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ABSTRACT:

The study was designed to assess the frequency of different phenotypic features and congenital or systematic clinical complicacy of the Down syndrome patients in West Bengal. Karyotype pattern was also studied for confirmation. Eighty five cases diagnosed as Down syndrome patients in the Genetics Department and 30 healthy individuals as control were taken from the pediatric department of Ramakrishna Mission Seva Pratishthan, Kolkata, India. Clinical features observed in more than 90.0% of the Down syndromepatients were flat facial profile, simian crease in palm, low set years, dysmorphic facial features and abnormal distance of eyes. Congenital heart disease was present in 56.5% of the patients, 41.2 % had jaundice at birth.These characteristics were significantly different from the healthy controls.Efforts to establish early diagnosis and a proper screening for high association with clinical features should be undertaken among the Down syndrome patients.

Key Words: Down syndrome, karyotype, physical examination, clinical complicacy, genetic counselling.

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INTRODUCTION:

Down syndrome (DS) is recognizable at birth. It is a relatively common congenital malformation having physical abnormalities of the face, eyelids, tongue and other parts of the body with retarded physical and mental growth [1]. It has been estimated to occur in approximately 1 in 732 infants [2].The affected individual has either a somatic cell of 46 chromosomes involving translocation of chromosome 21or 47 chromosomes with trisomy 21.As reported by Nussbaum et al. [3] over 95% of DS individuals

possess free trisomy 21 resulting from non-disjunctional error of chromosome 21 during gametogenesis in one of the parents. While about 2-4% result showed a translocation, in the rest of 1-2% of persons with the DS showed mosaicism [3]. These individuals may be phenotypically less severely affected than those in the first two types, but their conditions are generally indistinguishable in all other aspects.

Dr Langdon Down (1828-1896) was the first to describe the clinical features of DS children precisely [4,5,1]. The knowledge of clinical manifestations of DS by physicians and other health professionals is important for an early diagnosis in order to reduce morbidity and mortality of these children (e.g. early operation of heart defects). Furthermore, proper clinical diagnosis of DS children is important to avoid normal children being investigated for DS based on only few clinical features [6].Apart from the Karyotype, the most common characteristic features of DS are facial features, development delay, hearing and visual abnormalities, gastrointestinal anomalies, congenital heart defects, and leukemia particularly acute mega-karyoblastic leukemia [7]. As DS is associated with many congenital abnormalities and health problems molecular mapping of the Down-critical region(DCR), of chromosome 21 has been carried out [7, 8]. The mapping provided evidence that the DCR which spans 0.4 to 3 million bases on 21q22.2 is involved in the pathogenesis of DS [7, 8].

Earlier clinical diagnosis allows parents to begin to accept the diagnosis at an earlier stage, and in some instances, make medical decisions about life threatening events [9].

This study on referral cases of DS was undertaken to correlate the cytogenetic profile with the clinical features in the patients. The objectives of this study were to identify problems in reaching clinical diagnosis and provide some recommendations for improvement.

PATIENTS AND METHODS:

Study groups

The study group comprised of 115 individuals, which include both DS patients (n=85) and healthy control (n=30). The age range was from 2 days to 30 years. Gender distribution of the DS patients indicates 55 (64.7%) males and 30 (35.3%) females. Among the control group, 17 (56.7%) were males and 13 (43.3%) were females.The studied population came from mainly the lower socioeconomic strata of the society. Thus, most of the parents of the patients were illiterate and the family incomes were very low. They had limited knowledge, and were not properly aware about genetic abnormality. The distribution of the DS patients according to age groups and gender is presented in Table 1.

Table 1: Distribution of Down syndrome patients (n = 85) according to age groups and gender

Age groups	Males n (%)	Females n (%)	Total n (%)
< 12 months	32 (37.7)	12 (14.1)	44 (51.8)
1-4 yrs	13 (15.3)	11 (12.9)	24 (28.2)
5 – 9yrs	2 (2.4)	4 (4.7)	6 (7.1)
10 – 14 yrs	2 (2.4)	0	2 (2.4)
15 – 19yrs	4 (4.7)	1 (1.2)	5 (5.9)
>20 yrs	2 (2.4)	2 (2.4)	4 (4.7)
Total	55 (64.7)	30 (35.3)	85 (100)

The studies involving human subjects were reviewed and approved by the ethical Committee of the Vivekananda Institute of Medical Sciences. All individuals were included in this study only after the Informed Consent of their parents.

Physical examination

Examinations of nose, eyes, mouth, tongue and palate were performed and recorded either as apparently normal or abnormal as observed from the clinical examination [7]. Abnormalities were recorded in specially prepared examination forms. Established clinically as an abnormally enlarged tongue this was protruding maximum time, when the ears were small, low set with an over folded helix, as a dysmorphic faces, instead of two creases across their palms, people with Down syndrome frequently have a single crease and abnormal distance present between two eyes [7,8]. The examination also included, if the individuals have any clinical complication like congenital heart disease at present and jaundice at birth time.

