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Comprehensive cytogenetic analysis of cases referred for suspected chromosomal abnormalities: A Five-year study at Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia, Penang, Malaysia.

***Abstract*—**Chromosomal abnormalities (CA) can affect numerical or structural compositions of chromosomosal DNA leading to a diversity of clinical phenotypic presentations. Awareness of prenatal diagnosis and genetic counselling have improved with advancing medical research but CA remain prevalent as its aetiology is unknown. The objective of this study is to determine the frequencies of various CA in the principle region of north-western Malaysia and compare this data to previous reports to ascertain if statistical differences exist. Karyotype analyses performed at the Genetics Laboratory, Advanced Diagnostic Laboratory (ADL) during the first 5-years of cytogenetic services, totalling 1461 cases, were assessed in this report. Cases suspected of CA were initially diagnosed by clinicians and detailed clinical and family histories were recorded. Peripheral blood lymphocytes of patients were collected and cultured *in vitro* for acquisition of karyotype by standardized G-banding technique. Fluorescence *in situ* hybridization (FISH) was conducted in cases suspected ~~of~~ to be DiGeorge, Prader-Willi, Angelman and Williams syndrome. Of the total samples (1805) received and cultured, 1669 (92.46%) successfully yielded results. Abnormal outcomes were observed in 495 cases (29.66%) whereby pronounced majority of cases 299 (68.42%) were Down syndrome. This is followed by Edward, Turner and Patau syndrome, in order of frequency. Numerical CA appears to be prevalent accounting for 85.86% of cases. Structural CA accounted for 14.14% of total positive cases whereby the most common was deletions (34.29%) followed by translocations (20%), ring chromosomes (5.71%), Fragile X syndrome (4.29%), duplications (5.71%) and marker chromosomes (7.14%). The remainder of cases (22.86%) consisted of derivative chromosomes and other complex aberrations. The number of polymorphic variant cases were 27 (1.62%). The number of peripheral blood samples received has significantly increased from 14.3 per month in 2006 to 32.17 per month in 2011. Comparative analysis of our study to previous reports reveal statistical differences in the occurrence of several CA including Edward, Patau, Klinefelter and Fragile-X syndrome. Our experience with peripheral blood samples for cytogenetic analysis demonstrated a success rate of 92.46%. This showed an increase in clinicians validating patients' diagnoses with karyotyping which is essential in confirming genetic anomalies with the goal to substantiate genetic counselling.

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1 INTRODUCTION

Chromosomal abnormalities (CA) are responsible for a diversity of clinical phenotypic presentations that could range from severe mental retardation to apparent asymptomatic conditions which may manifest disease in future generations. Even though awareness of prenatal diagnosis and genetic counselling have improved with advancing medical research, CA remains prevalent as its aetiology is unknown. Eradication of causal factors is improbable in the near future thus, patients with CA will remain an important medical problem. This study was performed to determine the frequencies of various CA in the principle region of north-western Malaysia. Karyotype analyses for the first five (5)-years duration of cytogenetic services provided by the Genetics Section of the Advanced Diagnostic Laboratory (ADL), totalling 1805 cases, was assessed in this report. Patients' samples were mainly from Sultanah Bahiyah Hospital, Seberang Jaya Hospital, Sultan Abdul Halim Hospital,

Penang Hospital, Kulim Hospital, Kuala Nerang Hospital, Sungai Bakap Hospital, Kepala Batas Hospital, Bukit Mertajam Hospital, Yan Hospital, Taiping Hospital and this institution. Data from a previous study conducted in Korea which adopted similar study methodologies where cases were grouped into almost the same type of cytogenetic categories was also compared to determine if statistical differences exists.

2 RESULTS

Cytogenetic analysis of 1805 cases referred were analysed for chromosomal abnormalities (CA). Of the 1805 samples received and cultured, 1669 successfully yielded results. There were 136 samples that could not be analysed due to failure in culture or yielded poor metaphase spreads. The number of peripheral blood samples received has significantly increased from 14.3 per month in 2006 to 32.17 per month in 2011. Of the cases referred, 495 samples (27.42%) were tested positive for CA (Table I). 27 cases of polymorphic variants were also observed (Table IV). The CA were predominantly autosomal related, amounting to 437 cases (88.28%) (Table II), while sex chromosome abnormalities were found in 58 referrals (Table III). The rate of CA detected was similar to the report of Verma et al. (1980) [2] and Choi et al (1984) [3] which were 27.2% and 29.3% respectively. To determine if our present study found any significant statistical difference from previous reports, we have selected the publication of Kim et al. (1999) [4] for comparison as they had a similar study methodology where cases were grouped into almost the same type of cytogenetic categories as our present study. Their study also reported a similar relative frequency of various chromosomal aberrations when compared to studies conducted in Korea in the early 1980s.

