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Review

Efficacy and Safety of Letrozole in the Management of Constitutional Delay in Growth and Puberty: A Systematic Review and Meta-analysis

Dutta D et al. Letrozole in Short Stature

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What is already known in this topic?
Small RCTs have suggested beneficial role of letrozole in CDGP with regards to growth and pubertal progression.

What this study adds?
This is the first meta-analysis to holistically analyze the efficacy and safety of letrozole in CDGP
Letrozole is superior to placebo with regards to improvement in predicted adult high, increase in testicular volume, and improving hormone parameters. Letrozole is superior to testosterone with regards to increase in testicular volume and delaying bone age progression.

Abstract
Background: No meta-analysis is available which has analysed role of letrozole in constitutional delay in growth and puberty (CDGP).
Methods: Electronic databases were searched for RCTs involving children with CDGP receiving letrozole. Primary outcome were changes in predicted adult height (PAH) and pubertal progression. Secondary outcomes were alterations in bone age, hormonal markers of puberty, bone mineral density and side-effects.
Results: One hundred-thirty articles were reviewed, from which 7 RCTs which fulfilled all criteria were analysed. Letrozole was superior to placebo [mean difference (MD) 4.63cm (95% CI: 3.90 – 5.36); P<0.01; I^2=0%] but not testosterone [MD 2.21cm (95% CI: -1.71 – 6.16); P=0.27; I^2=98%] with regards to improvement in PAH after 12-months use. Letrozole was superior to both placebo [MD 4.80ml (95% CI: 0.57 – 9.03); P=0.03] and testosterone [MD 3.36ml (95% CI: 0.58 – 6.75); P=0.02; I^2=0%] with regards to improvement in testicular volume after 12-months use. Letrozole was superior to testosterone [MD -0.84 years (95% CI: 2.83 – 8.18); P=0.06; I^2=0%] with regards to slowing in bone age progression after 12-months use, which approached statistical significance. Serum LH, FSH, testosterone and inhibin-B were significantly higher after 6-months letrozole use compared to active as well as passive controls. No increased occurrence of adverse events, spinal deformities were noted with letrozole.
Conclusion: Letrozole is safe and effective for improving height and pubertal outcomes in CDGP, and is better than testosterone with regards to improvement in testicular volume and delaying bone-age progression.
Keywords: letrozole, meta-analysis, safety, constitutional delay in growth and puberty, short stature

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Introduction
Constitutional delay in growth and puberty (CDGP) is perhaps the most common cause of short stature in both the sexes; and for not yet determined reasons, is much more common in boys than girls (1). The diagnosis of CDGP is often a diagnosis of exclusion (2). Children with CDGP have delayed pubertal growth spurt and usually have catch-up growth with the late onset of puberty, by 18 years age (2). Although reassurance and watchful waiting is recommended in CDGP, many children with CDGP in teens have associated significant psychosocial stress, negative interaction with peers, anxiety or depression warranting medical intervention (3). Suggested medical interventions include medications which will promote sexual maturation (4). Traditionally low dose testosterone injections/oxandrolone and ethinyl estradiol/estradiol patches have been tried to accelerate puberty in boys and girls respectively for many decades now (4). Recently few trials have been published which have suggested that letrozole may have a role in activation of the hypothalamic-pituitary-gonad (HPG) axis, faster testicular growth, resulting in accelerated height growth and pubertal progression in CDGP (5,6).
Theoretically, aromatase inhibitors are uniquely suited to manage different aspects of CDGP. Letrozole would delay bone maturation by inhibiting conversion of testosterone to estradiol and thereby lowering blood estradiol concentrations (7). Letrozole also has potential to induce maturation of HPG axis by decreasing negative feedback loop from estradiol to hypothalamus. Some safety concerns include reduced bone density, which have primarily been documented in adult cancer survivors (8)
However, to date no meta-analysis is available which has holistically analysed and summarized the clinical efficacy and safety of letrozole in CDGP. Hence the aim of this meta-analysis was to evaluate the efficacy and safety of letrozole in the management of CDGP.

Methods

Methodology
The meta-analysis was carried out according to the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions (9). The predefined protocol has been registered in PROSPERO having Registration number of CRD42021250345. All randomized controlled trials (RCTs) published till March 2021 were considered for this meta-analysis. This meta-analysis has been reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), the filled checklist of which can be found at the end of the manuscript

(9). Since ethical approval already exists for the individual studies included in the meta□analysis, no separate approval was required for this study.

The PICOS criteria was used to screen and select the studies for this meta-analysis with patients (P) being children diagnosed with constitutional delay in growth and puberty (CDGP); intervention (I) being use of letrozole for managing CDGP; control (C) being patients either on placebo or any other approved medication for managing CDGP (like testosterone, oxandrolone or estradiol); outcomes (O) being evaluated were impact on predicted adult height (PAH), height standard deviation score (HSDS), bone age, clinical and hormonal measures of puberty and any adverse effects noted; and (S) being studies included which were RCTs. Only children with CDGP were considered for this meta-analysis. Children with other forms or causes of short stature like familial short stature, growth hormone deficiency, panhypopituitarism, syndromic short stature, and idiopathic short stature (ISS) were excluded. Only those studies were included in this meta-analysis which had at least 2 treatment groups of children with CDGP, with one of the groups receiving letrozole and the other group receiving either placebo or any other medication in place of letrozole.

The primary outcomes were to evaluate the changes in predicted adult height (PAH) and pubertal progression as determined by testicular volume. The secondary outcomes of this study was to evaluate the alterations in height standard deviation score (Ht-SDS), final height (FH), bone age (BA), hormonal markers of puberty (testosterone, LH, FSH, estradiol, inhibin-B, anti-mullerian hormone (AMH)), bone mineral density, body composition changes (lean mass, fat mass), and any side effects reported. Analysis of the growth and puberty outcomes was done based on whether the control group received an active comparator (like testosterone, oxandrolone or any other approved medication for use in CDGP) – labelled here as the active control group (ACG) or a placebo/nothing – labelled as passive/placebo control Group (PCG).

Search method for identification of studies

A detailed search was done of electronic databases of Medline (Via PubMed), Embase (via Ovid SP), Cochrane central register of controlled trials (CENTRAL) (for trials only), ctri.nic.in, clinicaltrials.gov, global health and Google scholar using a Boolean search strategy: (letrozole) AND [(delayed puberty) OR (short stature)].

Data extraction and study selection

Data extraction was carried out independently by two authors using standard data extraction forms. In cases where more than one publication of a single study group were found, results were grouped and relevant data from each report were used in the analyses. Data on the primary and secondary outcomes as stated above was extracted. Patient characteristics (including demographic information and comorbidities) from the different studies included and excluded from the analysis were noted in a tabular form (Table-1,2). All disagreements were resolved by the third and fourth authors.

Assessment of risk of bias in included studies

Three authors independently assessed the risk of bias using the risk of bias assessment tool in Review Manager (Revman) Version 5.3 (The Cochrane Collaboration, Oxford, UK 2014) software. The following points were taken into consideration namely, was there adequate sequence generation to rule out selection bias. We looked for if the allocation adequately concealed again to rule out selection bias. We also looked for whether the knowledge of the allocated interventions were adequately prevented during the study or not. Participants and personnel blinding was specifically looked for to rule out performance bias. We also looked for the blinding of the outcome assessors to rule out detection bias. We looked for whether the incomplete outcome data issue was adequately addressed or not to rule out attrition bias. We also looked for if the reports of the study were free of suggestion of selective outcome reporting to rule out reporting bias. Lastly we also looked for whether the study was apparently free of other problems that could put it at risk of bias. Any disagreements were resolved by the fourth author.

