SHORT COMMUNICATION

Cytogenetic abnormalities associated with reproductive failure in Pakistani population: Experience of a tertiary care hospital

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Abstract

Constitutional chromosomal abnormalities play a significant role in causing reproductive anomalies in individuals of reproductive age. With the rapid advancement of genome engineering techniques, it has now become possible to cure different genetic disorders. However, very limited data is available regarding the prevalence of such aberrations in the Pakistani population. Considering this factor, this retrospective analysis was undertaken to elucidate the type and prevalence rate of such abnormalities in our population. A total of 241 individuals, who were referred to the Liaquat National Hospital, from January 2017 to December 2021, with a history of infertility or miscarriages, were evaluated using the standard GTG banding technique. The results revealed a notably high percentage 44(18.2%) of chromosomal abnormalities in our population. Surprisingly, the frequency of these anomalies was observed to be higher in males than in females. However, further research is needed using a larger sample size to confirm the findings of this investigation.

Keywords: Karyotyping, Infertility, Miscarriages.

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Introduction

Reproductive failure is deemed to be a major health concern worldwide and includes infertility, miscarriage, recurrent miscarriages, abortion, and recurrent abortions.[1] Infertility is defined as an inability to conceive even after one year of unprotected sex, while the term abortion or miscarriage means spontaneous loss of pregnancy before the 20th week of gestation.[1] Although the percentage of infertility varies from country to country, globally 12-15% of couples experience infertility when they want to conceive.[1,2] However, available data shows that in Pakistan, around 22% of individuals experience this obstacle in their lives.[3]

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In developing countries like Pakistan, having an offspring is considered an essential aspect of maintaining marital satisfaction.[4] Children, especially boys, are considered breadwinners for the family; hence, not having children is regarded as a big curse.[4] Although in olden times, only females were deemed responsible for reproductive failure, with the advancement of knowledge and scientific research, the contribution of males towards reproductive failure is evident. In fact, males and females are equally susceptible to reproductive issues.[2] Studies reflect that in couples with reproductive failure, 40% of the problems are attributed to males, 40% to females, while mutual or unknown factors may account for the remaining 20% of instances.[5]

The frequent causes of reproductive failure are hormonal imbalance,[6] environmental factors,[7] uterine dysfunction,[2] and genetic factors.[2,8] Although in the general population, the percentage of chromosomal anomalies is around 0.37% to 1.86%, it is relatively as high as 3.95% to 14.3% in infertile individuals.[9] Hence, chromosomal abnormalities are a principal genetic factor behind reproductive failure. Although previously cytogenetic abnormalities were considered irremediable, rapid advancement in the field of genetic manipulations has made it possible to cure different genetic alterations.[10] With the discovery of CRISPR/CAS9 technology, we have entered an era where one can think about the treatment of even aneuploidies, as currently researchers are focusing on the elimination of a whole extra chromosome using this novel technology.[11] However, it is unfortunate that there is a scarcity of proper data indicating the percentage and types of most common cytogenetic anomalies in our population. Considering this factor, this study was undertaken to unveil the frequency and types of major cytogenetic aberrations associated with the absence of puberty and reproductive failure in Pakistan's population.

Patients/Methods, and Results

This retrospective study included 241 individuals who visited the Molecular Pathology Laboratory, Liaquat National Hospital & Medical College, Karachi, for chromosome analysis from January 2017 to December 2021 from different parts of the country (Table S1 and S2 [supplementary data]). Only married individuals of

reproductive age (18 to 50 years) were included in this study. These patients either failed to conceive even after one year of unprotected sex or had a history of recurrent, consecutive pregnancy losses (two or more). Individuals with a history of tumours, reproductive organ surgery, or with previous successful fertility treatments were excluded from the study. There were 129 males and 93 females who faced infertility issues, whereas 7 male and 12 female patients had experienced miscarriages. The male infertility patients harboured a history of azoospermia or oligospermia, whereas female patients of this group mostly had a history of primary amenorrhoea, secondary amenorrhoea, or irregular periods. A detailed history along with written informed consent was taken from all the study participants. The institutional ethical committee approval number for this study is: 0833-2022 LNH-ERC.

Supplementary Data
Table S1: Distribution of chromosome analysis samples by year and region.