Numerical CA (Table I) appears to be prevalent in our study with 425 positive cases (85.86%). The majority of numerical aberrations (383) were trisomy cases (91.19%). Structural CA accounted for 14.14% of total positive cases whereby the most common was deletions (34.29%) followed by translocations (20%), ring chromosomes (5.71%), Fragile X syndrome (4.29%), duplications (5.71%) and marker chromosomes (7.14%). The remainder of cases (22.86%) consisted of derivative chromosomes and other complex aberrations.

Among the autosomal CA found in our study, Down syndrome recorded the highest incidences with 299 cases (68.42%). Of these cases, 282 (94.31%) were of trisomy 21 and 7 were mosaics (2.34%). Structural CA leading to Down syndrome were also found in 9 unbalanced Robertsonian translocation (3.01%) and 1 ring chromosome case (0.33%). The 9 translocation cases were identical with a karyotype of 46, XX(Y), rob(21) +21, 46, XY, rob(14;21) +21, 46, XX, der(21) +21 and 46, XX, der(13;21) +21. The karyotype of the ring chromosome case was determined to be 46, XX r(21)(p13;q22).

Table I. Distribution of numerical and structural CA in the present study of 495 cases.

Chromosomal Abnormality	Number of Cases
Numerical	
Trisomy 21	299
Trisomy 18	57
Trisomy 13	20
Monosomy X	36
Klinefelter's syndrome	3
47, XXX	2
47, XYY	2
Others	6
Total	425 (85.86%)
Structural	
Deletion	24
Translocation	14
Ring chromosome	4
Fragile-X syndrome	3
Duplication	4
Marker chromosome	5
Others	16
Total	70 (14.14%)

Edward syndrome was found to be the second most common autosomal aneuploidy with 57 cases (13.04%). There were 54 trisomy 18 incidences and 3 mosaics. No reports of structural aberrations leading to Edward syndrome was found in our study. Patau syndrome recorded 20 cases and was the third most common autosomal CA in our study. There were 18 trisomy 13 cases and 2 cases with translocations leading to this disorder. Other autosomal aberrations of structural origin consists of deletions (5.26%), translocations (2.52%), ring chromosomes (0.92%), duplications (0.69%) and marker chromosomes (0.92%). Of the deletion cases, there were 8 cases of DiGeorge syndrome, 5 cases of Prader Willi/Angelman syndrome, 3 cases of William syndrome and 8 at other chromosomal sites. The remaining 16 cases of autosomal aberrations include less well defined numerical and complex structural CA.

Of the sex chromosome abnormalities detected, Turner syndrome was the most common with 36 cases whereby 13 were monosomy 45, X and 23 were mosaics.

Table II. Distribution of autosomal chromosomal abnormalities by karyotype.

Karyotype	Present study			Kim et al. 1999
	Number	Sub-categories	Relative frequency (%)	Relative frequency (%)
Down syndrome (Trisomy 21)	299		68.42	55.98
47, XX(Y), +21		282		
Mosaic:				
47, XX(Y), +21/46, XX(Y)		7		
Translocation:				
46, XX(Y), rob(21) +21		6		
46, XY, rob(14;21) +21		1		
46, XX, der(21) +21		1		
46, XY, der(13;21) +21		1		
Ring chromosome 21		1		
Edward syndrome (Trisomy 18)	57		13.04	4.36
47, XX(Y), +18		54		
Mosaic:				
47, XX(Y)+18/46, XX(Y)		3		
Patau syndrome (Trisomy 13)	20		4.58	0.95
47, XX(Y), +13		18		
Translocation:				
46, XY, rob(13;13), +13		1		
46, XX, rob(13;14), +13		1		
Other autosomal aberrations				
Deletion	24		5.26	5.50
DiGeorge syndrome		8		-
Prader Willi/Angelman syndrome		5		-
William syndrome		3		-
Macrodeletion		8		
Translocation	11		2.52	16.70
Ring chromosome 21	4		0.92	1.52
Duplication	3		0.69	8.16
Marker chromosome	4		0.92	3.04
Miscellaneous	16		3.66	3.80
Total	**438**		**100.00**	**100.00**