Measures of treatment effect

For continuous variables, the outcomes were expressed as mean differences (MD). SI (International System) units were used for analysis, and all studies reporting results in conventional units were converted to SI units for analysis. For dichotomous outcomes (treatment success) results were expressed as risk ratios (RR) with 95% confidence intervals (CI). For adverse events, results were expressed as post treatment absolute risk differences. RevMan 5.3 was used for comparing MD of the different primary and secondary outcomes between letrozole and the control groups of the included studies.

Assessment of heterogeneity

Heterogeneity was initially assessed by studying the forest plot generated for the primary and secondary outcomes of this study. Subsequently heterogeneity was analysed using a Chi^2 test on N-1 degrees of freedom, with an alpha of 0.05 used for statistical significance and with the I^2 test (10). The interpretation of I^2 values is as follows: 0% to 40%: might not be important; 40% to 60%: may represent moderate heterogeneity; 60% to 90%: may represent substantial heterogeneity; 90% to 100%: considerable heterogeneity. The importance of the observed value of I^2 depends on the magnitude and direction of treatment effects and the strength of the evidence for heterogeneity (e.g. P-value from the Chi^2 test, or a confidence interval for I^2) (10).

Grading of the results

An overall grading of the evidence (certainty of the evidence) related to each of the primary and secondary outcomes of the meta-analysis was done using the GRADE (Grades of Recommendation, Assessment, Development and Evaluation) approach (11). The GRADE approach defines the quality of a body of evidence as the extent to which one can be confident that an estimate of effect or association is close to the true quantity of specific interest. The quality of a body of evidence involves consideration of within-trial risk of bias (methodological quality), directness of evidence, heterogeneity, precision of effect estimates and risk of publication bias (11). The GRADEpro Guideline Development Tool (GDT) software (McMaster University and Evidence Prime Inc, 2015) was used to create the Summary of Findings (SoF) table in this meta-analysis (Table-3). The "certainty of evidence" has been graded into 4 categories, namely "high" (there is a lot of confidence that the true effect lies close to that of the estimated effect), "moderate" (there is moderate confidence in the estimated effect: The true effect is likely to be close to the estimated effect, but there is a possibility that it is substantially different), "low" (there is limited effect in the estimated effect: The true effect might be substantially different from the estimated effect) and, "very low" (there is very little confidence in the estimated effect: The true effect is likely to be substantially different from the estimated effect) (11).

Publication bias was assessed by plotting the Funnel Plot, which specifically targets small study bias, in which small studies tend to show larger estimates of effects and greater variability than larger studies [9]. Presence of one or more of the smaller studies outside the inverted funnel plot was taken as evidence of presence of significant publication bias (12).

Data synthesis

Data was pooled as random effect model for the analysis of primary and secondary outcomes. The outcomes were expressed as 95% confidence intervals (95% CI). Forrest plots were plotted with the left side of the graph favouring letrozole and the right side of the graph favouring control using RevMan 5.3 software. $P<0.05$ was considered statistically significant.

Results

A total of 357 articles were found after the initial search (Figure-1). Following the screening of the titles, and abstracts, the search came down to 173 articles. Thirty eight duplicates were removed. One hundred-thirty articles were reviewed in details from which 7 RCTs which fulfilled all

inclusion and exclusion criteria were included in the meta-analysis (Figure-1) (5,6,13-17). One study was removed due to lack of a valid control group (18). Three studies evaluating impact on growth of letrozole in children with precocious puberty were excluded. (19-21)
Of the 7 studies included in this meta-analysis, analysis was done based on the nature of the control group. The control was testosterone in the studies by Kohva et al (2020) (15) and Varimo et al (2019) (5) and hence the results from these studies have been analysed in the ACG. The controls were placebo in the studies by Hero et al (2006) (13), Hero et al (2010) (14), and Wickman et al (2001) (17), and hence the results from these studies have been analysed in the PCG. In the study by Rohani et al (2019), the control group received nothing and hence its results have been analysed in the PCG (16). The study by Salehpour et al (2010) (6) has 3 groups comparing the outcomes of children with CGDP receiving letrozle, oxandrolone or placebo. Hence the results of comparison between children receiving letrozole vs oxandrolone in this study has been presented as Salehpour 2010a under the ACG. The results of comparison between children receiving letrozole vs placebo in this study has been presented as Salehpour 2020b under the PCG.
The details of the studies included in this meta-analysis have been elaborated in Table-1. The studies which were evaluated but were excluded have been summarized in Table-2.

Risk of bias in the included studies

The summaries of risk of bias of the 7 studies included in the meta-analysis have been elaborated in Figure 2a and Figure 2b. Random sequence generation, reporting bias and other bias were judged to be at low risk of bias in all the 7 studies (100%). Source of funding, especially pharmaceutical, authors from the pharmaceutical organizations and conflict of interests were looked into the "other bias" section. Allocation concealment bias (selection bias) were judged to be low risk in 2 studies (28.57%). Performance bias (blinding of participants and investigators) and detection bias (blinding of outcome assessors) were judged to be at low risk of bias in 4 out of 7 studies (57.14%). Attrition bias was judged to be at low risk in 6 out of 7 studies (85.71%).

Effect of letrozole on primary outcomes

Predicted adult height (PAH)

Data from 2 studies involving 88 children with CDGP was analysed to find out the impact of letrozole on PAH after at least 12 months of treatment, when compared to those receiving testosterone in the control group (ACG). Individuals receiving letrozole had a greater improvement in PAH by statistically not significant when compared to those in the ACG [mean difference (MD) 2.21cm (95% CI: -1.71 – 6.16); P=0.27; I^2=98% (considerable heterogeneity); figure-3a; moderate certainty of evidence (MCE); table-3]. Data from 3 studies involving 84 children with CDGP was analysed to find out the impact of letrozole on PAH after at least 12 months of treatment, when compared to those receiving placebo (PCG). Individuals receiving letrozole had a significantly greater improvement in PAH when compared to PCG [MD 4.63cm (95% CI: 3.90 – 5.36); P<0.01; I^2=0% (low heterogeneity); figure-3b; high certainty of evidence (HCE); table-3]

Testicular volume

Data from 2 studies involving 57 children with CDGP was analysed to find out the impact of letrozole on testicular volume after 6 and 12 months of treatment, when compared to those receiving testosterone in the control group (ACG). After 6 months of treatment, children receiving letrozole had a significantly greater increase in testicular volume when compared to those in the ACG [MD 5.51ml (95% CI: 2.83 – 8.18); P<0.01; I^2=0% (low heterogeneity); figure-3c; HCE; table-3], which persisted even after 12 months of treatment [MD 3.36ml (95% CI: 0.58 – 6.75); P=0.02; I^2=0% (low heterogeneity); figure-3d; HCE; table-3].
Data from only 1 study (Wickman et al) involving 19 children with CDGP was analysed to find out the impact of letrozole on testicular volume after 12 and 18 months of treatment, when compared to those receiving placebo (PCG). Children receiving letrozole had significantly higher testicular volume after 12 months [MD 4.80ml (95% CI: 0.57 – 9.03); P=0.03] but not after 18 months of therapy [MD 1.90ml (95% CI: -2.61 – 6.41); P=0.41], when compared to those receiving placebo.

