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META-ANALYSIS

Relative hyperestrogenism in Klinefelter Syndrome: results from a meta-analysis

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Abstract

Objective Klinefelter Syndrome (KS) is classically described as characterized by hyperestrogenism, although solid evidence is lacking. This study aims to test the hypothesis that men with KS have higher serum estradiol than normal controls.

Design Meta-analysis of all studies extracted by MEDLINE from 1942 to 31 January 2018. All studies reporting serum estradiol measurement were considered, among them only case-control studies were included in the meta-analysis.

Methods Meta-analysis was conducted according to the PRISMA statement using RevMan.

Results Out of 4120 articles, 23 case-control studies, 14 case series, and 19 case reports reported data on serum estradiol. A total of 707 KS and 1019 controls were included in the meta-analysis. Serum estradiol was slightly, but significantly higher in KS than controls (mean difference 4.25 pg/mL; CI: 0.41, 8.10 pg/mL; $p = 0.030$). This difference was lost considering only studies using estradiol assays with good accuracy (5.48 pg/mL, CI: −2.11, 13.07 pg/mL; $p = 0.160$). Serum testosterone and estradiol/testosterone ratio were significantly lower and higher in KS than controls, respectively. Data from KS case series and case reports confirmed that serum estradiol is within the normal ranges.

Conclusions Serum estradiol is not increased in KS although slightly higher than controls. However, the meta-analysis that included only studies using a serum estradiol assay with good accuracy showed no difference in serum estradiol between KS and controls. The traditional belief that KS is associated with elevated serum estradiol should be reconsidered. This meta-analysis shows that men with KS have relative hyperestrogenism (increased estradiol/testosterone ratio) compared to controls.

Keywords XXY aneuploidy · Estrogens · Sex steroids · Male · Estrogen to testosterone ratio · Testosterone

Introduction

Since the first description in 1942 Klinefelter Syndrome (KS) has been classically described as a disease characterized by gynecomastia, eunuchoid body proportions, feminine distribution of body adipose tissue, and reduced body hair, most of these signs being considered as feminized physical characteristics [1–4]. This feminized body habitus has been traditionally considered to be due to estrogen excess and elevated estradiol serum levels [1, 5–7]. The high prevalence of gynecomastia, especially as described in pioneering studies [5, 8], further substantiated this concept [9–11], thus definitively coupling the development of a phenotypic female appearance to the excess of circulating estrogens in men with KS [6, 12, 13]. Accordingly, two authoritative and extensive reviews on this disease report that '*on average, the oestradiol concentration is higher than in normal men*' and '*a typical patient with Klinefelter Syndrome will present often with elevated estradiol*', respectively [12, 14]. At present, however, there is no consensus about the real amount of circulating estradiol (normal or increased) in KS [15], since data available in the literature comparing serum estradiol in men with and without KS are scanty and show conflicting results. Estradiol serum levels do not differ between men with KS and controls in most of the studies [16–21], while other studies show higher estradiol serum levels in Klinefelter compared to healthy men [6,

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22–26]. Anyway, serum estradiol falls within the normal male range in a great percentage of cases [6, 14]. The finding of lower serum estradiol in men with KS compared to controls in few studies further complicates this issue [27–29]. Of note the lack of well-designed controlled studies investigating the degree of estrogenization of men with KS, as well as the limit concerning the choice of the assay employed for the measurement of estradiol in serum leave this issue controversial. The detection of serum estrogens in men, in fact, is challenging due to the poor accuracy of commercially available assays in measuring serum estradiol within the low male range [30–33]. Besides, it remains to be elucidated in detail, if a decrease of the estradiol to testosterone ratio occurs in men with KS when the hypogonadism worsens [3, 6, 7, 10, 11, 14, 17, 34, 35].

The aim of this study is to test the hypothesis that men with KS have higher serum concentrations of estradiol than normal controls. In order to establish whether serum estradiol is really increased in men with KS, we used a meta-analytic approach applied to all studies available in the literature, which describe the gonadal status of these men, including the measurement of serum estradiol.

Materials and methods

We performed a two-phase review of the literature. In the first phase, the average estradiol serum level in KS men was described through a wide search of all studies available. In the second phase, a meta-analysis of hormonal status available by controlled-studies was performed, according to the Cochrane Collaboration and PRISMA statement [36, 37].

Data sources and searches

MEDLINE was searched from 1942 to 31 January 2018 using 'PubMed'.

Search key words were KS, testosterone, estradiol, sex steroids, androgens, estrogens, and gonadal function connected by the Boolean functions AND and OR.

