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SHORT STATURE IN FEMALES OF NORTH KERALA - A CYTOGENETIC ANALYSIS

Genetics

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ABSTRACT

The present study was aimed to find out the frequency of chromosomal anomalies in phenotypic females with short stature, who consulted the Paediatric, Gynecology and GeneralMedicine Departments of Government Medical College, Kozhikode, which is a Tertiary care centre of North Kerala.

Study Settings And Design: A retrospective study of karyotypes of 407 females with short stature was done. The results of cytogenetic analysis over a period of 7 years and 6 months (2013- June 2020) were assessed.

Methods: Peripheral blood lymphocytes were cultured according to standard protocol and karyotypes were analysed using Cytovision Software Version 7 and the expertise of the staff.

Results: Out of 407 patients, 76 (18.67%) showed chromosomal anomalies which includes structural and numerical aberrations. Remaining 331 females (81.33%) had apparently normal karyotype.

Conclusion: This study shows the importance of karyotyping in females with short stature after excluding other pathological causes to obtain valuable information regarding genotype-phenotype correlation associated with X chromosome.

KEYWORDS

Females with short stature,Autosomal translocations and short stature, iso(X) chromosome.

INTRODUCTION

Short stature is a term applied to a child when height is less than 3rd or 4th percentile for that age [1]. It may be either a variant of normal growth or pathological.

The most common causes of short stature beyond the first or second year of life are genetic (familial) short stature and delayed growth, which are considered normal, non pathologic variants[2].

The aim of evaluation of a child with short stature is to identify the pathologic causes and to intervene, if necessary. Pathologic causes of short stature include under nutrition, steroid therapy, gastrointestinal diseases such as Crohn's disease, chronic renal disease, malignancies, lung diseases, congenital heart diseases, metabolic and immunologic disorders.

Endocrine disorders like hypothyroidism and Growth hormone deficiency are important causes of short stature.

Intracranial tumours, cranial irradiation and head injuries can also interfere with linear growth.

As majority of patients with short stature referred to the cytogenetic lab were females, the authors decided to evaluate the cytogenetic causes in them. They belonged to the age group of 3-20 years.

OBSERVATION AND RESULTS

A total of 407 cases of phenotypic females were evaluated for chromosomal anomalies. 331(81.33%) of them had 46,XX karyotype , while 76 (18.67%) had chromosomal anomalies which included numerical as well as structural abnormalities. The details of these are shown in Table 1.

Table 1: Types Of Chromosomal Anomalies Detected In Females With Short Stature

Sl No	Type of chromosomal anomaly	No: of cases	Percentage
1	45,X	20	4.91
2	46,XY	4	0.98
3	Mosaics	15	3.69
4	Isochromosome X	11	2.7
5	Ring Chromosome X	1	0.25
6	Deletion of X chromosome	3	0.74
7	Translocation of autosomes	3	0.74
8	47,XYY	1	0.25
9	Inversion (chromosome 8,9)	2	0.49
10	Marker	2	0.49
11	Derivative	2	0.49
12	Polymorphic Variants	12	2.94

DISCUSSION

Growth is a complex process which is influenced by genetic, endocrine and environmental factors including nutritional status of mother during pregnancy. Many genotype-phenotype analysis have shown that there is strong genetic cause for stature . Autosomal, sex chromosomal or both anomalies together can affect the linear growth and result in short stature.

Stature and sexual development are influenced bythe X and Y chromosomes. Sex chromosome anomalies are common and lead to several phenotypic abnormalities. Absence of or structural abnormalities of autosomes usually lead to severe abnormalities and can end up in abortion.

Prevalence of X chromosomal anomalies like monosomy, trisomy, XY gonadal dysgenesis is 1 per 1000 live births [3,4,5].The distal end of short arm of X chromosome (Xp) had been considered to play an important role in somatic development. Short stature is the result of haploinsufficiency of SHOX (Short Stature Homeobox - containing gene located at Xp22.33;Yp11.3)which codes for a transcription factor responsible for skeletal growth [6,7]

Turner Syndrome:

Turner syndrome is considered to be one of the important genetic causes of short stature in females affecting 1 in 2000 girls[8]. Classicmonosomy X was detected in 20 patients (4.91%). Short stature was the main presenting feature in all these females. Shailaja CM, Shobha*et al*[9]studied 62 cases of suspected Turner syndrome and 43 of them turned out to be Turner or its variants. Most common among them was 45,X(62.79%), followed by 45,X/46,XX(23.25%), 45,X/46,X i(6.97%) and 2.32% with 45X/46,XY. Probably the high incidence of chromosomal anomalies is due to selection of patients with features highly suggestive of Turner syndrome.

