Original Article

Analysis of Data Obtained From Chromosomal Studies Performed During the Period from 2000-2007 A Retrospective Study

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Summary:

Background:
Generally, genetic disorders are a leading cause of spontaneous abortion, neonatal death, increased morbidity and mortality in children and adults as well. They a significant health care and psychosocial burden for the patient, the family, the healthcare system and the community as a whole. Chromosomal abnormalities occur much more frequently than is generally appreciated. It is estimated that approximately 1 of 200 newborn infants had some form of chromosomal abnormality. The figure is much higher in fetuses that do not survive to term. It is estimated that in 50% of first trimester abortions, the fetus has a chromosomal abnormality.

Aim of the study:
This study aims to shed some light on the results of chromosomal studies performed during 7 year-period as these represent a sample of the only registered data available on genetic disorders in Iraq.

Patients and Methods:
For the period extending from Jan. 1st, 2000 till Jan. 1st, 2007, among all cases referred to the Genetic Clinic, Consultation Clinic, Medical City in Baghdad, Iraq, only those cases indicated for chromosomal study for diagnosis and then genetic counseling were included in this study; they were grouped and then subgrouped accordingly.

Results:
During the study period, 1720 cases needed chromosomal study for the sake of genetic counseling out of around 5000-8000 cases referred to the clinic during the same period. Mothers having an abnormal child or adverse pregnancy outcome constituted 30.79% of all cases included, followed by the group of children with multiple congenital abnormalities (20.14%), and then cases with primary amenorrhoea (13.97%) and ambiguous genitalia (13.5%). The overall positive findings in the chromosomal studies were 217/1720 (12.61%).

Conclusions:
Genetic disorders have a great impact on the practice of medicine in all specialties in Iraq. There is a need for a new policy for indications of karyotyping, especially at times of stress.

Keywords: *Genetic disorders, chromosomal study, Iraq*

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Introduction:

Genetic abnormalities are a common cause of disease, handicap, and death among infants and children. The primary diagnosis of 11.16% of patients admitted to the pediatric units of teaching hospitals is a genetic disease. One to 2% of newborn infants have a hereditary malformation and 0.5% have an inborn error of metabolism or an abnormality of the sex chromosomes which causes no physical abnormalities and can be detected only by specific laboratory tests [1]. About 3% of pregnancies result in a child with a genetic disease or birth defect and around 10% of all pediatric and adult hospitalization admissions involve genetic diseases. This number would increase substantially if one included complex, multifactorial genetic diseases, such as diabetes or cardiovascular disease. The prevalence of genetic diseases, combined with their severity and chronic nature, imposes a great financial, social, and emotional burden on society [2].

Generally, these conditions are a leading cause of spontaneous abortion, neonatal death, increased morbidity and mortality in children and adults as well. They are a significant health care and psychosocial burden for the patient, the family, the healthcare system and the community as a whole [3].

Nearly, one-third of genetic disorders in Arab individuals result from congenital malformations and chromosomal abnormalities (34.4%) [4].

Chromosomal abnormalities occur much more frequently than is generally appreciated. It is estimated that approximately 1 of 200 newborn infants had some form of chromosomal abnormality. The figure is much higher in fetuses that do not survive to term. It is estimated that in 50% of first trimester abortions, the fetus has a chromosomal abnormality [5].

In Iraq, although many studies have dealt with some genetic disorders [6, 7], there is no accurate measure of the burden of genetic disorders in Iraqi people, as there is no formal registration of these disorders.

This study aims to focus on results of chromosomal studies performed in 7 year-period as these represent a major sample of the only registered data available on genetic disorders.

Patients and Methods:

For the period extending from Jan. 1st, 2000 till Jan. 1st, 2007, among all cases referred to the Genetic Clinic, Consultation Clinic, Medical City in Baghdad, Iraq, only those whom were considered indicated for chromosomal study for diagnosis and then genetic counseling were included in this study.

We believe that the ratio of cases not included in this study (diagnosed clinically ad thus not registered) to those included (diagnosed with the help of karyotyping and thus reported) was in the range of 3-5:1.

Karyotyping is the basic tool of the cytogeneticist. The technique used in our laboratory is Giemsa Trypsin stain (G-banding).

All karyotypes were performed in the Genetic Laboratory, The Teaching Laboratories, Medical City in Baghdad during the study period *.

Those cases were grouped into 8 categories; some were divided into subcategories whenever needed.

