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Free-androgen Index in Women with Polycystic Ovarian Syndrome: A Meta-Analysis

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Abstract

Objectives Hyperandrogenism, a key feature of polycystic ovarian syndrome (PCOS), is caused by excess androgen secretion, most commonly of ovarian origin. Although the serum total testosterone (TT) levels have long been used as a traditional measure of hyperandrogenemia in women with PCOS, it is associated with many fallacies due to the fact that a component of TT is linked to sex hormone-binding globulin (SHBG). Recent research has discovered that measuring free testosterone levels and computing the free androgen index (FAI), which is a ratio of TT and SHBG, are better predictors of androgen excess in PCOS. The aim of this meta-analysis is to determine the association of FAI in diagnosing hyperandrogenism and its ability to discriminate PCOS from controls.

Materials and Methods The publicly available databases PubMed, Scopus and Web of Science were searched using MeSH terms, 'Polycystic Ovarian Syndrome' OR 'PCOS' OR 'PCOD' AND 'Testosterone' AND 'Sex Hormone Binding Globulin' OR 'SHBG' to collect the full-text articles for the retrieval of related data of case–control and cross-sectional studies. The studies quality was assessed using the Newcastle–Ottawa scale, and a subgroup analysis and publication bias between the studies was evaluated by funnel plot.

Statistical Analysis The R program (v4.0.3) and R packages 'metafor' and 'dmetar' were used for statistical analyses of quantitative data and the plots were generated using 'ggplot2' package through a comparison of pooled SMD by Egger's linear regression and Beggs-Mazumdar tests.

Results Twenty-four studies involving 7,847 participants including 3,290 controls and 4,557 PCOS were included in the meta-analysis. The pooled data analysis of the included studies showed that the PCOS women had higher FAI than controls, with SMD of 1.56 (95%CI 1.08–2.04; $p < 0.01$). The publication bias was tested using a funnel plot

Keywords
- free-androgen index
- meta-analysis
- polycystic ovarian syndrome
- testosterone
- sex hormone-binding globulin

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and Egger's regression asymmetry test, which revealed no risk of publishing bias ($p = 0.1727$). Additionally, the sub-group meta-analysis of geographic region revealed that FAI levels were more significant in PCOS subjects of Asia and Europe, compared with the American region.

Conclusion Overall, this meta-analysis indicates that FAI could be a reliable marker to differentiate PCOS patients from controls in Asian and European ethnicities.

Introduction

Polycystic ovarian syndrome (PCOS) is a prevalent endocrine illness that affects 4 to 20% of reproductive age women worldwide.[1] Since its characterization, there has been a vast advancement in the understanding of complex pathogenetic mechanisms but, the diagnosis of PCOS is fraught with controversies.[2] Several criteria have been proposed to diagnose PCOS, including the presence of oligo/amenorrhea and the absence of ovulation, which were established in 1990 by the National Institutes of Health (NIH).[3,4] Later in 2003, the Rotterdam Consensus proposed a third criterion of ultrasound appearance of polycystic ovarian morphology to be added to the two NIH criteria.[5] Finally, according to the androgen excess society (AES) criteria published in 2006, ovulatory dysfunction with clinical or biochemical hyperandrogenemia remains the cornerstone for PCOS diagnosis.[6] However, the evaluation of biochemical hyperandrogenemia itself is challenging due to uncertainty about what androgens should be measured and the ideal techniques to be used for analysis.[7] Total testosterone (TT) levels have traditionally been used to assess biochemical hyperandrogenism in PCOS women; however, there are numerous flaws associated with it. In the circulation, TT is composed of 0.5% to 3.0% free testosterone (FT) unbound to plasma proteins, 30% to 44% sex hormone-binding globulin (SHBG)-bound testosterone, and 54% to 68% albumin-bound testosterone.[8,9] The FT combining with albumin bound testosterone make up the bioavailable testosterone.[8–10] In PCOS women, circulating levels of SHBG are reduced by ~50% secondary to obesity and hyperinsulinemia.[10,11] Moreover, increased testosterone in PCOS itself causes decreased SHBG. Hence, the measurement of TT in serum includes a portion bound to SHBG and is therefore not an accurate reflection of hyperandrogenemia. The use of TT can identify only 20 to 30% of PCOS women as hyperandrogenemic.[12]

Recent studies have demonstrated that FT is a better predictor of androgen excess when compared with TT in PCOS.[13,14] In addition, free-androgen index (FAI), the ratio of TT to SHBG multiplied by 100, is another entity that can be used as a clinical indicator of hyperandrogenemia in PCOS.[15] Although previous individual studies have shown that FAI has a good diagnostic value for PCOS, the conclusions were based on relatively smaller sample sizes.[14,16–18] Another recent study, in contrast, indicated that FAI is not a trustworthy measure of FT when the SHBG concentration is low, and advised against using it.[19] With this background, the study aimed to conduct an updated systematic review on the relevance of FAI in PCOS focusing on the case–control studies to study the FAI accuracy in the diagnosis of hyperandrogenemia and its ability to discriminate PCOS from healthy individuals in different ethnic subgroups.

Materials and Methods

Bibliography Search Strategies

According to the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines,[20] this systematic review and meta-analysis study was conducted after the prospective registration of the protocol on PROSPERO (reg# CRD42021249417). The publicly available databases such as PubMed, Scopus, and Web of Science were comprehensively searched to collect the original articles with no restrictions on date until February 2022. The main MeSH terms for search are as follows: "Polycystic Ovarian Syndrome" OR "PCOS" OR "PCOD" AND "Testosterone" AND "Sex Hormone Binding Globulin" OR "SHBG" with a selected filter to search articles that published in English language and included female subjects only.