In a study conducted by Tahir.M.Malla, Fayaz A *et al*[10]inKashmir on 108 females with short stature and primary amenorrhoea, two unique cases were reported. Onepatient has 46,XX,dup2q(13)and another female had 46,XX,t(2;5)(p11.2;q34).

Autosomal translocations were detected in our study also. There were three cases (0.74%) of translocation

1.46,XX,t(2;7)(q31;q11.23)
2.46,XX,t(2;10)(q21;q11.2)
3.46,XX,t(1;7)(p32;q22)

RashmiTalwarSethi*et al*[11]have reported a case of balanced

translocation. The karyotype was 46,XX,t(2;9)(p23;13). They suggest that genes for human height could be mapped to short arm of chromosome 2 and/or long arm of chromosome9.

Filippis, L.Fatti*et al*[12] reported a case of balanced reciprocal translocation, 46,XX,t(10;15)(q22.3;q26;1)interrupting ACAN gene.RajasekharMoka[13]*et al*reported a case of t(12;14) translocation in a female.

Isochromosomes Of Long Arm Of X Chromosome i(Xq)

This phenomenon occurs due to abnormal transverse cleavage of the centromereof X chromosome during cell division. As a result of this, the X chromosome will contain either two short or two long arms resulting in unbalanced genetic constitution. This leads to monosomy for the missing and trisomy for the duplicated arms[14]. This process of isochromsome may occur in premeiotic, meiotic gametes or post zygotic cell divisions[15]. Females with isochromosomes usually present with short stature. They do not have Turner Syndrome features.

ShanoliGhosh, Pritha Pal *et al*[16]studied 50 females with short stature and reported 4 cases of(8%) isochromosome X (i(Xq)). Sybert and McCauley[17] have reported 46,X,i(Xq) karyotype in 7% of patients with short stature. In our study, there were 11 cases of isochromosomes (2.7%). In 10 patients , the karyotype was 46,X ,i(Xq) showing that short stature in these patients is a consequence of haploinsufficiency of SHOX. However, one patient presented with the genotype 46,X,i(Xp).

Mosaics

There were 15 cases of mosaics(3.69%) which are shown in **Table 2.**

Table 2: Types Of Mosaics Detected

Sl No:	Type of mosaic	Number
1	45,X/46,XX	3
2	45,X/46,XY	2
3	45,X/46,der X	2
4	45,X/46,r(X)	1
5	45,x/46,i(Xq)	3
6	46,X,del(X)/45,X	2
7	45,X/46,X mar	1
8	45,X/46,X,der(X),add(X)(p22.3/47,X,der(X),add (X)(p22.3;.der(X),add(X)(p22.3)	1

Rajasekhar Moka*et al* have reported 9.58% cases of mosaic karyotypes in their study. Shailaja C M, Shobha*et al* have reported 15 cases out of 62 (24.19%) with mosaic karyotypes in patients with Turner Syndrome features.

Shanoli Ghosh*et al* detected 6% cases of mosaics in their Cytogenetic study. Uzma Iqbal, Sequib Mehmood[18]*et al* report a high incidence of mosaics (35%) in their study which belonged to the category 45,X/46,XX. The number of cases studied by these authors is only 25. More varieties of mosaics are obtained as the sample size increases.

46, XY females

In our study, there were 4 cases of XY females (0.98%). Several authors have reported this anomaly in their cytogenetic study of females with short stature and primary amenorrhoea. Mohajer Tehran *et al*[19](18.2%),Wong *et al*[20] (8.4%), Kong *et al*[21](30%), Vijayalakshmi *et al*[22](17.9%), Ramirez[23] (7.85%),Butnarieu[24](5.2%), and Ten[25]*et al* (13.7%) are some of the authors who have reported XY females. There is a wide variation in the number of cases reported by each author.

Androgen insensitivity is one of the main causes of female phenotype in such cases. These patients should be investigated radiologically to detect the presence and location of testes. These gonads can undergo malignant changes later in life.

We had already reported a case of a phenotypic female with XY karyotype, who, after investigation, proved to be a case of 17 α hydroxylase deficiency[26]. This leads to decreased synthesis of cortisol and sex steroids. Absence of male sex hormones leads to feminine features in the affected individual.

Structural Abnormalities

Deletions were detected in 3 cases (0.74%) - 46,X,del(X)(p11.2pter), 46,X,del (X)(p11.22) and 46,X,del(X)q21.2) were the karyotypes.

