Estimation of Common Chromosomal Aneuploidies in the Population of Bahrain: 20 Years of Findings in the Genetic Laboratory

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Objective: To evaluate the incidence of common chromosomal aneuploidies in Bahrain during the last 20 years (1999-2019).

Design: A Retrospective and Descriptive Study.

Setting: Genetic Laboratory (now National Genome Center) at Salmaniya Medical Complex, Bahrain.

Method: A total of 5,153 patients' karyotypes and clinical reports were reviewed retrospectively. Conventional cytogenetic analysis was accomplished for karyotype reporting at the 400-band resolution level by using Giemsa staining technique. Fluorescence in situ hybridization (FISH) assay was used as well on selected cases.

Results: Out of 5,153 patients we uncovered 758 cases with various structural and numerical abnormalities. Numerical abnormalities (aneuploidies) were uncovered in 529 (69.7%) subjects. This includes 476 (62.8%) cases with autosome aneuploidies and 53 (6.9%) cases with sex-chromosome aneuploidies. Trisomy 21 (Down's syndrome) is the most common autosomal aneuploidy at 404 (53.3%) with an estimated population incidence rate of 1:701 live births. Trisomy 18 (Edward's syndrome) found in 53 (6.9%) patients, and trisomy 13 (Patau's syndrome) at 4% (19 patients) of autosomal aneuploidies. Incidence rate indicates 1:5343 for trisomy 18 and 1:16660 for trisomy 13. In contrast, our findings for the sex-chromosome aneuploidies includes 17 (2.2%) patients with Klinefelter's syndrome (47, XXY) and 28 (3.7%) patients with either standard Turner's syndrome (45, XO), 9 (1.2%), or other various mosaic Turner's syndrome, 19 (2.5%). Estimated incidence rate for Turner's syndrome is 1:10115 and for Klinefelter's syndrome is 1:16660. Finally, we uncovered 8 (1.1%) patients with rare sex chromosome aneuploidies or combined sex chromosome and autosome aneuploidies with an estimated overall incidence of 1:35402.

Conclusion: The most common autosomal aneuploidies in Bahrain is attributed to trisomy 21 (Down's syndrome) followed, with significantly less frequency, by trisomy 18 and 13. For sex-chromosome aneuploidies, various types of Turner's syndrome (including mosaics) are the most common followed by the Klinefelter's syndrome.

INTRODUCTION

Cytogenetics have evolved over the years as a laboratory means to investigate cellular heredity to uncover chromosomal aberrations. This entails many milestone discoveries in the field, including identifying the correct number of chromosomes in human as 46 in 1956 and deciphering the chromosomal basis of Down's syndrome as trisomy 21 in 1958[1]. Chromosomal abnormalities, or anomalies, can be broadly categorized as either structural or numerical. Numerical abnormalities, also known as aneuploidies, display a missing or extra chromosome for a pair of chromosomes. The aneuploidy might affect autosomes (chromosome 1 -22) or sex chromosomes (X and Y) or a combination of both. In contrast, structural abnormalities affect structural buildup of the chromosomes in various ways including deletion, duplication, translocation, etc[2,3].

Since chromosomal abnormalities affect large segments of chromosomes in the case of structural abnormalities, or whole chromosome altogether in the case of aneuploidies, its phenotypic consequences is grossly large and global[3,4]. For instance in the case of Down's syndrome, the most common chromosomal abnormalities, a whole extra chromosome 21 is introduced giving abnormal karyotypes of 47,XX,+21 or 47,XY,+21 rather than the karyotypes of 46,XX or 46,XY for normal female and male, respectively.

In this report, we are specifically summarizing the findings of chromosomal aneuploidies, rather than structural abnormalities, in our population for the last 20 years (1999-2019). Since Salmaniya

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Medical Complex is the main tertiary public hospital for the country, this retrospective study would be a good representation of the overall population in Bahrain. To our knowledge, this is the largest systematic investigation pertinent to patients with suspected chromosomal aneuploidies in the population of Bahrain that have been referred to the Genetic Laboratory at Salmaniya Medical Complex.