	Punjab	Sindh	KP	Balochistan	AKJ	GB
2017	2	23	7	5	1	0
2018	5	56	17	21	2	4
2019	7	16	8	3	1	1
2020	4	21	1	4	0	0
2021	5	17	3	5	0	2

KP:Khyber Pakhtunkhwa; **AJK**: Azad Jammu and Kashmir; **GB**: Gilgit-Baltistan.

Supplementary Data
Table S2: Distribution of chromosome analysis positive samples by year and region.

	Punjab	Sindh	KP	Balochistan	AKJ	GB
2017	0	4	2	1	0	0
2018	0	12	1	3	0	1
2019	0	0	1	2	0	0
2020	0	7	0	1	0	0
2021	1	7	1	0	0	0

KP:Khyber Pakhtunkhwa; **AJK**: Azad Jammu and Kashmir; **GB**: Gilgit-Baltistan.

Peripheral blood specimens were used to prepare standard GTG (G-banded using trypsin and Giemsa) banding chromosomes, followed by the analysis of the metaphases using a bright-field microscope (Leica, DM500) and Cyto Vision software. In all cases, a minimum of 20 well-spread metaphases were counted to detect aneuploidy, whereas at least five high-resolution metaphases were karyotyped to investigate any structural anomalies. Moreover, in the case of mosaicism, a total of 30 cells were counted to confirm the presence of different genetic cell lines.

Chromosomal abnormalities were classified as numerical anomalies or structural anomalies. Chromosomal polymorphic variants were also considered structural chromosomal anomalies. Overall, chromosomal abnormalities were observed in 28 (20.6%) male patients