Study Selection Criteria

After the removal of identified duplicate studies, two reviewers (P.P and L.N) independently evaluated the study titles and abstract for their eligibility to include in this systematic review and meta-analysis. The studies that were cross-sectional or case–control, conducted in adults, determined the FAI using testosterone and SHBG levels in women with and without PCOS, clearly defined criterion applied for control and cases, and reported diagnostic criteria (Rotterdam or NIH or AEPCOS) for PCOS were included. The disagreement raised between reviewers was resolved by consensus and if consensus could not be reached, the studies were added to the full-text selection. The full-texts were retrieved and independently assessed for eligibility using the same inclusion and the quality of data analysis and representation.

Data Extraction and Quality Assessment

The information and data on general characteristics including author, year, study design, study population especially number of cases and controls, age, body mass index (BMI), and FAI were collected from the eligible studies. If there were any identical populations in several studies, only the article with complete information and large sample size was

considered. The quality of the selected studies for the extraction of data was assessed using the Newcastle–Ottawa scale (NOS) with a maximum of nine stars by the two independent reviewers (H.B.G. and N.D.), which was subsequently checked by a third reviewer (L.N.).[21] The assessment criteria include the participants in PCOS or control groups (selection), comparability of groups based on age and BMI (comparability), and the type of method used to determine FAI in both groups and its outcome significance (exposure). A maximum of four stars was assigned to selection, two stars to comparability, and three stars to exposure components. The included studies were categorized as poor, fair, and good based on the stars obtained for each component (►**Supplementary Table S1** [online only]). Any discrepancies raised between reviewers in data extraction and study quality assessment were resolved through consensus and if consensus could not be reached, a fourth reviewer (P.P.) was consulted.

Sub-group Analysis and Publication Bias

A sub-group analysis was performed to find any bias between the studies of poor or fair or good quality and also studies conducted at different parts of the world. This analysis was performed to know whether the study quality and location have an effect on PCOS diagnostics based on the FAI determination. The publication bias between the study qualities and the different country regions of world was evaluated by funnel plot, which was done by plotting standardized mean difference (SMD) and standard error of the natural log of SMD.

Statistical Analysis

The statistical analyses were performed using R version 4.0.3 and R packages 'metafor' and 'dmetar'. The quantitative data were analyzed using 'metafor' package and plots were generated using 'ggplot2' package. The inverse variance approach was used to calculate pooled SMD and 95% confidence interval (CI) for studies that reported means of FAI among groups. Statistical heterogeneity was determined using the Cochrane chi-square and I^2 tests with cut-off $> 50\%$ for I^2, considered to be significantly heterogeneous. If clinical heterogeneity observed, $I^2 > 50\%$ random effects model was used. The variance between studies was measured using the Tau.[2] The publication bias was assessed qualitatively using the funnel plot by comparing the SMD and quantitatively using Egger's linear regression and Beggs–Mazumdar tests. To identify any sources of heterogeneity in studies, sensitivity analysis was done by omitting one study at a time, i.e., leave-one-out approach.

Results

Identification of Relevant Studies

The search strategy yielded 380 potentially relevant articles from PubMed, Scopus, and Web of Science databases after the removal of 415 duplicated articles and 2,609 studies of non-relevance, such as reviews, case reports, and non-English for this meta-analysis. Additionally, 264 articles were excluded based on screening the titles and abstracts of studies, the remaining 116 articles were subjected to full-text assessment and eligibility. Further evaluation of these full-text articles resulted in the exclusion of 92 articles, of which 64 were for improper case–control selection, 13 for incomplete reporting of FAI data, 8 for inadequate data analysis, 6 for study designs other than case–control or cross-sectional, and 1 for duplicated data. Finally, 24 studies were selected for this systematic review and meta-analysis, which is presented as a PRISMA plot showing the detailed process of inclusion and exclusion adopted (►**Fig. 1**).

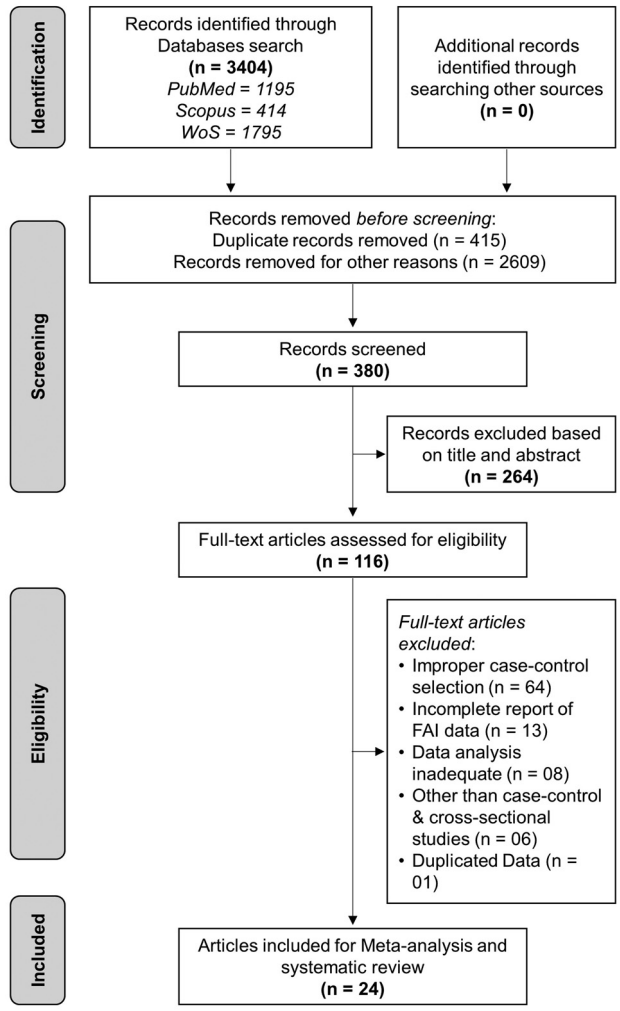

Fig. 1 PRISMA plot showing the study inclusion and exclusion for the systematic review and meta-analysis.