Klinefelter syndrome recorded a very low incidence count with only 3 cases. Other sex chromosome related aberrations detected from our study include Fragile X, triple X and 47, Jacob syndrome (XYY) with an incidence of 3, 2 and 2 case respectively. The remaining 6 cases consisted of numerical and complex structural anomalies which are uncommon and less well defined in terms of association with clinical features.

27 polymorphic variants were detected in our study (1.62%). There were 4 each of 46, XY, 21ps+ and 46, XY, 16qh+, 3 46, XY, 22pstk+, 2 each 46, XY, variant chromosome 9 and 46, XX, 1qh+, and 1 each of 46, XX, 22ps+, 46, XY, 22pstk+, ps+, 46, XY, 21pstk+, 46, XY, 21pstk+, ps+, 46, XX, 16qh+, 46, XY, 15cenh+, ps+, 46,XX, 14ps+, 46, XY, 14pstk+, ps+, 46, XX, 13ps+, 46, XY, 13ps+, 46, XY, 13ps+, 15pstk+ and 46, X, Yqh-. The variants found in our study were different from that in Kim's study which detected 152 cases from a total of 4117 samples (3.69%). The individuals in our study with polymorphic variants consisted of 21 males (77.78%) and 6 females (22.22%).

3 DISCUSSION

Our study observed an occurrence of CA similar to several studies including Verma *et al*. (1980) [2] and Choi *et al*. (1984) [3], however some reports have disclosed lower incidence rates [5]. Many factors could lead to statistical differences including application of cytogenetic tests, presence of risk factors and social practice.

In the present study, the most common CA was Down syndrome (Table II) which accounted for 299 of the positive cases (68.42%). Compared to the findings of Kim et al. (1999) [4] which reported 40.92% of positive cases were Down syndrome, our study reported a much higher relative frequency. However, we found significantly lower incidences of translocations leading to Down syndrome with only 3.01% compared to 5.08% in Kim's study. We also observed 1 case of ring chromosome (involving chromosome 21) leading to Down syndrome which was not found in Kim's report. Although it is noted that most cases of aneuploidy are not inherited, structural aberrations leading to these disorder, including translocations and ring chromosomes, can be passed down to future

generations. In the general population, it is observed that Robertsonian translocations are responsible for between 3 to 4% of Down syndrome cases while ring chromosome and segmental trisomy 21 rarely occur [6].

An interesting feature of our study is the identification of significantly higher relative frequencies for the incidences of Edward and Patau syndrome compared to other reports [4,7,8]. At the time of diagnosis, the Edward syndrome patients range from 1 day old to 1 year old while Patau syndrome patients were new-born to 5 months old. Patients of both syndromes have short life expectancies as a result of several life-threatening medical problems. Edward syndrome patients normally do not survive past their first month while Patau syndrome infants die within their first days or weeks of life. However, in both syndromes, about 5 to 10 percent of patients do live past a year [9,10].

Among the cases of sex chromosome abnormalities, Turner syndrome was found to be the most common with similar relative frequency to the report of Kim et al. (1999) [4] (Table III). Also in agreement is that mosaicism is more common than monosomy 45, X. Of the 23 mosaic cases, 6 (33.33%) had Y chromosome components (including presence of SRY gene, 45, X/46, X+mar(Y) and 45, X/46, XY), which is proportionally more than double of that reported by Kim (15.52%). Of these cases, 4 were reportedly 45, X/46, XY, whereby 2 were females (aged 19 and 25 years old), 1 was male (9 years old) and the remaining patient had ambiguous genitalia with the presence of the SRY gene later confirmed (4 months old). It is observed that in the general population, the majority of mosaic 45, X/46, XY patients are externally normal males, while about 5% are females with Turner syndrome and around another 5% are born with ambiguous genitalia [11].

