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RESEARCH ARTICLE

CYTOGENETIC STUDIES IN BAD OBSTETRIC HISTORY (BOH) AND INFERTILITY-A RETROSPECTIVE STUDY FROMA STAND-ALONE LABORATORY

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Abstract

Introduction: Cytogenetic abnormalities are one of the important causes of recurrent pregnancy loss or bad obstetric history and Infertility. Almost 50% of first trimester pregnancy loss and upto 20% of second trimester loss can be due to cytogenetic cause. At the same time, cytogenetic abnormalities are also detected in2-3% of cases with infertility especially in males.

Methods: In this paper, wepresent our detailed analysisof13,618 of Bad Obstetric History (BOH) and infertility cases,referred for cytogenetics studies at Metropolis Healthcare Ltd, with an aim to look at chromosomal abnormalities observed in our huge dataset. This is the largest ever reported study of cytogenetic studies in BOH and infertility cases.

Results: We detected chromosome abnormalities in 470 (3.45%) of 13,618 cases. Out of the 470 cases, reciprocal translocations were the highest abnormality noted in 136 (28.94%) cases, followed by chromosome 9 inversion seen in 125 (26.6%) cases and three (0.64%) cases of insertion were also observed. Inversions were seen in 76 (16.17%) cases. Robertsonian translocations were seen in 19(4.04%) cases and complex translocations were seen in 3 (0.64%) cases. Numerical abnormalities were detected in 84 (17.87%) cases where as Mosaicism was seen in 17(3.62%) cases.

Conclusion: This study reinstates the importance of cytogenetic analysis in BOH and infertility cases in order to provide effective genetic counseling to guide the patients to prevent the birth of cytogenetically affected babies by informing them about the prenatal diagnostic testing options and preimplantation genetic testing methods.

Introduction:-

Spontaneous abortions and infertility, both primary and secondary are a major cause of concern to couples who are keen to start a family of their own and cytogenetic abnormities are one of the major causes responsible for them. Critical aspect of detecting cytogenetic abnormalities as causes is that there is no treatment to resolve this problem as of now. The only way to avoid these is prenatal diagnosis and pre implantation genetic testing to ensure the birth of cytogenetically normal babies.

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WHO estimates approximately 48.5 million people may have infertility (Mascarenhas MN et al. 2012).Chromosomal aberrations are one of the major causes of concern,as other causes are being managed well due to increasing awareness and available treatment options. More and more and more efforts need to be put in cytogenetic area of concern for prevention and management of affected babies. Identification of the cytogenetics abnormality as a cause can help people focus on methods of prevention and select an appropriate method of choice to have their own biological child.

It is a known fact that almost 5%-10% of all abortions and primary and secondary infertility have a cytogeneticabnormality (Sayee R et al. 2007, IyerPet al. 2007, Poornima S 2020). Cytogenetic abnormalities are one of the first genetic and well-established causes for abortions and infertility and a lot of studies have also been published(Demirhan Oet al. 2016, Pande S et al. 2017, Rawal Let al. 2020).

In this article, we have analysed a huge data of study performed in our lab and looked for variability, if any, in our results.Aim of this study was to look at the cytogenetic profile of BOH and infertility cases referred to our laboratory.

Materials and Methods:-

A retrospective study was conducted for a period of 3 years, from January 2018 till February 2021at Metropolis Healthcare Ltdlaboratory based in Mumbai Maharashtra, India.

Peripheral blood samples received in Sodium heparin vacutainers of total of 13,618 cases, referred for recurrent abortions, primary or secondary infertility or even with history of having birth of affected child with known or unknown genetic conditions of which 4131 were couples and 5356were individual males and females (Table 1). Samples were processed for karyotyping studies. Seventy-twohours' cultures were set up, processed, slides were prepared and G banding using GTG banding method was performed. Minimum of 20 metaphases were analyzed and if necessary 30 and beyond metaphases have alsobeen screened and analyzed. Metaphases from minimum of 550 banding resolution were analyzed to ensure maximum detection of chromosomal abnormalities. ISCN 2016 nomenclature was followed for accurate detection and characterization of chromosomal abnormalities.

Descript Statistics is used in the study. Results of Qualitative Variables Age group, Gender, Couple/Single, Clinical indication,Karyotype are expressed as frequency and percentage.

