Trend of Chromosomal Disorders in Patients Referred for Chromosomal Analysis in Cytogenetic Lab of BPKIHS: An Institutional Based Retrospective Study

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ABSTRACT

Introduction: Chromosome abnormalities are the results of alterations in the number or structure of chromosomes causing significant human morbidity and mortality. Cytogenetic analysis is crucial in identifying these abnormalities and guiding genetic counseling and clinical management. The main objective is to describe the patients having chromosomal analysis on the basis of their demographic profile, cause of referral and referral central; and to estimate the distribution of different chromosomal aberration among those patients who underwent chromosomal analysis.

Methods: All the patient referred to the cytogenetic lab of B.P Koirala Institute of Health Sciences, Nepal between 2004 and 2015 for chromosomal analysis were assessed from the record of lab. Demographic data, referring department/ clinic, patients karyotype and frequency of distribution of various karyotype was assessed.

Results: Most of the patients were between 20 and 50 years old, and 64% were male. The most common reasons for referral were suspected Chronic myeloid leukemia (CML) (n=215), Down's syndrome (n=27), amenorrhea, and ambiguous genitalia. BPKMCH was the most common referral center to refer the cases in cytogenetic lab (63.7% of cases). Philadelphia-positive CML, Down's syndrome, and Turner's syndrome were the most common cytogenetic diagnoses made.

Conclusion: Suspected CML was the primary reason for referral. Most patients were aged between 20 to 50 years, with males comprising 64% of the cohort. Philadelphia positive CML was the most common cytogenetic diagnosis. Referrals peaked in 2005 AD, primarily from BPKMCH.

Keywords: Cytogenetic; Karyotyping, Leukemia; Chromosome abnormalities.

INTRODUCTION

Since many genetic abnormalities can be directly related to the chromosomal pattern, the characterization of chromosomes is of considerable diagnostic importance. This can be achieved through a process called "cytogenetics", which involves a photographic representation of a stained preparation in which the chromosomes are arranged in a standard manner. The term "karyotype" refers to the constitution of chromosomes of an individual.[1]

Genetic disorders, including congenital malformations, cancer, and metabolic disorders, are often associated with chromosomal abnormalities.[2] These abnormalities, caused by alterations in chromosome number or structure can lead to significant morbidity and mortality, with around 7.5% of conceptions affected.[3,4] Chromosome analysis is essential for the diagnosis and evaluation of genetic disorders, including developmental delays and intellectual disability.[5] Numeric and structural chromosomal aberrations, such as aneuploidies and translocations, can be identified through conventional cytogenetic analysis.[6] Most chromosomal diseases result in mental retardation or fetal malformations and are often associated with specific or general body features, such as the simian crease seen in Down syndrome.[7]

The incidence and trend of chromosomal disorders among patients referred for chromosomal analysis of

Nepal remain inadequately understood. Children with Down syndrome (DS) have recognizable physical characteristics, limited intellectual abilities, and ocular abnormalities dueto the extra chromosome 21. Nepalese children with DS have a high prevalence of refractive error and nystagmus. Regular eye exams are recommended for early diagnosis and proper management of ocular disorders to improve vision and quality of life.[8] Congenital malformations are a common cause of morbidity and mortality, not only in newborns but also in childhood and beyond, with many having a calculated recurrence risk. Identifying congenital malformations at birth is crucial for providing timely counseling to parents. For instance, the risk of a female carrier producing another Down syndrome child is 15%.[9]

Chromosomal aberrations cause diverse functional problems in various organs and are usually associated with clinical and intellectual problems that require medical and social support.[10] Information about the most common cause of referral for chromosomal analysis and the frequency of chromosomal abnormalities among individuals who show some pathological or clinical features suggesting the presumable presence of some chromosomal abnormalities is scarce in Nepal. The authors believe that awareness of these frequencies will help clinicians determine the priority of requesting a cytogenetic study in individual cases. It should also help recognize the most common presentations of prevalent chromosomal abnormalities in the area, allowing proper genetic counseling to be offered.

METHODS

A retrospectice quantitative study was conducted to examine the baseline variables including the patient's demographic characteristics such as age and gender, cause of referral, referral institution/department, and the patient's karyotype. All of these variables were noted from the patient's record file of cytogenetic lab of BPKIHS. The outcome variables of the study include the frequency of different chromosomal aberrations, the temporal pattern of referral of patients for chromosomal analysis, and the pattern of referral from different departments and institutions for chromosomal analysis.