Study selection and inclusion criteria

Types of studies

All studies, which provided data on estradiol serum levels in subjects with KS were considered for eligibility. In the first phase of cohort description, all studies were considered, irrespective to the number of patients enrolled, the study design, the main aims, and endpoints. Thus, randomized clinical trials, control studies, case-control studies, case series, and case reports were all collected (Fig. 1). In the second phase, only controlled-studies, in which men with KS were compared to control subjects, were included in the meta-analytic process (Fig. 1).

Type of participants

Men with genetic diagnosis of KS based on the results of karyotype assessment were considered. However, whether

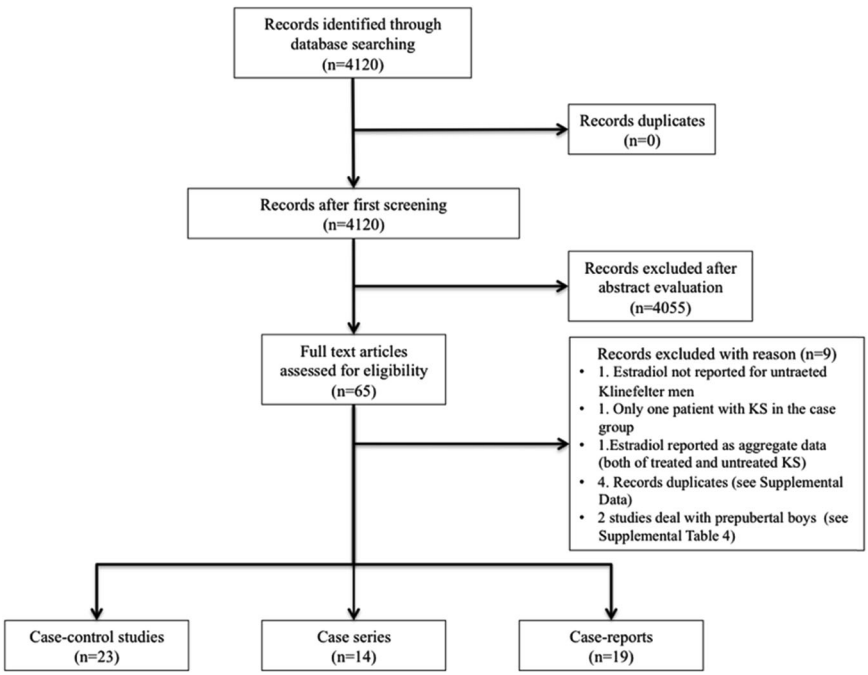

Fig. 1 Flow-chart of the meta-analysis

studies considered KS men treated with testosterone, only untreated patients, or baseline hormonal values were considered in the meta-analysis. Studies on prepubertal and pubertal patients with KS were excluded due to the wide variation of sex steroids in this period of life (see Supplemental Data for details) [38]. Healthy men represented the control groups in case-control studies during the meta-analytic approach, except for one study in which the control group was composed by patients with hypogonadotropic hypogonadism with documented normal serum testosterone during testosterone replacement therapy [29].

Type of interventions

This meta-analysis did not include any type of treatment and do not evaluate therapy efficacy.

Data collection process and quality

Three authors (D.S., S.S., and V.R.) extracted the abstracts from all studies found through the literature search. All abstracts were evaluated for inclusion criteria and data were extracted from the full text of each study considered eligible, with regard to study design, year of publication, number of included/excluded subjects and the presence of control group. Estradiol and testosterone values were collected (see Supplemental Table 1 in Supplemental Data) [38].

In the first phase a description of the average estradiol and testosterone serum levels was addressed (see Supplemental Data for details) [38]. In the second phase a meta-analysis considering all studies evaluating serum estradiol in KS compared to control subjects was performed (for details see Supplemental Table 1 in Supplemental Data) [38].

Where it was possible, we collected the assay employed for the hormonal measurements, in order to do group studies, according to the accuracy of the assay used for estradiol measurement (see the paragraph below and Supplemental Table 5 for further details) [38].

The quality of trials was independently assessed by three of the authors (D.S., S.D.V., and V.R.) using Cochrane risk-of-bias algorithm [39]. The following quality criteria and methodological details were evaluated for each trial included in the meta-analysis: (i) method of randomization, even if the randomization was not an inclusion criterion, (ii) concealment of allocation, (iii) presence or absence of blinding to treatment allocation, (iv) estradiol assays used, (v) type of treatment and follow-up phases (if present), (vi) number of participants and controls recruited and analyzed, (vii) timing of trial, (viii) source of funding, and (ix) criteria for including participants and assessing outcomes.