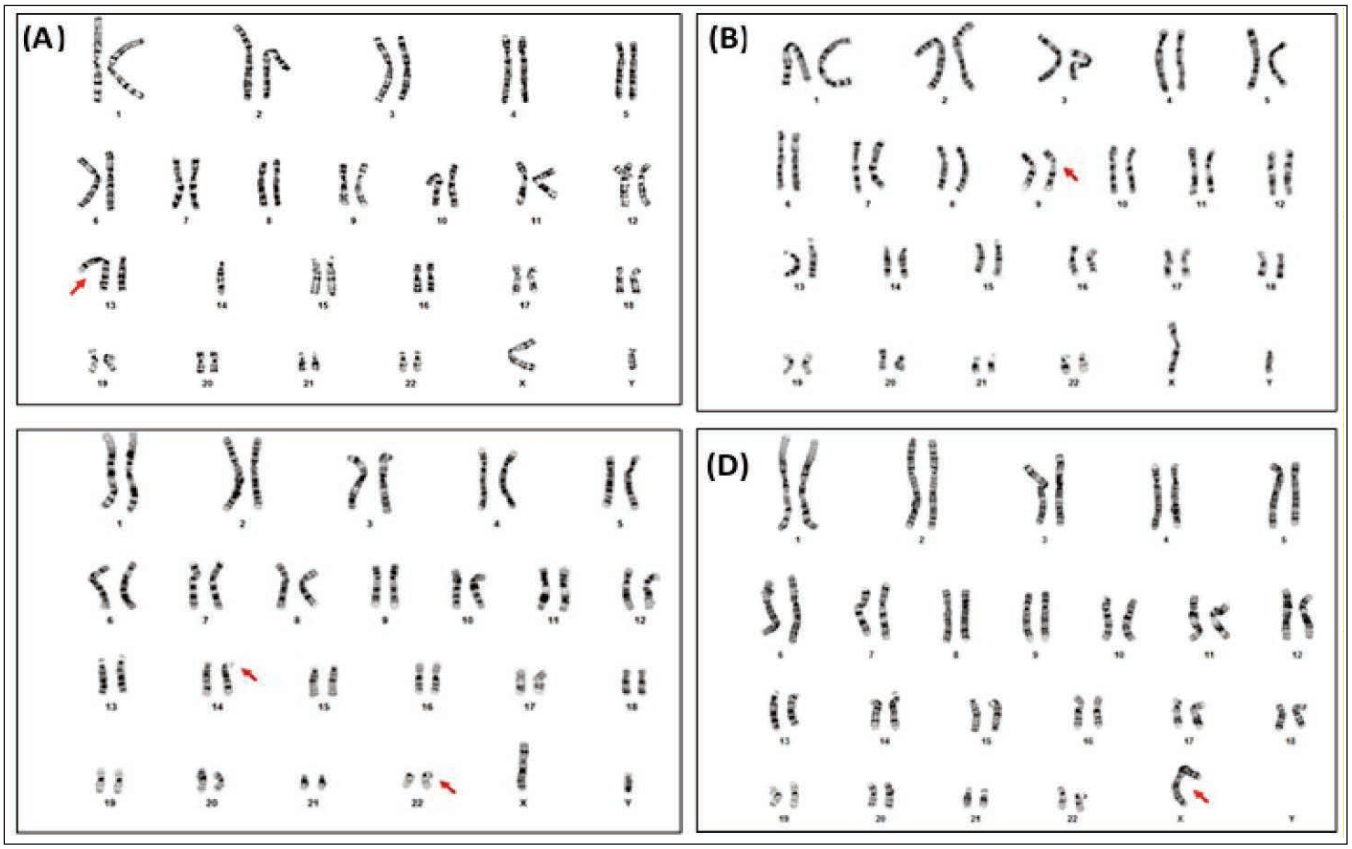

Figure: Abnormal karyograms showing (A) 45,XY,der(13;14)(q10;q10), (B) 46,XY,9qh+, (C) 46,XY,14ps+,22ps+ (D) 46,X,i(X)(q10).

Table-1: Types of chromosomal abnormalities observed in the study population.

Gender	Clinical Diagnosis	Types & Frequency of Abnormal Karyotype			n (%)
Male	Miscarriages or Abortions	**Types**		**Frequency**	7 (14.2)
		Numerical	NOB	NOB	
		Structural	45,XY,der(13;14)(q10;q10)	1	
		Chromosomal variants	NOB	NOB	
	Infertility	**Numerical**	47,XXY	20	129 (20.9)
			47,XXY[45]/46,XY[5]	1	
			47,XXY[38]/46XY[12]	1	
		Structural	46, XX	1	
		Chromosomal variants	46,XY,9qh+	3	
			46, XY,14ps+,22ps+	1	
	Total			28	136 (20.6)
Female	Miscarriages or Abortions	**Type**		**Frequency**	12 (17.2)
	Infertility	**Numerical**	45, X	8	
			45,X [31]/46,XX[19]	1	
		Structural	46,X,i(X)(q10)	1	
			46, XY	4	
		Chromosomal variants	46,XX,9qh+	2	
	Total			16	105 (15.2)

NOB= Not observed

and 16 (15.2%) female patients. Among the 129 males from the infertility group, there were 27 (20.9%) patients with chromosomal abnormalities, whereas out of 7 males in the miscarriages group only 01 (14.2%) had chromosomal abnormality. In the case of females, out of 93 in the infertility category 16 (17.2%) were afflicted cases, while there were no afflicted females in the miscarriages category (Table 1).

Numerical chromosomal anomalies were observed in 31(12.8%) of the total affected patients (22 males and 9 females). In men, the most common numerical disorder was Klinefelter syndrome (47,XXY) (n=20) (Table 1); however, the most frequent aneuploidy observed in women was Turner syndrome (45,X) (n=8) (Table 1), which was only observed in females with a history of primary amenorrhoea. Moreover, in two male individuals of the infertility group, mosaic Klinefelter syndrome (47,XXY[45]/46,XY[5], 47,XXY[38]/46,XY[12]) was detected (Table 1), while in case of female individuals, only one case with Turner syndrome mosaicism (45,X [31]/46,XX[19]) was observed (Table 1).

On the contrary, structural chromosomal aberrations were observed only in 5.3% (n=13) of total patients (6 men and 7 women). All of the women with a history of miscarriages (n=12) were cytogenetically normal, whereas, in one man out of seven, a derivative chromosome (45,XY,der13;14)(q10;q10)) was noted (Figure 1 A). Furthermore, three men from the infertility group harboured heteromorphism (46,XY,9qh+) (Figure 1 B). In women, this type of heteromorphism was observed in one primary amenorrhoea and one secondary amenorrhoea group. Besides, one male patient in the infertility group (n=129) experienced pseudo satellites (46,XY,14ps+,22ps+) (Figure 1 C) in his chromosome complement. Furthermore, only 1 female patient was observed to have isochromosome abnormality (46,X,i(x)(q10)) (Figure 1 D) in the primary amenorrhoea group.