Characteristics of Included Studies

The baseline characteristics of the included studies ($n = 24$) with a total of 7,847 participants (3,290 controls and 4,557 PCOS) are summarized in ►**Table 1** and ►**Supplementary Table S1** (online only). All studies were observational including case–control and cross-sectional designs, and followed the same method of FAI determination and also compared these values between cases and controls. All studies adopted the Rotterdam 2003 international diagnostic criteria of PCOS except two studies, in which NIH criteria was followed. This

Table 1 Baseline characteristics of studies included in the meta-analysis

Author and year	Study type	Country region	PCOS criteria	PCOS		Control	
				N	FAI (mean ± SD)	N	FAI (mean ± SD)
Mehrabadi et al (2020)[28]	Cross-sectional	Asia	Rotterdam	53	1.26 ± 0.99	50	0.82 ± 0.69
Demiïr et al (2019)[29]	Case-control	Asia/ Europe	Rotterdam	80	8.21 ± 1.74	80	2.48 ± 0.11
Sun et al (2019)[30]	Case-control	Asia	Rotterdam	654	9.78 ± 7.07	535	3.4 ± 2.11
Kogure et al (2018)[31]	Case-control	Americas	Rotterdam	45	8.3 ± 6.3	52	5.6 ± 4.6
Haydardedeoglu et al (2018)[32]	Cross-sectional	Asia/ Europe	Rotterdam	51	1.76 ± 0.9	47	0.72 ± 0.38
Temur et al (2017)[33]	Cross-sectional	Asia/ Europe	Rotterdam	40	7.8 ± 1.75	40	2.47 ± 0.09
Borghi et al (2017)[34]	Case-control	Europe	NIH	30	11.14 ± 8.65	30	2.41 ± 1.37
Shaikh et al (2016)[35]	Case-control	Asia	Rotterdam	301	5.54 ± 4.61	205	2.19 ± 1.36
Jedrzejuk et al (2015)[36]	Case-control	Europe	Rotterdam	92	8.1 ± 4.5	85	2.5 ± 1.5
Pinola et al (2015)[37]	Case-control	Europe	Rotterdam	681	4.4 ± 3.8	230	2.1 ± 1.3
Abu-Hijleh et al (2015)[38]	Case-control	Asia	Rotterdam	242	7.7 ± 4.1	238	3.2 ± 2.2
Song et al (2014)[39]	Case-control	Asia	Rotterdam	432	7.5 ± 4.9	927	1.9 ± 1.2
Bottcher et al (2013)[40]	Case-control	Europe	Rotterdam	30	5.86 ± 4.42	32	1.95 ± 1.49
Kahal et al (2013)[41]	Case-control	Europe	Rotterdam	21	4.3 ± 1.9	19	2.7 ± 1.3
Hendriks et al (2013)[42]	Case-control	Europe	Rotterdam	10	5.12 ± 2.46	8	1.5 ± 1.06
Paltoglou et al (2013)[43]	Case-control	Europe	NIH	142	7.37 ± 1.07	112	2.2 ± 1.28
Chen et al (2012)[44]	Cross-sectional	Asia	Rotterdam	135	9 ± 7.1	38	3.3 ± 2.6
Golbahar et al (2012)[45]	Case-control	Asia	Rotterdam	50	7.38 ± 3.1	50	2.77 ± 1.7
Misichronis et al (2011)[46]	Case-control	Europe	Rotterdam	1087	9 ± 7.4	206	2.6 ± 1.8
Mukherjee et al (2009)[47]	Case-control	Asia	Rotterdam	180	4.2 ± 2.96	144	1.82 ± 0.76
Lujan et al (2009)[48]	Case-control	Americas	Rotterdam	98	12.3 ± 9.4	51	5.4 ± 2.3
Sverrisdottir et al (2008)[49]	Case-control	Europe	Rotterdam	20	5.1 ± 3.1	18	1.9 ± 0.6
Verit et al (2008)[50]	Case-control	Asia/ Europe	Rotterdam	63	3.7 ± 2	58	1 ± 1.1
Codner et al (2007)[51]	Cross-sectional	Americas	Rotterdam	20	11.8 ± 8.7	35	5.1 ± 3.9

Abbreviations: N, Number; NIH, National Institutes of Health; PCOS, polycystic ovarian syndrome; SD, standard deviation; WoS, Web of Science.