Criteria used for rating case-control studies according to the accuracy of the assay used for estradiol measurement

The meta-analysis focusing on serum estradiol was repeated after grouping or excluding the case-control studies according to the accuracy of the assay used for serum estradiol measurement. The details concerning the assay used in case-control studies are listed in Supplemental Table 5 [38], where the studies excluded from the meta-analysis due to the lack of information on the estradiol assay are marked in gray [27, 40].

In order to define the accuracy of estradiol assays the following considerations were taken into account:

1. Assay for the measurement of serum estradiol are often inaccurate when serum estradiol is low (<40 pg/mL) and becomes less useful especially for serum estradiol <20 pg/mL; their accuracy depending largely from the type of assay used [30, 32, 41–43].
2. The gold standard for the measurement of serum estradiol in the normal range of men, prepubertal children, and postmenopausal women is liquid chromatography–tandem mass spectrometry (LC–MS/MS) [30, 32, 42, 44].
3. Direct radioimmunoassays (RIA) have low sensitivity that makes them inaccurate for measuring serum estradiol in the low range (<40 pg/mL). Furthermore, they tend to overestimate the real amount of circulating estradiol [30, 41, 42].
4. Indirect RIAs correlate better than direct RIAs with the results obtained by the gold standard LC–MS/MS [45] and this correlation makes them more adequate than commercially available direct RIAs and immunoassays to be used both in research and clinical setting. Accordingly, values obtained by indirect RIAs highly correlate with and are very close to that of LC–MS/MS [30, 45, 46] and usually fall in the same quartile, notwithstanding differences in absolute values [46]. However, even indirect RIAs tend to overestimate serum estradiol [30, 45, 46].
5. At serum concentrations of men and postmenopausal women, direct immunoassays for the determination of serum estradiol show a great variability among different types of assays when compared each other [43, 47], and usually tend to overestimate the amount of serum estradiol compared to LC–MS/MS [42, 43]. The degree of variability from LC–MS/MS is largely different among various assays [31, 42, 48], some immunoassay reaching serum estradiol values closer to that of the gold standard [49].

For all the above-mentioned reasons we decided to repeat the meta-analysis on serum estradiol after

grouping the studies as follows: (1) Group 1: assays with poor accuracy and (2) Group 2: assays with good accuracy. Studies using direct RIAs or immunoassays having a poor accuracy (defined as an accuracy >25% compared to LC–MS/MS) were included in Group 1 (assays with poor accuracy). Studies using LC–MS/MS, indirect RIAs or immunoassays having an accuracy close to or less than the 25% of that of LC–MS/MS were included in Group 2 (assays with good accuracy). When analyzing the accuracy of immunoassays the studies using the immunoassay based on ARCHITECT (Abbott, Laboratories) were considered as having a good accuracy, since there are many data showing that this assay is reliable for serum estradiol >15 pg/mL [42, 48, 49] (Supplemental Table 5) [38]. Conversely, the studies using the Elecsys 2010 (Roche Diagnostics, USA) were included in the group with poor accuracy, since the performance of this assay is not reliable in the low male range [42, 48] (Supplemental Table 5) [38].

Summary measures

The primary outcome was estradiol serum levels, evaluated as mean ± SD either in KS or control subjects. Secondary outcome was total testosterone serum levels, evaluated as mean ± SD either in KS or control subjects.

Finally, the mean ± SD of the estradiol to testosterone ratio was calculated for each study.

Data synthesis and analysis

The meta-analysis was conducted using the Review Manager (RevMan) 5.3 software (Version 5.3.1 Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Data were combined considering the mean differences of each endpoint. Heterogeneity between results of different studies was examined by inspecting the scatter in the data points and the overlap in their confidence intervals and by performing chi-square tests and I^2 statistics. The fixed effect model was used when low heterogeneity was detected (i.e. $I^2 < 50\%$), whereas the random effect model was used when high heterogeneity among studies was found (i.e. $I^2 > 50\%$).

Additional analyses were conducted using the 'Statistical Package for the Social Sciences' software for Macintosh (version 20.0; SPSS Inc., Chicago, IL). In particular, the hormonal pattern of Klinefelter patients was evaluated considering the entire group of studies included.

Correlations were evaluated using Spearman's analysis.

Values of $p < 0.05$ were considered statistically significant and 95% confidence intervals are presented.