Effect of letrozole on secondary outcomes

Height standard deviation score (Ht-SDS)

Data from 1 study (Salehpour et al) involving 61 children was available comparing the changes in Ht-SDS after at least 12 months of therapy in ACG. Data from 2 studies (Salehpour et al and Rohani et al) involving 65 children were available comparing the changes in Ht-SDS after at least 12 months of therapy in PCG. Children receiving letrozole had a significantly greater improvement in Ht-SDS when compared to those receiving placebo in PCG [MD +0.63 (95% CI: 0.52 – 0.74); P<0.01; I^2=0% (low heterogeneity)], but not when compared to those receiving testosterone (ACG) [MD 0.00 (95% CI: -0.16 – 0.16); P=1.00].

Bone age

Data from 2 studies involving 88 children was analysed comparing the changes in bone age after 12 months of therapy with letrozole, as compared to those receiving testosterone in the ACG. When compared to ACG, children receiving letrozole has a slower progression in bone age, which approached statistical significance [MD -0.84 years (95% CI: 2.83 – 8.18); P=0.06; I^2=0% (low heterogeneity); figure-3e; low certainty of evidence; table-3].
Data from 3 studies involving 88 children was analysed comparing the changes in bone age after 12 months of therapy with letrozole, as compared to those receiving placebo (PCG). When compared to PCG, children receiving letrozole has similar progression in bone age [MD 0.06 years (95% CI: -0.88 – 0.99); P=0.91; I^2=90% (considerable heterogeneity); figure-3f].

Luteinizing hormone

Data from 2 studies involving 55 children with CDGP was analysed to find out the impact of letrozole on serum LH after 6 and 12 months of treatment, when compared to those receiving testosterone in the control group (ACG). Serum LH was significantly higher after 6 months [MD 5.28 IU/L (95% CI: 3.16 – 7.40); P<0.01; I^2=0% (low heterogeneity); figure-4a], but not after 12 months [MD 0.05 IU/L (95% CI: -0.69 – 0.79); P=0.90; I^2=0% (low heterogeneity); figure-4b] of treatment with letrozole, when compared to ACG.
Data from 1 study (Hero et al 2006) involving 17 children with CDGP was analysed to find out the impact of letrozole on serum LH after 6 and 12 months of treatment, when compared to those receiving placebo (PCG). Serum LH was significantly higher after 6 months [MD 6.50 IU/L (95% CI: 3.40 – 9.60); P<0.01], and 12 months [MD 5.10 IU/L (95% CI: 2.14 – 8.06); P<0.01] of treatment with letrozole.

Follicle Stimulating Hormone

Data from 2 studies involving 55 children with CDGP was analysed to find out the impact of letrozole on serum FSH after 6 and 12 months of treatment, when compared to those receiving testosterone in the control group (ACG). Serum FSH was significantly higher after 6 months [MD 3.59 IU/L (95% CI: 1.42 – 5.76); P<0.01; I^2=0% (low heterogeneity); figure-4c], but not after 12 months [MD -0.20 IU/L (95% CI: -1.21 – 0.61); P=0.63; I^2=0% (low heterogeneity); figure-4d] of treatment with letrozole, when compared to ACG.
Data from 1 study (Hero et al 2006) involving 17 children with CDGP was analysed to find out the impact of letrozole on serum FSH after 6 and 12 months of treatment, when compared to those receiving placebo (PCG). Serum FSH was significantly higher after 6 months [MD 7.70 IU/L (95% CI: 4.37 – 11.03); P<0.01], and 12 months [MD 2.70 IU/L (95% CI: 0.42 – 4.98); P=0.02] of treatment with letrozole.

Testosterone

Data from 2 studies involving 57 children with CDGP was analysed to find out the impact of letrozole on serum total testosterone after 6 months of treatment, when compared to those receiving testosterone in the control group (ACG). Serum total testosterone was significantly higher after 6 months [MD 22.73 mmol/L (95% CI: 14.96 – 30.50); P<0.01; I^2=0% (low heterogeneity); figure-4e], treatment with letrozole, when compared to ACG. Data from 3 studies involving 118 children with CDGP was analysed to find out the impact of letrozole on serum total testosterone after 12 months of treatment, when compared to those receiving testosterone in the control group (ACG). Serum total testosterone was not significantly different after 12-24 months [MD -0.60 mmol/L (95% CI: -2.35 – 1.15); P=0.50; I^2=0% (low heterogeneity); figure-4f; HCE; table-3], treatment with letrozole, when compared to ACG.

Data from 1 study (Hero et al 2006) involving 17 children with CDGP was analysed to find out the impact of letrozole on serum total testosterone after 6 months of treatment, when compared to those receiving placebo (PCG). Serum testosterone was significantly higher after 6 months [MD 49.20 mmol/L (95% CI: 22.05 – 76.35); P<0.01], of treatment with letrozole. Data from 3 studies (Hero et, Salehpour et al and Wickman et al) involving 89 children with CDGP was analysed to find out the impact of letrozole on serum total testosterone after 12 months of treatment, when compared to those receiving placebo (PCG). Serum total testosterone was significantly higher after 12 months [MD 32.37 mmol/L (95% CI: 10.58 – 54.16); P<0.01; I^2=97% (considerable heterogeneity); MCE; table-2] treatment with letrozole, when compared to PCG.

Inhibin-B

Data from 2 studies involving 57 children with CDGP was analysed to find out the impact of letrozole on serum inhibin-B after 6 and 12 months of treatment, when compared to those receiving testosterone in the control group (ACG). Serum inhibin-B was significantly higher after 6 months [MD 62.97 ng/L (95% CI: 24.50 – 101.43); P<0.01; I^2=0% (low heterogeneity); figure-5a], and 12 months [MD 25.40 ng/L (95% CI: 1.51 – 49.29); P=0.04; I^2=0% (low heterogeneity); figure-5b] of treatment with letrozole, when compared to ACG.

Data from 1 study (Wickman et al) involving 19 children with CDGP was analysed to find out the impact of letrozole on serum inhibin-B after 5, 12 and 18 months of treatment, when compared to those receiving placebo (PCG). Serum inhibin-B was significantly higher only after 5 months [MD 64.00 ng/L (95% CI: 27.84 – 100.16); P<0.01], but not 12 month [MD 35.00 ng/L (95% CI: -6.01 – 76.01); P=0.09], and 18 months [MD -5.00 ng/L (95% CI: -43.46 – 33.46); P=0.80] of treatment with letrozole, when compared to PCG.

Insulin like growth factor-1

Data from 2 studies (Kohva et al and Varimo et al) involving 57 children with CDGP was analysed to find out the impact of letrozole on serum IGF-1 after 6 of treatment, when compared to those receiving testosterone in the control group (ACG). Serum IGF-1 was significantly lower after 6 months [MD -11.86 nmol/L (95% CI: -18.08 – -5.64); P<0.01; I^2=0% (low heterogeneity)], of treatment with letrozole, when compared to ACG. Data from 3 studies (Kohva et al. Salehpour et al and Varimo et al) involving 118 children with CDGP was analysed to find out the impact of letrozole on serum IGF-1 after 12-24 months of treatment, when compared to those receiving testosterone in the control group (ACG). Serum IGF-1 was not significantly different after 12-24 months [MD -1.24 nmol/L (95% CI: -8.70 – 6.22); P=0.74; I^2=0% (low heterogeneity)] of treatment with letrozole, when compared to ACG.

