Prevalence and associated factors of nonalcoholic fatty liver disease in women with polycystic ovary syndrome: A Prospective cross-sectional (Fatty LIver in PolyCystic Ovary Syndrome "FLIPCOS") study and a systematic review and meta-analysis

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Table of Contents

List of figures

Thesis

Manuscripts

Manuscript A

Manuscript B

List of tables

Manuscripts

Manuscript A

Manuscript B

List of supplemental materials

List of abbreviations

AES	Androgen excess and PCOS society criteria
AIC	Akaike information criterion
ALT	Alanine aminotransferase
aOR	Adjusted odds ratio
APRI	Aspartate aminotransferase (AST) to platelets ratio index
ASCVD	Atherosclerotic cardiovascular disease
AUC	Area under the curve
BIC	Bayesian information criterion
BMI	Body mass index
CAP	Controlled attenuation parameter
CI	Confidence interval
CKD	Chronic kidney disease
CVD	Cardiovascular disease
FAI	Free androgen index
FFA	Free fatty acid
FIB-4	Fibrosis 4 index
FLIPCOS	Fatty LIver in PolyCystic Ovary Syndrome
HA	Hyperandrogenism
HOMA-IR	Homeostatic model assessment of insulin resistance
HSI	Hepatic steatosis index
IR	Insulin resistance

LSM	Liver stiffness measurement
LT	Liver transplant/ation
MetS	Metabolic syndrome
MRI	Magnetic resonance imaging
MRS	Magnetic resonance Spectroscopy
MUHC	McGill University Health Center
NAFL	Nonalcoholic fatty liver
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NIH	National Institute of Health
NOS	Newcastle-Ottawa scale
OR	Odds ratio
PCOM	Polycystic ovarian morphology
PCOS	Polycystic ovary syndrome
PNPLA3	Patatin-like phospholipase domain-containing protein 3
SD	Standard deviation
SHBG	Sex hormone binding globulin
T2DM	Type 2 diabetes mellitus
TE	Transient elastography
US	Ultrasonography

Abstract

BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) is the most frequent liver disease worldwide. Identifying populations at-risk is pivotal for case finding and resource optimization. Polycystic ovary syndrome (PCOS) patients seem at higher risk for NAFLD. Data on NAFLD in such population are scarce and inconsistent.

OBJECTIVES

To estimate primarily the prevalence and associated factors of NAFLD among patients with PCOS through a cross-sectional study and a systematic review and meta-analysis.

METHODS

For FLIPCOS study, South Asian women diagnosed with PCOS according to Rotterdam criteria were prospectively included. This ethnicity was selected as both PCOS and metabolic comorbidities are very frequent. Prevalence and cofactors of NAFLD and liver fibrosis were investigated by transient elastography (TE) with controlled attenuation parameter (CAP). NAFLD and significant liver fibrosis (stage ≥2 out of 4) were defined as CAP ≥288 decibels per meter (dB/m) and TE measurement ≥8 kilopascals (kPa), respectively. Predictors of NAFLD were determined by multivariate regression analysis.

In the meta-analysis, studies that have reported the association between NAFLD and PCOS were systematically identified. Pooled odds ratio (OR) using random effect model was calculated and heterogeneity was addressed through I^2. Subgroup analyses and meta-regression were performed to explore the effect and impact of certain variables and moderators on the outcome, respectively.

RESULTS

101 PCOS patients (mean age 36.3 years) were included in FLIPCOS study. Prevalence of NAFLD and significant liver fibrosis were 39.6% and 6.9%, respectively. Elevated ALT was observed in 40% of patients with NAFLD and 11.5% in those without NAFLD. After adjusting for duration of PCOS, and insulin resistance (IR), independent predictors of NAFLD were higher body mass index (BMI) (adjusted odds ratio [aOR] 1.30, 95%CI 1.13-1.52), Hyperandrogenism (aOR 5.32, 95%CI 1.56-18.17) and elevated ALT (aOR 3.54, 95%CI 1.10-11.47). Calculated lifetime atherosclerotic cardiovascular (ASCVD) risk was higher in PCOS patients with NAFLD, compared to those without (mean 0.31, SD 0.11 vs. 0.26, 0.13).