Results

The first work published in the literature on KS back to 1942 [1]. From 1942 to January 31, 2018, 4120 studies on KS were published (Fig. 1). These studies were designed to address different endpoints, thus in our review we considered only trials in which information on serum estradiol was provided. A total of 65 articles were considered (see Supplemental Table 1, Supplemental Table 2, Supplemental Table 3, and Supplemental Table 4 for further details) [38]. Of these, 23 case-control studies [6, 16, 17, 19, 22–29, 40, 50–58], 14 case series [14, 34, 59–70], and 19 case reports (all listed in Supplemental Data) [38] reported data on serum estradiol and were included in the analyses (Fig. 1). Details on case series and case reports are reported in Supplemental Data (see Supplemental Table 2 and Supplemental Table 3, respectively) [38].

All trials included in the meta-analysis had enrolled men with a confirmed diagnosis of KS obtained by cytogenetic analysis. All available cytogenetic analysis were considered useful, and all genotype forms of KS, both non-mosaic and mosaic KS patients, were considered eligible. However, this variability in the genetic cause of KS could represent one important source of heterogeneity for this meta-analysis.

As expected, different assays had been used for the measurement of both serum estradiol and testosterone measurements with different sensitivity and reference range. In addition, different systems of measurement were used for both serum estradiol and testosterone. In order to perform a correct and comprehensive meta-analysis, we converted all hormonal results into conventional unit, such as pg/mL for estradiol (3.671 was used to convert pmol/L) and ng/dL for total testosterone (0.0347 was used to convert nmol/L).

Average serum levels of reproductive hormones in KS

In the first phase-analysis, a total of 37 studies, 23 case-control and 14 case series studies, reporting the estradiol serum levels were included (for details see Supplemental Tables 1 and 2, respectively) [38]. Considering case-control studies and case series, the overall evaluation of sex steroids in 1504 men with KS enrolled in these studies showed an age of 29.92 ± 24.77 years, serum estradiol of 30.54 ± 35.22 pg/mL, serum total testosterone of 318.00 ± 572.00 ng/dL, and a serum estradiol (E_2) to total testosterone (T) ratio (E_2/T) of 0.0736 ± 0.0788 (data are expressed as mean ± SD). Considering the normal range for estradiol serum levels between 20 and 40 pg/mL, mean serum estradiol of patients with KS felt within the normal range, mainly in the highest quartile. However, the great variability in standard deviation does not allow to establish with certainty if serum estradiol is always in the highest quartile (Supplemental Data; Supplemental Tables 1 and 2) [38].

Gonadotropins measurements were available in 32 studies (86.48%), with serum LH of 24.26 ± 19.81 IU/L, and serum FSH of 37.43 ± 36.2 IU/L (mean ± SD). As expected, both LH and FSH were constantly significantly higher than normal range for adult men ($p < 0.001$ and $p < 0.001$, respectively).

Serum estradiol was neither related to total serum testosterone ($R = -0.174$, $p = 0.340$), nor to serum LH ($R = 0.246$, $p = 0.174$), and FSH ($R = 0.315$, $p = 0.096$). Similarly, total serum testosterone was not significantly related to serum LH ($R = -0.184$, $p = 0.347$) and FSH ($R = -0.054$, $p = 0.798$). FSH and LH were significantly directly correlated each other ($R = 0.773$, $p < 0.001$). Finally, the E_2/T ratio showed a direct and inverse relationship with both serum estradiol ($R = 0.613$, $p < 0.001$) and testosterone ($R = -0.719$, $p < 0.001$), respectively. Moreover the E_2/T ratio was not related to serum FSH ($R = 0.499$, $p = 0.0.061$) and LH ($R = -0.314$, $p = 0.118$). Finally, age was not related to LH ($R = -0.105$, $p = 0.579$), FSH ($R = -0.135$, $p = 0.501$), serum estradiol ($R = -0.182$, $p = 0.319$), and total serum testosterone ($R = -0.098$, $p = 0.614$).

Differences between KS and control subjects

Considering only 23 case-control studies, a total of 707 KS and 1019 control subjects were included in the meta-analysis. The mean age of men with KS (28.21 ± 21.14 years) was not significantly different compared to controls (29.30 ± 24.76 years) ($p = 0.743$) (Supplemental Table 1) [38]. The analysis has been also stratified according to the period of publication of the studies (Fig. 2).