The study was conducted at the department of human anatomy, as the Cytogenetic lab is one of its units, making it a feasible site for the research. The study population comprised all patients who were referred to the Cytogenetic lab from 2004-2015 AD for chromosomal analysis. A convenience sampling method was used to select all cases referred from different departments and institutions for chromosomal analysis, and the minimum sample size required was determined to be 167 by using the prevalence of chromosomal aberrations from previous study[5]. At the end the patients who were who were referred to the Cytogenetic lab from various departments or institutions for chromosomal analysis were included in the study. Two types of samples were taken for the purpose of cytogenetic analysis. Bone marrow was taken from most numbers of cases (67.4%). Peripheral venous blood was taken from the 32.6 % of cases.Samples were first cultured for 72 hours using RPMI media, calf serum and phytohemagglutinin. After this stage colchicine was added and samples were harvested. Samples were stained with Giemsa stain. The preparation was then screened under 10X objectives using Zeiss light microscope. Well spread metaphase were further analyzed under 100X oil immersion objectives. Minimum of 20 spreads were analyzed for determining the karyotype.

The data collection technique involved assessing the record files of all the patients who were referred to the Cytogenetic lab from 2004 to 2015, with permission from the department of anatomy. The relevant information was entered into a preformed proforma and then transferred to an Excel sheet for further analysis. Data from the case sheets of the record section were entered into Microsoft Excel Chart and checked for consistency. The data were then entered into SPSS version 22 for statistical analysis. Descriptive analysis was performed by calculating the percentage, arithmetic mean or median. Prior to the commencement of the study, ethical clearance was obtained from the Institutional Review Committee (IRC/2187/022), in accordance with the objective of the study, which involved using data of the human subjects who were referred to the Cytogenetic lab.

RESULTS

The present study constitutes a retrospective cross-sectional analysis encompassing 322 cases referred from diverse clinics, departments, and wards to undergo cytogenetic analysis at the Cytogenetic Laboratory within the Department of Human Anatomy. The age range of the referred patients spanned from under one month to over 50 years. The majority of patients fell within the age bracket of 20 to 50 years. Among the participants, 206 individuals (64%) were identified as male. Cause of referral: suspected CML (66.8%), suspected Downs's syndrome (8.4%), amenorrhea (4.3%), ambiguous genitalia (3.4%), missed conception and miscarriage (2.8%), and delayed physical and intellectual development (1.9%) were the major causes of referral. Other less frequent causes of referral are presented in (Table 1). A lack of documented cause for

referral was observed in 22 cases, accounting for 6.8% of the total cases examined.

Referral by year: The study spans referrals from 2004 AD to 2015 AD. The highest number of cases were referred in 2005 (25.2%), followed by 2006 (18.9%), 2007 (18.3%), 2009 (9%), and 2004 (7.1%). Only three cases were referred in 2015 (Figure 1). Karyotype of the patients: The predominant karyotype observed among the cases subjected to karyotyping was the presence of Philadelphia positive chromosomes, accounting for 64.6% of the studied cases. Approximately 19% exhibited a normal karyotype, characterized by either 46 XX or 46 XY configurations. Notably, twenty-nine cases presented with a karyotype indicating trisomy 21. Additionally, less frequently observed karyotypes included 45 (XO) and Philadelphia negative chromosomes, as detailed in Table 2 and Figure 2.

Table 1: Frequency distribution of causes of referral for the cytogenic analysis

Causes of referral	Frequency	Percent
Not in record	22	6.8
Suspected downs syndrome	27	8.4
Delayed physical and intellectual development	6	1.9
Suspected CML	215	66.8
Ambiguous genitalia	11	3.4
Amenorrhea	14	4.3
Missed conception and miscarriage	9	2.8
Missing uterus	2	0.6
Drug therapy for CML	1	0.3
Bloom syndrome	1	0.3
Anophthalmic socket	1	0.3
Small or no testicle	2	0.6
Enlarged testis	1	0.3
Infertility	2	0.6
Missing ovaries	1	0.3
Small neck	2	0.6
Large tongue	2	0.6
Low set ear and flat nose	1	0.3

Cytogenetic diagnosis: The most prevalent cytogeneticdiagnosis made was Philadelphia-positive Chronic Myeloid Leukemia (CML), accounting for 64.6% of the diagnoses. Other frequently identified diagnoses included complete trisomy Down syndrome, mosaic forms of Down syndrome and Turner syndrome, as well as Philadelphia negative CML. Further details regarding the distribution of these diagnoses are presented in Table 3.