For the meta-analysis, of the 1833 studies retrieved in the initial search, 29 studies were eligible for the systematic review and 23 studies were qualified for quantitative synthesis. The pooled result showed that PCOS patients have 2.5-fold increase in risk of NAFLD, compared to controls [OR 2.49, 95%CI 2.20-2.82]. When stratified by geographic location, the result indicates that South American and Middle East populations have greater risk of NAFLD [OR 3.69, 95% CI 1.94-7.02] and [OR 3.89, 95% CI 2.12-7.14], respectively, compared to European [OR 2.22, 95% CI 1.85-2.67] and Asian [OR 2.63, 95% CI 2.20-3.15] populations. Study quality and BMI were the only moderators showed relationship with the outcome in meta-regression (regression coefficient -2.219, 95%CI -3.927--0.511 and regression coefficient -1.929, 95%CI -3.776--0.0826, respectively).

CONCLUSION

Our meta-analysis indicates that NAFLD is a prevalent condition among women with PCOS across all ethnicities, with a greater risk being identified in women from South America and the Middle

East. Besides, FLIPCOS study revealed that South Asian PCOS patients are at increased risk as well. BMI is strongly associated with NAFLD in both studies.

Résumé

CONTEXTE

La stéatose hépatique non alcoolique (NAFLD) est la maladie du foie la plus fréquente dans le monde. L'identification des populations à risque est essentielle pour la recherche de cas et l'optimisation des ressources. Les patients atteints du syndrome des ovaires polykystiques (SOPK) semblent plus à risque de NAFLD. Les données sur la NAFLD dans cette population sont rares et incohérentes.

OBJECTIFS

Estimer principalement la prévalence et les facteurs associés de la NAFLD chez les patients atteints de SOPK grâce à une étude transversale et une revue systématique et méta-analyse.

MÉTHODES

Pour l'étude FLIPCOS, les femmes sud-asiatiques diagnostiquées avec le SOPK selon les critères de Rotterdam ont été incluses de manière prospective. Cette origine ethnique a été choisie car le SOPK et les comorbidités métaboliques sont très fréquentes. La prévalence et les cofacteurs de la NAFLD et de la fibrose hépatique ont été étudiés par élastographie transitoire (TE) avec un paramètre d'atténuation contrôlé (CAP). La NAFLD et la fibrose hépatique significative (stade ≥2 sur 4) ont été définies comme une CAP ≥288 décibels par mètre (dB / m) et une mesure TE ≥8 kilopascals (kPa), respectivement. Les prédicteurs de la NAFLD ont été déterminés par une analyse de régression multivariée.

Dans la méta-analyse, les études qui ont rapporté l'association entre la NAFLD et le SOPK ont été systématiquement identifiées. Le rapport de cotes (OR) groupé à l'aide d'un modèle à effets

aléatoires a été calculé et l'hétérogénéité a été traitée via I2. Des analyses de sous-groupes et une méta-régression ont été effectuées pour explorer l'effet et l'impact de certaines variables et modérateurs sur le résultat, respectivement.

RÉSULTATS

101 patients SOPK (âge moyen 36,3 ans) ont été inclus dans l'étude FLIPCOS. La prévalence de la NAFLD et de la fibrose hépatique significative était de 39,6% et 6,9%, respectivement. Une ALAT élevée a été observée chez 40% des patients atteints de NAFLD et 11,5% chez ceux sans NAFLD. Après ajustement de la durée du SOPK et de la résistance à l'insuline (IR), les prédicteurs indépendants de la NAFLD étaient un indice de masse corporelle (IMC) plus élevé (odds ratio ajusté [aOR] 1,30, IC à 95% 1,13-1,52), l'hyperandrogénie (aOR 5,32, 95% IC 1,56-18,17) et ALT élevée (aOR 3,54, IC 95% 1,10-11,47). Le risque cardiovasculaire athérosclérotique (ASCVD) à vie calculé était plus élevé chez les patients atteints de SOPK atteints de NAFLD, par rapport à ceux sans (moyenne 0,31, ET 0,11 vs 0,26, 0,13).