Short time leucocyte culture

About 5ml of peripheral blood samples were taken from each subject in heparinized vials by venipuncture. The blood samples were coded and used to carry out lymphocyte culture for chromosomal aberrations (CA) analysis by the method of Sharma and Talukder [10]. For each subjects duplicate cultures were maintained. Leucocyte rich plasma (0.5ml) was added to 5 ml culture media supplemented with 20% fetal bovine serum and Phytohaemagglutinin M (0.04ml/ml of culture media). The cultures were incubated at 37º C. The harvesting was done at 72 hrs after initiation of the culture. At 70 h of culture colchicine was added. Two hours later cells were centrifuged at 1000rpm for 10 min, treated with pre-warmed KCl (0.075M) for 15 min, centrifuged at 1000rpm for 10 min and fixed in methanol: acetic acid (3:1).Fixatives were removed by centrifugation and two more changes of fixative were performed. Fixed cell

suspension was laid on clean grease-free glass slide and air-dried. The preparation was stained with aqueous Giemsa. All slides were coded and 100 metaphase plates were scored randomly for chromosomal aberrations per individual. In all cases, the data was analyzed statistically following the chi-square test.

Genetic counseling was carried out with the help of counselors of Ramakrishna Mission Seva Partishthan Hospital.

RESULTS:

Chromosomal study

Of the 85 patients with Down syndrome 68 (80.0%) were below five years of age. A total of 44 (51.8%) of the 85 cases were in the less than 12 months age group. The age and gender distribution are presented in Table 1. The mean age at referral did not differ for the different categories of karyotypic abnormalities. A small number of patients were referred from neonatal ward while the remaining was referred for delayed development and mental retardation.

Cytogenetic testing results of all the DS patients revealed that 78 (91.8 %) had pure trisomy 21; two (2.4 %) had translocation, one of which was a denovo 21, 21 translocation, and 5 (5.9 %) showed mosaic pattern. All the individuals in the control group showed normal karyotype.

Clinical complications of the studied population

Clinical complications like congenital heart defect, jaundice at birth time of the DS patients and control were compared. The result shows 56.5% of DS patients had different types of congenital heart disease like inter-atrial communication, patent ductusarteriosus, atrioventricular septum, tetralogy of Fallot and valve insufficiency. Among them two cases are not properly specified about the type of the heart disease; more than 30.0% DS patients had more than one type of congenital heart defect. In the control group, only 3.3% had congenital heart defect ($p \leq 0.001$).Among them, two had atrioventricular septum and one had non specified artery defect. Jaundice occurred at the birth time of 41.2% DS patients compared to 16.7% in the control group ($p \leq 0.05$).Thus, the incidence of jaundice at birth time is significantly higher among DS patients compared to the control.

Table 2 shows the distribution of the DS patients with clinical complication according to age groups. No statistically significant differences were found when the age groups were compared. Some kids with Down syndrome need a lot of medical attention, others lead healthy lives.

Table 2: Distribution of Down syndrome cases according to their clinical complications

Age	DS patients (n = 85)	Heart Problem (n = 48)	Jaundice at birth time (n = 34)
		N (%)	N (%)
< 12 months	44	30 (35.3)	21 (24.7)
1-4 yrs	24	10 (11.8)	8 (9.4)
5 – 9yrs	6	4 (4.7)	2 (2.3)
10 – 14 yrs	2	2 (2.3)	1 (1.2)
15 – 19yrs	5	1 (1.2)	1 (1.2)
>20 yrs	4	2 (2.3)	1 (1.2)

Physical features of the studied population

In our study, Physical features like protruding tongue, small and low set ears, dysmorphic (mongoloid) faces, simian crease in the palm in hand or toe or both and abnormal distance between two eyes of the DS patients and control were compared (Table. 3). Among the control group, none had protruding tongue and mongoloid faces ($p \leq 0.001$), only 6.7% had low set ears, one had simian crease in left hand, and three had flat nasal bridge with upward slanting eyes. 94.6% Down syndrome patients had three or more defective physical features. Prevalence of the defective physical features was significantly higher among the DS patients compared to the control. Age wise Physical features of the DS patients were also observed (Table 4).The physical features associated with Down syndrome can vary widely from child to child, but can't vary among different age group of patients. At birth, infants with DS appear maximum physical features.