Study	PCOS Mean	PCOS SD	PCOS Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI
Mehrabadi et al (2020)	1.26	0.99	53	0.82	0.69	50	4.3%	0.51 [0.12; 0.90]
Demir et al (2019)	8.21	1.74	80	2.48	0.11	80	3.9%	4.63 [4.03; 5.23]
Sun et al (2019)	9.78	7.07	654	3.40	2.11	535	4.6%	1.17 [1.05; 1.30]
Kogure et al (2018)	8.30	6.30	45	5.60	4.60	52	4.3%	0.49 [0.09; 0.90]
Haydardedeoglu et al (2018)	1.76	0.90	51	0.72	0.38	47	4.2%	1.47 [1.02; 1.92]
Temur et al (2017)	7.80	1.75	40	2.47	0.09	40	3.4%	4.26 [3.45; 5.07]
Borghi et al (2017)	11.14	8.65	30	2.41	1.37	30	4.0%	1.39 [0.82; 1.96]
Shaikh et al (2016)	5.54	4.61	301	2.19	1.36	205	4.6%	0.91 [0.73; 1.10]
Jedrzejuk et al (2015)	8.10	4.50	92	2.50	1.50	85	4.4%	1.64 [1.29; 1.98]
Pinola et al (2015)	4.40	3.80	681	2.10	1.30	230	4.6%	0.69 [0.53; 0.84]
Abu-Hijleh et al (2015)	7.70	4.10	242	3.20	2.20	238	4.6%	1.36 [1.16; 1.56]
Song et al (2014)	7.50	4.90	432	1.90	1.20	927	4.6%	1.91 [1.77; 2.04]
Bottcher et al (2013)	5.86	4.42	30	1.95	1.49	32	4.0%	1.19 [0.64; 1.73]
Kahal et al (2013)	4.30	1.90	21	2.70	1.30	19	3.8%	0.95 [0.30; 1.61]
Hendriks et al (2013)	5.12	2.46	10	1.50	1.06	8	2.7%	1.75 [0.61; 2.88]
Paltoglou et al (2013)	7.37	1.07	142	2.20	1.28	112	4.2%	4.42 [3.96; 4.88]
Chen et al (2012)	9.00	7.10	135	3.30	2.60	38	4.3%	0.89 [0.51; 1.26]
Golbahar et al (2012)	7.38	3.10	50	2.77	1.70	50	4.2%	1.83 [1.36; 2.30]
Misichronis et al (2011)	9.00	7.40	1087	2.60	1.80	206	4.6%	0.94 [0.78; 1.09]
Mukherjee et al (2009)	4.20	2.96	180	1.82	0.76	144	4.5%	1.05 [0.81; 1.28]
Lujan et al (2009)	12.30	9.40	98	5.40	2.30	51	4.4%	0.89 [0.53; 1.24]
Sverrisdottir et al (2008)	5.10	3.10	20	1.90	0.60	18	3.6%	1.37 [0.65; 2.08]
Verit et al (2008)	3.70	2.00	63	1.00	1.10	58	4.3%	1.64 [1.23; 2.06]
Codner et al (2007)	11.80	8.70	20	5.10	3.90	35	3.9%	1.09 [0.50; 1.68]
Total (95% CI)			**4557**			**3290**	**100.0%**	**1.56 [1.08; 2.04]**
Prediction interval								**[0.07; 3.05]**

Heterogeneity: $Tau^2 = 0.4647$; $Chi^2 = 587.30$, df = 23 (P < 0.01); $I^2 = 96\%$

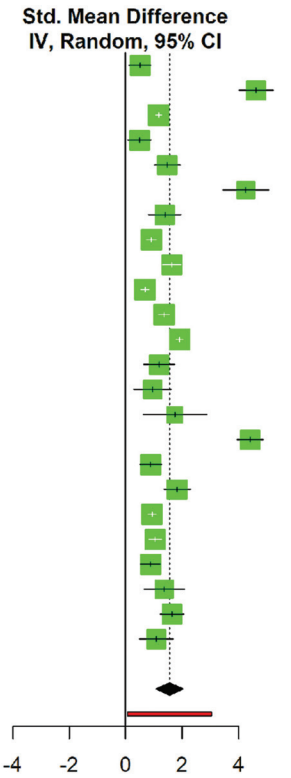

Fig. 2 Forest plot for all included studies using random effect models examining FAI values in cases and controls.

indicates that two of the three clinical features, oligomenorrhea or anovulation, hyperandrogenism, and polycystic ovaries were observed in the recruited PCOS patients. Among the geographic locations, nine studies were from Europe, eight from Asia and Middle-East, four from Asia/Europe, three from Americas, and no studies from Africa region. The NOS assessment for studies quality showed that 20 were good and 4 were of poor quality (►**Supplementary Table S2** [online only]).

Relevance of FAI in PCOS

Analysis of the 24 eligible studies comparing the FAI in cases and controls showed that the FAI values were consistently higher in PCOS patients than in controls (►**Table 1**). The pooled analysis showed that PCOS patients had higher FAI than controls, with an SMD of 1.56 (95%CI 1.08–2.04; $p < 0.01$) (►**Fig. 2**). However, the included studies were significantly heterogeneous ($I^2 = 96\%$) and the funnel plot indicates the presence of an asymmetry with studies lying outside of 95% CI. Eggers' test showed no risk of publication bias as it did not indicate the presence of funnel plot asymmetry ($p = 0.1727$). However, the Begg and Mazumdar test showed a risk of publication bias ($p = 0.0530$) because it observed the presence of funnel plot asymmetry. In addition, the publication bias was also assessed with the use of funnel plots, which were visually inspected for asymmetry (►**Fig. 3**).

Sensitivity Analysis

The sensitivity analysis was conducted by successively eliminating each study to analyze the influence of each study on the conclusions reached. When individual studies were eliminated, the results of analysis did not change significantly, and associated pooled SMD values did not change significantly, indicating the statistical stability of findings (►**Supplementary Table S3** and ►**Supplementary Figs. S1** and **S2** [online only]).

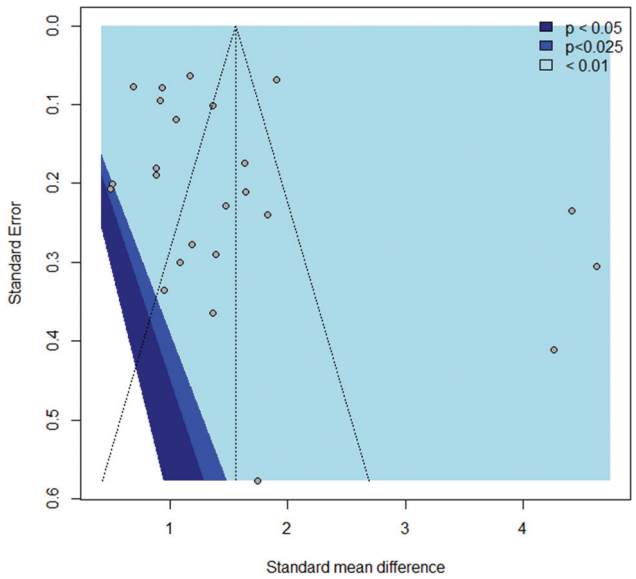

Fig. 3 Publication bias analysis of all included studies using the contour-enhanced funnel plot.

