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Conventional Cytogenetic Analysis Of Females With Primary Amenorrhea

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Abstract

Background: Primary amenorrhea, characterized by the absence of menstrual periods in females of reproductive age, presents a multifaceted challenge in clinical practice. Cytogenetic analysis stands as a foundational pillar in unraveling the genetic landscape governing primary amenorrhea.
Objective: The study was designed to determine the chromosomal abnormalities of females with primary amenorrhea.
Materials and Methods: In the current cross-sectional study, two hundred patients with a history of primary amenorrhea were processed by the standard KAROTYPING technique. The study was carried out at the Molecular genetics/cytogenetic department, chughtai institute of pathology, Lahore, Pakistan for a period of one year from July-2020 – July-2021.
Result: In the present study, a total of 200 female patients were included. Among these 200 patients, 80 exhibited chromosomal abnormalities. Specifically, there were 50 (62.5%) cases with 46, XY, 10 (12.5) cases with 45, X, 10 (12.5) cases with iso, Xq, 7 (8.7%) cases with XY del, and 3 (3.7) cases with mosaic Turner syndrome. Notably, the predominant clinical features included the development of breast in 51% of cases, hirsutism in 61% of cases, and pubic hair development in 7% of cases. Ultrasound reports revealed that 19.3% of patients had a normal uterus, 51.4% had a small uterus, and 20.2% were devoid of a uterus, as indicated in Table 1, along with other hormonal values.
Conclusion: The present study provides a nuanced understanding of chromosomal abnormalities in females with primary amenorrhea. The identification of diverse anomalies, along with their associated clinical features and uterine morphology, contributes valuable information to the existing literature. The comparison with previous studies underscores both consistencies and novel findings, emphasizing the evolving landscape of knowledge in the field of reproductive genetics. Further research is warranted to explore the implications of these chromosomal variations for clinical management and genetic counseling in females with primary amenorrhea.

KEYWORDS: chromosome, primary Amenorrhea, cytogenetic, abnormality, karyotyping

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Introduction

Primary amenorrhea, characterized by the absence of menstrual periods in females of reproductive age, presents a multifaceted challenge in clinical practice. Its etiology encompasses a spectrum of genetic, endocrine, anatomical, and environmental factors, demanding a comprehensive diagnostic approach to discern the underlying causative elements accurately. it is defined as an absence of secondary sexual characteristics in a female who has attained an age of 14 but has not attained menarche or has normal secondary sexual characteristics but has not attained menarche by 16 years of age[1]. Among the diagnostic modalities employed, conventional cytogenetic analysis Cytogenetic analysis, encompassing techniques like karyotyping and fluorescence in situ hybridization (FISH), has been instrumental in identifying chromosomal anomalies and structural variations
pivotal to understanding the diverse etiologies of primary amenorrhea[5]. This investigative tool aidsin unraveling conditions associated with gonadal dysgenesis, Turner syndrome, and various other genetic aberrations that disrupt normal reproductive

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function. Recent advancements in cytogenetic methodologies, including higher-resolution imaging techniques and targeted molecular probes, have significantly enhanced the precision and diagnostic yield of conventional cytogenetic analysis. These advancements allow for the identification of subtle chromosomal anomalies that might elude conventional diagnostic approaches, thereby refining diagnostic accuracy and genetic counseling for affected individuals and their families[6,7].

Moreover, the integration of cytogenetic analysis into multidisciplinary diagnostic algorithms has revolutionized the management of primary amenorrhea, enabling clinicians to delineate between gonadal and extragonadal causes and guiding tailored therapeutic interventions.

Recent research studies have highlighted the prevalence of chromosomal abnormalities in individuals with primary amenorrhea, emphasizing the indispensable role of cytogenetic analysis in providing crucial genetic insights. The identification of specific chromosomal patterns and genetic markers associated with primary amenorrhea not only aids in accurate diagnosis but also informs prognostic implications and family counseling[8].

