

Phenotypic differences in mosaic Klinefelter patients as compared with non-mosaic Klinefelter patients

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Objective: To determine whether men with Klinefelter syndrome (KS) have the same phenotype as men with mosaic KS.

Design: Subject identification via prospectively collected database.

Setting: Male infertility specialty clinic.

Patient(s): Men undergoing a fertility evaluation from 2005 to 2012 at a single male infertility specialty clinic and having a karyotype demonstrating KS (mosaic or non-mosaic).

Intervention(s): None.

Main Outcome Measure(s): Testicular size, and semen and hormone parameters, genetic evaluation, and signs of testosterone (T) deficiency using validated questionnaires.

Result(s): Of 86 men identified with KS, 6 (6.7%) were mosaic KS, and 80 (93.3%) were non-mosaic KS. Men with mosaic KS had lower baseline luteinizing hormone (LH) levels ($10.31 \text{ IU/L} \pm 5.52$ vs. $19.89 \text{ IU/L} \pm 6.93$), lower estradiol levels ($58.71 \pm 31.10 \text{ pmol/L}$ vs. $108.57 \pm 43.45 \text{ pmol/L}$), larger mean testicular volumes ($11 \pm 7.3 \text{ mL}$ vs. $6.35 \pm 3.69 \text{ mL}$), and a higher mean total sperm count ($4.43 \pm 9.86 \text{ M/mL}$ vs. $0.18 \pm 1.17 \text{ M/mL}$). A higher proportion of men with mosaic KS had sperm in the ejaculate: 3 (50%) of 6 versus 3 (3.75%) of 80. The Sexual Health Inventory for Men (SHIM) and Androgen Deficiency in the Aging Male (ADAM) questionnaire scores were not different between groups.

Conclusion(s): Men with mosaic KS seem to be more well androgenized than their non-mosaic KS counterparts, both with respect to hormones and sperm in the ejaculate. (Fertil Steril® 2014;101:950–5. ©2014 by American Society for Reproductive Medicine.)

Key Words: Hypogonadism, Klinefelter(s) syndrome, male infertility, spermatogenesis, XXY

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Klinefelter syndrome (KS) is the most common genetic cause of human male infertility (1). About 80% to 85% of cases are due to the congenital numerical chromosome aberration 47,XXY (1, 2). Approximately 15% to 20% of KS men are mosaics, usually with two cell lines: 47,XXY/46,XY (3). The true prevalence of mosaic forms may be underestimated due to different

chromosomal mosaicism levels in different tissues. In addition, popular belief holds that men with mosaic KS are more androgenized than their non-mosaic counterparts (1, 4). These two factors, in addition to others, may result in underdetection of men with mosaic KS.

The most consistent phenotypic abnormalities in men with KS are

small testicular volume, increased gonadotropin levels, and adult azoospermia (1, 4). However, whether the same phenotype can be applied to men with mosaic KS remains unknown. In general, men with mosaic KS are reported to be less affected than non-mosaic KS (1, 4), but there has never been any report directly comparing the phenotypes of mosaic or non-mosaic KS men. We examined the phenotypic differences between mosaic and non-mosaic KS men.

MATERIALS AND METHODS

Men undergoing a fertility evaluation from 2005–2012 at a single male

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infertility specialty clinic and having a karyotype demonstrating KS (mosaic or non-mosaic) were identified via a prospectively collected database. These data were reviewed in a retrospective manner. The collection of data and the analysis of the data in this database was approved by the research ethics board of Mount Sinai Hospital. Men were excluded from the hormone analysis if they were being treated with exogenous testosterone.

Data were analyzed for testicular size by physical examination, semen and hormone parameters, genetic testing results (cystic fibrosis, karyotype, Y chromosome microdeletion), and clinical signs of testosterone deficiency using the Sexual Health Inventory for Men (SHIM) and the Androgen Deficiency in the Aging Male (ADAM) questionnaires (5). The ADAM questionnaire was defined as positive when a “yes” answer was given to questions 1 or 7 (relating to libido and erections) or any three other questions.

