Cytogenetic Analysis in Cases with Sexual Anomalies in Albania

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Abstract- Cytogenetic analysis is a valuable investigation in the diagnostic work up of children with suspected sexual chromosomal disorders. The objective of this study was to describe the prevalence of various types of sexual chromosomal abnormalities in Albanian children undergoing cytogenetic analysis for the time period 1984-2015. The frequencies of sex chromosomal abnormalities were calculated for comparison with data reported in similar previous studies.

Cytogenetic analysis was carried out for all 228 patients. The study was performed in the Cytogenetic Laboratory of Medical Genetics Service in the University Hospital Centre "Mother Teresa", Faculty of Medicine, Tirana, Albania according to the International System for Human Cytogenetics Nomenclature (ISCN 2009).

Turner syndrome was the most common sex chromosome abnormality among diagnosed cases (63,1%). In 144 Turner patients, 40,2% were with classic karyotype and 59,8% were with the other forms of Turner syndrome. The second frequent sex chromosome abnormality in our study was Klinefelter syndrome. Among 81 (35%) cases with Klinefelter syndrome, 73 (32%) cases showed 47/XXY classic karyotype. Triple X syndrome or trisomy X was detected in two patients (0,8%) with 47, XXX karyotype. We also found 47,XYY karyotype in only one patient. In 3 cases (1,2%) had discordance between the chromosomal sex and the phenotype.

We hope that the data gathered by this study will provide a basis for a multidisciplinary team to oversee management of these patients from diagnosis through to adult life.

The types of chromosomal abnormalities identified in this study were similar to those found in other studies.

Index Terms- Aneuploidy, chromosome aberrations, karyotypes, Turner syndrome.

I. INTRODUCTION

Sex chromosomes abnormality (SCAn) is one of the most important causes of disordered sexual development and infertility. In frequency of occurrence SCAs formed by numerical or structural alteration in X and Y chromosomes, are slightly less common than autosomal abnormalities [1].

However, they are usually much less severe in their effects.

Sex chromosome abnormalities are gender specific. Normal males inherit an X and a Y chromosome while females have two X's. A single Y chromosome is sufficient to produce maleness while it's absence is necessary for femaleness. Female abnormalities are due to variations in the number of X chromosomes. Male abnormalities are the result of irregular numbers of either the X or the Y chromosome or both [2].

Cytogenetic analysis is an essential component in the diagnosis and evaluation of children with various sexual chromosomal aberrations with congenital abnormalities, dysmorphic features, developmental delay and/or intellectual disability [3]. Studies found a wide range of sex chromosomal aberrations in children with suspected chromosomal disorders who were referred for cytogenetic analysis [4, 5, 6, 7].

The present study is designed to investigate the frequency and types of sexual chromosomal abnormalities in Albanian patients with suspected genetic disorders for the time period 1984-2015 such as: Turner syndrome (TS, the most frequent sex chromosome anomaly in females); Klinefelter syndrome (KFS, the most frequent sex chromosome anomaly in males [about 14-20% of cases with azoospermia]); sex reversal **(**female 46,XY**);** trisomy X; XYY syndrome etc. Most of them are with mosaicism karyotype who denotes the presence of two or more populations of cells with different genotypes in one individual who has developed from a single fertilized egg.

Our focus was to identify precisely the role of cytogenetic investigation in confirming the clinical diagnosis, thus allowing a multidisciplinary team to oversee management of these patients from diagnosis through to adult life. Additionally, the frequencies of sex chromosomal abnormalities were calculated for comparison with data reported in similar previous studies.

II. MATERIAL AND METHODS

In this retrospective study, the cases are evaluated newborns and children for suspicion of sex chromosomal abnormality for the time period 1984-2015, postnatally. 228 patients were subjected to a full genetic analysis. Complete genetic examination and pedigree construction was done to exclude known non chromosomal causes of the anomaly.

Firstly, children have been seen and diagnosed clinically by the Service of Medical Genetics in the University Hospital Centre "Mother Teresa", Faculty of Medicine, Tirana, Albania and then the cytogenetic analysis was performed in the Cytogenetic Laboratory of this service.

Cytogenetic analysis was carried out for all the patients. The study included peripheral lymphocyte culture determined by a standard method of G-banding and carried out according to the International System for Human Cytogenetics Nomenclature (ISCN 2009). The frequencies of our study were compared with results of similar studies.

III. RESULTS

In the present study, results of cytogenetic investigations of 228 cases with only sex chromosome abnomalities are reported.