The aim of this study is to define the various type of chromosomal aneuploidies on the population level in Bahrain, including the estimation of incidence rate, by using a retrospective approach. The data was extracted from our findings in the Genetic Laboratory at Salmaniya Medical Complex for the last 20 years (1999-2019). The main methodology employed for these investigations is conventional karyotype analysis for suspected cases of chromosomal abnormalities. Our main findings divided broadly between autosomal aneuploidies and sex chromosome aneuploidies. The majority of the cases attributed to autosomal aneuploidy and specifically trisomy 21 (Downs Syndrome).

A core message been forwarded for the need of implementing a preventive strategy, especially for high-risk cases, based on parents' attitudes toward pregnancy screening with emerging noninvasive prenatal screening methods. Finally, we stress the need for an effective national registry for such type of diseases in the country.

METHOD

A total of 5,153 patients were recruited retrospectively for this study during the last 20 years (1999-2019). These patients presented with various clinical manifestations including, among others, dysmorphic features, recurrent abortion, infertility and ambiguous genitalia.

Karyotype analysis was accomplished according to established protocols[5]. Briefly, 0.7 ml of heparinized blood was incubated with RBMI 1640 media supplemented with bovine serum albumin (BSA) as a source of nutrients and gentamycin as an antibacterial growth agent. Cell Culture supplemented as well with phytohemagglutinin (PHA) (Gibco, Life Technologies) as a powerful mitogen to induce cells entering cell division (mitosis). Cell culture extended for 72 hours at 37 °C in CO2 incubator (5%) and the cell division were arrested at the metaphase stage by using the spindle formation inhibitor Colcemid (Gibco, Life Technologies). After a brief treatment with pre-warmed hypotonic solution, cell culture suspension treated with fixative solution (methanol: acetic acid [3:1]) followed by meticulously optimized chromosomal spread onto microscopic glass slides. Chromosome staining was accomplished with the standard G-banding using Trypsin digestion and Giemsa staining (GTG technique) as described before[5]. Karyotype analysis consisted of two stages: chromosomes counting under light microscope (of at least 20 nuclei) and full karyotype analysis of 5 different nuclei by using software-assisted karyotype system. Karyotype images was captured and analyzed with the Leica or Zeiss microscope karyotype systems and the karyotype designations was recorded as of the ISCN recommendations[3].

For fluorescence in situ hybridization (FISH) analysis, the protocol was accomplished according to the kit's manufacturer's recommendations (Vysis, Abbott Co.). FISH results were obtained within 2-3 days after analyzing cell spreads by using a fluorescent microscope.

RESULT

We analyzed a total of 5,153 patient samples during the last 20 years (1999-2019). An average of 258 patients per year that are indicated for suspected chromosomal abnormalities. The total number of patients uncovered with chromosomal abnormalities during this period, including both chromosomal aneuploidies and structural abnormalities, is 758 cases. Structural abnormalities presented in 229 (30%) cases whereas aneuploidies, including both autosomal and sex chromosomes aneuploidies, uncovered in 529 cases (70%). Structural abnormalities would not be discussed further in this report.

Autosomal aneuploidies were found in 476 (62.8%) of the cases with aneuploidies, whereas 53 (6.9%) of the cases have sex-chromosome aneuploidies. Down's syndrome (trisomy 21) represents the vast majority of autosomal aneuploidies at 404 (53.3%) and with gender distribution showing more male cases 230 (30%) than females 174 (22.9%) indicating a sex ratio of 1.3:1 (male-to-female), see Table 1. Edward's syndrome (trisomy 18) represents the second most common autosomal aneuploidies during the last 20 years with significantly fewer numbers in comparison with Down's syndrome. We uncovered 53 (6.9%) of the cases having trisomy 18 with almost two-fold proportion for female 36 (4.7%) cases versus male 17 (2.2%) cases indicating a sex ratio of 1:2.1 (male-to-female), see Table 1. The third type of autosomal aneuploidies is Patau's syndrome (trisomy 13) and found in 19 (0.5%) of the cases with gender distribution resembling that of trisomy 18 with more than double for the female 13 (1.7%) cases versus male 6 (0.8%) cases; revealing a sex ratio of 1:2.2 (male-to-female), see Table 1.