Data from 1 study (Wickman et al) involving 19 children with CDGP was analysed to find out the impact of letrozole on serum IGF-1 after 6 and 12 months of treatment, when compared to those receiving placebo (PCG). Serum IGF-1 was significantly lower after 6 months [MD -11.30 nmol/L (95% CI: -19.39 – -3.21); P<0.01], and 12 months [MD -9.00 nmol/L (95% CI: -14.52 – -3.48); P<0.01] of treatment with letrozole, when compared to PCG.

Safety

Data from 7 studies (235 children) was analysed to evaluate the impact of letrozole on the occurrence of adverse events [(total adverse events (TAEs) and severe adverse events (SAEs)], over 1-4 years of treatment. The occurrence of TAEs [Risk ratio (RR) 0.61 (95% CI: 0.17 – 2.17); P=0.45; I^2=0% (low heterogeneity); figure-6a; HCE; table-3] and SAEs [Risk ratio (RR) 1.22 (95% CI: 0.28 – 5.28); P=0.79; I^2=0% (low heterogeneity); figure-6b; HCE; table-3] was not statistically different in children receiving letrozole as compared to the control group.

Vertebral deformity and end-plate deformity was studied in the study by Hero et al (2010). In that study data from 12 children (6 receiving letrozole with testosterone vs 6 receiving placebo with testosterone) was analysed after 4.2 years follow up. The occurrence of vertebral deformity [Risk ratio (RR) 0.28 (95% CI: 0.01 – 8.42); P=0.47] and end-plate deformity [Risk ratio (RR) 0.50 (95% CI: 0.05 – 5.15); P=0.56] was not statistically different in children receiving letrozole as compared to the control group.

Changes in the bone mineral density (BMD) Z-scores, before and after therapy was noted in the study by Salehpour et al. A significantly lower (improvement) in lumbar spine BMD Z-score [MD -2.59 (95% CI: -2.88 – -2.30); P<0.01; n=61] and femoral neck BMD Z-score [MD -2.2 (95% CI: -2.55 – -1.85); P<0.01; n=61] was noted in children receiving letrozole as compared to those receiving testosterone in the control group (ACG). Changes in lumbar spine BMD Z-score [MD 0.11 (95% CI: -0.35 – 0.57); P=0.64; n=61] and femoral neck BMD Z-score [MD 0.10 (95% CI: -0.25 – 0.45); P=0.58; n=61] was not significantly different when comparing children receiving letrozole to those receiving placebo (PCG).

A small decline in high density lipoprotein cholesterol (HDL-C) was noted in children receiving letrozole as compared to those receiving placebo in a cohort of 69 patients from 2 studies [MD -0.77 mmol/L (95% CI: -2.00 – 0.47); P=0.22; I^2=99% (considerable heterogeneity); figure-6c], which was however statistically not significant.

Discussion:

This is the first meta-analysis to highlight the efficacy and safety of letrozole in children with CDGP. An important observation from this meta-analysis is letrozole use in children with CDGP is associated with a significantly greater improvement in PAH when compared to receiving placebo. This improvement in PAH with letrozole is comparable to the improvements seen with the use of testosterone in CDGP. In accordance with the previous observation, children receiving letrozole had a significantly greater improvement in Ht-SDS when compared to those receiving placebo but not testosterone. Six months and 12 months letrozole use was associated with a significantly greater improvement in testicular volume in CDGP when compared to both placebo and testosterone. All studies have used letrozole in dose of 2.5 mg/day for the duration of the study.

Use of letrozole in CDGP was associated with a slower progression in bone age when compared to those receiving placebo or testosterone. Serum LH, FSH, total testosterone and inhibin-B were significantly higher after 6 months of use of letrozole in children with CDGP, when compared to those receiving placebo or testosterone. After 12 months of use, the difference for LH, FSH and testosterone persisted only when compared to placebo, but not with regards to those receiving testosterone. Inhibin-B continued to be significantly higher in children receiving letrozole as compared to testosterone after 12 months of use. Serum IGF-1 was significantly lower after 6 months use of letrozole when compared to those receiving placebo or testosterone. Oestrogen has a trophic impact on growth hormone through paracrine effects, which indirectly has a trophic impact on IGF-1 levels (22). Letrozole is an aromatase inhibitor associated with lower oestrogen levels. Testosterone in contrast is aromatized to oestrogen to some extent in the body, having a trophic impact on GH release from pituitary, explaining the higher IGF-1 levels at 6 months (22). Pubertal bone age progression is associated with higher IGF-1 levels (23). A lower bone age progression may contribute to the marginally lower IGF-1 levels in the first 6 months use of letrozole. It is important to note that this observation is transient and

the difference did not persist after 12 months of clinical use. After 12 months use, IGF-1 levels were comparable in children receiving letrozole or testosterone.

This meta-analysis provided assuring data regarding the long term safety of letrozole use in CDGP. No increased occurrence of TAEs and SAEs were noted with use of letrozole. The occurrence of vertebral deformities was not significantly increased. No significant decline/change in BMD z-scores were noted in children receiving letrozole as compared to placebo. Bone health outcomes were better in children receiving testosterone as compared to letrozole cause of the anabolic impact of testosterone on bone mineral density. Testosterone is aromatized to estrogen in the body which has a direct impact on increased bone formation (24). A mild but statistically significant decline in the good cholesterol HDL-C was noted in children receiving letrozole. This is an observation and its impact on long term cardiovascular outcomes needs further evaluation. Advantages of letrozole over testosterone also includes its oral administration in contrast to monthly injections with regards to testosterone.

To conclude, it may be said that this first meta-analysis on the efficacy and safety of letrozole in CDGP, provides us with reassuring data on the good efficacy and tolerability of this molecule on height outcomes and pubertal progression. Letrozole in comparable to testosterone and superior to placebo with regards to improving height outcomes in CDGP. Letrozole has a much better slowing effect on bone age progression, when compared to both testosterone and placebo. This may have an additional impact on improving height outcomes. Letrozole is superior to both testosterone and placebo with regards to improvement in testicular volume (an important marker of pubertal progression in boys). Letrozole has a better short term impact on hormonal markers of pubertal progression (LH, FSH, testosterone and inhibin-B).

