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Original Article

Cytogenetic spectrum and clinical presentation of Klinefelter syndrome: A comprehensive study from South India

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ABSTRACT

Background: Klinefelter syndrome (KS) is a common sex chromosome abnormality that mostly presents as testicular failure and a myriad of other clinical features. The phenotypic features and their severity vary between individuals, age groups and cytogenetic subtypes. Karyotyping remains the method of choice for the diagnosis of KS despite advanced molecular testing in this modern era. The objective of this study was to determine the cytogenetic subtypes, clinical presentation and hormonal profile of patients with KS.

Methods: Retrospective observational study comprised patients with KS determined by cytogenetic analysis at the tertiary care centre in south India from 2001 to 2019. Clinical details including testicular size, hormone levels and semen analysis were obtained from the medical records.

Results: There were 147 KS (145 post-natal and 2 pre-natal) patients ranging in age from two days to 51 years, 116 (74.8%) patients being ≥18 years. Classic (cKS), mosaic (mKS) and variant KS (vKS) accounted for 126 (85.7%), 9 (6.1%) and 12 (8.2%) cases and their mean ages were 26.6, 29.6 and 12.8 years, respectively. cKS and mKS had diverse clinical presentations including several co-morbidities of which small testes were most common (94.8%). vKS presented with behavioural problems (58.3%) without any comorbidities. Testosterone ($p < 0.05$), FSH and LH levels were abnormal in >90% of patients.

Conclusion: All three cytogenetic subtypes of KS were seen in our patients. cKS was seen in all ages, mKS was common in adults and vKS was seen predominantly in children. Early diagnosis of KS and timely medical intervention may alter the clinical outcomes and improve the quality of life.

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Introduction

Klinefelter syndrome (KS) is the most common sex chromosome abnormality and is an important cause of testicular failure.[1,2] Testicular dysfunction in KS is characterised by impaired spermatogenesis and Leydig cell dysfunction resulting in primary (hypergonadotropic) hypogonadism characterised by low/mid-normal levels of testosterone and increased levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).[2] The testes show progressive loss of germ cells due to replacement of germinal epithelium by Sertoli cells, tubular atrophy, Leydig cell hyperplasia and interstitial fibrosis, becoming small and firm.[1,2]

This syndrome was first described in 1942 by Klinefelter et al in nine males with tall eunuchoid stature, gynecomastia, small testes, azoospermia and elevated urinary gonadotropins.[3] These patients were found to be Barr body (sex-chromatin) positive, but it was only in 1959 that Jacobs and Strong demonstrated the presence of an additional copy of the X chromosome and a 47,XXY karyotype.[4] Its prevalence in newborn screening studies from the West is 1–2.2 per 500–1000 and 0.6 per 1000 in Japan.[5,6] It is estimated that 50–75% of KS with less severe or mild forms are undiagnosed.[7–10]

Classic KS (cKS) (47,XXY) accounts for 80–85% of patients.[1] The subtypes of KS include variants with two or more additional sex chromosomes and mosaic KS (mKS).[7,8,10,11] Variant KS (vKS) are infrequent: 48,XXYY and 48,XXXY karyotypes are estimated to occur in 1 in 17,000 and 1 in 50,000 male births, respectively, while 49,XXXXY is even rarer, occurring in 1 in 85,000 to 100,000 male births.[1,12,13] Less commonly, (vKS) may be due to 49,XXXYY or gain of a structurally abnormal X chromosome resulting in a 47,X,i(X) (q10),Y karyotype.[2,10,11,14] mKS is characterised by the presence of a normal cell line, most commonly 47,XXY/46,XY, and is result of mitotic non-disjunction post fertilisation or loss of an X chromosome because of anaphase lag.[10]

Therefore, KS may present with a wide variety of phenotypes which include features associated with androgen insufficiency, neurodevelopmental, behavioural and psychosocial disorders.[1,7,8,10,15] The presenting features and their severity vary between individuals, age groups and cytogenetic subtypes. The phenotypic abnormalities worsen with the number of additional X chromosomes, therefore vKS have more severe phenotypes than cKS, while mKS have a milder form of the disorder depending upon the degree of mosaicism and the tissues affected.[1,7–11,16]

The clinical features associated with KS usually become evident at puberty or in adulthood.[1,7,12] Some patients may have overt features of hypogonadism including sexual dysfunction while others have normal virilisation or only mild signs of androgen deficiency and be diagnosed only when investigated for infertility.[1,2,8,10,17]

KS may also be associated with co-morbidities that appear during adulthood and worsen with advancing age, increasing morbidity and reducing life by about two years.[6,10,15] These include diabetes, obesity, dyslipidemia, osteoporosis, predisposition to venous thrombosis and susceptibility to breast carcinoma and extragonadal germ cell tumours.[2]

Despite the increasing use of advanced molecular testing for the diagnosis of genetic disorders, conventional cytogenetic analysis (CCA) of peripheral blood is still the method of choice for establishing a diagnosis of KS.[2] We describe the cytogenetic subtypes, clinical presentation and hormonal profile of patients with KS.

Materials and methods

This retrospective observational study included patients with KS determined by CCA at the tertiary care centre in south India from 2001 to 2019. Details of the clinical presentation including testicular size, hormone levels and results of semen analysis were obtained from medical records.