Table 1: Number of patients and their gender identity uncovered with common autosomal chromosomal aneuploidies in the Genetic Laboratory at SMC during the period 1999-2019

No.	Aneuploidy Type	Number Identified	Male-to-Female Ratio
1	Down's syndrome:		~ 1.3:1
	Males (47, XY, +21):	230	
	Females (47, XX, +21):	174	
2	Edward's syndrome:		~ 1:2.1
	Males (47, XY, +18):	17	
	Females (47, XX, +18):	36	
3	Patau's syndrome:		~ 1:2.2
	Males (47, XY, +13)	6	
	Females (47, XX,+13)	13	

We estimated the incidence rate for Down's syndrome in our population and we found, on average for the last 20 years, one case per 701 live births of the population, see Table 2. In comparison, the overall estimation of live births incidence for trisomy 18 and 13 per year were 1:5343 and 1:16906, respectively. Finally, we found various clinical indications in relevance with suspected autosomal aneuploidies. Some of the most common request indications for autosomal aneuploidies found in this study are summarized in Table 3.

Table 2: Average Incidence Rate for the Last 20 Years of the Different Common Chromosomal Aneuploidies in the Population of Bahrain

Aneuploidy Syndrome	Designated Karyotype(s)	Incidence rate (per live birth) **
Down's Syndrome	47,XX,+21 or 47,XY,+21	1:701
Edward's Syndrome	47,XX,+18 or 47,XY,+18	1:5343
Patau's Syndrome	47,XX,+13 or 47,XY,+13	1:14906
Turner's Syndrome	Various*	1:10115
Klinefelter's Syndrome	47,XXY	1:16660
Rare aneuploidies***	Various	1:35402

*Standard Turner's karyotype is (45,X), but this rate includes mosaic cases (see table 4)
**Live birth statistics obtained from Bahrain Health Statistics portal, Ministry of Health Website (www.moh.gov.bh)
***Various types, see table 4

Table 3: The Most Common Clinical Indications for Autosomal Aneuploidies Uncovered in This Study

Down's syndrome	Edward's syndrome	Patau's syndrome
To R/O Trisomy 21	To R/O Trisomy 18	To R/O Trisomy 13
Clinical features of Down's syndrome	Multiple congenital anomalies	Multiple congenital anomalies
Heart murmur	Congestive heart failure (CHD)	CHD
Congenital Heart Disease	Low set ears	Clift lip and palate
Atrioventricular canal (AVC) defect	Wide space nipples	Undescended testis
Hypotonic infant	Abnormal index finger	Polydactyly
Epicanthal fold	Micrognathia	Microcephaly
Upward slanting eye	Rocker bottom feet	
Low set ears	Features of trisomy 18	
Depressed nasal bridge	Depressed nasal bridge	
Prominent forehead	Hypoplastic aortic arch	
Rocker bottom feet	Persistent hypocalcemia	
Short neck	Dysmorphic features	
Dysmorphic child with ambiguous genitalia	Pulmonary artery stenosis	
	Hypertrichosis	
	Low set ears	
	Colonic atresia	
	Corpus collosum agenesis	

In regard of sex chromosome aneuploidies, we uncovered 53 out of 529 total aneuploidies (10%). Seventeen (2.2%) patients presented with Klinefelter karyotype (i.e., 47,XXY) whereas 9 (1.2%) patients presented with standard Turner's syndrome karyotype (i.e., 45,X), see Table 4. In addition, we uncovered various type of mosaic Turner's syndrome in a total number of 16 (2.1%) cases, see Table 4; this includes 9 (1.2%) cases with mosaic for (45,X) and (46,XX) and 3 (0.4%) cases with mosaic for (45,X) and (46,XY). The other mosaics are for X-isochromosome (i.e., 45,X and 46,X,i(Xq)) that is found in 4 (0.5%) cases with Turner's syndrome phenotype, one (0.1%) mosaic case found with ring X-chromosome (i.e., 45,X and 46,X,r(X)) and one (0.1%) mosaic case found with marker chromosome (i.e., 45,X and 46,X,+mar). Finally, we uncovered one (0.1%) structural case presented with partially deleted X-chromosome (46,XX,del(X)(q26;q28)), see table 4. In estimation of incidence rate for these common sex chromosome aneuploidies in Bahrain, we found an incidence rate of 1:10115 for Turner's syndrome and 1:16660 for Klinefelter's syndrome, see Table 2.