Semen samples and blood samples for measurements of serum testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol were collected at several laboratories, based on patient convenience. Semen samples were collected at least 48 hours but not more than 7 days after the time of last ejaculation. The semen samples analyzed at Mount Sinai Hospital were assessed for volume and then analyzed for sperm count, sperm concentration, and motility by computer-assisted semen analysis following the 1999 World Health Organization (WHO) criteria (6). All andrology laboratories used validated methodologies and performed their own quality control procedures. Sperm morphology was assessed using the WHO criteria. These criteria are based on microscopic high-power evaluation of 200 sperm for intactness of membranes of acrosome, head, neck, midpiece, and tail.

When comparing variables between mosaic KS and non-mosaic KS men, the nonpaired, two-tailed Student's *t*-test was used for continuous variables, and $P < .05$ was considered statistically significant. For contingency tables, Fisher's exact test was used, and $P < .05$ was considered statistically significant.

RESULTS

From 2005 to 2012, 116 men were identified with KS presenting to the male infertility clinic. Thirty men taking exogenous testosterone at the time of presentation were excluded. Of the remaining 86, 6 (6.7%) were mosaic KS, and 80 (93.3%) were non-mosaic KS (Table 1). The mosaic KS patients were all pattern 47,XXY/46,XY. The mean age at presentation was 37 years.

For the mosaic KS, the reported proportions of XXY:XY were: 9:21 (30% XXY), 2:28 (6.7% XXY), 4:16 (20% XXY), 2:98 (2% XXY), and 28:4 (87.5% XXY). One man had duplicate karyotype analyses, which were slightly different, showing 7:43 (14% XXY) and then 7:13 (35% XXY).

Testicular Volume

The overall mean testicular volume by physical exam was 6.59 ± 4 mL (range: 1–20). Men with mosaic KS had larger testicular volume than men with non-mosaic KS, 11 ± 7.3

mL (range: 5–20) versus 6.35 ± 3.69 mL (range: 3–12) ($P < .001$).

Comorbidities

The only urologic comorbid condition present in mosaic KS men was bilateral undescended testicles ($n = 2$): 2 (33.3%) of 6. One of these men was 47,XXY[28]/46,XY[4] and underwent orchidopexy at 6 years of age; the other was 47,XXY[9]/46,XY[21] and underwent orchidopexy in infancy. Non-mosaic KS men had a series of other urologic conditions, including bilateral undescended testicles, 8 (10%) of 80; inguinal hernia repair, 4 (5%) of 80; and posterior urethral valves, 1 (1.3%) of 80. Other medical issues present in non-mosaic KS patients included asthma, 4 (5%) of 80; type 2 diabetes, 3 (3.8%) of 80; hypertension, 3 (3.8%) of 80; hyperlipidemia, 1 (1.3%) of 80; anxiety, 1 (1.3%) of 80; and obsessive compulsive disorder, 1 (1.3%) of 80. There were no reported other significant medical conditions present in mosaic KS patients. Men with mosaic KS had an incidence of undescended testicles, 2 (33.3%) of 6, higher than that of non-mosaic KS men 8 (10%) of 80, although this difference did not reach statistical significance ($P = .142$).

SHIM and ADAM Scores

The overall mean SHIM score for all KS men was 20.99 ± 4.9 . For non-mosaic KS, the mean SHIM score was 20.8 ± 5.03 , which was not statistically significantly different from that of mosaic KS (22.8 ± 2.17 , $P = .395$). For all the men with KS, 25 (29.1%) of 86 tested positive on the ADAM questionnaire. For ADAM scores, although 1 (16.7%) of 6 mosaic KS men scored positive, and 25 (29.8%) of 84 non-mosaic KS men scored positive, this did not reach a statistically significant difference ($P = .667$).