All patients were suspected for sex chromosomal aberrations according to their clinical features. After clinical examinations and cytogenetic analyses they were grouped according to their karyotyping results and the details of sex chromosome aberrations are summarized in table 1.

Table 1. Sex chromosome abnormalities in patients referred for cytogenetic analysis

Cases	Karyotype	No	Frequency %
Turner syndrome		**144**	**63,1**
	45,X	58	25,4
	45,X/46,XY	9	3,9
	45,X/46,XX	45	19,7
	45,X/47,XXX	6	2,6
	45,X/46,XX/47,XXX	2	0,8
	45,X/46,X,i(Xq)	12	5,2
	45,X/46,XX/47,XX i(Xq)	1	0,4
	45,X/46,X r(X)/46,X,i(Xq)	1	0,4
	45,X/46,X,i(Xq)/47,XX,i(Xq)	1	0,4
	46,X,i(Xq)	8	3,4
	46,XX/46,X,i(Xq)	1	0,4
Klinefelter syndrome		**81**	**35**
	47,XXY	73	32
	46,XY/47,XXY	5	2,1
	46,XY/47,XXY/48,XXXY	1	0,4
	46,XY/47,XYY/47,XXY/48,XXYY	1	0,4
	46,XY/48,XXYY	1	0,4
Trisomy X	47,XXX	**2**	**0,8**
47,XYY syndrome	47,XYY	**1**	**0,4**
Sex reversal	46,XY	**3**	**1,2**

TS was the most common sex chromosome abnormality among diagnosed cases (63,1%). In 144 Turner patients, 40,2% were with classic karyotype and 59,8% were with the other forms of Turner syndrome.

TS abnormalities were either numerical abnormalities (120 cases or 83,4%) or structural X abnormalities (24 cases or 16,6%). In cases with numerical abnormality (78 cases), 9 patients (3,9%) had a Y chromosome component (45,X/46,XY). Six cases from numerical abnormalities were mosaicism with two cell lines of 45,X/47,XXX and two cases were mosaicism with three cell lines of 45,X/46,XX/47,XXX.

Among 24 (16,6%) TS patients with structural abnormalies, 3,4% were with 46,X,i(Xq) karyotype. The other TS variants with mosaicism form included 12 patients (5,2%) with 45,X/46,X,i(Xq) karyotype; 45,X/46,XX/47,XX,i(Xq) [0,4%]; 45,X/46,X r(X)/46,X,i(Xq) [0,4%]; 45,X/46,X,i(Xq)/47,XX,i(Xq) [0,4%]; 46,XX/46,X,i(Xq) [0,4%]. The second frequent sex chromosome abnormality in our study was Klinefelter syndrome. Among 81 (35%) cases with KFS, 73 (32%) cases showed 47/XXY classic karyotype, while 5 (2,1%) cases showed 46,XY/47,XXY karyotype, one case (0,4%) 46,XY/47,XXY/48,XXXY with mosaicism karyotype, one patient (0,4%) with 46,XY/47,XYY/47,XXY/48,XXYY karyotype and only one case with 46,XY/48, XXYY karyotype.

Triple X syndrome or trisomy X was detected in two patients (0,8%) with 47, XXX karyotype. Karyotypes of two patients with Turner syndrome, one patient with 47,XXX and one patient with Klinefelter syndrome which were confirmed by cytogenetic analysis are showed below (Fig 1a,b,c,d).