Cytogenetic and fluorescence in-situ hybridization (FISH) analysis of phytohaemagglutinin-stimulated peripheral blood and prenatal samples (one each of chorionic villous sample and amniotic fluid) was performed using standard protocols and an automated karyotyping system (2.8.0/1.2.5, 5.5.10, 5.8.8, 5.8.10 and 5.8.14 versions of Ikaros, MetaSystems, GmbH, Altlussheim, Germany).[18] The results were reported as per available editions of An International System for Human Cytogenetic/Cytogenomic Nomenclature (ISCN) during the reporting period.[19–21]

At least 25 G-banded metaphases were studied in all patients, and extended to at least 50 metaphases when mosaicism was suspected, which is estimated to exclude <10% of mosaicism with a 0.99 confidence level.[22] FISH analysis was performed using probes for the centromeres of chromosomes X and Y to confirm mosaicism. At least 500 interphase cells and all available metaphases were analysed by two or more observers for the confirmation of mosaicism. Cut-off values for low-level mosaicism were determined to be 5% for interphase FISH analysis in our laboratory, and at least three metaphases with monosomy or two metaphases with trisomy as per the ISCN definition for different cell lines. Polymerase chain reaction (PCR) for Y chromosome microdeletions in the a, b, and c loci of the AZF region was performed in a subset of patients with azoospermia.[23] Hormone levels were assessed by chemiluminescent immunoassay done on Siemens Immulite 2000 using Immulite kits. The flowchart depicts the methodology followed in the study (Fig. 1). All guidelines as per the Declaration of Helsinki and good clinical practice guidelines were followed.

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Statistical analysis

The distribution of study variables was summarized using descriptive statistical methods. Kruskal–Wallis rank sum test was used to determine the statistically significant ($p < 0.05$) difference between the medians of three or more independent groups.

Results

There were 147 KS, comprising 145 (98.6%) post-natal and two (1.4%) pre-natal samples. Post-natal patients ranged in age from two days to 51 years, 116 (74.8%) being ≥18 years. Of the 29 (19.7%) who were ≤17 years old, 17 (11.6%) were <12 years old.

cKS accounted for 126 (85.7%), mKS for 9 (6.1%) and vKS for 12 (8.2%) each. The two prenatal samples comprised one cKS with concurrent trisomy 21 (double aneuploidy) and one mKS. The distribution of subtypes by age is shown in Fig. 2 and Table 1.

cKS was seen in all age groups (two months–48 years) with 84.1% being ≥18 years, and 47.6% in the 18–30-year age group. Most (77.8%) mKS were seen in those ≥30 years, but the youngest (two days) and oldest (51 years) patients overall were seen in this subtype. vKS (1-28 years) was most common (75) in those ≤17 years.

The mean ages of diagnoses of classic, mosaic and vKS were 26.6, 29.6 and 12.8 years, respectively.

Presenting features according to cytogenetic subtypes

Clinical data was available for 139/145 (95.9%) patients including 110 adults. All six adults for whom details of clinical presentation were not available had cKS. The overall frequencies of common presenting features, findings and co-morbidities by age and cytogenetic subtype are shown in Table 1.

All three cytogenetic subtypes presented with one or more features of hypogonadism and/or disordered neurologic development. cKS which was seen in all age groups had the most diverse clinical presentation which included several co-morbidities. mKS was also associated with diverse clinical presentations including some co-morbidities.

vKS presented with behavioural problems (58.3%), developmental delay (50%), dysmorphism and speech delay (33.3% each), micropenis (25%), and intellectual disability (16.7%). Other features included delayed secondary sexual characteristics and small testes (41.7% each) and cryptorchidism, gynaecomastia, skeletal abnormalities and a ventricular septal defect (8.3% each). vKS was not associated with co-morbidities.

Presenting features according to age

i) <12 years, n = 17

The most common presenting features in this category were developmental delay and dysmorphism in 10 (58.8%) each and behavioural problems in seven (41.2%). Other presenting features included speech delay in four (23.5%) and intellectual disability and seizures in three (11.8%) each. Of the four children (23.5%) with cryptorchidism, one sought attention for bilateral inguinal swellings, but it was an additional finding in the remaining three (17.6%). Three children (17.6%) who presented with dysmorphism or developmental delay were found to have ventricular septal defects, and one with a patent ductus arteriosus. One patient presented with obesity and another, a baby with a deformity of the left leg (fibular aplasia, campomelia, and oligosyndactyly consistent with FATCO syndrome) which was considered to be a co-incidental finding.

ii) 12–17 years, n = 12

The most common finding was small testes in 10 (83.3%) patients. Frequent presenting features were delayed/poorly developed secondary sexual characteristics in nine (75%) patients, gynecomastia and behavioural problems in six (50%) each and developmental delay in five (41.7%).