Table 3: Phenotypic features of the study groups

Types	Tongue protruded	Ear (low set)	Mongoloid Face	Palm (simian crease)	Distance of eye
	N (%)	N (%)	N (%)	N (%)	N (%)
Control (n = 30)	1 (3.3)	2 (6.7)	2 (6.7)	1 (3.3)	3 (10.0)
DS Patient (n = 85)	56 (65.9)*	61 (71.8)*	62 (72.9)*	39 (45.9)*	77 (90.6)*

*Statistically significant at $P \leq 0.001$

Table 4: Phenotypic features of the Down syndrome patients

Age	DS patients (n = 85)	Tongue protruded	Ear (low set)	Mongoloid Face	Palm (simian crease)	Distance of eye
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
< 12 months	44	34 (40.0)	39 (45.9)	35 (41.2)	23 (27.1)	42 (49.4)
1-4 yrs	24	14 (16.5)	13 (15.3)	18 (21.2)	11 (12.9)	22 (25.9)
5 – 9yrs	6	4 (4.7)	3 (3.5)	2 (2.3)	4 (4.7)	5 (5.9)
10 – 14 yrs	2	0	0	1 (1.2)	0	1 (1.2)
15 – 19yrs	5	2 (2.3)	4 (4.7)	4 (4.7)	0	3 (3.5)
>20 yrs	4	2 (2.3)	2 (2.3)	2 (2.3)	1 (1.2)	4 (4.7)

DISCUSSION:

The genetic diseases are divided into two categories: chromosomal abnormalities and gene abnormalities. Chromosomal abnormalities are caused by cells that have extra or missing chromosomes or parts of chromosomes. Gene abnormalities (gene mutations) occur when the genetic instructions stored in the DNA are altered so that the protein product coded for by the gene is less functional or nonfunctional [11]. Cells that have extra chromosomes or chromosomes missing are aneuploid. DS is one of the most common chromosomal anuoploidy. Free trisomy 21, translocation and mosaicisim are the three types of this disease. Nondisjunction (free trisomy 21) is the most common genetic defect found in Down syndrome [12]. In this present study, it was observed 91.8% of the children with DS have free trisomy 21, 5.9% had mosaicism, and while 2.4% have a translocation, where one had de novo 21, 21 translocation. High frequency of carriers of balanced Robertsonian translocations in a population could result in a higher frequency of cases with translocation trisomies since the risk of having a live born child with a translocation trisomy 21 is increased for the carriers. In the present study, the percentage of children having a translocation trisomy is even lower compared to a study from Kuwait [13]. All DS cases were cytogenetically confirmed. In our study 51.8% of DS children were diagnosed cytogenetically at the age of less than twelve month and 28.2% were diagnosed at the age between 1-4 years. Thus, more than 80.0% of DS cases were diagnosed below 5 years of age, which indicates that pediatricians and other medical professionals are aware of the clinical phenotype of DS and prompt the cytogenetic confirmation. Similar results were reported from Lebanon [14] and Estonia [15]

where 47.3% and 48.0% of DS diagnosis were confirmed cytogenetically during the child's first year of life. In contrast, registries from England and Wales showed that 90.0% of the DS were confirmed cytogenetically within 10 days after birth [16]. Comparison of the clinical complicacy and phenotypic features of the DS patients with those in the control group indicated significant statistical differences for most of the characteristics. In 1966, Hall described ten cardinal features of trisomy 21 in the newborn [17]. Hall looked at trisomy 21 only, without including mosaic or translocation DS. Our study includes translocation and mosaic DS and this may have accounted for the difference in the results. Abbag [18] reported that the incidence of congenital heart diseases in DS was about 60.0%. In our present study, 56.5% of the children presented with congenital heart diseases, similar frequency as in Brazilian studies, which ranges from 51.0 to 62.2% [19]. The most common characteristic features of DS are gastrointestinal anomalies [8] which may be congenital or non-congenital. Our result shows that 41.17% DS patients had jaundice in the time of their birth. This suggests that many DS children may have different types of congenital and non-congenital complicacy from the birth time, which may impact their physical and psychological development. The children in the control group were selected from among the low socioeconomic area where ground water is the only source of drinking water. Thus water pollution, other environmental factors and unhealthy lifestyles may affect the mothers of these children. Several other factors may contribute to the phenotype variability in DS, such as allelic heterogeneity for chromosome 21 genes present in three copies, the individual's genetic constitution and environmental factors like metallic contamination, excessive pollution [20]. India is one of the countries with the different ethnic heterogeneity, thus, mixed gene population may affect the phenotypic features. The frequency of the phenotypic features observed in the present study are different from those reported by Kava et al and Ahmed et al for DS children in India [21, 22]. The observation of simian crease, distance of eyes, abnormality of ears and mongoloid faces in more than 30% of the total DS cases in the present study are consistent with the values reported by Jones [23] and Fryns [24]. The early diagnosis of children with DS is important, because appropriate treatments for certain common diseases, such as, hypothyroidism and cardiac defects may commence. In addition, it will enable the parents to have access to supporting groups and make use of early intervention programmers for special education and training that aims to improve the quality of life for the children.

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