Abstract

Objective: To identify clinical and laboratory data which differentiate Klinefelter syndrome (KS) patients according to age group.

Methods: The study included all cases of hypogonadism, gynecomastia and/or infertility whose karyotype was performed at a university hospital from January 1989 to December 2011, in a total of 105 subjects. The following data were retrospectively analyzed: age at first visit, ratio of arm span to height, pubic hair, gynecomastia, testicular volume, luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone (T), and sperm analysis.

Results: During the study period, 33 patients were diagnosed with Klinefelter syndrome (KS+) and 72 were not (KS-). Out of all KS cases, only seven (21.2%) were diagnosed before 20 years old and two (6.1%) before 10 years old. Age at first consultation (in years) was similar in both groups (KS+ = 31.3±12.9 and KS- = 27.6±12.1), as were ratio of arm span to height and frequency of gynecomastia. However, in KS+ patients, pubic hair was less developed, testicular volume was smaller and testosterone levels were lower, while LH and FSH levels and frequency of azoospermia were higher.

Conclusions: Klinefelter syndrome is both an under and late diagnosed condition. The most important data for diagnosis are testicular volume, hormone levels and presence of azoospermia in sperm analysis, especially in puberty and adult life.


Introduction

Klinefelter syndrome (KS) is the most common sex chromosome abnormality in men, with an estimated prevalence of about one in 600 newborn males.1 It is characterized cytogenetically by the presence of an extra X chromosome (47,XXY), which occurs in around 90% of cases; however, KS variants, such as mosaicism (46,XY/47,XXY) and other much less frequent aneuploidies (48,XXXY, 48,XXYY, 49,XXXXY) have already been described.2

The main clinical findings, present in almost all individuals with KS, are small testes, azoospermia and increased gonadotropins, especially follicle stimulating hormone (FSH). However, other findings, such as gynecomastia, delayed puberty, sparse body and pubic hair, micropenis, tall stature, increased arm span to height ratio, learning disabilities, psychiatric illnesses, peripheral venous disease, abdominal obesity, metabolic syndrome, higher risk of...
autoimmune diseases and cancer, may be observed with different frequencies according to population, age group and karyotype.1,3-10

Although first described 70 years ago,11 KS remains as an under diagnosed disease, because patients do not seek doctors very often, and doctors not always recognize the diagnosis.8 Therefore, only about 25% of all adult patients with KS are diagnosed; most during the investigation of infertility and/or hypogonadism; and less than 10% of cases are diagnosed before puberty.1,12

Therefore, the aim of this study was to identify clinical and laboratory data that differentiate KS cases according to age group.

Methods

This is a retrospective clinical study including all individuals who sought care at the Outpatient Care of the Interdisciplinary Group for the Study of Sex Determination and Differentiation at the Hospital de Clínicas (HC) da Faculdade de Ciências Médicas (FCM) da Universidade Estadual de Campinas (UNICAMP), Brazil, in the period of January, 1989 to December, 2011, to investigate KS. It should be noted that the consultation in this outpatient care is the only possibility of performing the karyotype at the HC-UNICAMP when the suspicion is KS.

All patients were evaluated according to age at first consultation, main clinical complaint (infertility, gynecomastia, intellectual disability and/or neuromotor delay, evidence of hypogonadism, including delayed puberty, incomplete development of secondary sexual characteristics, microorchidism and micropenis), ratio of arm span to height (A/H), pubic hair by classification according to Marshall & Tanner;13 presence of gynecomastia, testicular volume (measured by Prader orchidometer), luteinizing hormone (LH), FSH, total testosterone (T), sperm analysis and karyotype. Testicular volume was calculated by the arithmetic mean between the volumes of both testicles of each patient.

All laboratory assessments (LH, FSH, T and sperm analysis) were performed at the Laboratory of Physiology of HC-UNICAMP. In relation to sperm analysis, results were categorized into normal, oligospermia or azoospermia. The examination of the karyotype was performed from culture of peripheral blood leukocytes with counting of at least 32 metaphases at the Cytogenetic Laboratory, Department of Medical Genetics of the FCM-UNICAMP. From the karyotype test results, patients were divided in carriers (KS+) and non-carriers (KS-) of KS.