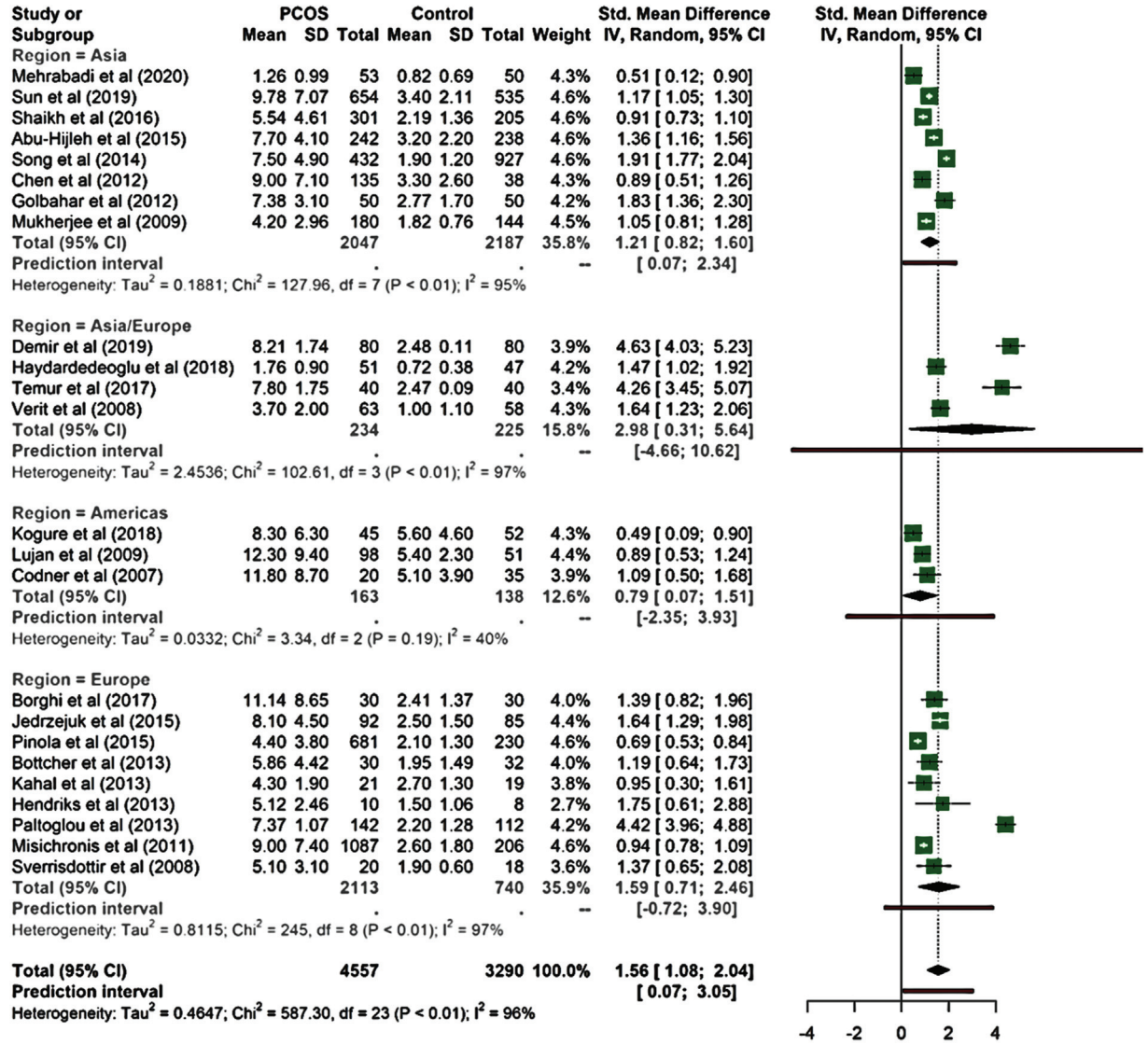

Fig. 4 Forest plot using random effect models examining FAI values in cases and controls based on region.

Subgroup Analysis

To find the source of heterogeneity, subgroup analysis was conducted according to geographic location and study quality. There were significantly higher FAI levels in PCOS patients of the Asia/Europe region (SMD = 2.98, 95%CI = 0.31–5.64, $p < 0.01$), Europe region (SMD = 1.59, 95%CI = 0.71–2.46, $p < 0.01$), and Asia region (SMD = 1.21, 95%CI = 0.82–1.60, $p < 0.01$), compared with controls. However, no significant difference was observed in FAI between the PCOS cases and controls of the American region (SMD = 0.79, 95% CI = 0.07–1.51, $p = 0.19$) (►**Fig. 4**). Moreover, FAI was significantly higher in PCOS cases in poor (SMD = 2.01, 95%CI = −0.59–4.61, $p < 0.01$) and good quality studies (SMD = 1.46, 95%CI = 0.98–1.93, $p < 0.01$), when compared with controls (►**Fig. 5**).