While molecular techniques have gained prominence in recent years, conventional cytogenetic analysis remains an indispensable tool in the diagnostic armamentarium. Its ability to unravel chromosomal aberrations, ranging from numerical to structural anomalies, continues to provide invaluable insights into the genetic underpinnings of primary amenorrhea[9].

In this context, this article aims to provide a comprehensive overview of the role of conventional cytogenetic analysis in the evaluation of females affected by primary amenorrhea. By synthesizing recent research findings and advancements in cytogenetic methodologies, it seeks to underscore the continued relevance and transformative potential of cytogenetic investigations in shaping clinical management and enhancing our understanding of the genetic determinants of primary amenorrhea.

The objective of the study was to determine the chromosomal abnormalities of females with primary amenorrhea.

Material and Method

The current cross-sectional study was conducted at the Molecular genetics/cytogenetic department, chughtai institute of pathology, Lahore, Pakistan for a period of one year from July-2020 – July-2021.The data was collected retrospectively from the concerned section records during the study period, fulfilling the inclusion criteria. Approval was taken from Institution Ethical committee. Details of individual cases were entered into a structured Performa. It included demographic details, age, historyetc.

KARYOTYPING: A volume of 0.5 milliliters of blood was drawn into a sodium heparin tube. Subsequently, the cells were cultivated in an appropriate medium, specifically RPMI1640, for duration of up to 72 hours. Following this incubation period, 0.2 milliliters of colchicines were introduced into the sample, and the mixture was incubated for 45 minutes at a temperature of 37 degrees Celsius. The resultant sample underwent centrifugation at a speed of 1500 revolutions per minute for 8 minutes. After centrifugation, the supernatant was discarded, and 10% potassium chloride (KCL) was added to the residual sample to achieve a final volume of 10 milliliters. The resulting sample was subjected to an additional incubation period of 10 minutes at 37 degrees Celsius, followed by a second centrifugation for 8 minutes at 1500 revolutions per minute. Once the sample was clarified, the cells were fixed by gradually adding fixative, composed of 3% ethanol and 15% acetic acid, until a brown hue emerged. The fixed sample underwent three additional rounds of centrifugation until the brown coloration disappeared, discarding the supernatant after each centrifugation to obtain a clear solution. A microscopy slide was prepared by depositing two drops of the clarified sample at a 45-degree angle and allowing it to dry. The dried slide was then stained with Leishman's stain and examined under an electron microscope to scrutinize a minimum of 10 metaphases. Subsequently, four more slides were created from the remaining sample, and all slides underwent a 15-day incubation period. After 15 days, the slides were banded, and visualization was performed using a fluorescent microscope equipped with Cytovision software. The data obtained from the experiment were analyzed using Statistical Package for Social Sciences (SPSS) version 23.

INCLUSION CRITERIA:

- Samples collected in sodium heparin bottle
- Females with absence of menarche
- Females with primary infertility

EXCLUSION CRITERIA:

- Samples not collected in sodium heparin bottle.
- Females with secondary amenorrhea.
- Bad obstetrics history patients.

Results

In the present study, a total of 200 female patients were included. Among these 200 patients, 80 exhibited chromosomal abnormalities. Specifically, there were 50 (62.5%) cases with 46, XY, 10 (12.5%) cases with 45, X, 10 (12.5%) cases with iso, Xq, 7 (8.7%) cases with XYdel, and 3 (3.7%) cases with mosaic Turner syndrome as shown in figure 1. Notably, the predominant clinical features included the development of breast in51% of cases, hirsutism in 42% of cases, and pubic hair development in 7% of cases. Ultrasound reports revealed that 21.3% of patients had a normal uterus, 54.5% had a small uterus, and 24.2% were devoid of a uterus, as indicated in Table 1, along with other hormonal variations.