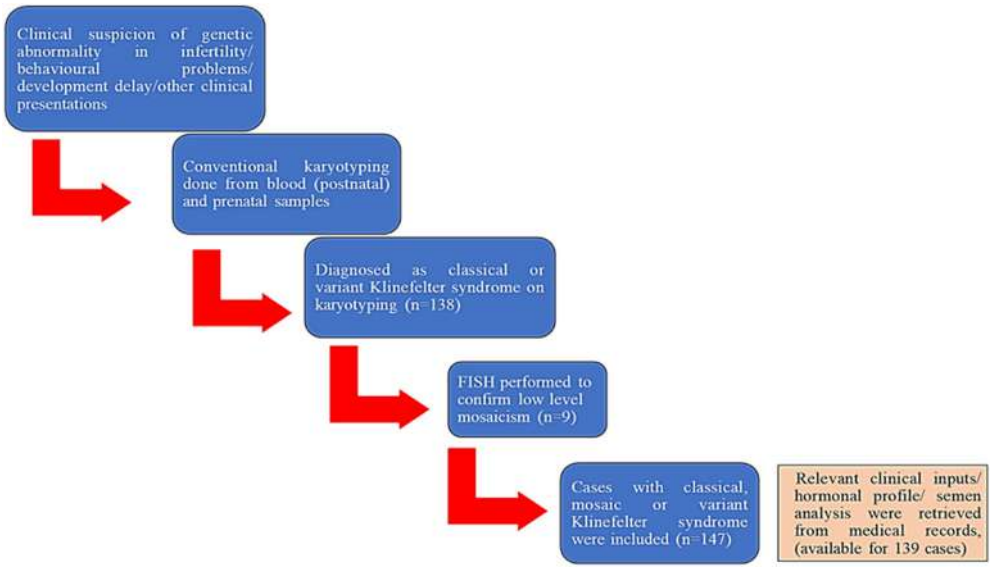

Fig. 1 – Flow diagram of the cohort.

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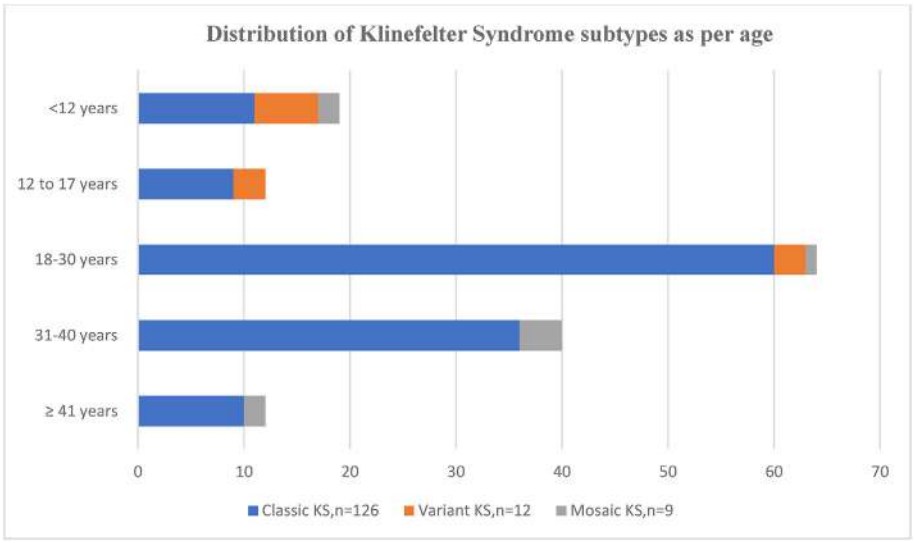

Fig. 2 – Distribution of Klinefelter syndrome subtypes as per age.