Data evaluated were compared between the cases of KS+ and KS- by chi-square test or Fisher exact for categorical variables (pubic hairiness, gynecomastia and sperm analysis) and Mann-Whitney test for continuous variables (age, A/H, testicular size, LH, FSH and T) with p < 0.05. The program used in the statistical analysis was the Statistical Package for the Social Sciences (SPSS, Chicago, IL, EUA) version 16.0.

Results

In total, 105 cases were included in the study, 72 with normal karyotype (46,XY), 31 with 47,XXY karyotype and two with 46,XY/47,XXY, totaling, therefore, 72 cases (68.6%) without KS (KS-) and 33 (31.4%) with KS (KS+).

Age at first consultation, in the KS- group, ranged from 1.5 to 67.5 years (27.6±12.1 years), and in the KS+ group, from 5.3 to 59.7 years (31.3±12.9 years), with no statistical difference between the groups (p = 0.13). Out of the 105 cases, 30 (28.6%) were evaluated before 20 years old: seven KS+ and 23 KS-; and only three before 10 years old: two KS+ and one KS-. Therefore, among the 33 cases of KS+, seven (21.2%) were diagnosed before 20 years old, and two (6.1%) before 10 years.

Table 1 shows the main complaint in the first consultation in relation to age (≤ and > 20 years) and KS+ and KS-diagnosis. Regardless of diagnosis, the main complaint above 20 years was infertility (77.5% in the KS- group and 57.7% in the KS+ group), while in patients under 20, hypogonadism and gynecomastia were the main complaints (91.3% in the KS- group and 71.4% in the KS+ group). The complaint of mental deficiency or neuromotor delay occurred only in patients below 20 years, regardless of diagnosis of KS; and all patients were under 10 years old.

There was no statistical difference between the SK+ and SK- groups regarding main complaint when separated by age group (≤ 20 years: chi-square(2) = 4.29; p = 0.12; and > 20 years: chi-square(2) = 5.10; p = 0.08).

Table 2 presents data on pubic hair staging and presence of gynecomastia only among patients above 10 years old (n = 102; 31 KS+ and 71 KS-), and sperm analysis in patients above 18 years (n = 79; 29 KS+ and 50 KS-). There was a statistically significant difference between the groups in pubic hair staging (chi-square = 26.22; p < 0.0001), with KS+ group presenting lower pubic hair. There was also a statistically significant difference in the outcome of sperm analysis (chi-square(2) = 25.16; p < 0.0001), with the KS+ group presenting only azoospermia. However, there was no statistically significant difference in the presence of gynecomastia (chi-square = 2.68; p = 0.10) between the two groups.

Table 3 shows data on A/H of all cases and data on mean testicular volume, LH, FSH, and T only of patients above 14 years (n = 92; 31 KS+ and 61 KS-). There was no statistically significant difference between the groups in A/H (p = 0.98); however, there was statistically significant difference on testicular mean volume (p = 0.001), on LH levels (p = 0.001), FSH levels (p = 0.001) and T levels
Table 1 - Reason for consultation in relation to age and diagnosis (KS- or KS+)

<table>
<thead>
<tr>
<th>Reason for consultation</th>
<th>KS- (n = 72)</th>
<th>KS+ (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 20 years</td>
<td>&gt; 20 years</td>
</tr>
<tr>
<td></td>
<td>≤ 20 years</td>
<td>&gt; 20 years</td>
</tr>
<tr>
<td>Infertility</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>Hypogonadism</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>Gynecomastia</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Mental deficiency</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>49</td>
</tr>
</tbody>
</table>

KS- = without Klinefelter syndrome; KS+ = with Klinefelter syndrome.