Table 4: Number of Patients with Common Sex-Chromosome Aneuploidies, and Rare Sex-Chromosome Aneuploidies That Have Been Identified in the Genetic Laboratory at SMC during the period 1999-2019

No.	Sex-Chromosome Aneuploidy	Number Identified
1	**Turner's syndrome:**	
	45,XO:	9
	Mosaic 45,XO/46,XX:	9
	Mosaic: 45,XO/46,XY:	3
	Others*:	7
2	Klinefelter's syndrome (47,XXY)	17
3	47,XXX syndrome	3
4	47,XYY syndrome	2
5	48,XXY,+21	1
6	48,XYY,+18	1
7	49,XXXXY	1

*including: four mosaic cases with isochromosome X (45,X/46,X,i(Xq)); one mosaic case with the ring X chromosome (45,X/46,X,r(X)) ; one mosaic case with marker chromosome (45,X/46,X,+mar); and one case with partial X chromosome deletion (46,XX,del(X)(q26;q28))

In addition, we uncovered low number of patients with rare sex-chromosome aneuploidies, including three (0.4%) cases with 47,XXX syndrome and two (0.3%) cases with 47,XYY syndrome. For the three cases with 47,XXX syndrome: one (0.1%) is for an adult presented with ovarian insufficiency and the other two (0.3%) are for one 10-year child and an infant and both presented with macroglosia. In contrast, the two cases with 47,XYY karyotype are for one (0.1%) adult presented with azoospermia and primary infertility, and the one (0.1%) case for an infant presented with multiple congenital anomalies. Moreover, we uncovered one (0.1%) single case with 49,XXXXY karyotype in a severely sick infant, see Table 4. Finally we uncovered two (0.3%) cases with a combination of sex chromosome and autosome aneuploidies, namely 48,XXY,+21 and 48,XYY,+18. Each of these cases are for severely sick infants presented with multiple congenital anomalies and dysmorphic features due to these complex aneuploidies. A collective incidence estimation for these rare aneuploidies shows a rate of 1:35402 live births of the population, see Table 2.

In relevance with clinical presentation several clinical indications being found for the various sex chromosome aneuploidies. The overall clinical presentations for the most common sex-chromosome aneuploidies are presented in table 5.

Table 5: The Most Common Clinical Presentations provided by Physicians for Major and Rare Forms of Sex-Chromosome Aneuploidies Uncovered in this Study

Turner's syndrome	Klinefelter syndrome	47,XXX syndrome	47,XYY syndrome
Primary amenorrhea	Primary infertility	Macroglosia	Infertillity
Short stature	Azoospermia	Suspected Beck with-Wiedemann syndrome	Azoospermia
Ovarian failure	Multiple congenital anomalies	Ovarian insufficiency	Multiple congenital anomalies
Absence of ovaries	Hypogonadism		
Hypogonadism	Amenorrhea		
Primary infertility	Testicular atrophy		
Coarctation of aorta	Severe oligospermia		
Coarse facial features	To R/O Klinefelter's syndrome		
To R/O Turner's syndrome			

DISCUSSION

Down's syndrome represents the most common aneuploidy uncovered in this study, which is in concordance with a previous report published

by Al Arrayed[6]. Our findings indicate an incidence level of 1 in 682 live births (~1.5 per 1000) from a consecutive seven years of observation, see table 2. In comparison, a regional findings from Saudi Arabia, and specifically in Riyadh area, indicate an incidence level for Down's syndrome at 1 in 554 (1.8 per 1000) live births, whereas the incidence level in the USA is 1 in 787 live birth babies (~1.3 per 1000)[7,8]. In contrast, findings from England and Wales indicate a further lower live birth incidence of 1 per 926 live birth babies (1.08 per 1000); however antenatal screening and subsequent pregnancy termination has been attributed in reducing incidence rate of Down's syndrome in that region[9]. It is worth noting that our results are solely relevant to referrals of Salmaniya Medical Complex and, hence, might be an underestimation of the true frequency of Down's syndrome in the whole population. Nonetheless, our findings would most likely reflect an actual incidence since SMC is indeed the main public hospital and referral center for such cases in the country.

Gender distribution in Down's syndrome revealed a slightly more male cases versus females (a ratio of 1.3:1 male-to-female); i.e., 56% males and 44% females, see table 1. In concordance with our findings, a large study from the UK revealed a male gender presentation at 54% versus 46% for females[10]. However, the indicated study from Saudi Arabia showed that 57% of Down syndrome cases are males whereas 43% are females[7]. In addition, a higher male-to-female ratio being uncovered in a large postnatal study from China indicating a ratio 1.5 (i.e., about 70% males and 45% females)[11].

