LABORATORY INVESTIGATION OF SUSPECTED CASES OF CHROMOSOMAL ABNORMALITIES AT THE KORLE BU TEACHING HOSPITAL.

C.N.B. Tagoe +, E.T. Sowah +, A.S. Ayettey +, F.N-L Engmann*

+Department of Anatomy, University of Ghana Medical School, P. O. Box 4236, Accra, Ghana
***Department of Anatomy, College of Medicine, King Faisal University, P. O. Box 2114, Damman 31451, Saudi Arabia.**
***Correspondence should be addressed to this author.**

SUMMARY

Results of cytogenetic analysis carried out on 434 patients referred with clinical history suggestive of chromosomal abnormality showed a frequency of 27.4% (119/434) Of this number (119), 66.4% (79/119) had trisomy 21, made up of 71 regular mongols [47,XX(Y), + 21] and 8 mosaics [46/47,XX(Y), +21]. There was a male/female ratio of 3:2. Of the remaining 40, five were trisomy 13 and five trisomy 18. 26 of these had sex chromosome abnormality, out of which twenty were of the Turner's syndrome, three of intersex, two of Klinefelter's syndrome, and one 47XYY. Among the 20 Turner syndrome patients fourteen were mosaic (46,XX/45,XO) and five homogeneous (45,XO); one patient had the 46,XY/45,XO pattern. Four structural chromosomal abnormalities were found. They consisted one each of 46,XX,D-,t(DqGq)+, 46,XX/46,XX,20q-(Philadelphia chromosome), 46,XX,q- and 46,XX/46,XXr types.

Key Words: Abnormal Chromosomes; Ghana.

INTRODUCTION

Chromosomal abnormalities are not infrequently encountered in clinical practice in West Africa. However, the occurence and pattern of these abnormalities have not been carefully studied, apart from a few case reports [1,2,3].

The inherent difficulty in screening large populations for chromosomal aberrations is recognized. Even in well developed countries, the information available on this subject is drawn largely from selected groups. These include reports from survey of consecutive live-born babies in which chromosomal anomaly is reported at 0.56%[4], and from study of spontaneously aborted foetuses where the abnormality is quite high[5] (50-60%).

In a study of selected patients with history suggestive of chromosomal problem, a 32% frequency of abnormality has been reported.

The information about frequency of occurence of chromosomal abnormalities in suspected individuals is of interest. Although it gives limited knowledge about actual incidence of the abnormality in society, it, nevertheless, indicates the degree of correlation between clinical and laboratory diagnosis.

In the present report we present the results of chromosomal studies on 434 patients referred mainly from the Korle Bu Teaching Hospital.

MATERIALS AND METHODS

A total of 657 patients were refered for investigation. Venous blood (5ml heparinised) was collected for study in all cases. The material obtained was cultured (in duplicate) as follows. 0.1ml of blood was added to 5ml of Ham F 10 or

TC 199 containing 1ml of fetal calf serum, 0.1ml phytohaemaglutinin and 0.1ml penicillin/streptomycin at a concentration of 500ug/ml. This material was incubated at 37^0C for a period ranging from 52 to 72 hours. Prior to harvesting 0.1 ml colcemid (0.2ug/ml) was added to the culture to arrest mitosis. The cultured cells were harvested first by centrifuging the material at 2000 rpm for 10 minutes. The supernatant was then discarded and the cell pellet was resuspended in hypotonic solution (0.07M KCl) and immediately centrifuged for a critical period of 5 minutes. The supernatant was discarded and the remaining cells were fixed for 10 minutes in Canoy's fixative of 1:3 glacial acetic acid and absolute methanol. The fixed material was centrifuged for 5 minutes and the supernatant was discarded. The residual cells were dehydrated in 3 rapid changes in the same fixative solution, centrifuging for 5 minutes after each change.

Chromosomes were prepared by the wet slide method. In this, a drop of cell suspension was placed on a wet slide. Excess fluid is carefully mopped with clean gauze. The slide is gently warmed to about 60^0C on a hot plate
The chromosomes harvested which are at the metaphase stage of cell division are stained in 2% Orcein solution for general examination of the chromosomes and in 1% Giemsa solution for G-banding for identification of individual chromosomes with structural defects.