Klinefelter syndrome was the second most common sex chromosome abnormality reported by Kim with a relative frequency of 30.41% which is almost 6 times higher than our present study of 5.17%. Although it is believed that Klinefelter syndrome is possibly one of the most common CA occurring in humans, affecting about 1 in 500 males [12], which is five times higher than Turner syndrome which affects 1 in 2500 females [13], our study found an extremely low occurrence of this aberration. This could be, in part, due to the varying degree of phenotypic presentations, rendering the condition not readily identifiable. Individuals with Klinefelter syndrome live near-normal lives especially in adulthood as many are asymptomic, although some cases may have complications pertaining to physical, language and social development earlier in life. The disorder may be more apparent after marriage as between 95% to 99% of XXY males are infertile [12].

The relative frequencies of 47, XXX and 47, XYY cases in our study (Table III) is in agreement with Kim et al. (1999) [4] which is significantly lower compared to previous statistic reports in new-born children [7,8]. As phenotypic manifestation of these CA are not usually apparent, many carriers are undiagnosed [4]. The incidences of Fragile-X syndrome in our study is also considerably low, with only 3 confirmed cases, although it is thought to be the second most common cause of genetically associated mental deficiencies after Down syndrome [14]. The incidences of Fragile-X syndrome are 1 in 4000 males and 1 in 8000 females [15]. All the Fragile X syndrome cases in our study are males.

Table III. Distribution of sex chromosome abnormalities by karyotype.

Karyotype	Present study			Kim et al. 1999
	Number	Sub-categories	Relative frequency (%)	Relative frequency (%)
Turner syndrome	36		62.07	58.76
45, XO		13		
Mosaic 45, X0/46, XX		23		
Fragile-X syndrome (FXS)	3		5.17	-
Klinefelter syndrome 47, XXY	3		5.17	30.41
47, XXX	2		3.45	1.55
Jacob syndrome 47, XYY	2		3.45	2.58
Macrodeletion	1		1.72	
Translocation	3		5.17	
Duplication	1		1.72	
Marker chromosome	1		1.72	
Others	6		10.34	6.70
Total	**58**		**100.00**	**100.00**

Table IV. Relative frequency of polymorphic variant karyotype among referred cases of the present study.

Karyotype	Number	Relative frequency
46, XY, variant chromosome 9	2	7.41
46, XX, 22ps+	1	3.70
46, XY, 22pstk+	3	11.11
46, XY, 22pstk+, ps+	1	3.70
46, XY, 21ps+	4	14.82
46, XY, 21pstk+	1	3.70
46, XY, 21pstk+, ps+	1	3.70
46, XY, 16qh+	4	14.82
46, XX, 16qh+	1	3.70
46, XY, 15cenh+, ps+	1	3.70
46,XX, 14ps+	1	3.70
46, XY, 14pstk+, ps+	1	3.70
46, XX, 13ps+	1	3.70
46,XY, 13ps+	1	3.70
46, XY, 13ps+, 15pstk+	1	3.70
46, XX, 1qh+	2	7.41
46, X, Yqh-	1	3.70
Total	**27**	**100.00**

Polymorphic variants are common cytogenetic heteromorphism detectable by conventional G-banding technique and are not known to be associated with phenotypic presentations [16]. They encompass prominent acrocentric short arms, satellites and stalks, as well as heterochromatin regions of chromosome 1, 9, 16 and Y [17]. Although previous studies have suggested the role of polymorphic variants in male infertility [18], none of the cases in our report were referred for this reason. However mounting evidence by researchers in this field are increasingly supportive of this association [16]. The most common polymorphic variant observed in our study was that of variant chromosome 9, which affected one female and three males, accounting for 0.27% of total referred cases. The female patient was referred for neonatal encephalopathy whilst the male patients were diagnosed each with congenital hypothyroidism, Pierre Robin syndrome and one unstated. Nevertheless it is not possible to confirm whether variant chromosome 9 is responsible for the associated clinical features.

In conclusion, our experience with peripheral blood samples for cytogenetic analysis demonstrated a success rate of 92.46% and showed an increase in clinicians validating their diagnoses with karyotyping which is essential in confirming genetic anomalies and able to substantiate genetic counselling. It is hoped that our report together with future studies will increase the awareness in the importance of prenatal diagnosis to reduce the recurrence of CA.

CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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