On the other hand, gender distribution for both Edwards and Patau syndromes showed consistently higher female presentations than male with more than two-fold increase for females versus males, see table 1. This higher trend for females in T13 and T18 has already been described in a previous study, although to a lesser extent[12]. In contrast, another study from nine states in the USA showed almost equal number of infants with T13 (50.1% males and 49.1%) whereas the ratio for T18 showed higher number of females versus males (38.4% males and 61.2% females)[13].

Regarding incidence rate for T18 and T13 we uncovered 1 case per 5,343 live births for T18 and 1 case per 16,536 live births for T13 in our population, see table 1. In contrast, previous study describing various world populations indicates an overall mean prevalence of 1.07 cases per 10,000 for T18 and 0.55 cases per 10,000 for T13[14]. The lower prevalence levels in these populations might reflect, at least in part, the effect of elective pregnancy termination in some of these populations[14]. Nonetheless, various clinical indications being mentioned for T18 and T13 by requested physicians with frequently indicated cardiac anomalies, see table 3. Cardiac anomalies, among others, represent a major congenital abnormality found in these syndromes[15]. For instance, the ventricular septal defect (VSD) and/or atrial septal defect (ASD) are commonly uncovered in patients having trisomy 13 or trisomy 18[15]. In a previous study, cardiac anomalies were uncovered in 80% of cases with T18 and 57% of cases with T13[15].

In regard of sex chromosome aneuploidies, Turner's syndrome stands as the most common followed by Klinefelter syndrome. Data for Turner's syndrome revealed various underlying karyotypes leading to this syndromic presentation that include structural X chromosome abnormalities as well, see Table 4. If we only consider X chromosome monosomy (i.e., 45,X) this would place Turner's syndrome in the second place of frequency after Klinefelter's syndrome, see Table 4. However, counting Turner-related karyotypes collectively would place Turner's syndrome at the first place of frequency for sex chromosome aneuploidies with only 32% of Turner-causing karyotype allocated to X chromosome monosomy (45,X), see Table 4. An overall trend was described for both Turner's and Klinefelter's from a previous study in Turkey showing a slightly higher level for Turner-causing karyotypes versus klinefelter syndrome, and 61% of Turner's allocated to X-chromosome monosomy[16]. In addition, a similar observation was described in a study from South Korea showing Turner's syndrome as the most common sex chromosome aneuploidy with various underlying karyotypes including X-chromosome monosomy at 28%[17].

With respect to the classic form of Klinefelter syndrome (i.e., 47,XXY karyotype), it is the most commonly described karyotype for this aneuploidy at 80-90% of the cases[18]. However, other rare forms do exist (like 48,XXXY karyotype) as well as some structural variants. We only uncovered a single case with atypical Klinefelter's karyotype (i.e., 49,XXXXY) in a severely sick infant. The KS cases we uncovered in our investigation are mostly from infertility clinic referrals with various clinical presentations, see Table 5. This finding is very much in concordance with the data showing that 3-4% of infertile men have the KS syndrome in comparison with only 0.1-0.2% of newborn male having KS syndrome[19].

CONCLUSION

The most common autosomal aneuploidies in Bahrain is attributed to trisomy 21 (Down's syndrome) followed, with significantly less frequency, by trisomy 18 and 13. Turner's syndrome (including mosaics) is the most common sex-chromosome aneuploidies followed by the Klinefelter's syndrome. From our study, the incidence of aneuploidies is higher than many other countries due, partly, to the fact that routine screening during pregnancy is not implemented. Therefore, we recommend further investigation on the attitudes of parents of children with chromosome aneuploidy regarding early diagnosis and the use of non invasive prenatal testing (NIPT). In addition, we strongly recommend establishing an efficient national registry for the various genetic abnormalities in the population including chromosomal anomalies, as such the country will have a unified and updated genetic database.

Author Contribution: The corresponding author made the whole effort contribution towards (1) conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content, and (3) final approval of the manuscript version to be published. Yes.

Potential Conflicts of Interest: None.

Competing Interest: None.

Sponsorship: None.

Acceptance Date: 20 October 2020

Ethical Approval: Approved by the Genetic Department, Salmaniya Medical Complex, Ministry of Health, Bahrain

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