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Delving into the impact of cytogenetics and karyotyping on precise diagnosis and quality of life preservation in Pakistan (2011-2022)

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ABSTRACT

Introduction: Conventional cytogenetics or Karyotyping is an essential tool to examine the banded pattern of chromosomes during metaphase of the cell cycle. It helps in diagnosis of specific chromosomal abnormalities and clinical syndromes. We tried to find the prevalence of different cytogenetic abnormalities and significance of this technique.

Methods: An observational, cross-sectional study with a focus on exploring the prevalence and significance of cytogenetic abnormalities was conducted during January 2011 and March 2022. This study was conducted at Islamabad Diagnostic Center, Islamabad, after approval from Ethical Committee. Patients were included who presented with clinical suspicion of genetic abnormalities. These abnormalities include those having mongoloid facies, gender ambiguities, primary amenorrhea (PA) in females, recurrent pregnancy loss (RPL) etc.

Results: A total of 1703 patients were included who presented with clinical suspicion of genetic abnormalities. As a part of disorders of sex development (DSD), those who were registered as females, 21% turned out to be genetically male (46, XY), while 17.3% were found to be genetically females (46, XX). Those who presented with primary amenorrhea, 5.2% were genotypically male. 171 out of 186 children presented with clinical suspicion of having Down syndrome (DS) were having Trisomy 21. 45 known cases of hematological disorders like Aplastic Anemia (AA) were also analyzed for chromosomal breakage studies (characteristic of Fanconi Anemia, FA) by karyotyping. Out of these 26.6% were reported for having chromosomal breakages. 74% of those having myeloid hyperplasia showed Philadelphia chromosome (t (9; 22), confirming the diagnosis of Chronic Myeloid Leukemia (CML).

Conclusion: Cytogenetic abnormalities are quite common but often remain undiagnosed due to unavailability of technical expertise needed for proper evaluation of different disorders. A multidisciplinary approach is needed for management and counseling of patients and their family members.

Keywords: Aneuploidy, Cytogenetic analysis, Karyotypes, Sex chromosomes.

Introduction

Chromosomal aberrations refer to any abnormality that involves alteration in the number or morphology of normal chromosomes.[1] Cytogenetic analysis has a significant role in diagnosis of different human diseases. Two types of techniques have been developed to detect the genetic imbalance namely conventional cytogenetics and molecular cytogenetics (fluorescence in situ hybridization, spectral karyotyping, and comparative genomic hybridization).[2]

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Conventional cytogenetics or Karyotyping is an indispensable tool to examine the banded pattern of chromosomes during metaphase of the cell cycle. It helps in diagnosis of specific chromosomal abnormalities and clinical syndromes such as genetic abnormalities (autosomal and sex chromosome abnormalities), developmental delay (DD), and mental retardation (MR).[3]

Approximately 0.5% of all living births are affected with congenital malformations and they may cause serious birth defects and contribute significantly towards neonatal mortality and morbidity. The incidence of autosomal chromosomal abnormalities is higher than sex linked and ratio is almost 3.2:1.[4] The most prevalent autosomal chromosomal defects include Trisomy 21 DS (Down syndrome),

Trisomy 18 (Edwards syndrome) and Trisomy 13 (Patau syndrome).[5]

Conventional cytogenetics technique has gained much importance in neonates born with gender ambiguity. The incidence of gender ambiguity is 1/4500 cases. Cytogenetics unfolds the various etiologies causing the gender being uncertain. Through this technique neonatologist can minimize the mental trauma and anxiety of parents by providing appropriate genetic counseling and can offer the available therapy to minimize and prevent gender ambiguity.[6]

Recognition of sex chromosome aneuploidies in people presenting with amenorrhea, infertility and no development of secondary sex characters is also becoming common with the help of cytogenetics. Sex chromosome abnormalities involve either gain or loss of any sex chromosome with Klinefelter syndrome (KS) (47, XXY) and Turner syndrome (TS) (45, XO) being the most common.

Cytogenetic studies have gained importance in recognizing the cause of recurrent abortions which is an emerging problem among the couples. The prevalence of chromosomal abnormalities reported in couples varied in different studies ranging from none to almost 21.4% with balanced autosomal translocation being the most common.[7] It helps couples to avoid expensive and extensive work up, assist to know the risk of recurrence and helps to understand if any prenatal testing is required.[8]

Correct diagnosis, prognostic evaluation and improved therapeutic options often require appropriate cytogenetic testing. The aim of our study is to find the prevalence of different cytogenetic abnormalities and significance of this technique, as limited studies have been published in our part of the world. Also the increased prevalence of common cytogenetic abnormalities are often undiagnosed and their early detection is necessary for medical and psychological treatment.[9] Karyotyping provides us with an opportunity to improve the management of various disorders. This study will create awareness among gynecologists, pediatricians, endocrinologists, surgeons, urologists and neonatologists and will also aid them in the management and counseling of patients.