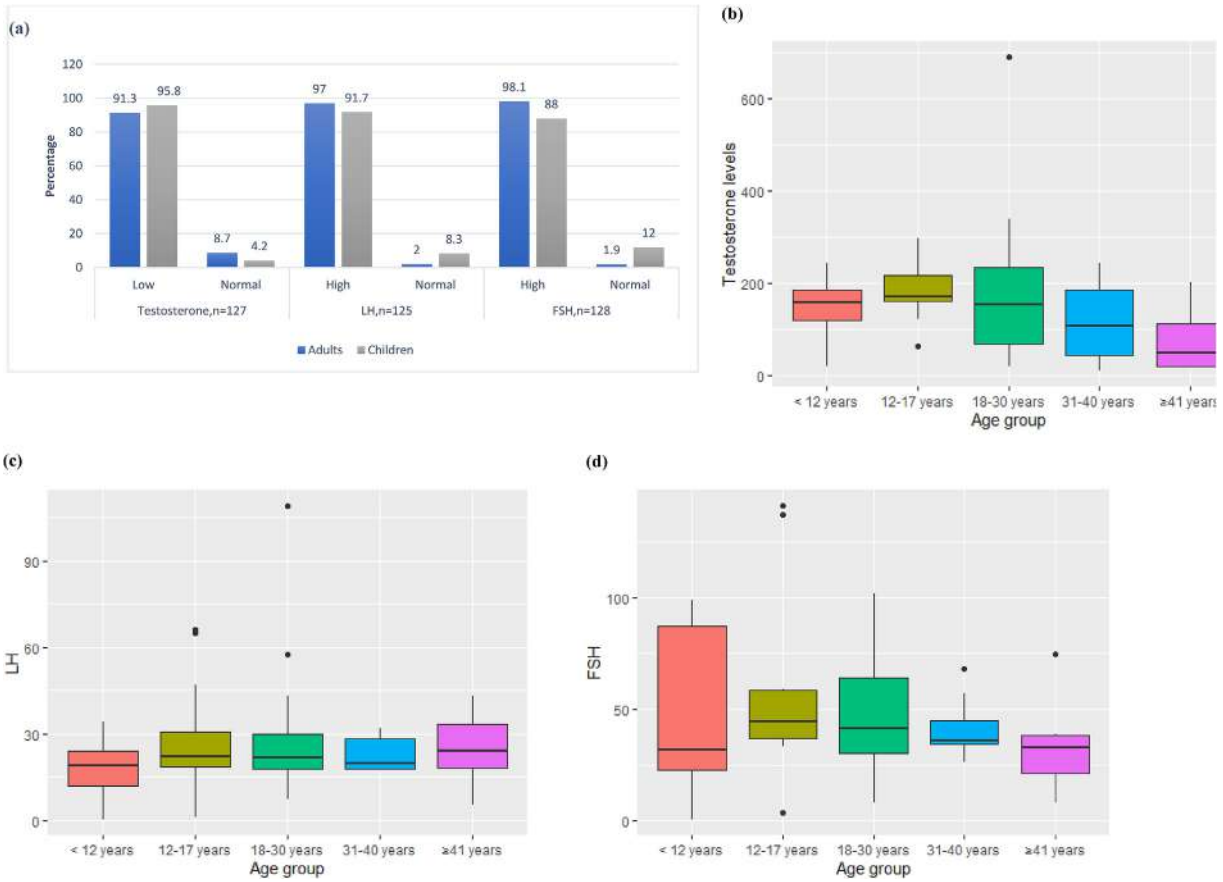

Fig. 3 – (a) Hormone levels in Klinefelter syndrome. (b) Testosterone levels according to age groups. (c) LH levels according to age groups. (d) FSH levels according to age groups.

iii) ≥18 years, n = 110

All the adults presented with one or more features related to hypogonadism. Common presenting features included delayed/poorly developed secondary sexual characteristics in 100 (91%) of patients and/or infertility in 48 (43.6%). Small testes were found in 99 (90%) patients. Azoospermia was seen in 27/28 patients tested (96.4%). These patients had

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Table 1 – Characteristics of 147 patients with KS.

Characteristic	Total	Age groups, years, n (147)					
		n(%)	<12	12–17	18–30	31–40	41–50	>51
Cytogenetic profile, n (147)			19 (12.9)	12 (8.1)	64 (43.5)	40 (27.2)	11 (7.5)	1 (0.7)
Classic KS, 2 months - 48 years	**126**	**126 (85.7)**						
Postnatal								
Classic KS		125 (85)	10 (6.8)[a]	9 (6.1)	60 (40.8)	36 (24.5)	10 (6.8)	–
Prenatal (amniotic fluid)								
Classic KS (48,XXY,+21)		1 (0.7)						
Variant KS, < 28 years	**12**	**12 (8.2)**						
Postnatal								
48,XXYY		1 (0.7)	–	–	1 (0.7)	–	–	–
48,XXXY		8 (5.4)	3 (2)	3 (2)	2 (1.4)	–	–	–
49,XXXXY		2 (1.4)	2 (1.4)	–	–	–	–	–
Prenatal (chorionic villus sample)								
48,XXYY		1 (0.7)						
Mosaic KS, 2 days-51 years	**9**	**9 (6.1)**						
47,XXY/46,XY		8 (5.4)	2 (1.4)	–	1 (0.7)	4 (2.7)	1 (0.7)	–
47,XXY/46,XX/46,XY		1 (0.7)	–	–	–	–	–	1 (0.7)
Presenting features of post-natal patients	**139**		**<12**	**12–17**	**18–30**	**31–40**	**41–50**	**>51**
		All n(%)	**17 (12.2)**	**12 (8.6)**	**61 (43.8)[a]**	**37 (26.6)[a]**	**11 (7.9)**	**1 (0.7)**
Small Testes	**139**	**110/116 (94.8)[b]**	1 (0.9)	10 (8.6)	55 (47.4)	32 (27.6)	11 (9.5)	1 (0.9)
Classic KS		98 (84.5)	–	8 (6.9)	52 (44.8)	28 (24.1)	10 (8.6)	–
Variant KS		5 (4.3)	1 (0.9)	2 (1.7)	2 (1.7)	–	–	–
Mosaic		7 (6)	–	–	1 (0.9)	4 (3.4)	1 (0.9)	1 (0.9)
Delayed/poorly developed secondary sexual characteristics, ≥ 12	**122**	**109 (89.3)**	–	**9 (7.4)**	**57 (46.7)**	**32 (26.2)**	**10 (8.2)**	**1 (0.8)**
