Original Paper

COMPUTER ASSISTED HUMAN KARYOTYPING – AN ANALYSIS OF 131 CASES

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Abstract

Introduction: Karyotyping is the process of pairing and ordering all the chromosomes of an organism, thus providing a genome-wide snapshot of an individual's chromosome. G-banded karyograms are routinely used to diagnose a wide range of chromosomal abnormalities in individuals. Although the resolution of chromosomal changes detectable by G-banded karyotyping is typically a few megabases, this can be sufficient to diagnose certain categories of abnormalities.

Objectives: The primary aim of this study was to investigate the different types of chromosomal aberrations and their relative frequencies in a group of referred patients with suspected genetic disorders.

Methods: This observational study was carried out at Armed Forces Institute of Pathology (AFIP) for a period of two years from January 2011 to December 2012. A total of 131 patients were included in this study. These patients were referred to AFIP from different Combined Military Hospitals (CMH) of Bangladesh Army and also from civil medical installations. All the patients were subjected to full genetic study; complete genetic examination and pedigree construction was done to exclude nonchromosomal causes of anomaly. Detailed history and physical findings were also noted in a prescribed format. The study included peripheral lymphocyte culture by a standard method using the G–banding technique.

Results: Out of 131 patients, 54 (42.2%) were male and 77 (57.8%) were female with male to female ratio 0.7:1. The age limit of the patient ranges from 04 days to 70 years. Most of the patients (32.1%) were in the age group of 0–10 years followed by 21–30 year age group (30.5%). Consanguineous marriage was found in 15 (11.5%) cases in which 3 (2.3%) cases had chromosomal aberrations. Recurrent abortion was the main clinical indication (18.3%) followed by infertility (15.3%). Chromosomal aberrations were detected in 26 patients (19.8%); of these, 20 (15.2%) involved autosomes, while only 6 (4.6%) involved gonosomes. Trisomy 21 was detected in 12 (9.1%) patients and Philadelphia chromosome was found in 8 (6.1%) patients. Turner syndrome was detected in 5 (3.8%) patients and Klinefelter syndrome was found in 1 (0.8%) patient.

Conclusion: The precise delineation of different types of chromosomal aberrations is only possible using clinical examination and advanced cytogenetic tools by experienced cytogeneticists.

Key-words: G-banded karyotyping, Chromosomal aberrations, Recurrent abortion, Infertility, Trisomy 21, Philadelphia chromosome, Turner syndrome, Klinefelter syndrome.

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Introduction

To date, around 20000 chromosomal abnormalities have been registered on laboratory data bases. While on individual basis most of these are very rare, together they make a major contribution to human morbidity and mortality. Chromosome abnormalities account for a large proportion of spontaneous pregnancy loss and childhood disability, and also contribute to the genesis of a significant proportion of malignancy in both childhood and adult life as a consequence of acquired somatic chromosome aberrations[1].

Chromosome abnormalities are present in at least 10% of all spermatozoa and 25% of mature oocytes[1]. Between 15% and 20% of all recognized pregnancies end in spontaneous miscarriage, and many more zygotes and embryos are so abnormal that survival beyond the few days or weeks after fertilization is not possible. Approximately 50% of all spontaneous miscarriages have a chromosome abnormality and the incidence of chromosomal abnormalities in morphologically normal embryos is around 20%. These observations indicate that chromosome abnormalities account for the loss of a very high proportion of all human conceptions. From conception onwards the incidence of chromosome abnormalities falls rapidly. By birth it has declined to a level of 0.5 – 1%, although the total number is much higher (5%) in stillborn infants[1].

The impact of chromosomal abnormalities is greatest during foetal life when they have their highest frequency and represent a major cause of fetal loss[2]. The frequency of chromosomal abnormalities is quite different in neonates (0.7%) as compared to abortuses (about 50%), some aneuploidies are lethal in utero[3]. The major autosomal abnormalities share a number of phenotypic features that are not distinctive or specific, including mental retardation, cardiac malformation and growth deficiency. While there is variability within every cytogenetic syndrome, neonatal death and serious congenital malformations are frequent manifestations. Most of the specific cytogenetic syndromes have a constellation of features that distinguish them and allow the clinician to suspect the condition[2].

Karyotyping is the process of pairing and ordering all the chromosomes of an organism, thus providing a genome-wide snapshot of an individual's chromosome. Karyotypes are prepared using standardized staining procedures that reveal characteristic structural features for each chromosome. Clinical cytogeneticists analyze human karyotypes to detect gross genetic changes– anomalies involving several megabases or more of DNA. Karyotypes can reveal changes in chromosome number associated with aneuploid conditions, such as trisomy 21 (Down syndrome). Careful analysis of karyotypes can also reveal more subtle structural changes, such as chromosomal deletions, duplications, translocations or inversions. In fact, karyotypes are becoming a source of diagnostic information for specific birth defects, genetic disorders and even cancer[4]. Today, G-banded karyograms are routinely used to diagnose a wide range of chromosomal abnormalities in individuals. Although the resolution of chromosomal changes detectable by G-banded karyotyping is typically a few megabases, this can be sufficient to diagnose certain categories of abnormalities[5]. The primary aim of this study was to investigate the different types of chromosomal aberrations and their relative frequencies in a group of patients with suspected genetic disorders and to identify precisely the role of cytogenetic investigation in confirming the diagnosis.