The groups included in this study were as follows:

1. Mothers of abnormal children (MAC):

a. Mothers with no living child but having recurrent abortions.

b. Mothers with recurrent child death, mainly neonatal deaths.

c. Mothers who have given birth to a child with a known chromosomal abnormality, mainly Down's syndrome.

d. Mothers who have given birth to primarily a mentally retarded or a child with brain lesion.

e. Others: including children with bone, eye, or heart lesion.

2. Child with multiple congenital abnormality (MCA): this group excludes all clinically diagnosed cases. This group was subdivided into:

a. Mental retardation with primary brain lesion.

b. Others: includes all lesions apart from (a) above.

* The cases were diagnosed by Dr. Selma AH Al-Taha prior to 2005 and by Dr. Ahmed Kamal – Genetics Lab. – Teaching Laboratories during and after 2005.

We also divided those cases into whether they were males or females.

3. Cases presented with ambiguous genitalia (AG). This group was further subgrouped according to the result of their karyotypes into:

a. Male name with a male genotype.
b. Male name with a female karyotype.
c. Female name with a male karyotype.
d. Female name with a female karyotype.
e. Cases found to be Turner's syndrome (including 45XO, 46XX, isoXq, mosaic pattern).
f. Cases found to be Klinefelter's syndrome (including all possibilities like 46XXY, 46XXXY, mosaic pattern).

4. Females presented with primary amenorrhoea: this group was subdivided according to the results of their karyotypes into:

a. Normal female genotype.
b. Male genotype.
c. Turner's syndrome (all possibilities).

5. Cases presented primarily with short stature (S.S.). The female patients were mostly sent for to exclude the possibility of Turner's syndrome. This group was further subdivided into:

a. Normal males.
b. Normal females.
c. Turner's syndrome (all possibilities).

6. Couples with either:

a. No living child but having recurrent abortion.
b. Recurrent child death.
c. Children with Down's syndrome or other chromosomal abnormalities.
d. Others.

7. Cases referred to as suspected Down's syndrome (? Down's). This group was subdivided into:

a. Males or b. Females.

Or subdivided according to the results into normal result or definitive Down's S. on karyotyping in any form (trisomy 21, translocation Down's, mosaic form, etc.).

8. Older males referred for different reasons, as follows:

a. Tall patient, obese, sparse facial hair, small genitalia, mental subnormality, or any combination of them (i.e. suspected as Klinefelter's syndrome).
b. Others: include father of an abnormal child, small genitalia).

Results:

During the study period, 1720 cases needed chromosomal study for the sake of genetic counseling. Around 5000-8000 cases (unfortunately not all registered) were omitted from the study as they were diagnosed either clinically or by some other laboratory or imaging method.

We divided the included cases into 8 categories and the results obtained each year and for each category were summarized in table (1).

Table (1): Summary of data obtained from chromosomal studies

	Category *	2000		2001		2002		2003		2004		2005		2006		Total	
		o.	%	o	%	o	%	o	%	N o	%	o	%	o.	%	N o.	%
1	MAC	14	3 4.75	52	3 7.90	28	3 4.59	4	4 9.43	3	4 2 3.11	8	1 8.94	0	1 2.04	5 29	3 0.79%
2	MCA	8	1 4.63	14	2 8.42	4	2 0	2	2 4.71	3	3 1 7.74	9	2 3 0.5	6	1 0.44	3 46	2 0.14%
3	A.G.	5	1 0.67	9	9 .72	4	1 4.5		7 .86	5	3 1 8.81	3	1 3.68	9	1 9.67	2 32	1 3.5%
4	Primary A.	2	1 8.90	1	7 .73	9	1 3.24		4 .49	8	2 1 5.05	3	1 3.68	3	2 1.28	2 40	1 3.97%
5	S.S.	6	7 .92	8	6 .98	5	6 .75		5 .61	2	1 6 .45		8 .42	2	8 .83	1 26	7. 34%
6	Couples		2 .43		1 .99		1 .35		0	2	1 6 .45		4 .21	2	4 .81	4 8	2. 80%
7	? Down's		2 .13		1 .74	1	2 .97		3 .37	0	1 5 .37		7 .36	7	1 4.85	8 3	4. 83%
8	Older male	8	8 .53	2	5 .48	4	6 .48		4 .49	3	1 6 .98		3 .15	0	8 .03	1 14	6. 63%
	Total	28	1 9.06	01	2 3.31	70	2 1.51	9	5 .17	86**	1 1 0.81	5	5 .52	49	1 4.47	1 720	1 00%

* Refer to the section of patients and methods for abbreviations used here.