Classic KS		98 (80.3)	–	7 (5.7)	53 (43.4)	29 (23.8)	9 (7.4)	0
Variant KS		5 (4.1)	–	2 (1.6)	3 (2.5)	0	0	1 (0.8)
Mosaic KS		6 (4.9)	–	0	1 (0.8)	3 (2.5)	1 (0.8)	–
Gynecomastia, age≥12	**122**	**50 (41)**	–	**6 (4.9)**	**30 (24.6)**	**8 (6.6)**	**6 (4.9)**	–
Classic KS		48 (39.3)	–	6 (4.9)	29 (23.8)	7 (5.7)	6 (4.9)	–
Variant KS		1 (0.8)	–	–	1 (0.8)	–	–	–
Mosaic		1 (0.8)	–	–	–	1 (0.8)	–	–
Infertility, age ≥18 years,	**110**	**48 (43.6)**	–	–	**17 (15.5)**	**24 (21.8)**	**7 (6.4)**	–
Classic KS		44 (40)	–	–	17 (15.5)	21 (19.1)	6 (5.5)	–
Mosaic KS		4 (3.6)	–	–	–	3 (2.7)	1 (0.9)	–
Azoospermia, age≥18	**27**	**27 (96.4)**	–	–	**9 (32.1)**	**16 (57.1)**	**2 (7.1)**	–
Classic KS		25 (89.3)	–	–	9 (32.1)	14 (50)	2 (7.1)	–
Mosaic KS		2 (7.1)	–	–	–	2 (7.1)	–	–
Micropenis	**139**	**24 (17.3)**	**2 (1.4)**	**3 (2.2)**	**13 (9.4)**	**4 (2.9)**	**2 (1.4)**	–
Classic KS		21 (15.1)	–	2 (1.4)	13 (9.4)	4 (2.9)	2 (1.4)	–
Variant KS		3 (2.2)	2 (1.4)	1 (0.7)	–	–	–	–
Cryptorchidism	**139**	**5 (3.6)**	**4 (2.9)**	–	–	**1 (0.7)**	–	–
Classic KS		3 (2.2)	3 (2.2)	–	–	–	–	–
Variant KS		1 (0.7)	1 (0.7)	–	–	–	–	–
Mosaic KS		1 (0.7)		–	–	1 (0.7)	–	–
Hypospadias	**139**	**1 (0.7)**	**1 (0.7)**	–	–	–	–	–
Classic KS		1 (0.7)	1 (0.7)	–	–	–	–	–
Behavioural Issues	**139**	**27 (19.4)**	**7 (5)**	**6 (4.3)**	**9 (6.5)**	**4 (2.9)**	**1 (0.7)**	–
Classic KS		20 (14.4)	3 (2.2)	3 (2.2)	9 (6.5)	4 (2.9)	1 (0.7)	–
Variant KS		7 (5)	4 (2.9)	3 (2.2)		–	–	–
Developmental Delay, <18	**139**	**15 (10.8)**	**10 (7.2)**	**5 (3.6)**	–	–	–	–
Classic KS		8 (5.8)	6 (4.3)	2 (1.4)	–	–	–	–
Variant KS		6 (4.3)	3 (2.2)	3 (2.2)	–	–	–	–
Mosaic KS		1 (0.7)	1 (0.7)	0 (0)	–	–	–	–
Dysmorphism, <18	**139**	**12 (8.6)**	**10 (7.2)**	**2 (1.4)**	–	–	–	–
Classic KS		6 (4.3)	4 (2.9)	2 (1.4)	–		–	–
Variant KS		4 (2.9)	4 (2.9)	–	–	–	–	–
Mosaic KS		2 (1.4)	2 (1.4)	–	–	–	–	–
Speech Delay	**139**	**9 (6.5)**	**4 (2.9)**	**4 (2.9)**	**1 (0.7)**	–	–	–
Classic KS		5 (3.6)	2 (1.4)	2 (1.4)	1 (0.7)	–	–	–
Variant KS		4 (2.9)	2 (1.4)	2 (1.4)	–	–	–	–