Discussion

This systematic review and meta-analysis study determines the accuracy of FAI in diagnosis of hyperandrogenemia in PCOS and its ability to discriminate PCOS from controls. Through a strict screening criteria, 24 eligible studies including 4,557 PCOS patients and 3,290 controls were included for the analysis. The primary finding is that FAI values were consistently higher in PCOS patients than in controls. Furthermore, pooled SMD values were not substantially altered, when individual studies were removed further validating the statistical stability of our findings. The only other meta-analysis on using FAI in PCOS diagnosis, which included 7 studies from Europe and Asia, concluded that FAI has a moderate diagnostic value for PCOS.[22] Furthermore, the study observed a significant ethnic and geographical heterogeneity in the clinical and biochemical manifestations of PCOS. This could be related to differences in lifestyle, prevalence of obesity, and insulin resistance across different ethnic groups.[23] Further, genetic variants and environmental toxins may also play a role.[24,25]

In this study, the results in terms of geographical heterogeneity indicates PCOS cases had significantly higher FAI compared with controls in both the European and Asian

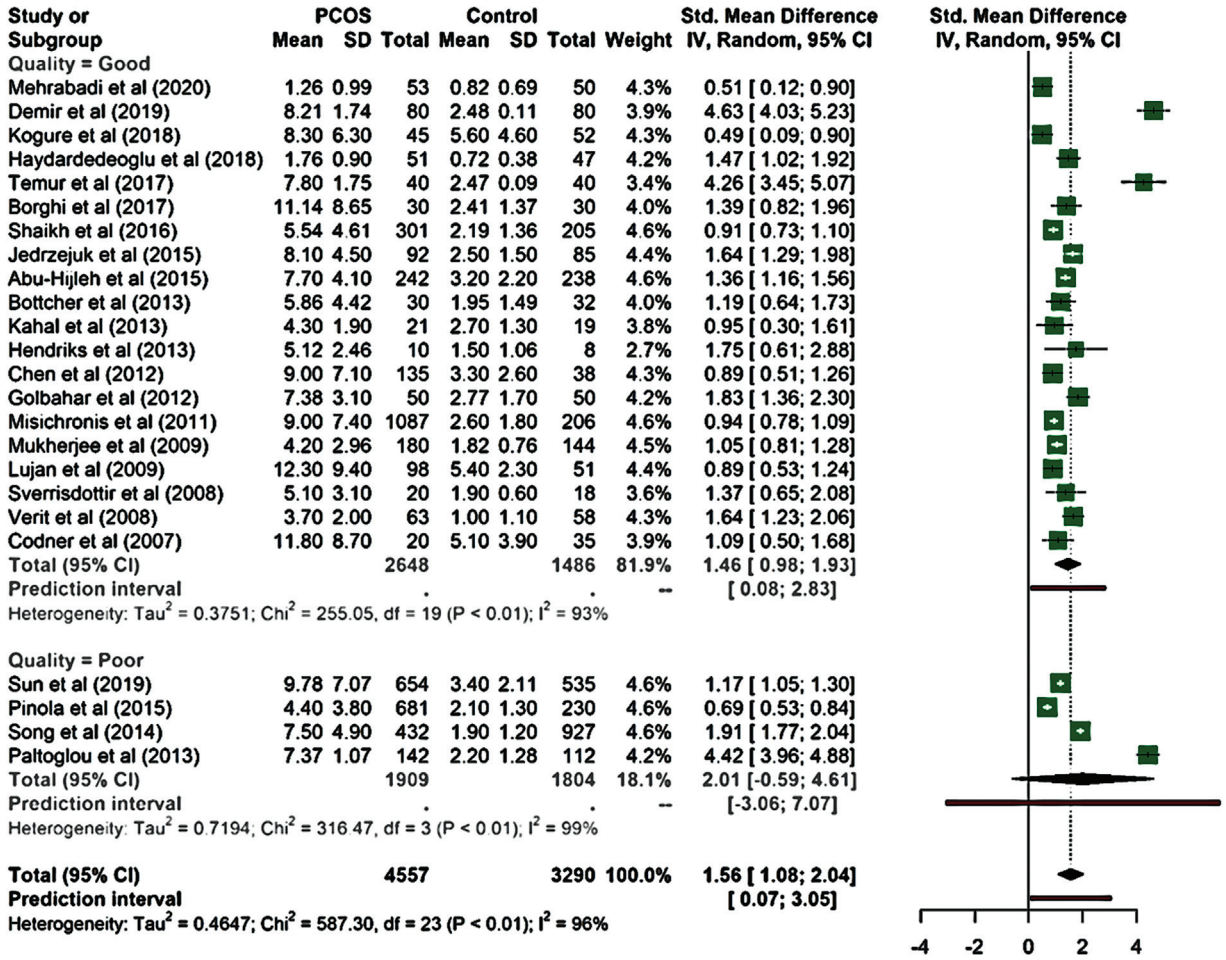

Fig. 5 Forest Plot using random effect models examining FAI values in cases and controls based on quality of the study.

ethnicities. However, there were no significant difference in FAI between the PCOS and controls of American region, attributable to the significant phenotypic differences in Asians, compared with Caucasians. This could be attributed to the fact that East Asians are generally less hirsute, and the Ferriman Gallwey score cut-off for hirsutism is lower than that of Caucasians.[26] Unlike East Asians, South Asians are reported to have an increased degree of hirsutism, early onset of symptoms, severe insulin resistance, and metabolic risks compared with Caucasians.[27] Overall, these results suggest that FAI can be used as a reliable marker of hyperandrogenism in both Asian and European ethnicities despite the reported phenotypic differences. There was no literature available on comparing androgen, SHBG levels, and FAI in the American and European subjects. Our study's limitations must be considered, such as the fact that we used data from 24 observational studies and did not include randomized control trials. Although randomized trials' participants may not be a true representative of those seen in practice; therefore, this study design is least prone to bias. Moreover, because observational studies are more likely to have biases than randomized trials, results from this study should be viewed with caution. Another limitation of our study is that Ferriman Gallwey scores in relation to FAI were not examined.