Rajasekhar Moka *et al* have reported 2 cases of deletions involving X chromosomes(0.68%) - 46,X,del(X)(pterp21) and 46,X, del(X) (qter>q28)

Partial deletions of short arm of X chromosome is known to produce significant ovarian insufficiency, features of Turner syndrome including short stature and gonadal dysgenesis[27] . Xp21 segment seems to cause short stature [28]

Inversions

2 cases (0.49%) were detected in our study. 46,XX,inv(9)(p12q13)and 46, XX, inv(8)(p21q22). Pericentric inversion in chromosome 9 is said to be a common heteromorphism in general population. Rajasekhar Mokha*et al* have also reported 2 cases of inversion with 46,XX, inv(9qh) karyotype.

A rare case of 47,XYY(Fig 1)

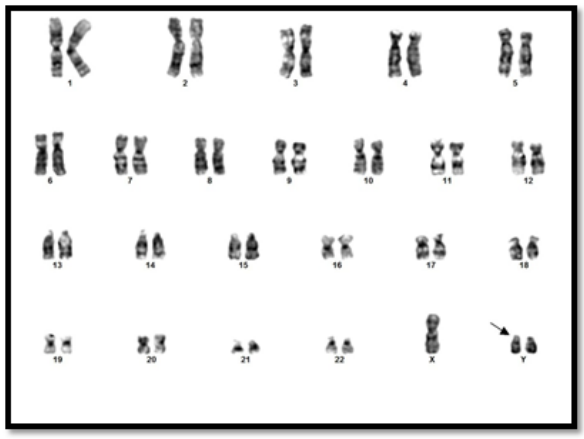

Fig 1: XYY karyotype

We had one case of a short female with 47,XYY karyotype. There were no signs of Turner Syndrome. The IQ was normal. 47,XYY is a chromosomal anomaly that is typically observed in patients with a male phenotype. Zhi-HuiLui[29]*et al* have reported a female with a rare karyotype mos46,XY/47,XYY. This female child was brought to the hospital with complaints of short stature. Karyotype analysis showed 47,XYY and chromosomal microarray examination revealed mos 46,XY/47,XYY.

May be during the post zygotic mitotic division, some cells might have got one additional Y chromosomes and some cells received 45,X only. Probably the Y chromosome is suppressed or silent. This explains the female phenotype and short stature.

Another case was reported by Grace H J, Campbell G D[30]. The 14 year old female had short stature and karyotype was 47,XYY. There was no sign of Turner Syndrome and mental and social abilities were normal.

The pathogenic mechanism is not clear. This genetic disorder may be the result of defects in certain signaling molecules in chromosomes X and Y or androgen signaling pathways. Molecular genetic screening may pinpoint the cause.

Pelvic imaging is compulsory in these patients and the immature gonads should be removed, because chances of malignancy in the gonad are quite high.

Parents have been raising the child as a girl. It can be continued. Meanwhile, sex hormone replacement therapy and growth hormone can be administered, so that the patient may gain more height and improve the quality of life.

Marker Chromosome

We came across 2 cases (0.49%) with marker chromosomes. mos 45,X/46,X,+mar and 46,X,+mar. Rajasekhar Moka *et al* have detected similar cases in their study(0.68%)

Ring Chromosome

Only one case (0.25%) was detected, as a mosaic 45,X/46,X,r(X). It is reported that females with a ring chromosome X have signs of Turner syndrome including short stature, dysmorphic facial features, ovarian

dysgenesis, endocrine disorders and auto immune condition[31]. These females are at a higher risk of mental retardation, learning disorders, autism, and structural anomalies of brain due to the loss of XIST region[32]

Polymorphic Variants

There were 12 cases (2.95%) showing polymorphic variants, as follows - 9 qh+
(3 cases), 9qh-(2cases), 15 ps+(2cases), 1qh+,1qh-,21ps+,22ps+,21 pstk+ one each

A Very Rare Case:

We have an extremely rare case to report. A female with short stature showed mos45, X/46,X,der(X)add(X) (p22.3)/47,X, der(X)add(X)(p22.3),der(X)add(X)(p22.3)(Fig2a,b,c) karyotype