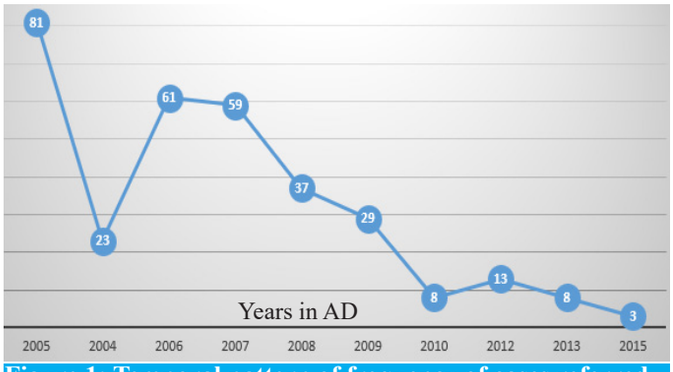

Figure 1: Temporal pattern of frequency of cases referred for cytogenetic analysis.

Table 2: Frequency distribution of different Karyotypes

Karyotype made	Frequency	Percent	Cumulative %
Not in record	1	0.3	0.3
46 XY	29	9.0	9.3
46 XX	32	9.9	19.3
47,XY+21	10	3.1	22.4
47, XX+21	8	2.5	24.8
47, XY+21 & 46 XY	8	2.5	27.3
47, XX+21 & 46 XX	3	0.9	28.3
45 XO	7	2.2	30.4
46 XX & 45 XO	8	2.5	32.9
46 XY & 45 XO	2	0.6	33.5
PH positive	208	64.6	98.1
PH negative	6	1.9	100.0

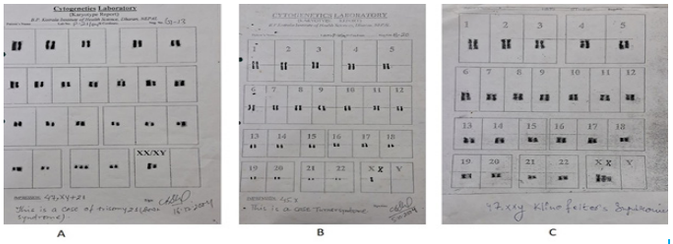

Figure 2: Karyotype of some of the syndromes suspected during cytogenetic analysis. A: Down's syndrome; B: Turner's syndrome; Klinefelter syndrome

Table 3: Frequency of different Cytogenetics diagnosis

Cytogenetic diagnosis made	Frequency	Percent
Not in record	1	0.3
Philadelphia positive CML	208	64.6
Mosaic Turner's syndrome	12	3.7
Down's syndrome (complete trisomy)	18	5.6
Mosaic Down's syndrome	5	1.6
Normal male	31	9.6
Normal female	32	9.9
Klinefelter syndrome	2	0.6
Complete Turner's s syndrome	7	2.2
Philadelphia negative CML	6	1.9

DISSCUSSION

Currently, karyotype analysis remains the primary and most commonly utilized method for diagnosing chromosomal disorders. This study investigated the prevalence of chromosomal abnormalities confirmed by karyotyping among referred cases with suspected genetic anomalies from various clinics and outpatient departments (OPDs). A total of 322 cases were included in this analysis. Notably, approximately 72% of the referred cases exhibited some form of chromosomal aberration. The predominant prevalence was attributed to a higher volume of patients referred for suspected Chronic Myeloid Leukemia (CML), constituting about 67% of the total referrals. During the initial years, due to a scarcity of other facilities equipped for Philadelphia chromosome detection, this center received referrals from various regions across Nepal. Even though data about role of genetics among many metabolic disorders and bleeding disorders are available from Nepal.[11,12]

The study about the chromosomal disorders among Nepalese population are very scant. Professor Dr. CB Jha had highlighted the importance of knowledge of human genetics among the school students during their reproductive age. The study conducted around 15 year back showed that knowledge about human genetics was poor. Majority of students were not aware of about the of various genetic disorders in the community.[13]