** Two more cases were referred as gender identity disorder (1 male and 1 female) and have not been included under any of the groups specified, thus making the total no. in 2004 188 instead of 186

Discussion:

If a child is born with abnormalities in more than one system (CNS, CVS, skeletal system, etc.), it should always be considered as possibly having a chromosomal abnormality and it is often necessary to study the parent's chromosomes as well on the off-chance that one of the parents carries a translocation [8]. Thus, group 1 (MAC) constituted the largest group within the included cases (30.70%). Mothers with recurrent abortion were the largest subgroup in this category (29.48%) followed by mothers with recurrent child death (24.38%), then mothers of a child with a known chromosomal abnormality (20.98%), tables (1, 2).

This is due to the fact that when a mother had recurrent abortions with no living child or gives birth to an abnormal child, she seeks for medical advice and she is motivated to consult many doctors and do whatever investigations required including the lengthy procedure of chromosomal study, especially when she is in her early family formation. In addition, when there is no obvious cause, chromosomal study is indicated.

In only 4 mothers an abnormal karyotype was found, 2 had R.A. 1 showed 46XX, 9q+ and the other 46XX, t(6p;7p); 1 had 2 babies with hydrocephalus and showed an accidental finding of 47XX+13/46XX; the last was a mother of Down's syndrome who was proved to be a translocation carrier [46XX, t(21;14)]. The rest were normal.

The other group was MCA, which constituted 20.14% from total. In fact, this group originally was the largest group among patients referred for genetic counseling but as most cases were diagnosed clinically or by another investigative method, they were not indicated for chromosomal study, and yet not included.

It is interesting to notice that primary brain lesion with mental retardation was the major cause for referral and counseling. In spite of the advances in diagnostic techniques, no specific cause can be found in most cases, and hence, are subjected to chromosomal study more than other groups for a chance of finding an abnormality although most cases are due to single gene disorders, table (2).

Males constituted (57.5%), while females the rest (42.5%). This is mainly due to a group of disorders mainly X-linked mental retardation and fragile X-syndromes that makes males referred more commonly than females.

In regard to category 3 (A.G.), it constituted 13.5% from total. For such cases, karyotyping was mandatory to determine the sex of the child, on which further management and rearing depend.

Sex reversal cases were found to be 76 (32.75% from total A.G. cases); These cases were diagnosed according to their clinical presentation, ultrasonographic findings and hormonal assessment, CT or MRI of pelvis, and even surgical exploration when indicated, as there was no gross structural or numerical abnormality in their chromosomes into either of the following: certain types of congenital adrenal hyperplasia, testicular feminization syndrome, microdeletion of *SRY*-gene on the Y-chromosome, translocation of this gene to another chromosome (mostly to the X-chromosome), or due to 5-α reductase deficiency.

Male genital ambiguity was noted to occur more frequently than in females (97 cases vs. 55 cases) after excluding cases of sex reversal. This may be due to the fact that some families tend to consider children with A.G. more frequently as males rather than females, table (2).

Turner's and Klinefelter's syndromes were presented unusually in 4 cases as genital ambiguity.

Cases of primary amenorrhoea (Group 4) were 13.97%. Males constituted only 22 cases (9.16%) but this figure is not small in regard to the significance of this category, as two (male) patients have already been married but considered as females with amenorrhoea!

The other subgroups were Turner's syndrome who present lately (as they usually present earlier as short stature) and only one case found to be a superfemale (47XXX). She was mentally subnormal. The rest (82.9%) had no detectable abnormality in their chromosomes and were referred back to their gynecologists, table (2).