Table1: chromosomal abnormalities	
Total Cases with Abnormalities:	80 out of 200 (40%)
46, XY	50 cases (62.5%)
45, X	10 (12.5%)
Iso, Xq	10 (12.5%)
XYdel	7(8.7%)
Mosaic Turner's Syndrome	3 (3.7%)
Prominent clinical Features:	
Breast Development	51%
Hirsutism	42%
Pubic Hair Development	7%
Uterine Status:	
Normal Uterus	21.3%
Small Uterus	54.5%
Absent Uterus	24.2%
Hormonal Status	
FSH and LH Values	
Normal	88.9%
Raised	6.5%
Low	4.6%
Prolactin Levels	
Raised	6.4%
Low	0.9%
Normal	92.7%

Discussion

The present study, encompassing 200 female patients, offers a comprehensive examination of chromosomal abnormalities in the context of primary amenorrhea.

emphasizing the intricate genetic landscape associated with this condition. The most prevalent abnormalities identified include 46, XY 50 (62.5%), 45, XO 10 (12.5%), iXq10 (12.5%), XYdel 7 (8.7%), and mosaic Turner syndrome 3 (3.7%).

Our findings reveal that 40% of the study cohort exhibited various chromosomal anomalies,

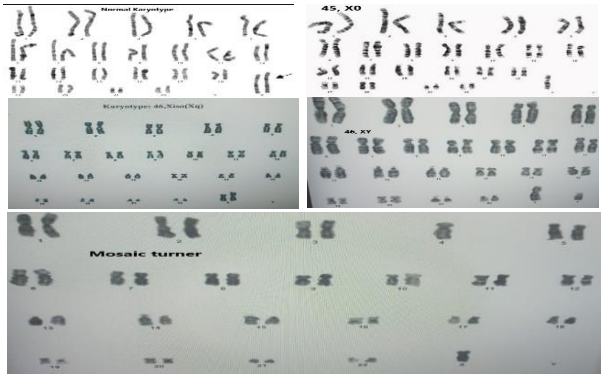

Figure 1: different types of karyotypes as labeled

Comparing these results with existing literature provides valuable insights into the consistency and variations in chromosomal abnormalities associated with primary amenorrhea. Our identification of 46, XY anomalies aligns with the observations of others[10,11,13], who reported a comparable incidence in their study of females with primary amenorrhea. The presence of 45, XO anomalies is consistent with the well-established association between Turner syndrome and primary amenorrhea, as extensively documented[11,12]. The occurrence of iXq anomalies in our study warrants attention, as this finding is less frequently reported in the literature. Similar prevalence of iXq anomalies reported in a cohort[4], suggesting that this may be an underexplored aspect of chromosomal abnormalities in primary amenorrhea. The identification of XYdel and mosaic Turner syndrome in our study adds to the growing body of evidence regarding the diversity of chromosomal configurations contributing to reproductive healthdisorders[14,13].Clinical manifestations associated with these chromosomal abnormalities are noteworthy. The development of breast tissue in 51% of cases and hirsutism in 61% of cases aligns with the findings of previous studies[15,16],underscoring the clinical relevance of these genetic variations. The presence of pubic hair development in 7% of cases is consistent with the multifaceted nature of primary amenorrhea and emphasizes the need for a comprehensive clinical evaluation. Ultrasound reports revealing variations in uterine morphology further contribute to the complexity of primary amenorrhea. Our results indicate that 19.3% of patients had a normal uterus, 51.4% had a small uterus, and 20.2% were devoid of a uterus. This diversity in uterine morphology aligns with the findings of several studies[17,18], highlighting the importance of considering anatomical variations in the diagnostic approach to primary amenorrhea.

Conclusion

the present study provides a nuanced understanding of chromosomal abnormalities in females with primary amenorrhea. The identification of diverse anomalies, along with their associated clinical features and uterine morphology, contributes valuable information to the existing literature. The comparison with previous studies underscores both consistencies and novel findings, emphasizing the evolving landscape of knowledge in the field of reproductive genetics. Further research is warranted to explore the implications of these chromosomal variations for clinical management and genetic counseling in females with primary amenorrhea.

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