(*continued on next page*)

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Table 1 – (*continued*)

Characteristic	Total	Age groups, years, n (147)						
		n(%)	<12	12–17	18–30	31–40	41–50	>51
Cytogenetic profile, n (147)			19 (12.9)	12 (8.1)	64 (43.5)	40 (27.2)	11 (7.5)	1 (0.7)
Presenting features of post-natal patients	**139**	**Age groups**	**<12**	**12–17**	**18–30**	**31–40**	**41–50**	**51**
		All, n(%)	**17 (12.2)**	**12 (8.6)**	**61 (43.8)[a]**	**37 (26.6)[a]**	**11 (7.9)**	**1 (0.7)**
Intellectual Disability	**139**	**7 (5)**	**2 (1.4)**	**3 (2.2)**	**1 (0.7)**	**1 (0.7)**		–
Classic KS		5 (3.6)	2 (1.4)	2 (1.4)		1 (0.7)	–	–
Variant KS		2 (1.4)	–	1 (0.7)	1 (0.7)	–	–	–
Seizures	**139**	**3 (2)**	**2 (1.4)**	**1 (0.7)**	–	–	–	–
Classic KS		**3 (2)**	2 (1.4)	1 (0.7)	–	–	–	–
Skeletal deformity	**139**	**5 (3.6)**	**1 (0.7)**	**1 (0.7)**	**2 (1.4)**	**1 (0.7)**	–	–
Classic KS		4 (2.9)	1 (0.7)	–	2 (1.4)	1 (0.7)	–	–
Variant KS		1 (0.7)	–	–	–	–	–	–
Cardiac Anomaly[a]	**139**	**4 (2.9)**	–	–	–	–	–	–
Classic KS		2 (1.4)	–	–	–	–	–	–
Variant KS		1 (0.7)	–	–	–	–	–	–
Mosaic KS		1 (0.7)	1 (0.7)	–	–	–	–	–
Co-Morbidities								
Obesity	**139**	**16 (11.5)**	**1 (0.7)**	–	**7 (5)**	**4 (2.9)**	**4 (2.9)**	–
Classic KS		16 (11.5)	1 (0.7)	–	7 (5)	4 (2.9)	4 (2.9)	–
Varicose veins	**110**	**6 (5.5)**	–	–	**2 (1.8)**	**1 (0.9)**	**2 (1.8)**	**1 (0.9)**
Classic KS		5 (4.5)	–	0	2 (1.8)	1 (0.9)	2 (1.8)	
Mosaic KS		1 (0.9)	–	–	–	–	–	1 (0.9)
Eye Disorders	**139**	**5 (3.6)**	**1 (0.7)**	–	**1 (0.7)**	**1 (0.7)**	**2 (1.4)**	–
Classic KS		5 (3.6)	1 (0.7)	0	1 (0.7)	1 (0.7)	2 (1.4)	–
Dyslipidaemia	**110**	**4 (3.6)**	–	–	**1 (0.9)**	**2 (1.8)**	**1 (0.9)**	–
Classic KS		3 (2.7)	–	–	1 (0.9)	1 (0.9)	1 (0.9)	–
Mosaic KS		1 (0.9)	–	–	–	1 (0.9)	–	–
Diabetes Mellitus	**110**	**9 (8.2)**	–	–	**1 (0.9)**	**6 (5.5)**	**2 (1.8)**	–
Classic KS		8 (7.3)	–	–	1 (0.9)	5 (4.5)	2 (1.8)	–
Mosaic KS		1 (0.9)	–	–		1 (0.9)	0	–
Acanthosis Nigricans	**110**	**4 (3.6)**	–	–	**2 (1.8)**	**1 (0.9)**	**1 (0.9)**	–
Classic KS		4 (3.6)	–	–	2 (1.8)	1 (0.9)	1 (0.9)	–
Kidney Disorders	**139**	**4 (2.9)**	**1 (0.7)**	–	**3 (2.2)**	–	–	–
Classic KS		3 (2.2)	–	0	3 (2.2)	–	–	–
Mosaic KS		1 (0.7)	1 (0.7)	–	–	–	–	–
Tumours = 3	139	3 (2.2)	–	–	–	–	–	–
Pituitary adenoma	**139**	**1 (0.7)**	–	–	–	**1 (0.7)**	–	–
Classic KS		1 (0.7)	–	–	–	1 (0.7)	–	–
Pineal germ cell tumour	**139**	**1 (0.7)**	–	–	**1 (0.7)**	–	–	–
Classic KS		1 (0.7)	–	–	1 (0.7)	–	–	–
Osteoid osteoma	**139**	**1 (0.7)**	–	–	**1 (0.7)**	–	–	–
Classic KS		1 (0.7)	–	–	1 (0.7)	–	–	–
Additional genetic abnormalities			–	–	–	–	–	–
Positive for AZFC deletion (PCR)	**19**	**1 (5.3)**	–	–	**1 (5.3)**	–	–	–
Classic KS		1 (5.3)	–	–	1 (5.3)	–	–	–
Incontinentia pigmenti (Biopsy)	**139**	**1 (0.7)**	–	–	–	–	–	–
Classic KS		1 (0.7)	–	–	–	–	–	–
Prader–Willi syndrome (MS-MLPA)	**139**	**1 (0.7)**	–	–	–	–	–	–
Classic KS		1 (0.7)	–	–	–	–	–	–
Down syndrome (Prenatal karyotype)	**139**	**1 (0.7)**	–	–	–	–	–	–
Classic KS		1 (0.7)	–	–	–	–	–	–

[a] No. of patients with available data
[b] Data available in 116 patients

several co-morbidities such as diabetes mellitus (10.9%), obesity (13.6%), varicose veins, (6.4%) and eye- disorders (4.5%). One patient had a pituitary adenoma which was resected elsewhere.

Additional genetic abnormalities

There were four patients (2.7%) with cKS who had additional genetic abnormalities.

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Table 2 – Hormonal profile of 139 post-natal KS patients.