Methods

An observational, cross-sectional study was conducted between January 2011 and March 2022 at Islamabad Diagnostic Center, Islamabad, after approval from Ethical Committee letter number IDCERB06223191 dated 12-Jan-2011. A total of 1703 patients with clinical suspicion of genetic abnormalities were included in the study. These abnormalities include those having mongoloid facies, gender ambiguities, primary amenorrhea in females, recurrent fetal loss etc.

8ml peripheral blood in adults and 4ml in children in heparinized test tubes was collected. After proper mixing, 1ml of blood was cultured in 10ml PB-MAX karyotyping medium and placed in incubator at 37^0C for 72 hours.

After incubation, 10µl colcemid was added to the sample for 30 minutes and then centrifuged at 800 RPM for 08 minutes. Supernatant was removed and hypotonic KCl was added. The tubes were incubated for 30 minutes at 37^0C and centrifuged. This was followed by addition of fixative (3:1, methanol:acetic acid). After three washings, 1-2 drops of fixed cell suspension were dropped on a glass slide to spread the chromosomes. The slides were then placed in oven at 90^0C for 1 hour and then treated with trypsin and stained with Giemsa stain. For chromosomal breakage studies in diagnosis of FA, Mitomycin C was also used in the culture medium. In case of CML, a non-stimulated blood/ bone marrow culture was established in RPM1 1640 medium.

Results

Total of 1703 individuals were enrolled in the study over a course of around 12 years. Out of total, 866 were registered as females and 837 as males.

Patients who were included in the study and had karyotyping for disorders of sex development (DSD), included those having a wide spectrum of clinical presentation like gender ambiguity, suspicion of Klinefelter syndrome (KF or KS), Turner syndrome (TS), delayed puberty, infertility, amenorrhea etc.

Out of a total 75 patients with DSD who were registered as females, 16 (21%) turned out to be genetically male (46, XY), while one had TS (45, X0). Similarly out of the 98 patients registered as males, 17 (17.3%) were found to be genetically females (46, XX). (Table 1)

Table 1: Patients who were enrolled for gender confirmation

Patient Registration	Number
Patients registered as females	**75**
karyotypically female	58
karyotypically male	16
Turner Syndrome	1
Patients registered as males	**98**
karyotypically male	78
karyotypically female	17
45,X0	1
45X0/46,XY (p-)	1
46,XY,t(1;4)	1
Grand Total	**173**

As a part of DSD, males who were clinically suspected of having KS (18 patients) and another 47 with semen analysis abnormalities, more than 14% were having KS. (Table 2)

Table 2: Male patients with Disorder of sex development

Clinical Indication	46,XYq-	47,XYY / 45,X0	KS	NF	NM	Grand Total
Infertility					50	50
Semen Abnormalities	1		7		39	47
Abnormal Genitalia		1	1		17	19
Suspicion of Klinefelter syndrome			3	1	14	18
Grand Total	**1**	**1**	**11**	**1**	**120**	**134**

Among the 96 females with DSD, patients presenting with primary amenorrhea, 5.2% were genotypically male and 02% having TS. (Table 3)

Table 3: Female patients with DSD

Diagnosis	45,XO /46,XX	46,X iso (xq)/45,XO variant TS	46,X,der (Y)	46,XX (Xp-q+)	Swyer syndrome	Males	Normal Females	TS	Grand Total
Primary amenorrhea			1	1		5	87	2	96
Secondary amenorrhea		1					36		37
Infertility						1	36		37
Menstrual Irregularities							16		16
Abnormal Genitalia (Internal & External)	1				1	1	10	2	15
Grand Total	**1**	**1**	**1**	**1**	**1**	**7**	**185**	**4**	**201**

186 children presented with clinical suspicion of having Down Syndrome(DS) (e.g monogoloid facies, delayed milestones, simian crease etc). After karyotyping, 171 were found to be karyotypically having trisomy 21 and 04 kids were having translocational DS.