Conclusion

Overall, we found that the FAI could be a reliable marker to identify PCOS women in Asian and European ethnicities.

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Conflict of Interest
None declared.

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References

1 Deswal R, Narwal V, Dang A, Pundir CS. The prevalence of polycystic ovary syndrome: a brief systematic review. J Hum Reprod Sci 2020;13(04):261–271
2 Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol 1935;29(02):181–191
3 Chang S, Dunaif A. Diagnosis of polycystic ovary syndrome: which criteria to use and when? Endocrinol Metab Clin North Am 2021; 50(01):11–23

4 Zawadzki J, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Ed. Polycystic Ovary Syndrome. Boston: Blackwell ScientificBoston, MA: Black-well Scientific; 1995:377–384
5 Fauser BCJMRotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81(01):19–25
6 Azziz R, Carmina E, Dewailly D, et al; Androgen Excess Society. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. J Clin Endocrinol Metab 2006;91 (11):4237–4245
7 Muraleedharan V, Jones TH. Testosterone and the metabolic syndrome. Ther Adv Endocrinol Metab 2010;1(05):207–223
8 Dunn JF, Nisula BC, Rodbard D. Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. J Clin Endocrinol Metab 1981;53(01):58–68
9 Vermeulen A. Physiology of the testosterone-binding globulin in man. Ann N Y Acad Sci 1988;538(01):103–111
10 Bulun SE. Physiology and Pathology of the Female Reproductive Axis. In: Williams Textbook of EndocrinologyElsevier; 2016: 589–663
11 Nestler JE. Obesity, insulin, sex steroids and ovulation. Int J Obes Relat Metab Disord 2000;24(Suppl 2):S71–S73
12 Rao P, Bhide P. Controversies in the diagnosis of polycystic ovary syndrome. Ther Adv Reprod Health 2020;14:2633494120913032. Doi: 10.1177/2633494120913032
13 Antonio L, Pauwels S, Laurent MR, et al. Free testosterone reflects metabolic as well as ovarian disturbances in subfertile oligomenorrheic women. Int J Endocrinol 2018;2018:7956951. Doi: 10.1155/2018/7956951
14 Hahn S, Kuehnel W, Tan S, et al. Diagnostic value of calculated testosterone indices in the assessment of polycystic ovary syndrome. Clin Chem Lab Med 2007;45(02):202–207
15 Carter GD, Holland SM, Alaghband-Zadeh J, Rayman G, Dorrington-Ward P, Wise PH. Investigation of hirsutism: testosterone is not enough. Ann Clin Biochem 1983;20(Pt 5):262–263
16 Al Kindi MK, Al Essry FS, Al Essry FS, Mula-Abed WAS. Validity of serum testosterone, free androgen index, and calculated free testosterone in women with suspected hyperandrogenism. Oman Med J 2012;27(06):471–474
17 Pinola P, Piltonen TT, Puurunen J, et al. Androgen profile through life in women with polycystic ovary syndrome: a nordic multicenter collaboration study. J Clin Endocrinol Metab 2015;100(09): 3400–3407
18 Cho LW, Kilpatrick ES, Jayagopal V, Diver MJ, Atkin SL. Biological variation of total testosterone, free androgen index and bioavailable testosterone in polycystic ovarian syndrome: implications for identifying hyperandrogenaemia. Clin Endocrinol (Oxf) 2008; 68(03):390–394
19 Keevil BG, Adaway J, Fiers T, Moghetti P, Kaufman JM. The free androgen index is inaccurate in women when the SHBG concentration is low. Clin Endocrinol (Oxf) 2018;88(05):706–710
20 Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71
21 Wells G, Shea B, O'Connell D, Peterson J. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Ottawa, ONOttawa Hospital Research Institute. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Published (2000). Accessed September 10, 2021
22 Wang L, Li J. The value of serum-free androgen index in the diagnosis of polycystic ovary syndrome: a systematic review and meta-analysis. J Obstet Gynaecol Res 2021;47(04):1221–1231
23 Wolf WM, Wattick RA, Kinkade ON, Olfert MD. Geographical prevalence of polycystic ovary syndrome as determined by region and race/ethnicity. Int J Environ Res Public Health 2018;15(11): E2589
24 Merkin SS, Phy JL, Sites CK, Yang D. Environmental determinants of polycystic ovary syndrome. Fertil Steril 2016;106(01):16–24
25 Rutkowska AZ, Diamanti-Kandarakis E. Polycystic ovary syndrome and environmental toxins. Fertil Steril 2016;106(04): 948–958
26 Ilagan MKCC, Paz-Pacheco E, Totesora DZ, Clemente-Chua LR, Jalique JRK. The modified Ferriman-Gallwey score and hirsutism among Filipino women. Endocrinol Metab (Seoul) 2019;34(04): 374–381
27 Kim JJ, Choi YM. Phenotype and genotype of polycystic ovary syndrome in Asia: ethnic differences. J Obstet Gynaecol Res 2019; 45(12):2330–2337
28 Mehrabadi S, Jahanian SadatmahallehS, Kazemnejad A, Moini A. Association of acne, hirsutism, androgen, anxiety, and depression on cognitive performance in polycystic ovary syndrome: A cross-sectional study. Int J Reprod Biomed 2020;18(12):1049–1058
29 Demiïr İ, Guler A, Alarslan P, et al. Fractalkine: an inflammatory chemokine elevated in subjects with polycystic ovary syndrome. Endocrine 2019;65(01):175–183
30 Sun Y, Li S, Liu H, et al. Association of GPx1 P198L and CAT C-262T Genetic Variations With Polycystic Ovary Syndrome in Chinese Women. Front Endocrinol (Lausanne) 2019;10(November):1–9
31 Kogure GS, Silva RC, Miranda-Furtado CL, et al. Hyperandrogenism enhances muscle strength after progressive resistance training, independent of body composition, in women with polycystic ovary syndrome. J Strength Cond Res 2018;32(09):2642–2651
32 Eksi HaydardedeogluF, Bagir GS, Haydardedeoglu B, et al. Serum betatrophin levels are reduced in patients with full-blown polycystic ovary syndrome. Gynecol Endocrinol 2019;35(03): 224–227
33 Temur M, Calan M, Akşit M, et al. Increased serum neuregulin 4 levels in women with polycystic ovary syndrome: A Case-Control Study. Ginekol Pol 2017;88(10):517–522
34 Borghi L, Leone D, Vegni E, et al. Psychological distress, anger and quality of life in polycystic ovary syndrome: associations with biochemical, phenotypical andsocio-demographic factors. J Psychosom Obstet Gynecol 2018;39(02):128–137
35 Shaikh N, Dadachanji R, Meherji P, Shah N, Mukherjee S. Polymorphisms and haplotypes of insulin-like factor 3 gene are associated with risk of polycystic ovary syndrome in Indian women. Gene 2016;577(02):180–186
36 Jedrzejuk D, Łaczmañski Ł, Milewicz A, et al. Classic PCOS phenotype is not associated with deficiency of endogenous Vitamin D and VDR gene polymorphisms rs731236 (TaqI), rs7975232 (ApaI), rs1544410 (BsmI), rs10735810 (FokI): A case-control study of lower Silesian women. Gynecol Endocrinol 2015;31(12): 976–979
37 Pinola P, Piltonen TT, Puurunen J, et al. Androgen profile through life in women with polycystic ovary syndrome: A nordic multicenter collaboration study. J Clin Endocrinol Metab 2015;100(09): 3400–3407
38 Abu-Hijleh TM, Gammoh E, Al-Busaidi AS, et al. Common Variants in the Sex Hormone-Binding Globulin (SHBG) Gene Influence SHBG Levels in Women with Polycystic Ovary Syndrome. Ann Nutr Metab 2016;68(01):66–74
39 Song DK, Lee H, Oh JY, Hong YS, Sung YA. FTO gene variants are associated with PCOS susceptibility and hyperandrogenemia in young Korean women. Diabetes Metab J 2014;38(04):302–310
40 Böttcher B, Stahlhofer KJ, Mattle V, Seeber B, Brezinka C, Wildt L. Ultrasonographic assessment of skin thickness in patients with PCOS-a case-control study. Gynecol Endocrinol 2013;29(04): 380–383
41 Kahal H, Aburima A, Ungvari T, et al. Polycystic ovary syndrome has no independent effect on vascular, inflammatory or thrombotic markers when matched for obesity. Clin Endocrinol (Oxf) 2013;79(02):252–258