Estradiol serum levels were slightly but significantly higher in KS than control subjects (mean difference 4.50 pg/mL, CI: 0.45, 8.55 pg/mL; $p = 0.030$) (Fig. 2). According to the wide spread of different study designs and patients enrolled, the heterogeneity of these studies was extremely high (chi-squared = 139.95, $I^2 = 84\%$). When the studies were analyzed according by decades of year of publication, this significant difference was found only when the studies published between 2010 and 2018 were considered (mean difference 2.80 pg/mL, CI: 0.74, 4.85 pg/mL; $p = 0.008$). The meta-analysis on serum estradiol was repeated after grouping the studies according to the accuracy of the estradiol assay used (Fig. 3) (see Supplemental Data) [38]. Estradiol serum levels did not differ between men with KS and control subjects when assays with a good accuracy were used (mean difference 5.48 pg/mL, CI: −2.11, 13.07 pg/mL; $p = 0.160$) (Fig. 3). Vice versa serum estradiol was slightly but significantly higher in KS than control subjects when assays with a poor accuracy were used (mean difference 6.81 pg/mL, CI: 1.70, 11.91 pg/mL; $p = 0.009$) (Fig. 3).

Total testosterone serum levels were significantly lower in KS than in control subjects (mean difference −260.29 ng/dL, CI: −325.28, −195.30 ng/dL; $p < 0.001$) (Supplemental Fig. 1) [38].

Finally, the estradiol/testosterone ratio was significantly higher in KS men compared to control subjects (mean difference 0.06, CI: 0.04, 0.08; $p < 0.001$, I^2: 92%) (Fig. 4).

Discussion

This study, based on a meta-analytic approach, overall indicates that serum estradiol in men with KS is slightly but significantly higher compared to control subjects, but this weak difference is lost when an accurate assay for estradiol measurement is used. Notwithstanding multiple and recurring statements claiming for the association between hyperestrogenism and KS are available in the literature [6, 7, 12–14], none among the studies investigating the status of reproductive hormones in men with KS had as primary endpoint the comparison of circulating estrogens between controls and 47-XXY men. Furthermore, the finding that only a total of 65 out of the 4120 studies on KS available in the literature reported data on serum estradiol is very surprising considering both the research (not clinical) context and the claimed hyperestrogenism in these patients. When considering that serum estradiol is indicated even in the clinical work-up of men with KS by most of the consensus and the expert panels [15, 71–73], this very low rate (1.6%) of studies that have investigated and reported serum estradiol in men with KS is quite disappointing.

With this in view, the aim of this study is to fill this gap of knowledge. Accordingly, this meta-analysis aims to analyze for the first time estradiol serum levels in men with KS through the collection of data available in all the case-control studies that have reported the measurement of circulating estradiol. The results of this meta-analytic study speak in favor of a slight condition of hyperestrogenism in men with KS compared to control subjects. However, some important issues should be considered in order to put the overall main result of this study into the appropriate context. First, in most of the studies (13 out of 29) the direct comparison of serum estradiol of men with KS with control subjects did not show significant differences. Second, less studies (11 out of 29) showed significant higher serum estradiol in men with KS and they included both studies on few patients [22, 23, 50, 51, 53], but also the two studies with the largest sample size [21, 26] (see Supplemental Table 1 for further details) [38]. These latter two studies might have influenced the weight of statistics having a greater impact on the results of this meta-analysis (Fig. 2). Third, the method employed for serum estradiol measurement might increase the variability among different studies and invalid, at least in part, the results provided by the meta-analysis. It is well known, indeed, that the results obtained