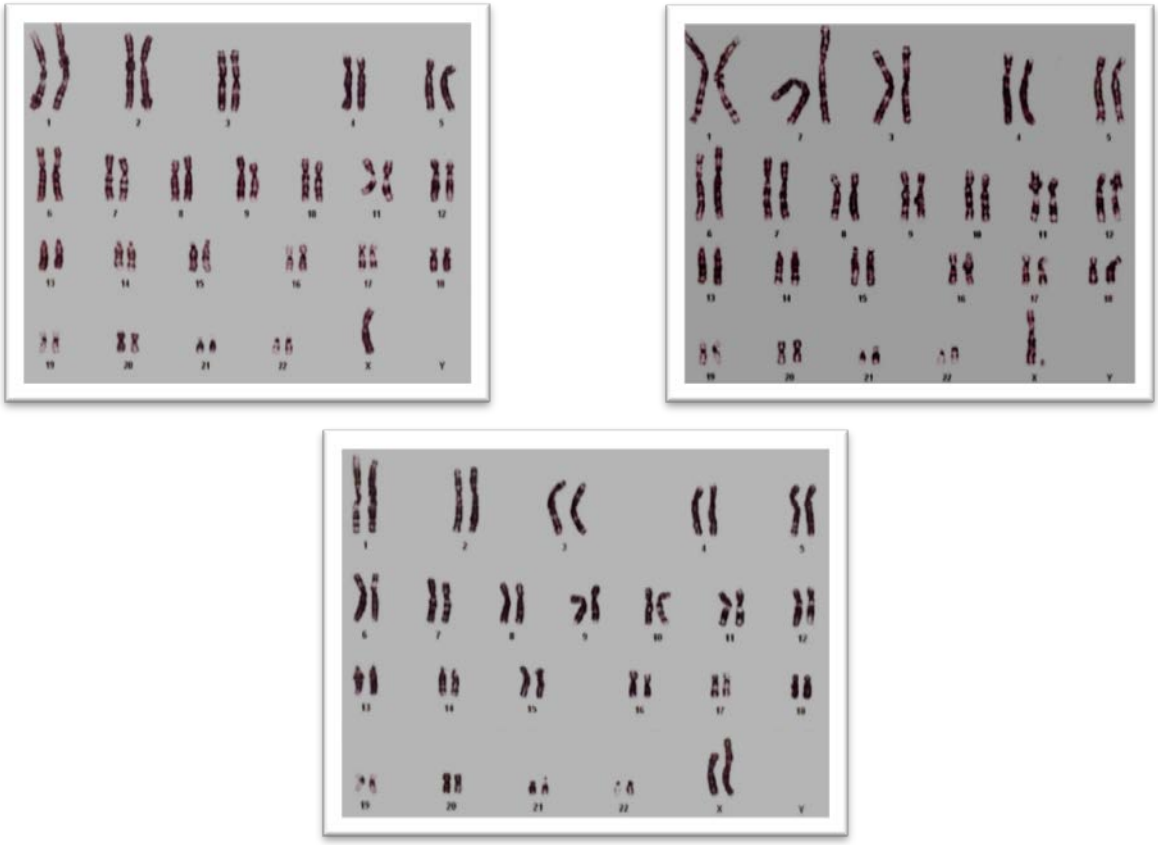

Figure 1a. A patient with 45,X/46,X r(X)/46,X,i(Xq) karyotype

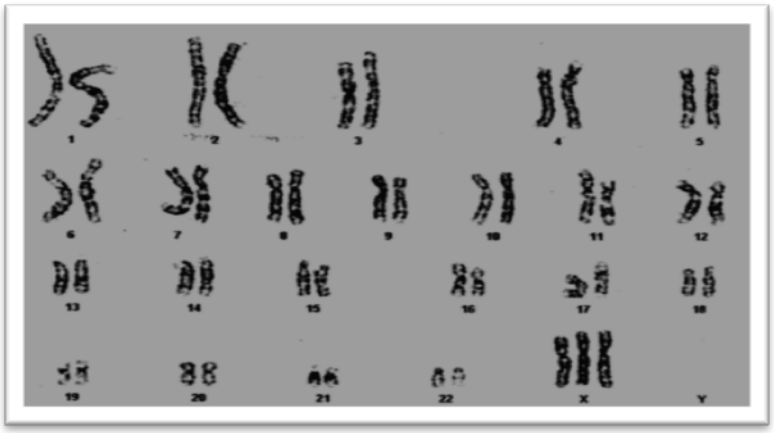

Figure 1b. A patient with 47,XXX karyotype

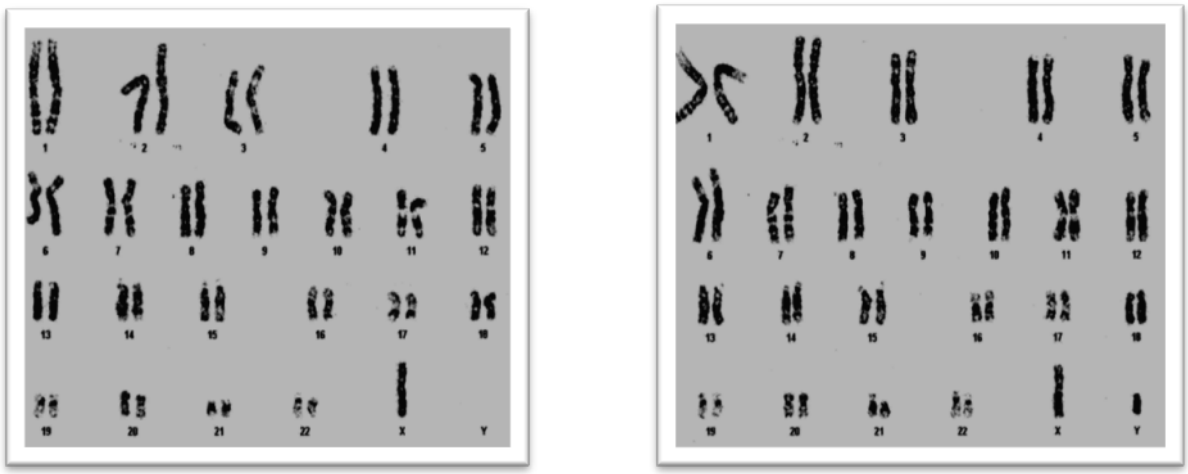

Figure 1c. A patient with 45,X/46,XY karyotype

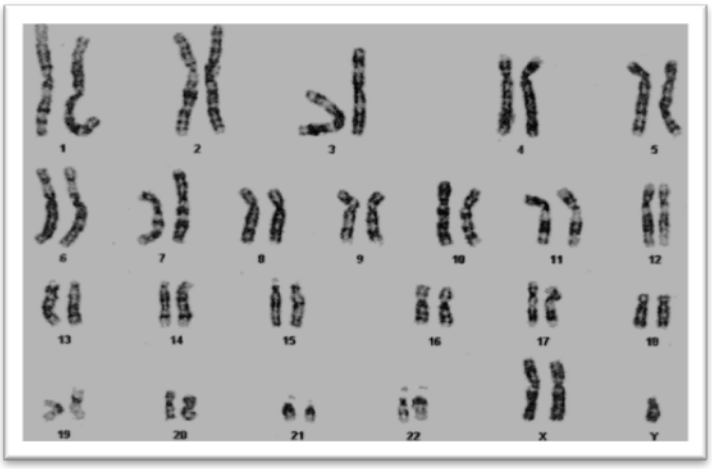

Figure 1d. A patient with 47,XXY karyotype

We also found 47,XYY karyotype in only one patient.

In 3 cases (1,2%) had discordance between the chromosomal sex and the phenotype. Another aim of this study is to compare the distribution of these aberrations with other similar reports performed in different countries (Table 2).

Table 2. Frequency of classic and others form of TS and KFS syndrome in some countries

Country	Classic Turner %	Others form of TS %	Classic Klinefelter %	Others form of KFS %
Brazil	28,6	71,4	20	80
	45	55	89,7	6
Iran	28,6	53,6	55	45
	32	68	66,6	33,4
India	34	66	80	20
	40,2	59,8	91,2	8,8

IV. DISCUSSION

The current study aimed to evaluate the cytogenetic findings in Albanian patients referred for suspected sex chromosomal anomalies that caused a variety of clinical disorders and secondly to compare the distribution of these aberrations with other similar reports performed in the other countries.

Chromosome abnormalities are important causes of lack of development in secondary sexual characteristics, delayed pubertal, miscarriage, infertility, etc. [8, 9]