Couples having history of recurrent pregnancy loss, previously having kids with congenital abnormalities or DS were also registered for karyotyping. (Table 4)

Known cases of hematological disorders like Aplastic Anemia were also analyzed for chromosomal breakage studies (characteristic of Fanconi Anemia) by karyotyping. Out of total 45 cases, 12 (26.6%) were reported for having chromosomal breakages. 27 cases of myeloid hyperplasia were subjected to karyotyping, out of them 20(74%) showed Philadelphia chromosome (t (9;22), confirming the diagnosis of Chronic Myeloid Leukemia.

Table 4: couples with RPL

Karyotype	Count
46,XX	315
46,XY	304
46 XY,t(3;13)	1
46,XX t(2;9)	1
46,XX,21p+	1
46,XX,22ps+	1
46,XY,t(4;5)	1
46,XY,yq-	1
46,XYp+	1
46,XYq-	1
47,XY,+mar	1
Grand Total	**628**
Parents having kids with genetic Abnormalities	
Karyotype	**Count**
46,XY	58
46,XX	28
Grand Total	**86**
Parents having DS children	
Karyotype	**Count**
46,XX	4
46,XY	3
Grand Total	**7**

Discussion

Down syndrome is the most prevalent chromosomal disorder with an overall incidence of 1 in 700 births. Limited studies have been published in developing countries for Down syndrome.

In our setup 171 out of 186 children with clinical suspicion of DS were found to have trisomy 21. Our findings concur with a study conducted in Mexico, where more than 85% of patients with clinical diagnosis of DS were also cytogenetically having it. In that study, 87% of the positive cases were non translocational DS, similar to that of our study (90%).[10]

Another study conducted by Amra Catovic and Sulejman Kendic et al also shows cytogenetic positivity for DS in 87% of suspected cases and out of positive cases >80% of cases were non-translocational DS,[11] likewise in our analysis.

Human sexual differentiation is a complex process under the influence of many genes and hormones.[12] People with DSD is a big concern for family. Proper and timely diagnosis needs cytogenetics as an integral part of evaluation and is essential for physical well-being and the physiological evolution of children with sexual obscurity.[13]

In our center among those registered as females, 21% turned out to be genetically male. Out of 98 patients registered as males, 17.3% were genetically females. These findings are in parallel with study conducted in India, where discrepancies between genetic gender and gender of rearing were found to be 27%.[14]

As a part of DSD, KS is the commonest chromosomal abnormality in humans but remain under diagnosed. This is because of wide spectrum of clinical presentation ranging from typical features of KS, developmental delay, dysmorphism to just infertility evaluation.In our study, among those having clinical suspicion of Klinefelter Syndrome and others with just semen analysis abnormalities, more than 14% were diagnosed with KS. Likewise a study conducted in Turkey revealed the incidence of KS to be 21.74%.[15]However a study conducted in South Indian Tertiary Care Center showed presence of 47XXY in 39% and 61% of considered pediatric and adult population respectively.[14] Another study conducted by Hiroshi Okada, Hitoshi Fujioka et al showed that those with clinical presentation of azoospermia only, 07% came out to be having KS[16], while in our study the percentage is 14%.

CML is a myeloproliferative disorder associated with pluripotent stem cells. Its diagnosis depends on molecular and cytogenetic analysis for the identification of the t (9;22)[17].

In Nepal, out of 35 patients with clinical suspicion of CML, 50% of patient were positive for Philadelphia chromosome.[18]

Another study conducted in Quetta and Faisalabad, 83% of the suspected cases of CML were found positive for t(9;22).[17] In our analysis of 27 cases, the percentage of positivity for t(9;22) is also high (74%) in those having clinical suspicion and myeloid hyperplasia.

Fanconi anemia is inherited bone marrow failure syndrome in hematopoietic stem cells leading to pancytopenia and aplastic anemia. In a study conducted in Delhi, considering 528 patients with aplastic anemia, 13.1% patients showed significantly increased chromosomal breakages.[19]At National Institute of Blood Diseases Karachi, 34% patients with diagnosis of Aplastic anemia were found to be positive for Fanconi anemia.[20]

Seema Korgaonkar, Kanjaksha Ghosh et al conducted a study on cases of AA, in whom chromosome breakage was detected in 21 (63.6%) patients.[21]

Conclusion

Proper assistance for children with a congenital disorder may include screening for cardiac defects, hearing and vision assessments and specialized education, which may not be adequately offered without a timely diagnosis. From our study, we conclude that cytogenetics provides us with an opportunity to improve the management of various disorders.

Conflicts of Interest: None declared.

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