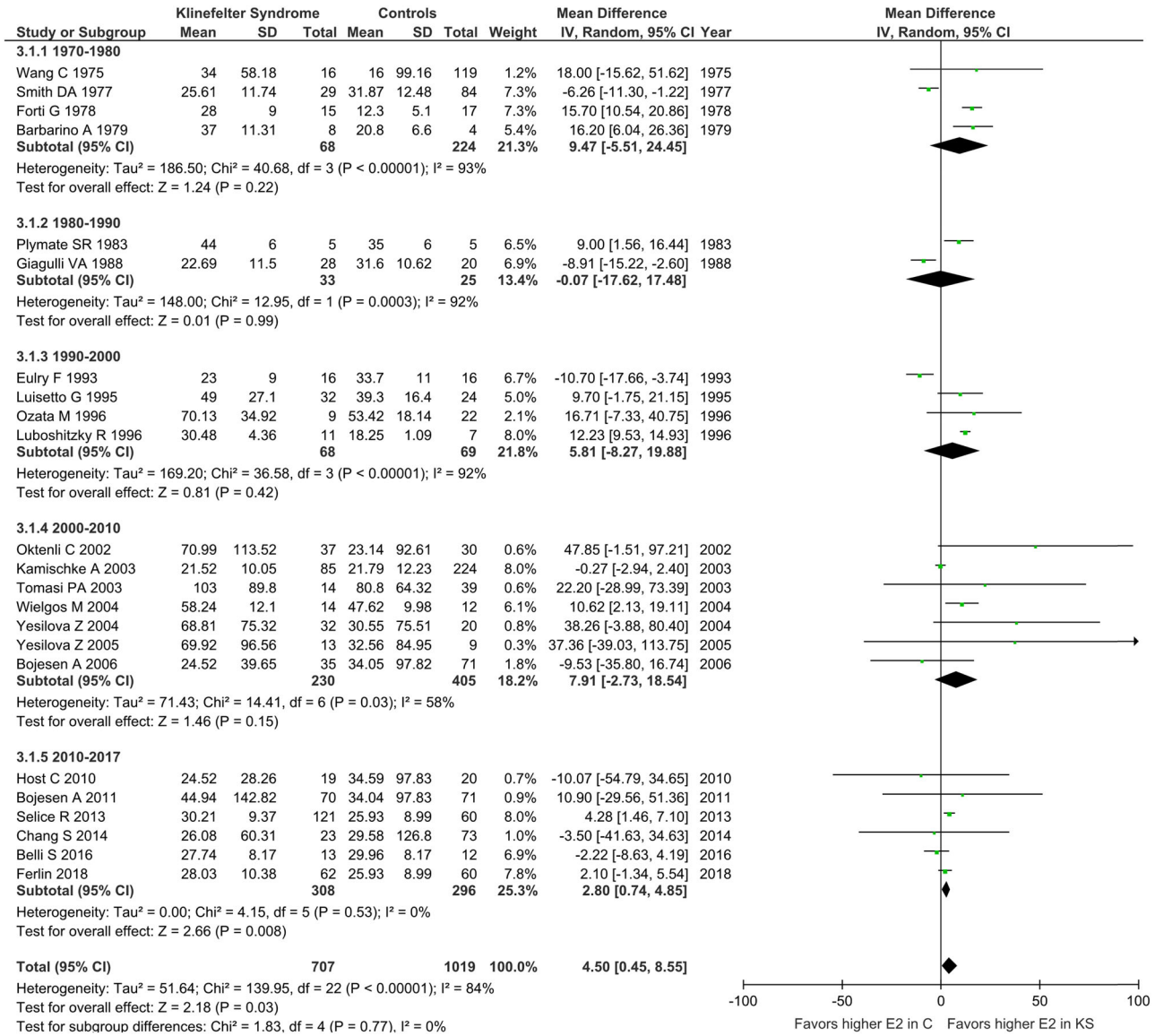

Fig. 2 Comparison of estradiol serum levels (pg/mL) between men with Klinefelter Syndrome and control subjects. Case-control studies have been also grouped according to decades of publication

by using different assays for estradiol measurement in serum are difficult to compare each other [47]. Furthermore, the measurement of serum estradiol within the male normal range is challenging due to lack of precision and accuracy of most of the commercially available assays [30–33, 42]. Only one study included in this meta-analysis used the gold standard LC–MS/MS for sex steroids measurement showing no difference in serum estradiol between men with KS and controls [19]. In order to limit the impact of poor accuracy of estradiol assays, case-control studies were grouped according to the quality of the assay (poor accuracy vs. good accuracy) and the meta-analysis restricted to the studies using an accurate assay for serum estradiol measurement did not show any difference in serum estradiol between men with KS and control subjects (Fig. 3).

If all the above-mentioned factors are taken into account the slightly but significant difference in serum estradiol found overall by this meta-analysis should be considered with caution. Hence, estradiol serum levels in men with KS are probably not different from that of control subjects. This assumption is further confirmed by the finding, in men with KS, of mean serum estradiol values almost constantly within the normal male range when both the thresholds reported in each study and the range extrapolated from the NHANES III US cohort were considered [74].