Total number of cells examined in each patient was 20-100 cells, the higher numbers being necessary for mosaics and for other equivocal cases.

RESULTS

Although 657 cultures were set up, only 434 were successful. The main reason for failure of cultures was unstable supply of electricity.

The results obtained in the remaining 434 successful cultures are summarized in Table 1. One hundred and nineteen samples of the total number showed abnormal karyotype. Nine samples from phenotypic females showed a normal karyotype with XY chromosomes, indicating testicular feminizing syndrome.

As shown in Table 2, the commonest abnormality encountered was trisomy 21, representing 66.4% of the abnormal karyotypes. This abnormality included 71 regular mongols and 8 mosaics and gave a male/female ratio of 3:2. Twenty cases of Turner's syndrome were found. They comprised 5 homogeneous types (45,XO), 14 with a mosaic complement (45,XO/46,XX) and 1 with 46,XY/45,XO pattern. The remaining 20 abnormalities were made up of five trisomy 13, five trisomy 18, three intersex (46,XX/46,XY), two Klinefelter's syndrome, one of the 47,XYY type and 4 with 46 chromosomes some of which showed structural

TABLE 1: INDICATION FOR REFERRAL AND NUMBER OF ABNORMAL CHROMOSOMES DETECTED.

INDICATION FOR REFERRAL	No. of Patients	No. of Abnormal Karyotype
Mongolism (including mosaics)	92	66
Abnormal External Genitalia	79	6
Congenital Anomalies	44	9
Abnormalities of Menstruation	40	11
Abnormal Facies	35	8
Subfertility	18	1
Abnormal Secondary Sexual Characteristics	17	4
Relatives of Patients studied	13	1
Mullerian Duct Underdevelopment	12	0
Neoplasia	11	1
Suspected Turner's Syndrome	9	3
Bad Obstetric History	9	0
Microcephaly	7	0
Delayed Developmental Milestone	7	1
Suspected Edward's Syndrome	4	2
Suspected Testicular Feminization Syndrome	9	0
Suspected Klinefelter's Syndrome	3	1
Mental Retardation	3	2
Delayed Motor Development	2	0
Suspected Patau's Syndrome	1	1
Others	19	2
Totals	434	119

defects.

DISCUSSION

The frequency of chromosomal abnormalities depends on the population studied. In a relatively unselected group of nearly 57,000 live consecutive newborns, values ranging from 0.5% to 0.8% have been obtained[4]. As expected a much higher figure (60%) is reported among spontaneously aborted foetuses[5]. Similarly, in a hospital-based study on mentally retarded patients 32% abnormality has been reported[6]. Our results which have been based on patients with histories suggestive of chromosomal abnormality revealed a frequency of 27%. This study also showed that there is a significant number of patients with congenital abnormalities of other aetiology.

TABLE 2: SUMMARY OF CHROMOSOMAL ABNORMALITIES DETECTED

Autosomal	No	% of Total
21 Trisomy (Down's Syndrome)	79	66.4
13 Trisomy (Patau's Syndrome)	5	4.2
18 Trisomy (Edwards Syndrome)	5	4.2
Sex Chromosomal		
Turner's Syndome	20	17
Intersex	3	2.5
Klinefelter's Syndrome (XXY)	2	1.7
47,XYY	1	0.8
Structural		
46,XX,D$^-$,t(DqGQ)+	1	0.8
46,XX/46,XX,Gq-	1	0.8
46,XX,q-	1	0.8
46,XX/46,XXr	1	0.8
Totals	119	100.0

The indications for referral of these patients are as given in Table 1. As shown in this Table, there is a fair correlation between patients referred with history of mongolism (92) and laboratory diagnosis (66) of chromosomal abnormalities in this group. It is important also to note that 27.5% of females with abnormalities of menstruation had chromosomal anomally and 20.4% of patients referred with congenital anomalies showed defective chromosome patterns.