42 Hendriks ML, Konig T, Soleman RS, et al. Influence of ovarian manipulation on reproductive endocrinology in polycystic ovarian syndrome and regularly cycling women. Eur J Endocrinol 2013; 169(04):503–510
43 Paltoglou G, Tavernarakis G, Christopoulos P, et al. PON1-108 TT and PON1-192 RR genotypes are more frequently encountered in Greek PCOS than non-PCOS women, and are associated with hyperandrogenaemia. Clin Endocrinol (Oxf) 2013;79(02):259–266
44 Chen MJ, Han DS, Yang JH, Yang YS, Ho HN, Yang WS. Myostatin and its association with abdominal obesity, androgen and follistatin levels in women with polycystic ovary syndrome. Hum Reprod 2012;27(08):2476–2483
45 Golbahar J, Al-Ayadhi M, Das NM, Gumaa K. Sensitive and specific markers for insulin resistance, hyperandrogenemia, and inappropriate gonadotrophin secretion in women with polycystic ovary syndrome: A case-control study from Bahrain. Int J Womens Health 2012;4(01):201–206
46 Misichronis G, Georgopoulos NA, Marioli DJ, et al. The influence of obesity on Androstenedione to Testosterone ratio in women with polycystic ovary syndrome (PCOS) and hyperandrogenemia. Gynecol Endocrinol 2012;28(04):249–252
47 Mukherjee S, Shaikh N, Khavale S, et al. Genetic variation in exon 17 of INSR is associated with insulin resistance and hyperandrogenemia among lean Indian women with polycystic ovary syndrome. Eur J Endocrinol 2009;160(05):855–862
48 Lujan ME, Bloski TG, Chizen DR, Lehotay DC, Pierson RA. Digit ratios do not serve as anatomical evidence of prenatal androgen exposure in clinical phenotypes of polycystic ovary syndrome. Hum Reprod 2010;25(01):204–211
49 Sverrisdóttir YB, Mogren T, Kataoka J, Janson PO, Stener-Victorin E. Is polycystic ovary syndrome associated with high sympathetic nerve activity and size at birth? Am J Physiol Endocrinol Metab 2008;294(03):E576–E581
50 Verit FF, Erel O. Oxidative stress in nonobese women with polycystic ovary syndrome: Correlations with endocrine and screening parameters. Gynecol Obstet Invest 2008;65(04): 233–239
51 Codner E, Iñíguez G, Villarroel C, et al. Brief report: Hormonal profile in women with polycystic ovarian syndrome with or without type 1 diabetes mellitus. J Clin Endocrinol Metab 2007; 92(12):4742–4746