Table 2 - Pubic hair, gynecomastia and sperm analysis in relation to diagnosis (KS- or KS+)

<table>
<thead>
<tr>
<th>Variable</th>
<th>KS-</th>
<th>KS+</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pubic hair</td>
<td>5</td>
<td>16</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>1 or 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 to 5</td>
<td>66</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Gynecomastia</td>
<td></td>
<td></td>
<td>0.10</td>
</tr>
<tr>
<td>+</td>
<td>31</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>40</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Sperm analysis</td>
<td></td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Oligospermia</td>
<td>21</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Azospermia</td>
<td>22</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

KS- = without Klinefelter syndrome; KS+ = with Klinefelter syndrome.

Table 3 - Ratio of arm span/height, mean testicular volume, luteinizing hormone, follicle stimulating hormone, and total testosterone in relation to diagnosis (KS- or KS+)

<table>
<thead>
<tr>
<th>Variable</th>
<th>KS- (M±DP)</th>
<th>KS+ (M±DP)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm span/height</td>
<td>1.02±0.03</td>
<td>1.01±0.03</td>
<td>0.98</td>
</tr>
<tr>
<td>Mean testicular volume (mL)</td>
<td>9.5±6.5</td>
<td>3.5±2.5</td>
<td>0.001</td>
</tr>
<tr>
<td>LH (U/L)</td>
<td>7.5±8.4</td>
<td>19.6±8.8</td>
<td>0.001</td>
</tr>
<tr>
<td>FSH (U/L)</td>
<td>14.9±7.4</td>
<td>43.8±25.4</td>
<td>0.001</td>
</tr>
<tr>
<td>T (ng/mL)</td>
<td>8.7±7.3</td>
<td>4.3±2.9</td>
<td>0.01</td>
</tr>
</tbody>
</table>

FSH = follicle stimulating hormone; KS- = without Klinefelter syndrome; KS+ = with Klinefelter syndrome; LH = luteinizing hormone; M±DP = mean ± standard deviation; T = total testosterone.

Discussion

Data from this study show that KS is still little and late diagnosed in our area. The HC-UNICAMP serves the population of a region with more than 5 million inhabitants, and for such an amount of patients we should have verified many more than 33 diagnoses of KS in 23 years (about three cases diagnosed every 2 years), indicating that either the patients do not seek medical help very often or the doctors think too little about this diagnosis. Although apparently small, the sample of this study is similar to that of recent studies, where the highest sample was of 98 cases.

Regarding age at diagnosis, out of 105 cases included in the study, only 30 (28.6%) were assessed before age 20, seven of these (23.3%) with diagnosis of KS and only three (2.9%) were assessed below 10 years old, two of them diagnosed with KS. Therefore, data from this study confirm that the diagnosis of KS is rarely done before 20 years of age, especially before 10 years. Out of the 75 cases evaluated after age 20, the diagnosis of KS occurred more frequently, in the ratio of 1:2, that is, in every three cases, one had the diagnosis of KS. Similar results were found by Abramsky & Chapple, when they verified that 26% of the expected number of cases of KS in North Thames, United Kingdom, between 1990 and 1993, were diagnosed after birth, 28% of those diagnosed before age 20 and only 4% before 10 years old; similar data to those found by Bojesen et al. and by the present study.

All individuals under 10 years old were referred by intellectual deficiency and/or neuromotor developmental delay. Specific cognitive deficits in language and executive function are part of the KS phenotype. Although many studies reported only a slight global cognitive impairment...
(mild delay in motor development and language), KS has been identified in 0.4% of boys in special education programs and in 1.2% of patients with intellectual retardation of undetermined cause. In general, referred initially for the investigation of fragile X syndrome, the leading inherited cause of mental retardation, most common in males and with little expressive manifestations before puberty, except for intellectual impairment and behavioral changes. In puberty and adult life, the main clinical characteristic that differentiates KS syndrome from X fragile syndrome is testicular volume, which is small in the first and big in the latter.