Materials and Methods

This study was conducted at Armed Forces Institute of Pathology (AFIP) for a period of two years from January 2011 to December 2012. A total of 131 patients were included in this study. These patients were referred to AFIP from different Combined Military Hospitals (CMH) of Bangladesh Army and also from civil medical installations. All the patients were subjected to full genetic study; complete genetic examination and pedigree construction was done to exclude nonchromosomal causes of anomaly. Detailed history and physical findings were also noted in a prescribed format. The study included peripheral lymphocyte culture by a standard method using the G-banding technique according to Seabright[6].

The protocol for the preparation of the chromosomes for karyotyping was as follows: About 2 ml of heparinized blood was collected in evacuated test tube from peripheral veins of the referral patients. Lymphocytes were grown in PB Max karyotyping media containing antibiotics (penicillin/ streptomycin) and 15% serum supplementation. Phytohaemagglutinin (PHA) was added as a mitotic stimulant (0.5 ml of the inoculum) and the samples were incubated for 72 hours at 37^0C incubator. The cells were arrested at metaphase with 0.1% colchicine; chromosome elongation was accomplished by adding 1% ethydium. Hypotonic treatment was done with KCL solution and cells were fixed with 3 changes of fixative (3:1, methanol: glacial acetic acid). The prepared slides were stained with GTG (G-bands using Trypsin and Geimsa stain). Chromosome analysis was done under 100 x, magnification[7]. At least 30 metaphase spreads were screened for each patient and 5 metaphases were captured using a CCD (Charge Coupled Device) camera. The captured picture was further enhanced by adjusting the sharpness, brightness and contrast and the printout was taken[8].

Results

A total of 131 patients' karyotyping was analyzed in this study. Out of them, 54 (42.2%) were male and 77 (57.8%) were female with male to female ratio 0.7:1. The age limit of the patient ranges from 04 days to 70 years. Most of the patients (32.1%) were in the age group of 0–10 years followed by 21–30 year age group (30.5%) (Table-I).

Table-I: Age distribution of the patients (n=131).

Age (yrs.)	Frequency	Percentage (%)
0 – 10	42	32.1
11 – 20	30	22.9
21 – 30	40	30.5
31 – 40	14	10.7
41 – 50	02	1.5
51 – 60	01	0.8
61 – 70	02	1.5
Total	**131**	**100**

The clinical indication of karyotyping is shown in Table-II. Recurrent abortion was the main clinical indication (18.3%) followed by infertility (15.3%).

Table-II: Clinical indications of cases referred for karyotyping (n=131).

Clinical indication	Frequency	Percentage (%)
Recurrent abortion	24	18.3
Infertility	20	15.3
Congenital malformation	15	11.5
Developmental delay	13	9.9
Growth retardation	09	6.9
Short stature	08	6.1
CML	08	6.1
Delayed puberty	07	5.3
Mental retardation	07	5.3
Secondary infertility	06	4.6
Abnormal/Ambiguous genitalia	04	3.1
Gynaecomastia	03	2.3
Protruded tongue	02	1.5
Neonatal jaundice	02	1.5
Parents of Down's baby	02	1.5
Tall stature	01	0.8
Total	**131**	**100**

Out of 131 patients on whom chromosomal analysis was done, normal karyotyping was found in 105 patients (80.15%) and chromosomal aberrations were detected in 26 patients (19.85%). Among 26 patients of chromosomal aberrations 20 (76.92%) involved autosomes, while only 6 (23.08%) involved gonosomes (Table-III).

Table-III: Distribution of chromosomal aberrations (n=26)

Type of Chromosomes involved	Frequency	Percentage (%)
Autosomes	20	76.92
Gonosomes	06	23.08

Among autosomal aberrations, trisomy 21 was detected in 12 (60.0%) patients and Philadelphia chromosome was found in 8 (40.0%) patients. Among trisomy 21, 7 (58.3%) patients had classic trisomy 21 and 5 (41.7%) patients had mosaic trisomy 21 (Table-IV).

Table-IV: Distribution of autosomal aberrations (n=20)

Autosomal chromosome aberrations	Frequency	Percentage (%)
Trisomy 21	12	60.0
Classic trisomy 21(47, XX +21 = 03; 47, XY +21 = 02)	7	58.3
Mosaic trisomy 21(46, XX/47,XX +21=2; 46, XY/47, XY +21=3)	5	41.7
Philadelphia chromosome	8	40.0

Among gonosomal aberrations, Turner's syndrome was detected in 5(83.3%) patients and Klinefelter syndrome was found in 1(16.7%) patients. Two patients (40.0%) had classic Turner syndrome and 03 (60.0%) patients had mosaic Turner syndrome (Table-V).