Characteristic	n (%)	Age groups, years, n (%)				
		<12	12–17	18–30	31–40	≥41
		17 (12.2)	12 (8.6)	61 (43.9)	37 (26.6)	12 (8.6)
Testosterone						
Low	**117 (92.1)**	**13 (10.2)**	**10 (7.9)**	**48 (37.8)**	**34 (26.8)**	**12 (9.4)**
Classic	100 (78.7)	8 (6.3)	8 (6.3)	44 (34.6)	30 (23.6)	10 (7.9)
Variant	9 (7.1)	4 (3.1)	2 (1.6)	3 (2.4)	0	0
Mosaic	8 (6.3)	1 (0.8)	0	1 (0.8)	4 (3.1)	2 (1.6)
Normal	**10 (7.9)**	**0**	**1 (0.8)**	**9 (7.1)**	**0**	**0**
Classic	10 (7.9)	0	1 (0.8)	9 (7.1)	0	0
Data not available	**12 (9.4)**	**4 (3.1)**	**1 (0.8)**	**4 (3.1)**	**3 (2.4)**	**0**
Classic	9 (7.1)	2 (1.6)	0	4 (3.1)	3 (2.4)	0
Variant	2 (1.6)	1 (0.8)	1 (0.8)	0	0	0
Mosaic	1 (0.8)	1 (0.8)	0	0	0	0
Follicle Stimulating Hormone						
Normal	**5 (3.9)**	**2 (1.6)**	**1 (0.8)**	**1 (0.8)**	**0**	**1 (0.8)**
Classic	3 (2.3)	2 (1.6)	0	1 (0.8)	0	0
Variant	1 (0.8)	0	1 (0.8)	0	0	0
Mosaic	1 (0.8)	0	0	0	0	1 (0.8)
High	**123 (96.1)**	**11 (8.6)**	**11 (8.6)**	**56 (43.8)**	**34 (26.6)**	**11 (8.6)**
Classic	107 (83.6)	6 (4.7)	9 (7)	52 (40.6)	30 (23.4)	10 (7.8)
Variant	9 (7)	4 (3.1)	2 (1.6)	3 (2.3)	0	0
Mosaic	7 (5.5)	1 (0.8)	0	1 (0.8)	4 (3.1)	1 (0.8)
Data not available	**11 (8.6)**	**4 (3.1)**	**0**	**4 (3.1)**	**3 (2.3)**	**0**
Classic	9 (7)	2 (1.6)	0	4 (3.1)	3 (2.3)	0
Variant	1 (0.8)	1 (0.8)	0	0	0	0
Mosaic	1 (0.8)	1 (0.8)	0	0	0	0
Luteinizing Hormone						
Low	**1 (0.8)**	**1 (0.8)**	**0**	**0**	**0**	**0**
Classic	1 (0.8)	1 (0.8)	0	0	0	0
Normal	**4 (3.2)**	**1 (0.8)**	**1 (0.8)**	**1 (0.8)**	**0**	**1 (0.8)**
Classic	2 (1.6)	1 (0.8)	0	1 (0.8)	0	0
Variant	1 (0.8)	0	1 (0.8)	0	0	0
Mosaic	1 (0.8)	0	0	0	0	1 (0.8)
High	**120 (96)**	**11 (8.8)**	**11 (8.8)**	**56 (44.8)**	**31 (24.8)**	**11 (8.8)**
Classic	104 (83.2)	6 (4.8)	9 (7.2)	52 (41.6)	27 (21.6)	10 (8)
Variant	9 (7.2)	4 (3.2)	2 (1.6)	3 (2.3)	0	0
Mosaic	7 (5.6)	1 (0.8)	0	1 (0.8)	4 (3.2)	1 (0.8)
Data not available	**14 (10.9)**	**4 (3.2)**	**0**	**4 (3.2)**	**6 (4.8)**	**0**
Classic	12 (9.4)	2 (1.6)	0	4 (3.2)	6 (4.8)	0
Variant	1 (0.8)	1 (0.8)	0	0	0	0
Mosaic	1 (0.8)	1 (0.8)	0	0	0	0

Lab Reference ranges.
Testosterone: Adult male - 280 to 1000 ng/dL; Follicle-stimulating hormone (FSH): Children- 0.5 to 3.3 mIU/ml, Men - 0.7 to 11.1 mIU/ml; Luteinising hormone(LH) Children - 0.5 to 3.3 mIU/ml, Men- 0.8 to 7.6 mIU/ml.

Table 3 – Association of Testosterone, LH, and FSH levels with age groups.

Hormone	<12 years (n = 17)	12–17 years (n = 12)	18–30 years (n = 61)	31–40 years (n = 37)	≥41 years (n = 12)	*p-value*[a]
Testosterone levels (mean/SD)	142 (67)	185 (68)	164 (115)	114 (76)	76 (71)	**0.011**
LH (mean/SD)	18 (10)	29 (20)	25 (15)	23 (6)	25 (13)	0.6
FSH (mean/SD)	45 (36)	58 (41)	46 (21)	41 (14)	34 (21)	0.4

[a] Kruskal–Wallis rank sum test.

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A two year old male patient suspected to have Prader–Willi syndrome presented with developmental delay, dysmorphism, short stature and bilateral cryptorchidism. A concurrent microdeletion of chromosome 15 was identified by methylation-sensitive multiplex ligation-dependent probe amplification (MS-MLPA) in this study.

A one-year-old boy presented with pigmentary mosaicism and a skin biopsy consistent with incontinentia pigmenti. Cytogenetic testing of peripheral blood as well as affected and normal skin was done to confirm the diagnosis of KS because the additional X chromosome enables the survival of foetuses with autosomal dominant disorders such as incontinentia pigmenti which are otherwise lethal in males.[24]

From 2008-2019, over 323 patients underwent testing for microdeletions of AZF a, b and c loci on the Y chromosome in our institute. Only one of the 19 patients (5.3%) with azoospermia tested showed a microdeletion of AZFc locus (Table:1). One KS diagnosed prenatally had a concurrent trisomy 21 (double aneuploidy).

Hormone levels (Tables 2, 3), (Fig. 3a-d)

Serum testosterone was low in 92.1% (117/127) of patients and in 95.8% of children and 91.3% of adults ($p = 0.011$). LH and FSH were elevated in 96% (123/128 and 120/125, respectively) of patients. FSH was elevated in 88% of children and 98% of adults and LH in 91.6% of children and 97% of adults.

Discussion

We have described the cytogenetic profile and the clinical presentation of KS as well as the associated findings. Genetic changes that could contribute to the abnormal phenotype in KS include the parental origin of the additional X chromosome, skewed (non-random or preferential) X-inactivation and increased dosage of genes in the pseudoautosomal regions/regions of duplication due to escape from X-inactivation.[9,10,25]

Age at diagnosis

The age at diagnosis (two days to 51 years) was comparable to other studies (one month to 50–60 years).[26–30] KS was diagnosed prenatally in 1.5% of our patients, less commonly than in other reports based on karyotypes from Denmark (22%) and Argentina (4%) reflecting the differences in the use of prenatal diagnosis in these countries.[26,27] According to other reports, 21% of patients with KS are diagnosed prenatally by karyotyping and non-invasive prenatal testing.[2]