In a retrospective cytogenetic investigation conducted by Zhang et al, involving 5328 fetuses exhibiting abnormal sonographic findings in the first or second trimester, the study revealed a statistically significant probability of an abnormal karyotype across various anatomical systems.[6] Similarly, the present study underscores that several anatomical anomalies leading to patient referrals for cytogenetic testing might indeed stem from underlying chromosomal aberrations. Likewise, in a separate cytogenetic investigation involving 117 Korean patients referred due to suspected chromosomal abnormalities, the study revealed that chromosome aberrations were detected in 17.5% of cases. Among these abnormalities, Down syndrome and Turner syndrome were identified as the most common anomalies.[10] Moreover, in an additional retrospective study conducted among 859 patients referred for chromosomal analysis in India, findings indicated that 43.1% of these individuals displayed chromosomal abnormalities. Among the identified abnormalities, Down syndrome (DS) was prevalent in 81.4% of autosomal abnormalities, while Turner syndrome (TS) represented 13.7% of sex chromosomal abnormalities. Numerical abnormalities were responsible for 41.0% of cases, while structural abnormalities accounted for 2.0%.[3] The present study revealed an overall prevalence of chromosomal disorders at 72%, predominantly due to suspected cases of Chronic Myeloid Leukemia (CML) and Down syndrome referrals. Numerical chromosome disorders accounted for 13.7% of cases, with Down syndrome being the most prevalent. This syndrome, a well-known neurodevelopmental disorder with a recognized genetic basis, manifests with facial dysmorphologies, congenital and/or acquired medical conditions, intellectual disability, accelerated aging, and an increased predisposition to early onset Alzheimer's disease in adulthood.[14]

In a study conducted by Balkan et al, which included 4216 patients referred to the Cytogenetic Unit at Southeast Turkey between 2000 and 2009, Down syndrome and repeated abortion emerged as the primary reasons for referral for cytogenetic analysis. Among the identified chromosomal abnormalities, sexual chromosomal anomalies were observed in 239 cases (17.6%), with Klinefelter syndrome being the most frequent. Autosomal abnormalities were detected in 1119 cases (82.4%), with Down syndrome being the most prevalent autosomal chromosomal abnormality.[5]

The sex ratio analysis in Downs's syndrome consistently exhibited a higher prevalence among males compared to females.[15] In alignment with this trend, the present study observed a higher proportion of males (16 males and 7 females) within the studied population. Conversely, Turner syndrome (TS) cases predominantly comprised females (17 out of 19 cases). Generally, the sex ratio (SR) for Downs's syndrome tends to display a skew towards an excess of males across various studied populations, including those with high levels of case ascertainment in epidemiological studies or within selected groups.[16] Chronic Myeloid Leukemia (CML) constitutes approximately 15% of all leukemias affecting adults.[17] It predominantly affects males, with a ratio of about 3:2 compared to females.[18] Characteristically, CML is marked by the presence of the Philadelphia (Ph) chromosome or the breakpoint cluster region-Abelson murine leukemia 1 (BCR-ABL1) rearrangement located at t(9;22)(q34.1;q11.2).[19] Diagnosis of CML involves a combination of peripheral blood (PB) examination and identification of the Ph chromosome through karyotyping using bone marrow (BM) samples or detecting BCR-ABL1 through real-time quantitative polymerase chain reaction (RqPCR) in PB or BM samples.[20] In the present study, 66.8% (n=215) of cases were referred due to suspicion of CML. Among these cases, 208 were diagnosed with Philadelphia positive CML, while six cases were found to be Ph negative. Additionally, one case was referred specifically for assessing drug therapy for CML. In the

current study, cases referred due to ambiguous genitalia were notable (n=11). Alongside pelvic ultrasound, karyotyping stands as a crucial laboratory method for investigating such anomalies.[21] Several cases were also referred due to conditions such as small testes, enlarged testes, absence of ovaries, and absent uterus. The precise incidence of disorders of sex development (DSD), inclusive of ambiguous genitalia, remains incompletely understood. In 2000, Fausto- Sterling suggested that it corresponds to 1.7% of live births.[22] Furthermore, one case involved a referral for cytogenetic testing due to congenital anophthalmos, a rare condition resulting from arrested embryogenesis during optic vesicle formation.

CONCLUSION

This retrospective study of 322 patients referred for chromosomal analysis from 2004 to 2015 highlighted that suspected CML was the primary reason for referral (215 cases). Most patients were aged between 20 to 50 years, with males comprising 64% of the cohort. Philadelphia positive CML was the most common cytogenetic diagnosis (66.1%). Referrals peaked in 2005 AD, primarily from BPKMCH (63.7%). These findings emphasize the relevance of cytogenetic analysis in diagnosing CML and other chromosomal abnormalities, supporting the need for accurate diagnosis aiding in effective management and genetic counseling.

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