Table-V: Chromosomal aberrations involving onosomes (n = 6)

Gonosomal aberrations	Frequency	Percentage (%)
Turner syndrome	5	83.3
Classic Turner syndrome(45, X)	2	40.0
Mosaic Turner syndrome (45, X/46, XX)	3	60.0
Klinefelter syndrome (47, XXY)	1	16.7

In this study, consanguineous marriage was found in 15 (11.5%) cases in which 3 (20%) cases had chromosomal aberrations (Table-VI).

Table-VI: Correlation between consanguineous marriage and chromosomal aberrations.

Traits	Frequency	Percentage (%)
Consanguineous marriage (n=131)	15	11.5
Chromosomal aberrations (Down syndrome, n=15)	3	20.0

Discussion

In this study, out of 131 patients' chromosomal aberrations were found in 19.85% cases. There are wide variations in the frequency of chromosomal aberrations in individuals suspected of having genetic disorders[9,10]. Berry et al. studied 114 patients and found chromosomal aberrations in 18 (15.8%)[11]. Navsaria et al. evaluated 1000 patients and found chromosomal aberrations in 160 (16%)[12]. Al-Awadi et al. studied 472 patients and found 92 cases (19.5%)[13]. Al-Arrayed reported a frequency of 27% among 500 patients[14]. Verma and Dosik found a frequency of 27.1% among 357 patients[15] and Singh reported a frequency of 28.8% among 451 patients[16]. Incidence of chromosomal aberrations in this study correlates with different studies. In consecutive neonatal studies, autosomal abnormalities are usually as common as sex chromosome aberrations[17]. In studies based on a referred population with phenotypic abnormalities, such as the present study, autosomal abnormalities (76.92%) are much higher than those of the gonosomes (23.08%). This figure correlates with other studies[18,19]. This is mainly due to the fact that sex chromosome imbalance has a much less deleterious effect on the phenotype than does autosomal aneuploidy[20].

Trisomy 21 has been recognized for more than 100 years. Because it is a common and familiar disorder. Down syndrome has been studied much more thoroughly than other chromosomal disorders. The Down syndrome phenotype is due to triple amount of chromosome [21]. The frequency of Down syndrome in patients with abnormal chromosomes in the present study is 60%. This value was lower than other surveys[13,19,21]. This could be attributed to the karyotyping of only referred cases. The frequency of classic trisomy 21 amongst Down syndrome in this study was 58.3%. This value differs from other studies which ranged from 84.6% to 90%[17]. This difference could be due to low sample size and analysis of only referred cases. The incidence of mosaicism in Down syndrome patients in the present study is 41.7% which is much higher than reported range between 0% and 4%. This could be due to fact that actual level depends on the maternal age distribution and the rate of indication for prenatal diagnosis. In this study, Philadelphia chromosome was found in 8 diagnosed cases (40%) of chronic myeloid leukaemia among 20 autosomal chromosomal aberrations. Philadelphia chromosome was found in all referred cases of chronic myeloid leukaemia. This is due to high incidence of Philadelphia chromosome in chronic myeloid leukaemia. The incidence of Turner syndrome in consecutive neonates has been reported to be 0.04%[22]. Turner syndrome is one of the few chromosomal aberrations that can be recognized clinically during infancy or childhood based on short stature, broad shield chest, lymphedema of the lower limbs, webbed neck and multiple minor anomalies[22]. However, karyotyping is necessary to confirm the diagnosis. The present study included five (3.8%) patients with Turner syndrome. Their chromosomal patterns were variable: 45, X (two cases) and 45, X/46, XX (three cases). This frequency was more or less similar to Kenue et al[19] but it was lower than that reported by Guera et al[23].

In this study, consanguineous marriage was found in 15 (11.5%) cases in which 3 (20%) cases had Down syndrome. Down syndrome does not show any increase in the frequency of consanguineous marriages among their parents with respect to the general population, and therefore does not support the hypothesis of an autosomal gene controlling mitotic nondisjunction. Many studies do not show any increase in the frequency of consanguineous marriages even among paternal and maternal grandparents of the affected children, thus not supporting the other possible explanation of an autosomal recesive condition in one of the patient's parent's which would cause meiotic nondisjunction[24]. The findings in this study may be incidental.

Conclusion

Among the referred cases with suspected genetic disorders having phenotypic abnormalities, the frequency of autosomal chromosomal aberrations was found to be much higher than sex chromosome abnormalities. Trisomy 21 was the most frequent followed by Philadelphia chromosome.The precise delineation of different types of chromosomal aberrations is only possible using clinical examination and advanced cytogenetic tools by experienced cytogeneticists. Recognition of parents with chromosomal abnormalities is also important as the risk of recurrence is high in some cases. This cytogenetic knowledge allows proper genetic counseling to be provided.

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