Results:-

The demographic and characteristics details of patients have been described in table 1. In this study the age ranges of patients were 18 to 91 years and the mean age was 31.1465 + 5.6211.Female to Male ratio was almost same (1:1).Age group of <35 years had 10058 (74.76%) cases and 3395(25.24%)cases were equal and above 35 years and in 165 cases age was not mentioned.

Of 13,618 cases, 7014(76.26%) were referred for bad obstetric history and 2184 (23.74%) were referred for infertility and4449 cases had not provided a clear indication. Cytogenetic abnormalities were detected in 470 cases (3.45%) rest had normal karyotypes. Of 13,618 cases 256 cases had also got BOH matrix profile test done which consisted of TORCH infection, herpes infection, anti-phospholipid and anti-cardiolipin antibody and TSH studies.

Of the 256 cases who had got BOH matrix profile test done, only 1 case was CMV positive, 4 cases were positive active HSV1, 11(4.3%), for HSV2, 21(8.86%) revealed abnormal for TSH studies.

Table 1:- Characteristics and demographic distribution of patient.

Variables	Frequency	Percentage
Age group		
<35	10058	74.76%
>=35	3395	25.24%
Details not available	165	-
Gender		
Female	6872	50.46%
Male	6746	49.54%

Couple/ Single		
Couple	4131	43.54%
Single	5356	56.46%
Clinical indication		
BOH	7014	76.26%
Infertility	2184	23.74%
Details not available	4449	-
Karyotype Result		
Abnormal	470	3.45%
Normal	13148	96.55%

Out of the 470 cases with cytogenetic abnormalities, reciprocal translocations were the highest abnormality observed in this study 136 (28.94%) cases, followed by chromosome 9 inversion noted in 125 (26.6%) cases. Numerical abnormalities like noting a maker chromosome in 84 (17.87%) cases followed closely along with inversions in 76 (16.17%) cases. Robertsonian translocations were seen in 19(4.04%) cases and complex translocations were seen in 3 (0.64%) cases and mosaicism was seen in 17(3.62%) cases.Also, 3(0.64%) cases of insertion were noted in this study, one involving chromosome 14 and 11, second involving chromosome 5 and 6 and the third case involved chromosome 9 and 4 (Table 2).

Table 2:- Type of abnormal karyotype.

Type of Abnormal Karyotype	Frequency	Percentage
Translocation Reciprocal	136	28.94%
Inversion(9)	125	26.60%
Numerical Chromosomal	84	17.87%
Inversion	74	15.74%
Translocations Robertson	19	4.04%
Mosaicism	17	3.62%
Miscellaneous	9	1.91%
Translocation Complex	3	0.64%
Insertion	3	0.64%

For patients referred for BOH, 5230(77.73%) cases were less than 35 years of age and 1698 (71.37%) of cases were equal to or above 35 years of age whereas for infertility, 1498(22.27%) cases were less than 35 years of age and 681(28.63%) of cases were equal to or above 35 years of age. (Table 3)

Abnormal karyotypes were noted in 356(3.54%) of cases with less than 35 years of age and 100(2.95%) in patients with age equal to or above 35 years of age. (Table 3)

Table 3:- Frequency distribution of age group in patients with clinical indication and karyotype result.

	Age group			
	<35		>=35	
	Frequency	Percentage	Frequency	Percentage
Clinical indication				
BOH	5230	77.73%	1698	71.37%
Infertility	1498	22.27%	681	28.63%
Karyotype Result				
Abnormal	356	3.54%	100	2.95%
Normal	9702	96.46%	3295	97.05%

Clinical indication wise 220 (3.14%) patient with BOH had abnormal karyotypes in comparison to 99 (4.53%) of cases with infertility who showed abnormal chromosomes (Table 4).

Table 4:- Distribution of patient with clinical indicationand patients with karyotype result.

Clinical indication	Karyotype Result			
	Abnormal		Normal	
	Frequency	Percentage	Frequency	Percentage
BOH	220	3.14%	6794	96.86%
Infertility	99	4.53%	2085	95.47%

Chromosomal abnormalities have been noted almost equally both in males 262(8.22%) and in 208(6.19%) females (Table 5). The breakup of these abnormalities was seen as listed below in Table 6. To summarize, inversions have been observed more in males 63(48.35%) whereas inversion 9 was seen in 65 (61.12%) females. Mosaicism was noted more in females (15.4%). Numerical abnormalities were observed more in males 63(47.19%) and reciprocal translocations were seen more in females 77(73.14%). Robertsonian translocations were seen slightly higher in female 10(9.33%) and male 9(6.82%) and abnormalities from other's category were almost same both in males (10.62%) and females (14.27%). Complex translocations and Insertion were also seen in 3 cases respectively.