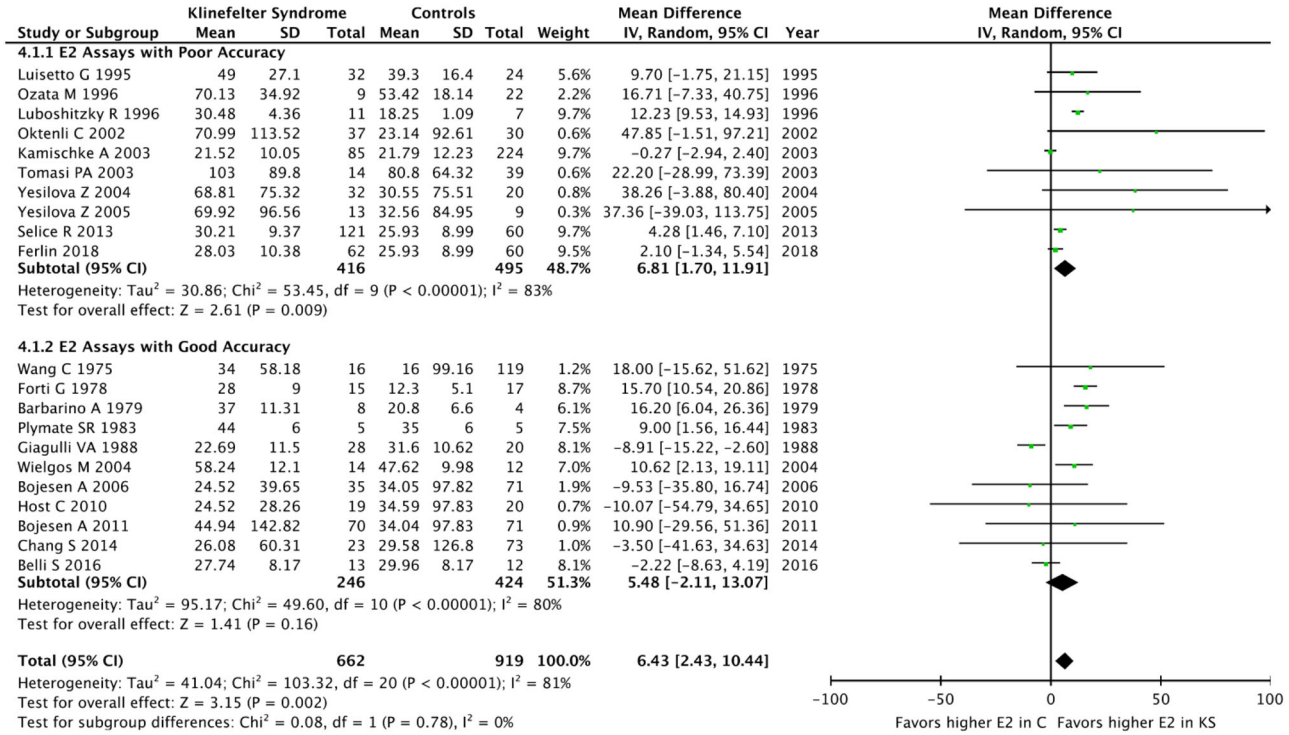

Fig. 3 Comparison of estradiol serum levels (ng/dL) between men with Klinefelter Syndrome and control subjects after grouping case-series studies according to the (poor or good) accuracy of the assay used for serum estradiol measurement

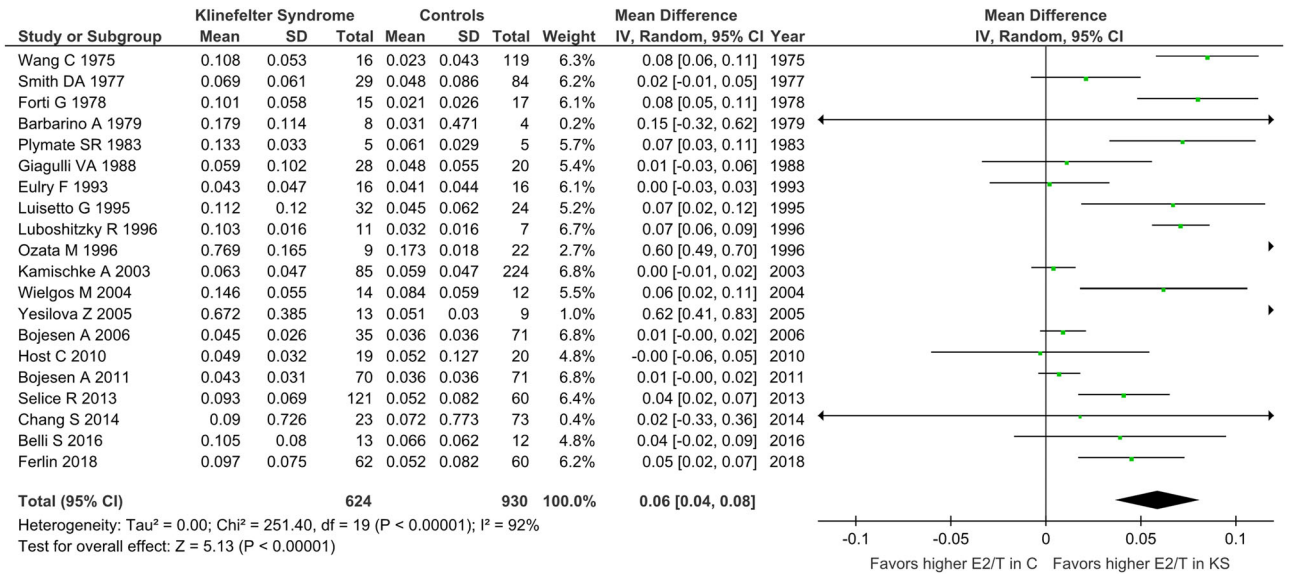

Fig. 4 Comparison of estradiol (E_2) [pg/mL] to testosterone (T) [ng/dL] ratio (E_2/T) between men with Klinefelter Syndrome and control subjects