Genetic Testing

For all 86 of the men with KS, 44 were tested for Y chromosome microdeletions. Of these, 2 (4.5%) of 44 men with non-mosaic KS tested positive for an AZFc microdeletion. Although cystic fibrosis is not a test ordered by our service for men with nonobstructive azoospermia, cystic fibrosis testing had been ordered by other centers for 31 of the men with KS, none of whom tested positive.

Hormones

There were statistically significant differences in the LH and estradiol levels between mosaic and non-mosaic KS men. For men with non-mosaic KS, the mean LH was 19.89 IU/L ± 6.93 compared with 10.31 IU/L ± 5.52 ($P = .002$) for the men with mosaic KS. Similarly, mosaic KS men had lower estradiol levels when compared with their non-mosaic KS counterparts: mean 108.57 ± 43.45 pmol/L versus 105.72 ± 44.23 pmol/L ($P = .027$).

The mean FSH, testosterone, and prolactin levels were not different between mosaic and non-mosaic KS men. The mean FSH for the non-mosaic KS men was 33.23 IU/L ± 14.22 versus 22.94 IU/L ± 15.65 for mosaics ($P = .113$), and the

TABLE 1

Mosaic versus non-mosaic Klinefelter men: clinical, semen, and hormone parameters.

Parameter	All KS men (N = 86)	47,XXY (n = 80)	47,XXY;46,XY (n = 6)	P value
Testicular volume (mL) ^a	6.59 ± 4	6.33 ± 3.67	11 ± 7.3	<.001
Undescended testes (no., %) ^b	10 (11.6%)	8 (10%)	2 (33.3%)	.142
SHIM ^a	20.9 ± 4.9	20.8 ± 5.03	22.8 ± 2.17	.395
ADAM (no. positive) ^b	25 (29.1%)	24 (30%)	1 (16.7%)	.667
FSH (IU/L) ^a	32.60 ± 14.40	33.23 ± 14.22	22.94 ± 15.65	.113
LH (IU/L) ^a	19.3 ± 7.19	19.89 ± 6.93	10.31 ± 5.52	.002
Testosterone (nmol/L) ^a	8.97 ± 5.57	9.02 ± 5.42	8.06 ± 8.39	.325
Prolactin (μg/L) ^a	8.73 ± 5.62	8.95 ± 5.72	5.51 ± 2.53	.188
Estradiol (pmol/L) ^a	105.72 ± 44.23	108.57 ± 43.45	58.71 ± 31.10	.027
Mean total sperm count (M/mL) ^a	0.48 ± 2.84	0.18 ± 1.17	4.43 ± 9.86	<.001
Azoospermic men ^b	80 (93%)	77 (96.3%)	3 (50%)	.003

Note: ADAM = Androgen Deficiency in the Aging Male questionnaire; FSH = follicle-stimulating hormone; LH = luteinizing hormone; SHIM = Sexual Health Inventory for Men questionnaire.

^a *P* < .05, two-tailed Student's *t*-test, statistically significant.

^b *P* < .05, Fisher's exact test, statistically significant.

Simplaski. Phenotype of Klinefelter syndrome. *Fertil Steril* 2014.

overall mean testosterone among the non-mosaic KS men was 9.02 ± 5.42 nmol/L compared with 8.06 ± 8.39 nmol/L for mosaics ($P = .325$). Finally, the overall mean prolactin for the non-mosaic KS men was 8.95 ± 5.72 μg/L, similar to that of mosaics, 5.51 ± 2.53 μg/L ($P = .188$).

Semen

There were statistically significant differences in the semen analyses between mosaic and non-mosaic KS men. Overall, 77 (96.3%) of 80 men with non-mosaic KS, and 3 (50%) of 6 men with mosaic KS ($P = .003$) were azoospermic. The overall mean total sperm count was 0.48 ± 2.84 M/mL. The mean total sperm count for the non-mosaic KS men was 0.18 ± 1.17 M/mL, statistically significantly lower than that of mosaics, 4.43 ± 9.86 M/mL ($P < .001$).