Table 5:- Frequency of karyotype result in couples/ single.

Karyotype Result	Couples/ Single			
	Couple (n=4131)		Single(n=5356)	
	Husband	Wife	Female(n=2741)	Male(n=2615)
Abnormal	128(3.10%)	114(2.76%)	94(3.43%)	134(5.12%)
Normal	4003(96.90%)	4017(97.24%)	2647(96.57%)	2481(94.87%)

Table 6:- Type of Abnormal Karyotype in Couples/ Single.

Type of Abnormal Karyotype	Couples/ Single			
	Couple		Single	
	Husband (n=128)	Wife (n=114)	Female (n=94)	Male (n=134)
Inversion	38(29.69%)	7(6.14%)	4(4.26%)	25(18.66%)
Inversion(9)	37(28.91%)	43(37.72%)	22(23.40%)	23(17.16%)
Mosaicism	1(0.78%)	3(2.63%)	12(12.77%)	1(0.75%)
Numerical Chromosomal	5(3.91%)	3(2.63%)	18(19.15%)	58(43.28%)
Translocation Reciprocal	41(32.03%)	47(41.23%)	30(31.91%)	18(13.43%)
Translocations Robertson	3(2.34%)	7(6.14%)	3(3.19%)	6(4.48%)
Translocation Complex	1(0.78%)	1(0.88%)	1(1.06%)	0(0%)
Insertion	0(0%)	2(1.75%)	0(0%)	1(0.75%)
Miscellaneous	2(1.56%)	1(0.88%)	4(4.26%)	2(1.49%)

In the miscellaneous category, we have included cases with not commonly reported chromosome abnormalities. In this groupwe havetwo interesting cases with the same rare abnormalities of mosaic fragile 16 along with deletion 16q, of which one had infertility and in the second one indication was not provided. Rest were sex chromosome abnormalities including three cases with isochromosome X for the long arm and one with isochromosome for the short arm of Y were noted. Besides, two with derivative Y and 1 with? deletion Y were noted. (Table 7)

Table 7:- Miscellaneous.

Abnormality	No
46,X der(Y)t(Y;?)(q12;?)	1
46,X,der(Y)	1
46,X,?del(Y)(q11.2)	1
mos 46,XX,fra(16)(q22)[20]/46,XX,del(16)(q22)[5]/46,XX[15]	1
mos 46,XX,fra(16)(q22)[21]/46,XX,del(16)(q22)[2]/46,XX[22]	1
46,X,i(X)(q10)	3
46,X,i(Y)(p10)	1

Discussion:-

Our study revealed chromosomal abnormalities in 470 (3.45%) cases which is almost equivalent to other studies published in literature. Of the total BOH cases referred, chromosomal abnormalities were seen in 3.14% whereas in infertility, chromosomal abnormalities were observed in 4.53% cases(Vidya H K et al.2017, Sayee R et al. 2007, Iyer P et al. 2007)

In our study, translocations were found to be (33.62%). In a study published by Leena et al 2020they got translocation in 13% of their abnormalkaryotypes. On the other hand, study reported by RajangamS et al in 2007had reported translocations as 48% of their total chromosomal abnormalities.

Balanced translocation in parents whether Robertsonian or reciprocal may result in unbalanced chromosomal rearrangement in the fetus, resulting in a genetic condition in the unborn child. Almost 2-4 % of couples with BOH have balanced structural chromosome rearrangement, with the most common being balanced reciprocal or Robertsonian translocations. Reciprocal translocation results due to exchange of chromosomal material between chromosomes. This is mediated because of fork stalling and template switching, microhomology-mediated break-induced repair, breakage–fusion–bridge cycles, or chromothripsis(Priya P K et al. 2018). Couples with balanced chromosomal rearrangement have 50% chances of recurrent pregnancy loss and 20% chances of having children with unbalanced rearrangement. There is also a good chance of 20-30% to have normal children.