Fig 2 . A,B,C. A Very Rare Karyotype In A Female With Short Stature

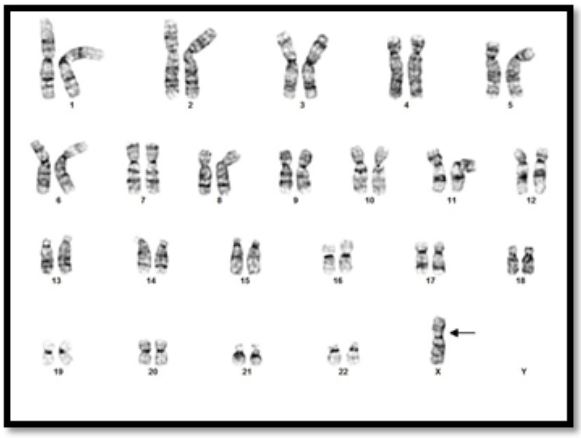

Fig 2a. Cell With 45X Karyotype

Fig 2 B Cell With 46,X,der(X)add(X)(p22.3) Karyotype

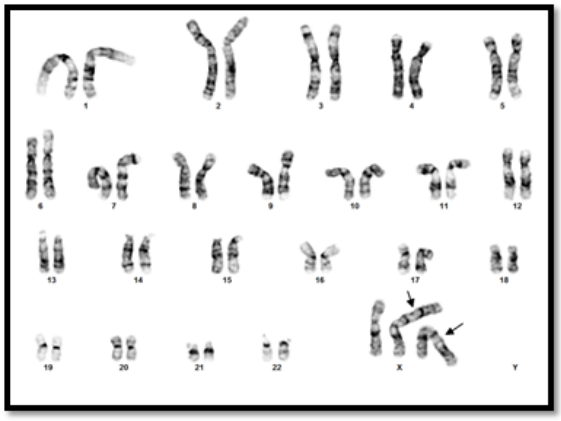

Fig 2c - Cell With 47,X,der(X)add(X)(p22.3),der(X)add(X)(p22.3) Karyotype

CONCLUSION

Growth is a complex process influenced by a lot of factors including genetic factors, endocrine factors and nutrition. After excluding endocrine factors and malnutrition, a genetic study must be conducted in females with short stature to obtain information about the genotype - phenotype correlations related to the sex chromosomes.

Patients with isochromosome X are at a high risk of mental retardation, learning disabilities and structural anomalies of brain. So, if i(X) is detected, all these should be specifically ruled out.

Early diagnosis of exact cause of short stature and timely intervention will enable the individual to lead a healthy fruitful life.