The mean age for the men who had sperm in their ejaculate was 43 years. Of this group of men, the mean testicular volume was 10.5 ± 6.09 mL (range: 3–20), FSH 27.63 ± 19.0 IU/L, LH 13.66 ± 6.37 IU/L, testosterone 12.09 ± 6.12 nmol/L, prolactin 8 ± 1.83 μg/L, and estradiol 86.33 ± 34.42 pmol/L. All of these men had similar sperm counts on repeat semen analysis, with the exception of one non-mosaic KS man who had a concentration of 0.016 M/mL on one semen analysis and was azoospermic on a second test.

DISCUSSION

The 47,XXY karyotype of KS arises from spontaneous nondisjunction of paired X chromosomes. This can occur either during stage I or II of germ-cell meiosis, or during oogenesis or spermatogenesis (7). Postfertilization nondisjunction is responsible for mosaicism in men with KS (4).

Groth et al. (8) pooled the data from eight different studies and estimated the incidence of KS to be 152 per 100,000 live-born males. The true incidence may be higher than this, as a Danish study found that only approximately one-fourth of adult males with KS were diagnosed and less than 10% were diagnosed before puberty (9). The incidence in the infertile male population has been reported to be higher than in the

general population (10), although studies in this population are scant.

Up to 20% of KS men are mosaic cases, usually with two cell lines: 47,XXY/46,XY (1, 3) (Table 2). The true prevalence of mosaic forms may be underestimated for two reasons. First, chromosomal mosaicism can be present only in the testes, with the karyotype of peripheral leukocytes being normal (1). Second, as we have found, men with mosaic KS appear to be more androgenized than their non-mosaic counterparts. They have a higher rate of sperm in the ejaculate, lower ADAM scores, and larger testicular volumes, and as such some of these men may not be tested for KS and thus not identified. In this respect, the group that we have evaluated may represent a “self-selected” subgroup of men with mosaic KS, having the most severe spermatogenic compromise.

There is considerable phenotypic variation among men with KS: the classic findings of eunuchoid body proportions, sparse facial and pubic hair, small testicles, and cognitive deficits are often absent (1). The most consistent phenotypic abnormalities are small testicular volume (<6 mL, present in >95% of men), increased gonadotropin levels (present in >95% of men), and adult azoospermia (present in >95% of men). Our findings were consistent with these, with an overall mean testicular volume of 6.59 mL, elevated gonadotropins, and 91% being azoospermic at presentation.

It is interesting that we found that men with KS had a relatively high incidence of undescended testicles: 10 (11.6%) of 86. This finding was also identified by Groth et al. (8), who found that the frequency of undescended testicles in men with KS was 27% to 37%; Kamischke et al. (2) found the rate to be 17%, higher than that of the general population of 1.6% to 2.2% (11). The mechanism of this may be related to low testosterone levels prenatally, as testicular descent is related to gubernacular growth, which is regulated by testosterone (12). The clinical relevance of this increased rate of undescended testicles remains unclear. Although undescended testicles are at a higher risk of developing malignancies, there is no known increased risk of testicular primary tumors in men with KS. Men with KS have been shown to be at higher risk for mediastinal germ cell tumors,

TABLE 2

Reported incidence of Klinefelter syndrome (KS) and mosaic KS.

Reference	Population	Men with KS	Men with mosaic KS
Nielsen and Wohlert 1991 (25)	Newborn children	31/17,872 (0.17%)	7/31 (22.6%)
Bojesen et al. 2003 (9)	Prenatal and postnatal	959/2,480,858 (0.04%)	63/959 (10.6%)
Kamischke et al. 2003 (2)	Adult men suspected of having KS	85/309 (27.5%)	3/85 (3.5%)
Taylor and Moores 1967 (26)	Infants	3/4,934 (0.06%)	1/3 (33.3%)
Hamerton et al. 1975 (27)	Infants	6/6,823 (0.09%)	3/6 (50%)
Maeda et al. 1991 (28)	Infants	7/7,608 (0.09%)	3/7 (42.9%)
Herlihy et al. 2011 (29)	Infants	639/85,650 (0.746%)	62/639 (9.7%)