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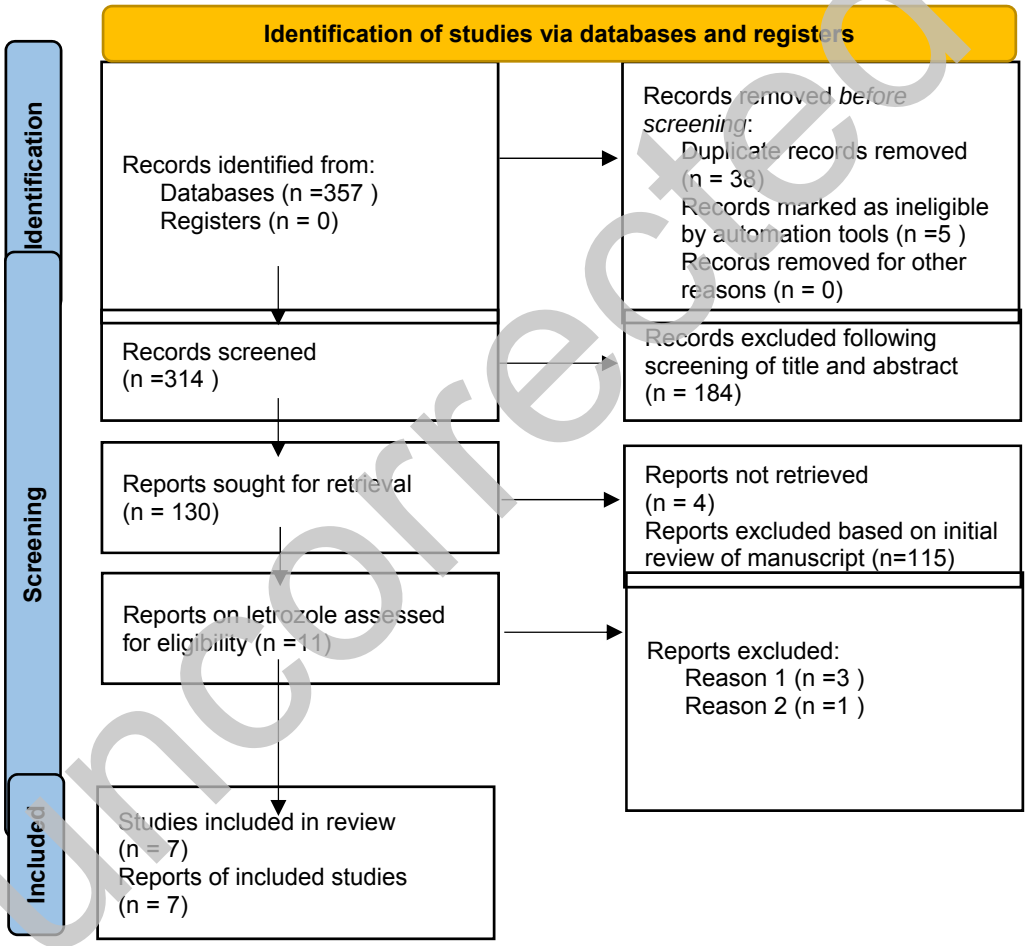
Figure-1: Flowchart elaborating on study retrieval and inclusion in the meta-analysis



Reason-1: lack if a valid control group; Reason-2: evaluated role of letrozole in precocious puberty
RCT: randomized controlled trial

Figure-2a: Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.
Figure-2b: Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

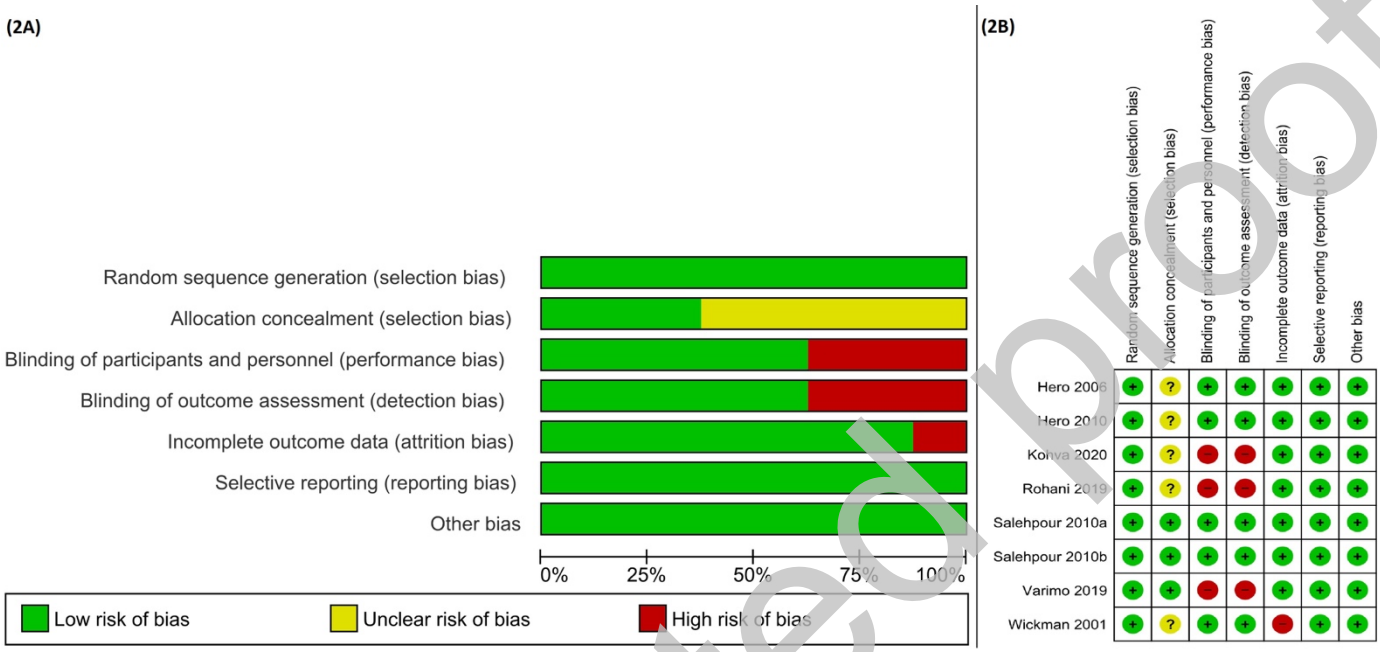


Figure-3: Forest plot highlighting the impact of letrozole on (a) Predicted adult height (PAH) in the ACG; (b) PAH in the PCG; (c) Testicular volume at 6 months in the ACG; (d) Testicular volume at 12 months in the ACG; (e): Bone age progression in ACG; (f): Bone age progression in PCG
ACG: active control group; PCG: passive/placebo control group

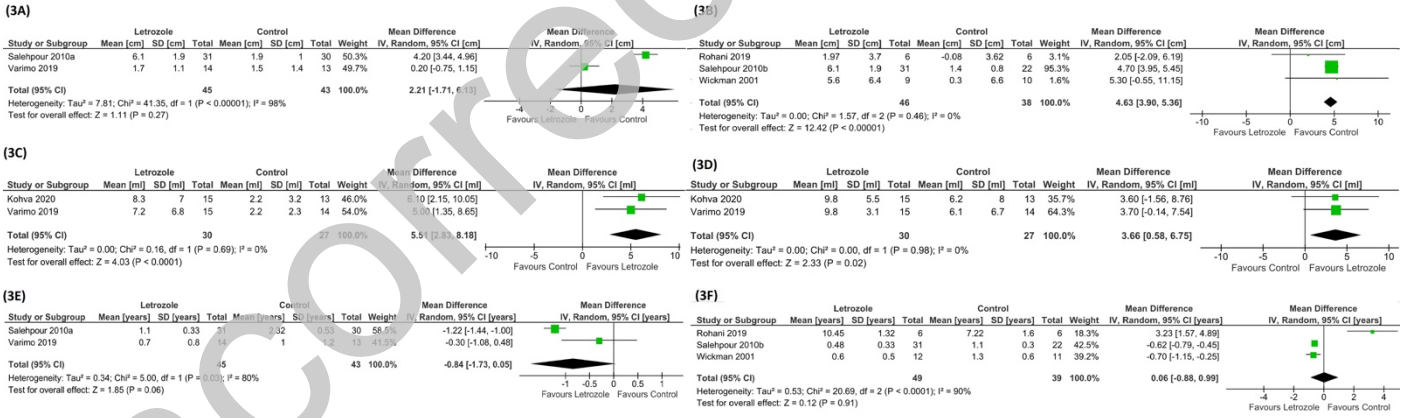


Figure-4: Forest plot highlighting the impact of letrozole vs the active control group on (a) Luteinizing hormone at 6 months; (b) Luteinizing hormone at 12 months; (c) Follicle stimulating hormone at 6 months; (d) Follicle stimulating hormone at 12 months; (e): Testosterone at 6 months; (f): Testosterone at 12 months