Above age 20, the main complaint in the suspicion of diagnosis was infertility, while in patients below 20 years, though already at the stage of puberty, over 10 years old, hypogonadism and gynecomastia were the main complaints. Reduced pubic hair and lower mean testicular volume were significantly more frequent in the KS+ group, which did not occur with gynecomastia and predominance of arm span in relation to height. Gynecomastia is a frequent finding in KS (50 to 88%) and in normal male puberty (60 to 70%), so it is a finding of little significance in the diagnosis of SK in puberty, once there is an overlap of diagnosis. Reduction of pubic hair and increased ratio of A/H are related to hypogonadism, which is often not suspected in puberty, once most patients with KS begins puberty at the normal stage.

Regarding laboratory tests, LH, FSH, T and the frequency of azoospermia in sperm analysis were significantly higher in the KS+ group. From the hormonal standpoint, patients with KS presented normal prepubertal values of T, LH, FSH, inhibin B and anti-müllerian hormone, with normal serum testosterone responses to human chorionic gonadotropin stimulation. During puberty, after a normal increase of T levels, they may, in some cases, remain within the low-normal range for the stage of sexual maturation, though sufficient to allow puberty progression and determine the satisfactory development of secondary sexual characteristics. From midpuberty onwards, in general, patients with KS already start to present a gradual increase in FSH (reflecting the dysfunction of the seminiferous tubules and the Sertoli cells) and LH (reflecting the Leydig cell dysfunction), and the increase in FSH generally precedes (about 1 year before) and is more intense than that of LH; such data may, from this moment on, assist in the suspicion of diagnosis, as verified in the present study. However, the main clinical data on the pubertal age group to justify the laboratory determination of LH and FSH and, if increased, the performance of the karyotype, is the disproportion of testicular volume for the stage of sexual maturation. Kamischke et al. showed that the mean measure of the testicular volume was the parameter with better sensitivity for the diagnosis of KS and that the mean volume of both testicles by ultrasound was 4.7 mL in cases of KS+ and 13.7 mL in cases of KS-, similar to our findings through clinical evaluation.

In adulthood, the main data that motivate the investigation of KS are hypogonadism (clinical) and hypergonadotropic (laboratory) and infertility. KS is the most frequent genetic cause of infertility, occurring in 11% of azoospermic men and 4% of infertile men. In the present study, these data are confirmed, and infertility and hypogonadism are the main complaints after age 20, and all KS+ patients above 18 years old were azoospermic.

Most clinical and laboratory findings present in KS occur due to the testicular degenerative process present throughout life. In fetuses and neonates, there is a decrease in the number of germ cells and an increased proportion of seminiferous tubules devoid of germ cells, progressing with the reduction of the seminiferous tubules, a greater decrease in the number of spermatogonia in the child and an acceleration of all this process in puberty, ending in adulthood with extensive fibrosis and hyalinization of the seminiferous tubules, absence of spermatogonia, predominance of Sertoli immature cells and hyperplasia of Leydig cells and interstitium.

It is noteworthy that among the 33 individuals with KS in the present study, 31 presented 47,XXY karyotype and two 46,XY/47,XXY, as, in general, patients with mosaicism presented a less severe phenotype, but without difference in morbidity and mortality, while the patients with 48,XXXXY and 49,XXXXXY karyotypes presented a more severely affected phenotype, in particular regarding intellectual impairment and association with malformations, although also without apparent increase in mortality.

As a result, we may conclude that the diagnosis of KS in our area is not being performed at the expected rate or at the appropriate time, and the main consequences may be the lack of treatment of motor and cognitive deficits at school age, of pubertal delay or incomplete puberty in adolescence or in early adulthood and of infertility in adulthood. It is appropriate that health professionals, especially those in the pediatric area, such as pediatricians in general, neurologists, pediatric endocrinologists, and speech therapists, be more alert to the diagnosis of KS.

References


Correspondence:
Gil Guerra-Júnior
Departamento de Pediatria - FCM - UNICAMP
Cidade Universitária “Zéferino Vaz”, sem número
CEP 13081-970 – Campinas, SP - Brazil
Tel.: +55 (19) 3521.7353
Fax: +55 (19) 3521.7322
E-mail: gilguer@fcm.unicamp.br