Samplaski. Phenotype of Klinefelter syndrome. *Fertil Steril* 2014.

but there are no data to suggest an increased risk of testicular primary tumors. In addition, the relative contribution of undescended testicles to fertility status in men with KS is also not known. Indeed, it is possible that in the two men with both mosaic KS and undescended testicles, the cause of their impaired fertility could be a result of either of these conditions, or a combination. For these two men, one had a higher proportion of XXY cells (28/32), and the other had a higher proportion of XY cells (9/30). From these small numbers, it is difficult to draw major conclusions about which is the true cause of the spermatogenic failure (Klinefelter versus undescended testicles).

Men with KS have been reported to have elevated serum gonadotropins, low testosterone, and elevated estradiol (1, 4). This is consistent with our findings. In general, for men with KS the decline in testosterone production is progressive, and not all men are symptomatically hypogonadal (4, 13). This is also consistent with our finding that sexual function was maintained, with the mean SHIM score being 20.65, and 29.1% of men testing positive on the ADAM questionnaire. Hormonally these men may have low testosterone levels (mean serum testosterone 8.89 nmol/mL), but from a functional standpoint they seem to maintain adequate sexual function.

How do we explain the less severe reproductive phenotype in the mosaic KS? Men with more than two X chromosomes (48,XXXY; 49,XXXXY) are more severely affected than men with the classic 47,XXY karyotype (14). The phenotype progressively deviates from normal as the number of X chromosomes increases (1). These men have been shown to function at a lower cognitive level and with more immature behaviors as compared with men with fewer X chromosomes (14). The presence of two active X chromosomes in animals and hybridoma models is lethal (15). In females, one X chromosome randomly undergoes inactivation in embryonic tissues (16); it appears that a similar mechanism occurs in men with X chromosome polysomy (1).

DNA sequencing of the X chromosome has identified over 1,000 genes involved in a variety of physiologic and pathologic disease states, particularly in brain and testicular function (17, 18). Genes located in the X chromosome inactivation center are responsible for the detection of an additional X chromosome and the subsequent activation of the X chromosome inactive-specific transcript promoter (19). In turn, this promoter binds to the supernumerary X chromo-

some and turns off gene transcription (16). In men having an extra X chromosome, the presence of the non-inactivated genes from the extra X-chromosome is thought to be the source of much of the pathology, although the exact mechanism remains unknown. Given the theory that much of the pathology of KS is due to the presence of the noninactivated extra genes from the X-chromosome, it would be a rational extension that men with mosaic KS would have a milder phenotype.

Karyotyping is the standard method for KS testing in the postnatal period. However, the test has been reported to have a relatively low sensitivity for 47,XXY/46,XY mosaicism (4). In addition, it has been reported that depending on the tissue sampled mosaicism may be missed, as men may have tissue-specific mosaic KS (1). Finally, chromosomal mosaicism may be present only in the testes, with the karyotype of peripheral leukocytes being non-mosaic KS (1). It is interesting to consider the true incidence of mosaicism in KS men, but this information is currently unknown.

We have found that men with mosaic KS do seem to be more well-androgenized than their non-mosaic KS counterparts. They have a lower percentage of positive ADAM scores (16.7% vs. 29.8%), lower baseline LH levels (10.31 IU/L \pm 5.52 vs. 19.89 IU/L \pm 6.93; $P=.002$), and lower estradiol levels (58.71 \pm 31.10 pmol/L vs. 108.57 \pm 43.45 pmol/L; $P=.027$). It is interesting to note that the mosaics had lower LH values but not lower FSH values. The reason for this is unclear.

The mean testicular volume of men with mosaic KS was almost double that of non-mosaics (11 \pm 7.3 mL vs. 6.35 \pm 3.69 mL; $P<.001$), which was consistent with their higher mean total sperm count (4.43 \pm 9.86 M/mL vs. 0.18 \pm 1.17 M/mL; $P<.001$) and higher proportion with sperm in the ejaculate: 3 (50%) of 6 versus 3 (3.75%) of 80 ($P=.003$).