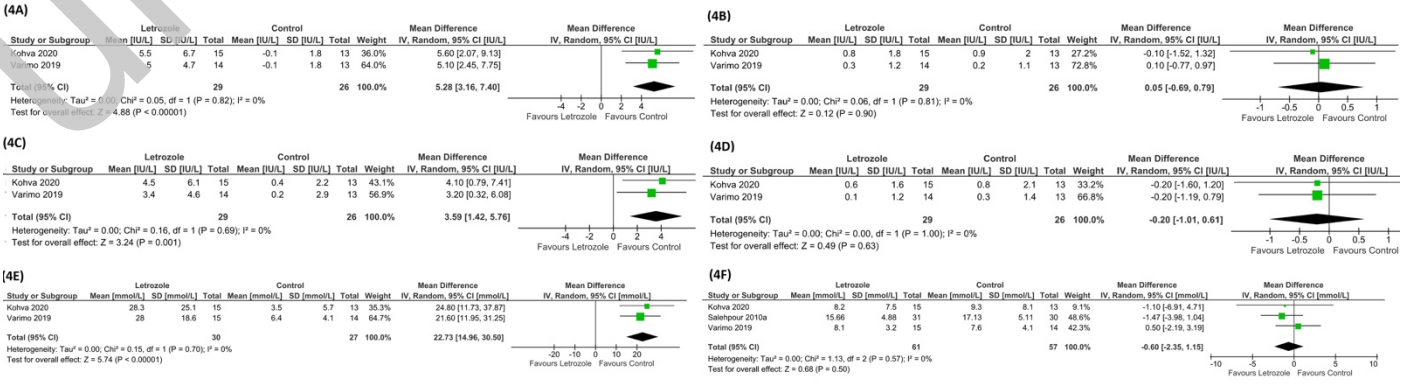


Figure-5: Forest plot highlighting the impact of letrozole vs the active control group on (a) Inhibin-B at 6 months; (b) Inhibin-B at 12 months

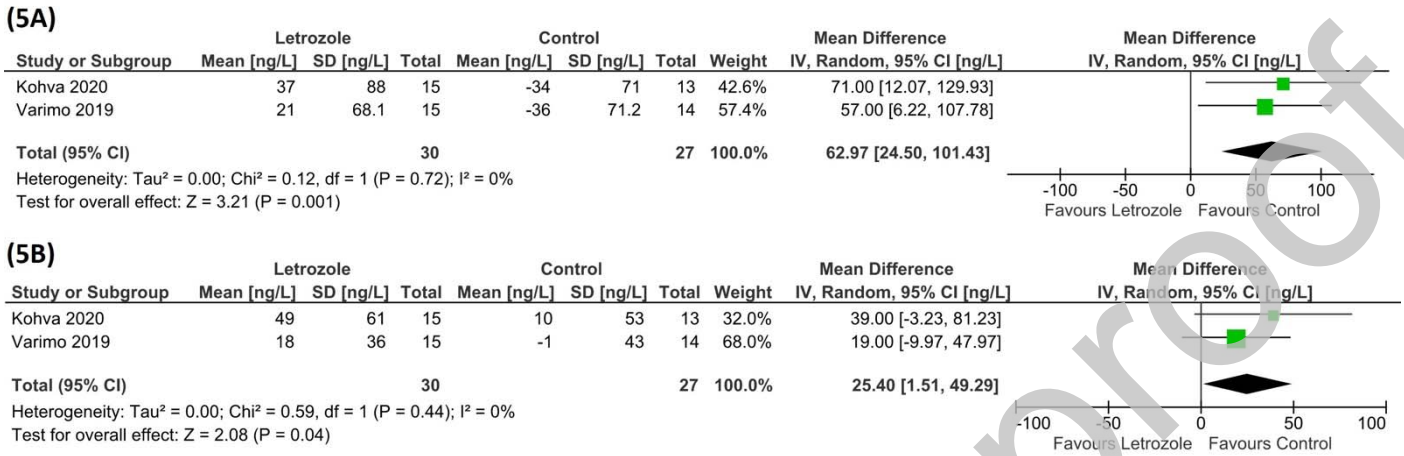


Figure-6: Forest plot highlighting the side effect profile of the use of letrozole as compared to controls focussing on (a): Total Adverse Events (TAEs); (b): Severe Adverse Events (SAEs); (c): High density lipoprotein cholesterol

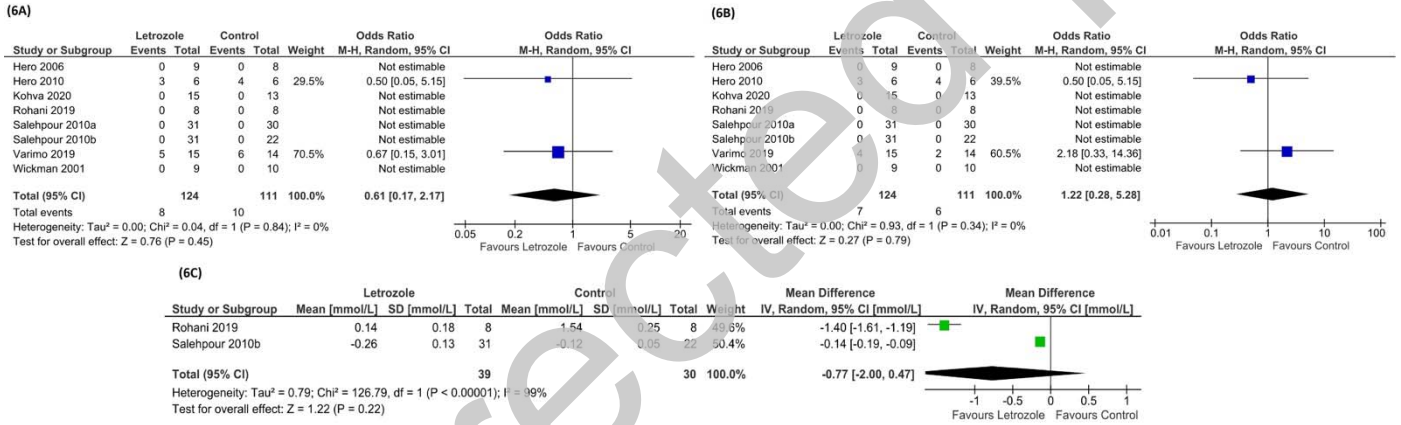


Table-1: Characteristics of patients in the different randomized controlled trials evaluated in this meta-analysis on use of letrozole in constitutional delay in growth and puberty

Study details	Number of patients in letrozole & control groups	Patient characteristics and nature of controls	Duration of study
Hero 2006 (13)	Lz+T (n=9) T+Placebo (n=8) Testosterone was used at a dose of 1 mg/kg i.m. every 4 weeks for 6 months	Children with testis volume <4 ml after 13.5 years of age.	Period of intervention 52 weeks; follow up ~ 4 years
Hero 2010 (14)	Lz+T (n=6) T+Placebo (n=6) Testosterone was used at a dose of 1 mg/kg intramuscularly every 4 weeks for 6 months	Children with testis volume <4 ml after 13.5 years of age.	52 weeks
Kohva 2020 (15)	Lz (n=15), T (n=13)	The inclusion criteria were testicular volume between 2.5 and 4 ml and serum T < 5 nmol/L or serum T ≥ 1 nmol/L, if the mean testicular volume was <2.5 ml, or Tanner genital stage 2 and serum T < 3 nmol/L. At the start of the trial, the boys were above 14 years of age. Controls were similar to patients	52 weeks
Rohani 2019 (16)	Lz (n= 8) Placebo (n=8)	PAH <1 SD MPH and Tanner pubic hair stage delayed by >SD or TV <= 3 ml	Follow up ~ 8 years