46, XX male and 46, XY female karyotypes were considered structural chromosomal anomalies. This was the second most frequent chromosomal abnormality noted in the females of the infertility group (4 out of 93 infertile females exhibited this problem). Contrastingly, 46, XX karyotype was observed in only 1 out of 129 male infertility patients.

Conclusion

The results of this study have highlighted a relatively high percentage (18.2%, n=44) of cytogenetic anomalies in the Pakistani population with a history of reproductive failure. Klinefelter syndrome is the leading cause of male infertility, followed by chromosomal heteropolymorphism, whereas in females, sex reversal syndrome was the second most frequent abnormality after Turner syndrome. The results clearly indicated that chromosomal aberrations were more common in males than females of our ethnicity. Typically, the prevalence of Klinefelter syndrome and Turner syndrome is considered a random event that occurs due to nondisjunction during meiosis. Like other genetic anomalies, consanguineous marriages and advanced maternal age stand as risk factors for these two chromosomal abnormalities; however, there are conflicting results in the existing literature. Therefore, it will be of great interest to look at the association of each risk factor with the occurrence of Klinefelter syndrome and Turner syndrome in Pakistan. Moreover, similar types of retrospective studies from some other healthcare institutions in Pakistan are needed to help in finalising the type and prevalence ratio of chromosomal anomalies in our population.

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Conflict of interest: None.

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References

1. Shibahara H, Wakimoto Y, Fukui A, Hasegawa A. Anti-sperm antibodies and reproductive failures. Am J Reprod Immunol 2021;85:e13337. doi: 10.1111/aji.13337.

2. Saha S, Roy P, Corbitt C, Kakar SS. Application of Stem Cell Therapy for Infertility. Cells 2021;10:1613. doi: 10.3390/cells10071613.
3. Kaya Z, Oskay U. Stigma, hopelessness and coping experiences of Turkish women with infertility. J Reprod Infant Psychol 2020;38:485-96. doi: 10.1080/02646838.2019.1650904.
4. Ali S, Sophie R, Imam AM, Khan FI, Ali SF, Shaikh A, et al. Knowledge, perceptions and myths regarding infertility among selected adult population in Pakistan: a cross-sectional study. BMC Public Health 2011;11:760. doi: 10.1186/1471-2458-11-76.
5. Antoine E, Chirila S, Teodorescu C. A Patented Blend Consisting of a Combination of Vitex agnus-castus Extract, Lepidium meyenii (Maca) Extract and Active Folate, a Nutritional Supplement for Improving Fertility in Women. Maedica (Bucur) 2019;14:274-9. doi: 10.26574/maedica.2019.14.3.274
6. Chang CW, Sung YW, Hsueh YW, Chen YY, Ho M, Hsu HC, et al. Growth hormone in fertility and infertility: Mechanisms of action and clinical applications. Front Endocrinol (Lausanne) 2022;13:1040503. doi: 10.3389/fendo.2022.1040503.
7. Skakkebæk NE, Lindahl-Jacobsen R, Levine H, Andersson AM, Jørgensen N, Main KM, et al. Environmental factors in declining human fertility. Nat Rev Endocrinol 2022;18:139-57. doi: 10.1038/s41574-021-00598-8.
8. Beke A. Genetic Causes of Female Infertility. In: Igaz P, Patócs A, eds. Genetics of Endocrine Diseases and Syndromes. Cham, Switzerland: Springer Nature Switzerland AG, 2019; pp 367-83. Doi: 10.1007/978-3-030-25905-1
9. Liu Y, Kong XD, Wu QH, Li G, Song L, Sun YP. Karyotype analysis in large-sample infertile couples living in Central China: a study of 14965 couples. J Assist Reprod Genet 2013;30:547-3. doi: 10.1007/s10815-013-9964-6.
10. Roth TL, Marson A. Genetic Disease and Therapy. Annu Rev Pathol 2021;16:145-66. doi: 10.1146/annurev-pathmechdis-012419-032626.
11. Zuo E, Huo X, Yao X, Hu X, Sun Y, Yin J, et al. CRISPR/Cas9-mediated targeted chromosome elimination. Genome Biol 2017;18:224.

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FN: Conceptualization, writing original draft, supervision.
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