Therefore, genetic counseling is extremely critical and important for these couples to plan their families. They are provided options of prenatal diagnosis through amniocentesis or chorionic villus sampling for detection of the chromosomal status in the fetus. Couples can also be advised preimplantation genetic diagnosis for selection of normal embryos in the in vitro fertilization (IVF)/assisted pregnancies. Use of donor gametes can be recommended in situation where the embryos will always result in abnormal/chromosomally aneuploid affected pregnancies like for couples with balanced Robertsonian translocation involving acrocentric chromosomes.

Approximately 2%–4% of recurrent pregnancy loss (RPL) is associated with a parental balanced structural chromosome rearrangement, with the most common being balanced reciprocal or Robertsonian translocations. A reciprocal translocation is an interchange of chromosomal material between specific chromosomes, and it may be the result of fork stalling and template switching, microhomology-mediated break-induced repair, breakage–fusion–bridge cycles, or chromothripsis.These are balanced when the exchange does not result in loss of genetic material and unbalanced when genetic material is gained and/or lost (Priya P K et al. 2018).

In case any couples were found to have marker chromosomes or uncharacterizablerearrangements, they were advised to go ahead with M-FISH or spectral karyotyping studies to understand them for management of future pregnancies.

Significance of polymorphic variants like 9qh+, inversion 9, 16qh+, Yqh+, Inversion Y including enlarged satellites and enlarged stalks on acrocentric chromosomes has always been a matter of discussion regarding their significance and role in BOH and infertility(FerideIffetSahinet al. 2008,Dr Elva Cortes-Gutierrez 2009, Ji Liang et al. 2014). In this study, polymorphic variants including inversion 9 were observed in the substantial no of (2030 - 15.44%) cases. Of these 172(1.31%) had shown double polymorphism and whereas 8(0.06%) cases have shown triple polymorphism, rest had shown single polymorphism i.e. 1850 (14.07%).

This does not include inversion 9 which was seen in 125 cases (0.91%), which we kept along with our abnormal karyotypes. Clinical significance of inversion 9 is not clear (Sasiadek M et al. 1997; Sipek A Jr, et al. 2015)but it has been associated with bad obstetric history and they have been reported in higher number in cases with BOH/infertility in comparison to normal population. In general population it has been reported to be present in 1-3% of population but in patients with BOH and infertility, it has been reported to be 2-7 %
(Mierla DANA, and Stoian V, PhDb 2012; Muthuvel Aet al. 2016).

Although inversion 9 has been largely accepted as normal polymorphic variant but studies have also stated that the carriers of such balanced structural aberrations have an increased possibility of having a foetus with an unbalanced chromosomal abnormality. The chance of them giving rise to abnormal gametes as a result of meiotic crossing-over ranges from 1% to 10%. Couples with inversion 9, either one of them or both have higher incidence of Down's syndrome and other chromosomal abnormalities in the progeny has been reported. Interestingly, the sperm DNA

integrity of a male patient with infertility and inversion (9) karyotype was studied by García-Peiró et al. 2011and found thatcases with inversion 9 have high sperm DNA fragmentation, significant meiotic alterations, anomalous aneuploidy, and altered seminogram parameters; all of these can result in chromosomal imbalance in the progeny (Muthuvel A et al. 2016).

Besides, in our study, there were also two cases with the same rare abnormalities of mosaic pattern with one cell line showing fragile 16 and deletion 16 at band position q22. Fragile site at 16q22 is a common fragile site and is not really associated with any specific clinical condition but have been reported in a male case with fragile site at 16q22 and also in a female case with h/o 4 spontaneous abortions(Martorell M R etal. 2014,KomnenicRadovanovic, M. et al. 2019).

Conclusion:-

In this paper, we have reviewed a huge data of 13,618 cases for chromosomal abnormalities in BOH and Infertility cases. Detection of these chromosomal abnormalities facilitates effective genetic counseling which helps guiding the patient accurately. Significance of all these cytogenetic study results lies in the successful prevention of birth of children with genetic defects using prenatal diagnosis and help affected couples in having their own biological children using advance assisted reproductive techniques like IVF, IUI and ICSI using preimplantation genetic screening/testing to select chromosomally normal embryos.

Conflict of interest:
We have no conflict of interest to disclose

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