Salehpour 2010a (6)	Lz (n=31) Oxandrolone (n=30)	12.6-14.6 years old boys with PAH <1 SD MPH and Tanner pubic hair stage delayed by >SD or TV <= 3 ml	Intervention 104 weeks; follow up 260 weeks
Salehpour 2010b (6)	Lz (n=31) Placebo (n=30)	12.6-14.6 years old boys with PAH <1 SD MPH and Tanner pubic hair stage delayed by >SD or TV <= 3 ml	Intervention 104 weeks; follow up 260 weeks
Varimo 2019 (5)	Lz (n=15) T (n=15) (testosterone (Sustanon 250; Aspen Nordic, Ballerup, Denmark) was injected intramuscularly every 4 weeks for 6 months (six injections in total)	The inclusion criteria were testicular volume between 2.5 and 4 ml and serum T < 5 nmol/L or serum T ≥ 1 nmol/L, if the mean testicular volume was <2.5 ml, or Tanner genital stage 2 and serum T < 3 nmol/L. At the start of the trial, the boys were above 14 years of age. Controls were similar to patients	Intervention 26 weeks; follow up 52 weeks
Wickman 2001 (17)	Lz+T (n=10) T+Placebo (n=12) (testosterone enanthate (Testoviron- Depot-250, Schering, Berlin, Germany) six times at a dose of 1 mg/kg intramuscularly every 4 weeks, and placebo orally once daily for 12 months) Placebo (n=10)	Children with testis volume <4 ml after 13.5 years of age.	18 months

Lz: letrozole; T: testosterone; LH: luteinizing hormone; FSH: follicle stimulating hormonel IGF: insulin like growth factor; IGFBP: insulin like growth factor binding protein: HDL: high density lipoprotein; BMD: bone mineral density; MPH: mid parental height; SD: standard deviation; TV: testicular volume; PAH: predicted adult height; letrozole was used at a dose of 2.5mg/day for the duration of the study in all the above RCTs; Oxandrolone was used at dose of 2.5mg/day in the study by Salehpour et al.

Table-2: Characteristics of patients in the different studies evaluated but excluded this meta-analysis

Study details	**Number of patients in study & control groups**	**Patient characteristics and nature of controls**	**Duration of study**	**Reasons for exclusion**
Karmazin 2005 (19)	6 children on androgen + Lz. No control group	Definition of case not clearly congruent with CDGP. There is also mention of GH replacement in 3 patients.	12 months	Lack of matched control group
Zhao 2014 (18)	22 children. No control group	Study done in children with idiopathic central precocious puberty	6 months	Study not done in children with CDGP
Neely 2014 (20)	Lz 17 Anastrazole 22	Short stature with reduced PAH in prepubertal boys. ISS and CDGP not differentiated	24 months	Lack of a matched control group. Not a randomized controlled trial
Xu 2021 (21)	GH + Lz single arm	Short stature with reduced PAH in prepubertal boys. ISS and CDGP not differentiated	12 months	Lack of a matched control group. Not a randomized controlled trial

Lz: letrozole; GH: growth hormone; PAH: predicted adult height; ISS: idiopathic short stature; CDGP: constitutional delay in growth and puberty

Table-3: Summary of findings:

Letrozole compared to Control in the management of constitutional delay in growth and puberty A systematic review and meta-analysis

Patient or population: Managing constitutional delay in growth and puberty A systematic review and meta-analysis
Setting:
Intervention: Letrozole
Comparison: Control

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)
	Risk with Control	**Risk with Letrozole**			
Predicted Adult Height (PAH) ACG	The mean predicted Adult Height (PAH) ACG was **175.5** cm	MD **2.21 cm higher** (1.71 lower to 6.13 higher)	-	88 (2 RCTs)	⊕⊕⊕◯ MODERATE [a]
Predicted Adult Height (PAH) PCG	The mean predicted Adult Height (PAH) PCG was **173.85** cm	MD **4.71 cm higher** (3.97 higher to 5.45 higher)	-	72 (2 RCTs)	⊕⊕⊕⊕ HIGH
Progression in bone age ACG	The mean progression in bone age ACG was **13.65** years	MD **0.84 years lower** (1.73 lower to 0.05 higher)	-	88 (2 RCTs)	⊕⊕◯◯ LOW [b,c]
Testicular Volume 6 months ACG	The mean testicular Volume 6 months ACG was **5.65** ml	MD **5.51 ml higher** (2.83 higher to 8.18 higher)	-	57 (2 RCTs)	⊕⊕⊕⊕ HIGH
Testicular Volume 12 months ACG	The mean testicular Volume 12 months ACG was **9.6** ml	MD **3.66 ml higher** (0.58 higher to 6.75 higher)	-	57 (2 RCTs)	⊕⊕⊕⊕ HIGH
Testosterone 12 months ACG	The mean testosterone 12-24 months ACG was **19.6** nmol/l	MD **0.6 nmol/l lower** (2.35 lower to 1.15 higher)	-	118 (3 RCTs)	⊕⊕⊕⊕ HIGH
Testosterone 12 months PCG	The mean testosterone 12 months PCG was **16.36** nmol/l	MD **32.37 nmol/l higher** (10.58 higher to 54.16 higher)	-	89 (3 RCTs)	⊕⊕⊕◯ MODERATE [c]
Total Adverse Events (TAEs)	90 per 1,000	**57 per 1,000** (17 to 177)	**OR 0.61** (0.17 to 2.17)	235 (8 RCTs)	⊕⊕⊕⊕ HIGH
Severe Adverse Events (SAEs)	54 per 1,000	**65 per 1,000** (16 to 232)	**OR 1.22** (0.28 to 5.28)	235 (8 RCTs)	⊕⊕⊕⊕ HIGH

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **MD:** Mean difference; **OR:** Odds ratio; **ACG:** Active control group; **PCG:** Passive/placebo control group

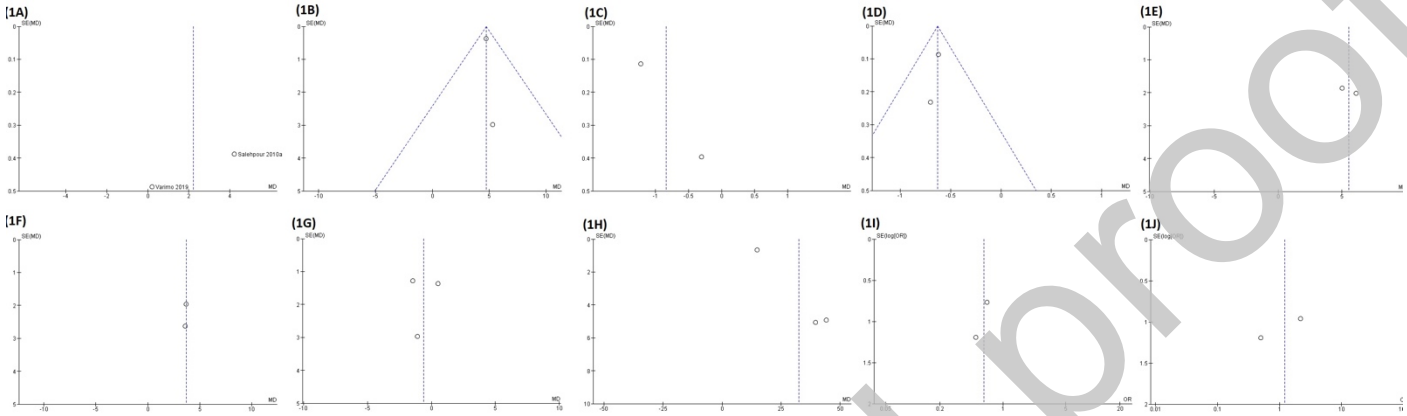
GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Explanations
a. Due to large variation in effect, confidence intervals do not overlap, P value for heterogeneity is <0.01 and I^2 is more than 90%
b. Due to large variation in effect, confidence intervals do not overlap, I^2 is more than 80%
c. High publication bias suspected as evidenced by the funnel plot (Supplementary Figure-1)