TS occurs approximately 1 in 2000 females and are the most common factor in infertile women. [10] Monosomy X may arise from meiotic non-disjunction or anaphase lagging during spermatogenesis, oogenesis or from postzygotic error. TS usually results from total or partial absence of one of the two X chromosomes normally present in females. It may also result from a structurally abnormal X chromosome in which deletion or duplication of genetic material has occurred.

TS was the most frequent sex chromosome abnormality (63,1%) of totall patients. These abnormalities were either numerical or structural X abnormalities, including mosaics with normal karyotype 46 XX, mosaics with 47 chromosomes, mosaics with long-arm isochromosome, mosaics with long arm isochromosome in the patients with 47 chromosomes, mosaic with X ring chromosome and 46,XY.

We found also patients with 46,X,i(Xq) and 46,XX/46,X,i(Xq) karyotypes. Isochromosome formation may occur in pre meiotic stage, in meiotic cell division or in post zygotic cell division. All individuals with i(Xq) show characteristics similar to individuals with classical 45,X.

The distribution of Turner's syndrome in our study showed that TS variants (59,8%) were found to be commoner than the classic 45,X karyotype (40.2%). Similar findings were reported by studies in Brazil (28,6%; 71,4%) [5] , in Tunisia (32%; 68%) [12] , in Denmark (45%; 55%) [13] , in India (34%; 66%) [6] and in Iran (28,6%; 53,6%) [15], (Table 2). However, in another Indian study [28] it was found that in 45 cases of TS, the most commonly observed karyotype was 45,X (44.4%), followed by 45,X/46,XX mosaicism (24.4%).