Table (2): Results of chromosomal studies in all study groups

	Category	2000	2001	2002	2003	2004	2005	2006	Total
1	MAC:	114	152	128	44	43	18	30	529
	-RA	36	36	34	9	19	4	18	156
	-RCD	35	38	31	12	5	7	1	129
	-Chrom. Abnorm.	18	25	27	7	10	4	11	111
	-Br. lesion	15	31	28	15	0	3	0	92
	-Others	10	22	8	1	0	0	0	41
2	MCA	48	114	74	22	33	29	26	346
	- Br. Lesion	16	15	31	3	10	11	12	108
	- Others	32	99	43	19	23	18	13	238
	- Males	33	65	43	12	18	14	14	199
	- Females	15	49	31	10	15	15	12	147
3	A.G.	35	39	54	7	35	13	49	232
	- M→M	15	14	23	1	15	7	22	97
	- M→F	3	8	5	3	4	2	6	31
	- F→M	8	9	15	1	4	0	8	45
	- F→F	7	7	11	2	12	4	12	55
	- Turner's	1	0	0	0	0	0	0	1
	- Klinefelter's	1	1	0	0	0	0	1	3
4	Primary amenorrhea	62	31	49	4	28	13	53	240
	- Female pattern	48	24	41	4	23	12	47	199
	- Male pattern	7	4	7	0	2	1	1	22
	- Turner's	7	3	1	0	3	0	4	19
5	Short stature	26	28	25	5	12	8	22	126
	- Normal male	2	3	5	2	0	2	1	15
	- Normal female	19	18	12	2	8	6	15	80
	- Turner's	5	7	8	1	4	0	6	31
6	Couples	8	7	5	0	12	4	12	48
	- RA	1	3	2	0	3	1	4	14
	- RCD	0	0	0	0	2	1	2	5
	- Chrom. Abnorm.	2	1	1	0	1	2	5	12
	- Others	5	3	2	0	6	0	1	17
7	? Down's	7	8	11	3	10	7	37	83
	- Normal results	5	4	8	3	3	0	2	25
	- Abnormal res.	2	4	3	0	7	7	35	58
	- Males	4	5	4	1	2	2	20	38
	- Females	3	3	7	2	8	5	17	45
8	Older males	28	22	24	4	13	3	20	114
	- Klinefelter's	3	4	6	2	2	0	1	18
	- Others	25	18	18	2	11	3	19	96
	Total	328	401	370	89	*186* *	95	249	1720

* The total cases encountered in 2004 were in fact 188; the other 2 cases were referred for gender identity disorders to determine their genotype (the first was male and the other was female).

Table (3): Number of positive karyotypes in each category in the study group

Category No.	Category	Positive karyotype	
		No.	%
1	MAC	4 / 530	0.755%
2	MCA	19 / 340	5.588%
3	A.G.	80 / 232 *	34.48% *
4	Primary A.	40 / 232	17.24%
5	S.S.	31 / 126	24.60%
6	Couples	0 / 48	0%
7	? Down's	25 / 83	30.12%
8	Older male	18 / 114	15.789%
	Total	217 / 1720 *	12.61% *

* if we considered all cases of A.G. as positive, as the aim was to determine the sex of the child, then the total no. would be 369 (21.453%)

Short stature, the next category, constituted only 7.34%. Most cases were referred from the endocrine clinics for suspicion of Turner's syndrome in females. Turner's syndrome cases were 31 (11.92% from total S.S. cases), as compared to 80 (63.49%) who were found to have no gross structural or numerical chromosomal anomaly.

Males were referred as short stature much less frequently than females do among our patients; this might be due to the bias resulting from the fact that females often were thought of as Turner's syndrome, thus referred for chromosomal study while in males, there is no 'famous' syndrome that require chromosomal study and affects males in particular, table (2).

In group 6, a relatively small group of couples (2.80%) who seek genetic advice for various reasons, the largest was due to recurrent abortion (29.1%), followed by a previous child with Down's syndrome (25%). In up to 10% of couples with R.A., 1 of the parents has a chromosomal abnormality which is related to the cause of R.A. [9]. Unfortunately, all couples were found normal simply due to the small size of this group, table (2).

Cases referred to the genetic clinic as suspicious Down's syndrome constituted 83 (4.83%). Most cases, were diagnosed clinically as there was a typical and convincing clinical presentation to make a firm diagnosis, especially when there was a shortage of materials encountered during most of the study period, thus only the most atypical or suspicious cases were tested.

There was no significant difference between males and females, but there was abundance of abnormal results (positive findings) confirming the diagnosis of Down's syndrome (69.87% vs. 30.13%). This is due to filtration of cases on clinical basis; but even clinically typical Down's syndrome may show a normal karyotype, thus making clinical diagnosis, unless in very clear cases, inadequate for definitive diagnosis [10].

The last group (Group 8), older males, constituted 6.63% from total study group. They were referred, in most instances, as cases of suspected Klinefelter's syndrome. The results confirmed the diagnosis in 15.78% of cases, while it was negative in the rest.

In summary, the sum of all positive findings in the study group was a bit disappointing, table (3).

In conclusion, we believe that a new policy for indications of karyotyping is needed in the light of the results of this study. All cases of A.G., primary amenorrhoea, suspected chromosomal abnormality, and only selected cases of MCA, a parent of such cases especially the mother are really indicated for karyotyping. Many of the rest require either non-genetic investigation or a very specific one (e.g. molecular cytogenetic or molecular DNA study) and not merely a chromosomal study.

In addition, we have determined that genetic disorders have a great impact on the practice of medicine in different specialties in Iraq, in the view that only one genetic clinic in one area and in extremely difficult circumstances, have dealt with between 5000-8000 cases in 7 years period and offered them genetic counseling.

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