SUPPLEMENTARY FIGURES:

Supplementary Figure-1: Funnel plot of all the included studies in the meta-analysis (assessing the publication bias) of the main outcomes assessed (a) predicted adult height (PAH) ACG; (b) PAH PCG; (c) Bone age progression ACG; (d): Bone age progression PCG; (e): Testicular volume at 6months in ACG; (f): Testicular volume at 12 months in ACG; (g) Serum testosterone at 12 months in ACG; (h): Serum testosterone at 12 months in PCG; (i): Total adverse events; (j): Severe adverse events

ACG: active control group; PCG: passive/placebo control group



Supplementary Table-1: Risk of bias assessment table

Hero 2006	**Risk Of Bias**	**Author Judgement**
Random Sequence Generation (Selection Bias)	Low Risk	Randomised, double-blind, placebo controlled study
Allocation Concealment (Selection Bias)	Unclear Risk	Details not available
Blinding Of Participants & Personal (Performance Bias)	Low Risk	Yes, double blinded RCT
Blinding Of Outcome Assessment (Detection Bias)	Low Risk	Yes, double blinded RCT
Incomplete Outcome Data (Attrition Bias)	Low Risk	Of the 19 boys initially evaluated in this study, 17 boys completed the study
Selective Reporting (Reporting Bias)	Low Risk	All pre-specified outcomes were reported
Other Biases	Low Risk	This work was supported by the Foundation for Paediatric Research, Helsinki, Finland, and the Hospital District of Helsinki and Uusimaa.
Hero 2010	**Risk Of Bias**	**Author Judgement**
Random Sequence Generation (Selection Bias)	Low Risk	Randomised, double-blind, placebo controlled study
Allocation Concealment (Selection Bias)	Unclear Risk	Details not available
Blinding Of Participants & Personel (Performance Bias)	Low Risk	Yes, double blinded RCT
Blinding Of Outcome Assessment (Detection Bias)	Low Risk	Yes, double blinded RCT
Incomplete Outcome Data (Attrition Bias)	Low Risk	No drop-outs; all patients completed the study
Selective Reporting (Reporting Bias)	Low Risk	All Pre-Specified Outcomes Were Reported
Other Biases	Low Risk	This study was supported by the Foundation for Paediatric Research, Helsinki, Finland
Kohva 2020	**Risk Of Bias**	**Author Judgement**
Random Sequence Generation (Selection Bias)	Low Risk	Randomised, controlled, open-label multicentric trial
Allocation Concealment (Selection Bias)	Unclear Risk	Details not available in the manuscript
Blinding Of Participants & Personel (Performance Bias)	High Risk	Open labelled study
Blinding Of Outcome Assessment (Detection Bias)	High Risk	Open labelled study
Incomplete Outcome Data (Attrition Bias)	Low Risk	All patient outcomes reported. NO drop-outs
Selective Reporting (Reporting Bias)	Low Risk	All Pre-Specified Outcomes Were Reported
Other Biases	Low Risk	The Academy of Finland, The Foundation for Pediatric Research, The Emil Aaltonen Foundation, Sigrid Juselius Foundation, Helsinki University Hospital Research Funds.Takeda Development Center
Rohani 2019	**Risk Of Bias**	**Author Judgement**
Random Sequence Generation (Selection Bias)	Low Risk	Randomized, open-label, parallel-group study
Allocation Concealment (Selection Bias)	Unclear Risk	Details not available
Blinding Of Participants & Personel (Performance Bias)	High Risk	Open labelled study
Blinding Of Outcome Assessment (Detection Bias)	High Risk	Open labelled study
Incomplete Outcome Data (Attrition Bias)	Low Risk	Outcomes of al the 16 randomized patients have been presented.

Selective Reporting (Reporting Bias)	Low Risk	All Pre-Specified Outcomes Were Reported
Other Biases	Low Risk	Nothing significant was noted
Salehpour 2010	**Risk Of Bias**	**Author Judgement**
Random Sequence Generation (Selection Bias)	Low Risk	a prospective, double-blind, randomized, placebo-controlled clinical trial
Allocation Concealment (Selection Bias)	Low Risk	Stratified randomization was done
Blinding Of Participants & Personel (Performance Bias)	Low Risk	Yes, double blinded RCT
Blinding Of Outcome Assessment (Detection Bias)	Low Risk	Yes, double blinded RCT
Incomplete Outcome Data (Attrition Bias)	Low Risk	83 out of the 91 randomized children completed the study. Hence attrition rate was 8.79%
Selective Reporting (Reporting Bias)	Low Risk	All Pre-Specified Outcomes Were Reported
Other Biases	Low Risk	This study was supported by the Genomic Research Center of Shaheed Beheshti University of Medical Sciences
Varimo 2019	**Risk Of Bias**	**Author Judgement**
Random Sequence Generation (Selection Bias)	Low Risk	Multicenter, open-labeled, randomized, placebo-controlled, parallel-group
Allocation Concealment (Selection Bias)	Low Risk	Patients randomly assigned in blocks of ten to receive either letrozole or testosterone for 6 months. The randomisation sequence was generated with a computer
Blinding Of Participants & Personel (Performance Bias)	High Risk	Open labelled study
Blinding Of Outcome Assessment (Detection Bias)	High Risk	Open labelled study
Incomplete Outcome Data (Attrition Bias)	Low Risk	30 children were randomized of which 29 completed the treatment and the study. Hence attrition rate was 3.33%
Selective Reporting (Reporting Bias)	Low Risk	All Pre-Specified Outcomes Were Reported
Other Biases	Low Risk	The study was funded by Helsinki University Hospital, Academy of Finland, and Finnish Foundation for Pediatric Research
Wickman 2001	**Risk Of Bias**	**Author Judgement**
Random Sequence Generation (Selection Bias)	Low Risk	Randomised, double-blind, placebo controlled study
Allocation Concealment (Selection Bias)	Unclear Risk	Details not available
Blinding Of Participants & Personel (Performance Bias)	Low Risk	Yes, double blinded RCT
Blinding Of Outcome Assessment (Detection Bias)	Low Risk	Yes, double blinded RCT
Incomplete Outcome Data (Attrition Bias)	High Risk	23 children were randomized of which 19 children completed the study. Hence attrition rate was (17.39%). An attrition rate of more than 15% was considered to be significant
Selective Reporting (Reporting Bias)	Low Risk	All Pre-Specified Outcomes Were Reported
Other Biases	Low Risk	This study was supported by the Foundation for Paediatric Research, Helsinki, Finland