Of the 92 patients referred with mongolism (Down's syndrome) in our study, 62 had chromosomal pattern of that abnormality. The results are therefore in agreement in only 67% of cases referred compared with nearly 100% correlation in other studies[3,8].

The proportion of mosaics found in the group of mongols studies is much higher than that reported by Mulcahy and Jenkyn[6]. In our study, there were 8 mosaics out of a total of 79 (10%) mongols but in the study of Mulcahy and Jenkyn[5], only two mosaics were found among 107 mongols representing 2%. Adeyokunnu[3] in a study in Nigerians and Al Awadi *et al.*[8] in a study among children in Kuwait also had 1.5% as mosaics. The estimated level of mosaicism in mongols is about 3%[7], the commonest karyotype being 47,XY(X)+21/46,XY(X).

Our study of the mongols revealed only one case of translocation 21 trisomy, 46,XX,D-,t (DqGq)$^+$. This was in a 7-year-old girl with mongoloid features. Another report[9] indicates that such translocations account for nearly 5% of trisomy 21.

The 5 samples which showed 13 trisomy came from four neonates with multiple congenital anomalies and a boy aged 11 years who was mentally retarded, and who had low set ears slanting eyes and rocker-bottom feet as expected. It is noted that the average age of survival of such individuals is 3 years although some may live longer[10].

The sex pattern in the 18 trisomies identified in our study is similar to that reported in the litera-

ture[9]. Of the five 18 trisomies, 4 were girls. Apart from one female aged 7, our patients ranged a few days to 21 weeks in age. The 7-year-old girl in our series was a mosaic, 46,XX/47,XX+18, hence her survival beyond infancy[11].

Turner's syndrome is the most frequently encountered sex chromosomal anomaly in man. Classically described, it includes features such as small stature, sexual infantilism with primary amenorrhoea and sterility, bilateral pterygium colli and cubitus valgus. Nine out of the 20 cases with Turner's type of chromosome presented with primary amenorrhoea. Eight were referred with problems which included Mullerian duct system underdevelopment, primary infertility and abnormal secondary sexual characteristics. Of the remaining 3, one was referred on account of Down's syndrome. This patient (a baby girl) did not otherwise show clinical evidence of the syndrome. The other two were referred with history of Down's syndrome and Klinefelter's syndrome. The point must be made that only three of the 20 diagnosed in the laboratory as Turner's syndrome were referred with history as Turner's.

70% of the group identified as Turner's were mosaics in form. This figure is much higher than that reported elsewhere[9].

The 3 cases of intersexuality had the 46,XX/46,XY karyotype. One was a young woman who presented with primary amenorrhoea and lacked secondary sexual characteristics as already reported[2]. The second was a 15-year-old boy with perineal hypospadias, and the third, a 6-month-old baby with hypospadias and bifid scrotum. This karyotype is found in a considerable proportion of true hermaphrodites and in patients with pure gonadal dysgenesis. In the case of the young woman (previously reported by Engmann[2]) no ovaries were found. The presence or absence of gonads in the other two patients have not been determined as yet.

The case of 47,XYY, recorded in our laboratory has already been reported and discussed by Engmann[1]. We have not found any since.

Of the group that showed structural abnormality one sample was obtained from a 22-year-old woman with chronic granulocytic leukemia (CGL). The investigation showed that she had Phildelphia or Ph1 chromosome. This abnormal chromosome is detectable in over 90% of patients with a clinical history of CGL[12].

Two other patients had structural aberrations of the X chromosome. These turned out to be cytogenetic variants of Turner's syndrome. One of the patients whose X chromosome showed deletion of a long arm presented with a history of congenital absence of the uterus. Usually, patients with this type of structural defect are normal in stature, have few abnormalities and, in a majority of cases, menstruate[13].

The 46, XX/46,XXr structural anomaly was found in a 43-year-old woman with hirsutism, deep voice and clitoral hypertrophy. This kind of virilization is sometimes also found in Turner's syndrome[9]

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