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REFERENCES

1. Freeman JV, Cole TJ et al. Cross sectional stature and weight reference curves for the UK, 1990. Arch Dis child.1995; 73:17-24
2. Erick J Richmond, Alan D Rogol, Causes of short stature. UpTo Date, April2020.
3. Ford CE, Jones KW, Polani PE, De Almeida JC, Briggs JH. A sex chromosomal anomaly in a case of gonadal dysgenesis (Turner's syndrome) Lancet. 1959;1:771–3.
4. Jacobs PA, Baikie AG, Brown WM, Macgregor TN, Maclean N, Harnden DG. Evidence for the existence of the human "superfemale". Lancet. 1959;2: 423–5.
5. Guidozzi F, Ball J, Spurdle A. 46, XY pure gonadal dysgenesis (Swyer-James syndrome) -Y or Y not : A review? ObstetGynecolSurv. 1994; 49:138–146.
6. Rao E, Weiss B, Fukami M, Rump A, Niesler B, Mertz A, et al. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. Nat. Genet. 1997;16:54–63.
7. Chen J, Wildhardt G, Zhong Z, Roth R, Weiss B, Steinberger D, et al. Enhancer deletions of the SHOX gene as a frequent cause of short stature: The essential role of a 250 kb downstream regulatory domain. J Med Genet. 2009;46: 834–9.
8. Donaldson MDC, Gault EJ, Tan KW, Dunger DB. Optimising management in Turner syndrome: from infancy to adult transfer. Arch Dis Child . 2006; Jun;91(6):513-20.
9. Shailaja CM, Shobha BJ, Vijayakumar NK, Pravinkumar, Clinical and Cytogenetic Study of Turner Syndrome and its variants. International Journal of Anatomy and Research. 2020; Apr. 2, 7440-44.
10. TahirM.Malla ,FayazA.DarArshadA.PandithMahrukhH.Zargar. Frequency and pattern of cytogenetic alterations in primary amenorrhea cases of Kashmir, North India Egyptian Journal of Medical Human Genetics, January 2016, 17: (1) 25-31.
11. RashmiTalwarSethi,Sunil Kumar Singh Vats,Mohit Kumar Verma, RohitAgarwal. A Novel Translocation t(2;9)(p23;q13) in Female with Short Stature. International Journal of Human Genetics, 2011, Vol: 11, Issue 4.
12. De Filippis T , Fatti L , Taurino J et alA balanced reciprocal translocation t(10;15)(q22.3;q26.1) interrupting ACAN gene in a family with proportionate short stature August 2018; J Endocrinol Invest, 41(8):929-936. Epub 2018 Jan 4.
13. Rajasekhar Moka, Kodandapani Sreelakshmi et al. Cytogenetic evaluation of patients with clinical spectrum of Turner syndromeJ Hum Reprod Sci. 2013 Apr;6(2):129-32.
14. Young ID 2005. Medical Genetics. Oxford: Oxford University Press.
15. Gardner RJM, Sutherland GR 2004. Chromosome Abnormalities and Genetic Counseling. 3rd Edition. Oxford Monograph on Medical Genetics No. 46. New York: Oxford University Press.
16. Shanoli Ghosh, Pritha Pal, AtreyeeDutta, Sanchita Roy, Ajanta Halder. Short Stature : The Only Morphological Stigmata for IsochromosomeXq VIMS Journal, July 2016.
17. Sybert VP, McCauley E. Turner's syndrome. N Engl J Med. 2004;351(12):1227–38.
18. Uzma Iqbal, Saqib Mehmood, Muhammad Faisal and Shahida Hasnain, 2007. Chromosomal Analysis of Girls with Short Stature and Puberty Failure. *Trends in Medical Research, 2: 204-207.*
19. Farnaz Mohajertehran, Kazem Ghodsi,et al. Frequency and the Type of Cromosomal abnormalities in Patients with Primary Amenorrhea in Northeast of Iran. Iran J Basic Med Sci. 2013, Apr 16(4): 643-47.
20. Wong MSF, Lam STS. Cytogenetic analysis of patients with primary amenorrhea and secondary amenorrhea in Hong Kong: retrospective study. Hong Kong Med J. 2005;11: 267–72.
21. Kong H, Ge YS, Wu Q, Wu HN, Zhou DX, Shen YY. Molecular and cytogenetic study on 18 cases of amenorrhea: the use of fluorescence in situ hybridization and high resolution comparative genomic hybridization. Chinese Journal of Medical Genetics, Jun 2007, 24(3):256-60
22. Vijayalakshmi J, Koshy T, Kaur H. Cytogenetic analysis of patients with primary amenorrhea. IntJ HumGenet . 2010; 10:71–76.
23. Ramírez G, Herrera C, Durango N, RamírezJ45, X/46, X, r (X) karyotype in patients with clinical diagnosis of Turner's syndrome. Iatreia. 2000; 13:161–66.
24. Butnariu L, Covic M, et al Clinical and cytogenetic correlation in primary and secondary amenorrhea: retrospective study on 531 patients. RevistaRomândeMedicin de Laborator. 2011; 19:149–60.
25. Ten SK, Chin YM, Noor PJ, Hassan K. Cytogenetic studies in women with primary amenorrhea. Singapore Med J. 1990;31: 355–59.
26. AshalathaPR , Narayan Govindaraj, Manju. Phenotype-karyotype correlation of patients with primary amenorrhoea in North Kerala. IndJ App Res. March 17, Vol.7 (3): p.1-6.
27. Kaiser P, Gerhard-Ratschow K, Zabel B, Daume E. Short-arm deletion of an X chromosome (45,XO/46,XX p-) Hum Genet. 1977;37:93–6.
28. Zinn AR, Tonk VS, Chen Z, Flejter WL, Gardner HA, Guerra R, et al. Evidence for a Turner syndrome locus or loci at Xp11.2-p22.1. Am J Hum Genet. 1998; 63:1757–66.
29. ZhiHui Liu, Shi Chao Zhou, Jun-Wen Du, Kun Zhang and Tao Wu. BMC Endocrine Disorders. A patient with 46,XY/47,XYY karyotype and female phenotype: A case report. 2020, Vol.20 Article 42.
30. Grace HJ, Campbell GD. XYY karyotype, female phenotype and gonadal dysgenesis, A case report. S Afr Med J. 1978;54:284–6.
31. Dennis N, Coppin B, Turner C, Skuse D, Jacobs P. A clinical, cytogenetic and molecular study of 47 females with r(X) chromosomes. Ann Hum Genet. 2000;64: 277–93.
32. Kuntsi J, Skuse D, Elgar K, Morris E, Turner C. Ring-X chromosomes: Their cognitive and behavioural phenotype. Ann Hum Genet. 2006;64:295–305.