What is clear is that a condition of relative hyperestrogenism is present in these patients, since the estradiol/testosterone ratio is altered thanks to the lower concentrations of total serum testosterone in presence of normal serum estradiol. Accordingly, the results of this study confirm unequivocally the significant reduction in total serum testosterone in men with KS compared to controls (Supplemental Fig. 1) [38], which seems to be also responsible for the significant, higher estradiol/testosterone ratio in men with KS compared to control subjects.

With this in view, the most important result of this study is the finding of a significant increase of the estradiol/

testosterone ratio in men with KS compared to control subjects (Fig. 4), suggesting a clinical condition of testosterone deficiency coupled with relative estrogen excess. Currently, only few studies provided direct evidence on the unbalanced estradiol/testosterone ratio in men with KS [10, 11, 17, 22, 26, 75]. This imbalance in circulating sex steroids is a peculiar characteristic of men with KS in whom serum estradiol is in the highest quartile of the normal range notwithstanding low circulating levels of its precursor testosterone. Probably, the activity and expression of the aromatase enzyme is increased in these patients, but information about the underlying mechanism is still not available in literature. It is known that elevated serum LH levels may increase aromatase activity leading to a relative increase of estradiol secretion from the testes [76], but to what extent this mechanism operates in men with KS is not known. There is only indirect evidence by in vitro study [11]. The unbalance of the estradiol/testosterone ratio may be also due to the high prevalence of visceral adiposity in men with KS [17], which accounts for increased peripheral conversion of testosterone into estradiol within the adipose tissue due to herein aromatase overexpression [17]. In addition, obesity in these patients may worsen the hyperestrogenism and the imbalance between testosterone and estradiol [17]. Hence, the increased estradiol/testosterone ratio more than hyperestrogenism per se probably accounts for the development of gynecomastia in men with KS [77]. This is also in line with recent advances in the pathophysiology of gynecomastia outside the context of KS [78].

This study has several limitations, which mainly belongs from that of the studies included in this meta-analysis. First, each study used different methods for estradiol measurement with all the above-mentioned limitations in the absence of the use of the gold-standard LC–MS/MS, which has been used only by one study [19]. Second, the results obtained by different methods and laboratories are difficult to compare each other [47]. Third, there are not studies properly designed to compare estradiol serum levels between men with KS and control subjects. This comparison, indeed, was extrapolated from studies designed according to different aims. Several potential modifiers were hypothesized, such as study design, sample size, year of publication, age and body mass index of study population, estradiol assay used, time of blood sample, type of outcome studied, and heterogeneity of genetic defect (e.g. mosaicism). All these aspect, alone or taken together, could explain the heterogeneity of the studies enrolled. However, we are unable to reduce the wide variability, as well as the limitations of all the studies included in this meta-analysis.

In conclusion, this meta-analysis challenges the traditional belief that KS is associated to elevated serum estradiol, which is not so higher than in control subjects and suggests condition of relative hyperestrogenism, supported by the constant increase of estradiol/testosterone ratio in these patients. Some clinical features of KS such as decreased bone mineral density, elevated serum LH, and eunuchoid body proportions indirectly speak against absolute values of elevated serum estradiol in these patients, since high serum estradiol is known to prevent all these clinical features [79, 80]. Rather than an increase of serum estradiol, the imbalance between testosterone and estradiol accounts for some features associated to KS such as gynecomastia.

Further studies using the gold standard LC–MS/MS for the measurement of serum estradiol are needed based on a proper design aiming to compare serum estradiol of men with KS to that of control subjects. This will allow overcoming both the main limit of this meta-analysis that includes only one study using LC–MS/MS and the gap of knowledge in the literature. The rapid diffusion of LC–MS/MS in clinical laboratories will further help to obtain more reliable data on serum estradiol in men and of course in men with KS. In this way, also data coming from the clinical setting will further elucidate the degree of estrogenization associated to this disease.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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