In patients with 45,X/46,XY karyotype the amount of Y chromosome material is not enough to cause the development of any male features, but is associated with an increased risk of developing a form of cancer known as gonadoblastoma. In such cases, it is recommended that the non-functioning ovaries be removed [29] .

From the studies in different countries up to 19,7-25 % of 45,X/46,XX patients are fertile and the likelihood to have follicles in their ovaries is highest among mosaic TS girls, so finding 46,XX line in these patients could offer hope toward natural pregnancies by receiving hormone replacement therapy [16,17] . These data show that TS in mosaic status condition is more compatible with life than pure TS [18,19] .

The second frequent sex chromosome aberrations was Klinefelter syndrome (35% or 81 patients) being more frequent in infertile males [10] . The extra chromosome is retained because of a nondisjunction event during paternal or maternal meiosis I (gametogenesis) or through a nondisjunction event during meiosis II in the egg.

In general, the findings of this study are in accordance with most investigations which confirm the XXY aneuploidy to be the most prevalent chromosomal aberrations [21, 22, 23, 24]. Here, in this study we found 91,2% of classic form (47,XXY) and 8,8% of mosaism form. These frequencies are close to those reported in Denmark (89.7%; 6%) [13], in India (80%; 20%) [6] , in Iran (55%; 45%) [15], in Tunisia (66,6%; 33,4%) [12]. However, in one Brazil's study it was found that in 80% of KFS cases, the most commonly observed patients were with mosaicism karyotype [28] (Table 2). Studies on mosaic Klinefelter syndromes 46,XY/47,XXY (we observed five cases) reveal that the germ cells with sex chromosomal abnormalities were capable of completing meiosis [14] and the individuals may reproduce with the aid of modern reproductive technology.

We found also some rare mosaic variants with KFS; one male with 46,XY/47,XXY/48,XXXY karyotype, one patient with 46,XY/47,XYY/47,XXY/48,XXYY karyotype and one patient with 46, XY/48,XXYY karyotype. In three last patients we found extra copies of the X chromosome (48,XXXY) or extra copies of both the X and Y chromosomes (48, XXYY) in some of their body's cells. The most likely etiology is the non disjunction of homologous chromosomes (during the first meiotic division) or sister chromatids (during the second meiotic division) in the parental germ cells. There is no known factor responsible favoring the development of this syndrome.

XXYY is still considered a variation of Klinefelter syndrome by some definitions, mainly because the pathophysiology of the testicular dysfunction has not been shown to differ from 47,XXY. The most current research does not suggest that there should be any differences in the evaluation and treatment of testosterone deficiency in 48, XXYY compared to 47, XXY [30].

In our study we found 2 patients with 47, XXX karyotype. It mostly derives from maternal non disjunctional errors during meiosis I (63%) or II (17,4%). Only one of three X chromosomes is activated and the others two are inactivated to Barr bodies.

We found only one patient with 47, XYY syndrome that occur almost always due to paternal meiosis II non disjunction and produce high levels of testosterone. These sexual abnormalities like XXX or XYY syndromes have such a mild symptoms that are out of clinical notice. Some of these individuals are unknown of their problems and almost don't refer to any genetic lab, therefore these cases were rare.

This study also indicates that karyotyping still plays an important role in gender assignment of patients with sex reversal. Gender identity is a complex process of differentiation that is affected by numerous variables. Female phenotype can occur in XY embryo when testis determining factor or other genes in the testis determining pathway are lost, mutated or otherwise compromised [11]. Only 1.2% of patients had a discrepancy between the genetic sex and the phenotypic sex. They had normal female phenotype with 46,XY chromosome complement suggestive of sex reversal conditions. This percent was relatively lower than those found in several Indian studies [6, 27]. Rajasekhar et al [26] reported a higher proportion (14.9%) of XY females among 1400 referral cases in India. Many of such cases become apparent in infancy due to sex reversal or during adolescence when they fail to have normal puberty.

V. CONCLUSION

In conclusion, cytogenetic analysis is one of the most useful approaches to investigate the individuals with sexual problems of unknown origin and to confirm the clinical diagnose in patients with a known cytogenetic syndrome or reject the chromosomal abnormality.

However we hope that the data gathered by this study will provide a basis for a multidisciplinary team to oversee management of these patients from diagnosis through to adult life.

The types of chromosomal abnormalities identified in this study were similar to those found in other studies.

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