Only 10–16% of KS have been reported to be detected in childhood or adolescence because the well-recognised clinical signs of hypogonadism are mild, or not evident.[2,10,15] However, nearly a quarter (23) of our patients, and 27–45% of KS in studies from Denmark, Argentina and other parts of South Asia were <18 years, reflecting the increasing use of cytogenetic analysis for the work-up of neurodevelopmental disorders and dysmorphism.[26,27,29,30]

Frequency and age

The frequency of cKS is reported to vary from 83 to 92% and that of mKS, from 5.5 to 11%, comparable to our findings (85.7% and 6.1%, respectively).[26–30] The frequency of our vKS was 8.2%, within the range of 2–13.6% in studies which included children; it was infrequently seen (<1) in adults with infertility.[26–30]

Frequencies of presenting features

Most of the adults (≥18 years) presented with one or more features related to hypogonadism. Secondary sexual characteristics were delayed/poorly developed in 91% of all patients, in 89.3% of the 122 patients ≥12 years of age and in 75% of the pubertal age group (12–17 years). The levels of testosterone, FSH and LH were abnormal in >90% of patients.

Small testes which are reported to be the most constant feature of KS were seen in 94.8% of the 116 patients in whom this data was available as compared to 78.5–100% of patients in some studies.[1,2,27,28] Infertility was a presenting feature in 48 (43.6%) patients within the range of 20.8–100% reported in other studies.[1,2,17] Azoospermia was found in 27/28 (96.2)% of our patients with infertility who were known to have undergone testing, within the range reported in the literature.[9,17,27,28] Germ cell loss begins in foetal life and worsens during puberty.[17,27] About 10% of males with KS have severe oligozoospermia or cryptozoospermia.[7–9] Although the majority of seminiferous tubules show azoospermia, foci of spermatogenesis may be present possibly because of mosaicism for a 46,XY cell line in the testis. Sperm retrieval rates of 40–60% have been reported, with live births with normal karyotypes occuring in up to 50% patients.[17]

Gynaecomastia was noted in 41% of our patients, within the range of 20–75% reported in other studies and was bilateral in 90%.[7,8,26–30] Gynaecomastia in KS has been linked to a high oestradiol/testosterone ratio which is equivalent to an increased oestrogen effect as well as a long CAGn repeat sequence on the AR gene.[7,10]

Genital abnormalities such as micropenis, undescended testis, bifid scrotum, hypospadias, chordee and penoscrotal transposition have been reported in KS.[7,8,10,31] The majority (80%) of our patients with cryptorchidism were <12 years of age, but the frequency was lower than in other reports (3.6% vs 14% of all patients and 13.8% vs 18–40% of those <18 years). It was bilateral in almost two-thirds (60%), comparable to the literature (57%).[7,8,25,27,30] The frequency of micropenis (17.3%) was comparable to the literature (6.1–25%), with 20.8% seen in those <18 years.[8,27,29,30] Hypospadias was infrequent (0.7%) but is reported to be a presenting feature.[10,15,31]

The factors that result in the formation of abnormal genitalia in KS are not well-understood but possible causes include low levels of testosterone during the first trimester when masculinisation of the genitalia occurs and incomplete inactivation of the DAX1 gene.[9,31]

Non-gonadal presenting features

Developmental delay, dysmorphism and seizures were presenting features only in those <18 years and were seen in all

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three subtypes. Developmental delay was slightly less common than in other reports (10.8% vs 11.8–55%) but dysmorphism was within the range reported in the literature (12.2% vs 5–23.5%).[26,30]

Speech delay, which is the most serious problem in children with KS, and behavioural problems were seen only in classic and vKS and were less common than in other reports (6.5% vs 40% and 19.4% vs 43.1–55%, respectively).[27,30] Behavioural problems were seen in all age groups, in slightly less than half (48%) of those <18 years of age and were slightly less common (18.3% vs 43.1–55%) than in other reports. Congenital heart disease has been reported to occur in KS; we had fewer patients (2.7%) than Asirvatham et al (22.7%).[30] The frequency of diabetes mellitus in our patients was comparable to the literature (10.9% vs 10–39%).[6,8]

Limitations of our study

This was a retrospective observational study of the cytogenetic profile and presenting features of patients with KS. Therefore, an ascertainment bias could be present with respect to the frequencies of the presenting features, especially if mild.

Conclusion

Despite the increasing use of advanced molecular tests to identify genetic abnormalities, CCA still remains the mainstay of diagnosis of KS. All three cytogenetic subtypes of KS were seen in our patients. cKS was seen at all ages but most of our mKS were seen in adults. cKS and mKS presented with a variety of features including infertility, signs of hypogonadism and co-morbidities. vKS was seen predominantly in children who sought medical attention for neurodevelopmental/neuro-cognitive/cardiac disorders and features of hypogonadism.

It is crucial to raise awareness about KS as up to three-quarters of cases may go undiagnosed. Recognizing its diverse modes of presentation at different ages allows for early diagnosis. Timely implementation of appropriate therapy and counselling with regard to outcomes including fertility can mitigate or correct some effects of KS, reduce the risk of age-related co-morbidities and enhance quality of life.

Patient/Guardians consent

Patients informed consent was obtained.

Ethical clearance

Institute/hospital ethical clearance certificate was obtained.

Source of support

Nil.

Disclosure of competing interest

The authors have none to declare.

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Nil.

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