) of the chromosome and include the centromere. Paracentric inversions occur in one arm of the chromosome and do not include the centromere.^[13] Unbalanced paracentric inversions produce gametes that have either no centromere (acentric) or two centromeres (dicentric) and are thus not viable. Any efforts in preimplantation genetic testing serve to improve reproductive efficiency and not to prevent the birth of an anomalous child. However, unbalanced pericentric inversions can result in live born children with birth defects due to the presence of partial trisomy or partial monosomy. The overall risk for a carrier of pericentric inversion to have a child with an unbalanced chromosome rearrangement is estimated at 5%–10%.^[12,13] Further risk estimates can be calculated based on the size of the inverted region, as the chance of meiotic imbalances correlates with the size of the inverted segment in proportion to the length of the chromosome. The risk of recombinant chromosomes becomes a factor if the inverted segment constitutes >30% of the total chromosome length and risks are considered significant once the inverted segment exceeds 50%.^[13,14]

The incidence of Inversion 9 is found to be about 1% - 3% in the general population.^[16] In this present study it was seen in 1.29% of the population. Inversion of chromosome 9 can be acknowledged as a reason of recurrent pregnancy loss in these women as we have ruled out other reasons of abortion in them thereby making its possibilities of association stronger.

CONCLUSION-

Maternal karyotyping alone has tremendous diagnosing potential for RPL cases. In conclusion, it may be said that further study with a large cohort of patients and analysis of all the three sets of genomes- maternal, paternal, and fetal (when available) can direct us with specific findings of chromosomal abnormalities as a definite cause of recurrent pregnancy loss.

This being a cross-sectional hospital-based study with a small number of participants, the need for a bigger community-based study is recommended that can expedient the understanding of RPL and its associated risk factors

ACKNOWLEDGEMENT-

We are grateful to the Dr. B. Baruah Cancer Institute, Guwahati, Assam, India for providing the laboratory facilities to carry out the study.

In addition to all who participated in the study and provided informed consent, the authors thank the Department of Obstetrics and Gynaecology, Gauhati Medical College, Guwahati, Assam, India for the outstanding co-operation while collecting samples for the study.

CONFLICT OF INTEREST- None

FUNDING-

The authors are thankful to Srimanta Sankaradeva University of Health Sciences, Guwahati, Assam, India for the funding received for this study.

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