In general, most men with KS are azoospermic. Occasionally, single foci of spermatogenesis do exist in the testes of men with KS, and subsequently rare men will have sperm in the ejaculate. In our series, 7% of men had some sperm in the ejaculate. The mean age of these men was 43 years, which would seem to be contradictory to reports that men with KS are born with a normal number of sperm precursors and that there may be a period in early puberty when spermatogonia undergo massive apoptosis (4). If this were the case, it would seem reasonable that men with sperm in the ejaculate would be younger than those without, but our findings did not support this notion.

There has been a traditional belief that all KS males who produce spermatozoa in any numbers are XY/XXY mosaics, as XXY cells are meiotically incompetent (20). However, Foresta et al. (21) demonstrated that there are some 47,XXY germ cells that can go through the meiotic and mitotic processes and mature to become spermatozoa. Another school of thought has held that the presence of sperm in the ejaculate is because the karyotype of the lymphocyte lineage does not predict the chromosomal constitution of testis cells or the presence or absence of spermatogenesis (22). This was demonstrated by fluorescence in situ hybridization (FISH) analysis of testicular biopsy samples from non-mosaic KS men, which showed that testicular mosaicism existed (46,XY; 47,XXY) with only the 46,XY cells undergoing meiosis (23). In reality, it is possible that both processes may be occurring simultaneously within the testes of some men with KS. Future studies should focus on identifying markers to determine which men could be expected to have sperm at the time of microsurgical testicular sperm extraction.

Of note, we did have two men with non-mosaic KS who tested positive for both an AZFc microdeletion and non-mosaic KS. As 44 men were tested for both AZFc microdeletions and karyotype, this resulted in a 4.5% (2 of 44) incidence of both conditions. Both of these men were azoospermic on repeat semen analysis. Neither underwent microsurgical testicular sperm extraction. It would seem to reason that the rates of successful sperm retrieval would be lower in men with both abnormalities, but this has never been shown. Mitra et al. (24) examined the incidence of KS and AZF microdeletion and found both to be present in 2 (14%) of 14 men (one AZFa and one AZFb microdeletion), which was higher than our findings.

Limitations of our study include the relatively small number of men included (six mosaic KS patients), the retrospective nature of the study, and variation among the laboratories used for semen and hormone testing. Future studies should focus on larger populations of both mosaic KS and non-mosaic KS men, which will likely emerge in the coming years with the use of multicenter databases. Nonetheless, we believe that important information can be gained from this study as it does demonstrate that men with mosaic KS are more androgenized than their non-mosaic counterparts. It is possible that karyotypically normal cells in the periphery function in a physiologically normal manner, allowing these men to appear more well virilized. As the number of men with mosaic KS increases with increased physician awareness and testing, it will be interesting to determine whether the proportion of cells being XXY:XY portends a more severe or less severe phenotype. Finally, as testing methods improve, it will be interesting to determine what proportion of men who currently test 47,XXY, non-mosaic KS are actually mosaic KS in other tissues.

CONCLUSION

We have found that men with mosaic KS do seem to be more well-androgenized than their non-mosaic KS counterparts. They have larger mean testicular volumes (11 ± 7.3 mL vs.

6.35 ± 3.69 mL; $P < .001$), lower baseline LH levels (10.31 IU/L ± 5.52 IU/L vs. 19.89 IU/L ± 6.93 ; $P = .002$), lower estradiol levels (58.71 ± 31.10 pmol/L vs. 108.57 ± 43.45 pmol/L; $P = .027$), a higher mean total sperm count (4.43 ± 9.86 M/mL vs. 0.18 ± 1.17 M/mL; $P < .001$), and a higher proportion with sperm in the ejaculate: 3 (50%) of 6 versus 3 (3.75%) of 80 ($P = .003$).

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