Pour la méta-analyse, sur les 1833 études récupérées lors de la recherche initiale, 29 études étaient éligibles pour la revue systématique et 23 études ont été qualifiées pour la synthèse quantitative. Le résultat combiné a montré que les patients atteints de SOPK ont un risque 2,5 fois plus élevé de NAFLD, par rapport aux témoins [OR 2,49, IC à 95% 2,20-2,82]. Lorsqu'il est stratifié par emplacement géographique, le résultat indique que les populations d'Amérique du Sud et du Moyen-Orient ont un risque plus élevé de NAFLD [OR 3,69, IC à 95% 1,94-7,02] et [OR 3,89, IC à 95% 2,12-7,14], respectivement, par rapport à l'Europe [OR 2,22, IC à 95% 1,85-2,67] et les populations asiatiques [OR 2,63, IC à 95% 2,20-3,15]. La qualité de l'étude et l'IMC étaient les seuls modérateurs qui ont montré une relation avec le résultat de la méta-régression (coefficient

de régression -2,219, IC à 95% -3,927-0,511 et coefficient de régression -1,929, IC à 95% -3,776-0,0826, respectivement).

CONCLUSION

Notre méta-analyse indique que la NAFLD est une condition courante chez les femmes atteintes de SOPK de toutes les ethnies, avec un risque plus élevé d'être identifié chez les femmes d'Amérique du Sud et du Moyen-Orient. En outre, l'étude FLIPCOS a révélé que les patients sud-asiatiques du SOPK courent également un risque accru. L'IMC est fortement associé à la NAFLD dans les deux études.

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Contribution of authors

Thesis

MS wrote, formatted, and revised the whole thesis. GS critically revised all thesis sections.

FLIPCOS study

Authors; Mohamed Shengir, Srinivasan Krishnamurthy, Peter Ghali, Marc Deschenes, Philip Wong, Tianyan Chen, Giada Sebastiani.

MS was involved in all steps, studies concept and design, recruitment, acquisition of data, interpretation of data, analysis and drafting of manuscripts. SK was involved in studies concept and design, critical revision of manuscripts. He also performed clinical examination and transvaginal ultrasound for participants at enrollment. PG, MD, and PW were involved in studies concept and critical revision of manuscripts. TC was involved in studies concept, critical revision of manuscripts and overall study supervision. GS was involved in studies concept and design, acquisition and interpretation of data, analysis and drafting of manuscripts, critical revision of manuscript and overall study supervision. GS and TC were providing hepatology follow-up care for participants who were identified positive for the outcome.

Systematic review & meta-analysis

Authors; Mohamed Shengir, Tianyan Chen, Elena Guadagno, Ramana Agnihotram, Peter Ghali, Marc Deschenes, Philip Wong, Srinivasan Krishnamurthy, Giada Sebastiani.

MS was involved in all steps from studies concept and design, acquisition of data, interpretation of data, analysis and drafting of manuscripts. TC was involved in studies concept, acquisition and interpretation of data, critical revision of manuscript and overall study supervision. EG has done the search strategy and was involved in the revision of manuscript. RA performed the meta-analysis and was involved in critical revision of manuscript. PG, MD, PW, and SK were involved

in studies concept and critical revision of manuscript. GS was involved in studies concept and design, acquisition and interpretation of data, analysis and drafting of manuscripts, critical revision of manuscript and overall study supervision.

Other contributions

MS has contributed to 4 other scholar projects as a coauthor:

1. ***"NASH in HIV"*** review article, recently published in *Current HIV/AIDS Reports* (Critical revision of the manuscript).
2. ***"Effective Detection of Compensated Cirrhosis using Machine Learning"***, paper under review in *The Lancet Digital Health* (Data collection).
3. ***"FIB-4 improves LSM based prediction of clinical decompensation in overweight and/or obese patients with compensated advanced chronic liver disease"***, paper in preparation (Data collection & drafting manuscript).
4. ***"Noninvasive diagnosis of nonalcoholic steatohepatitis in liver transplant recipient: a prospective study employing serum cytokeratin 18 and transient elastography (Fibroscan/CAP)"***, project in progress (Study design, methods, and drafting conference's abstracts).

1 INTRODUCTION & LITERATURE REVIEW

1.1 Nonalcoholic fatty liver disease

Definition

NAFLD is defined as a condition in which there is excessive deposition of fat in the liver parenchyma (hepatic steatosis) in the form of triglycerides in a proportion greater than 5%, either identified histologically in a liver biopsy or using imaging studies, in absence of other causes of hepatic fat accumulation(1, 2). In clinical practice, NAFLD is considered a diagnosis of exclusion, since substantial alcohol intake and secondary causes of steatosis (e.g. steatogenic medications, viral induced steatosis, hereditary disorders) need to be ruled out(3). Excessive alcohol consumption is defined according to U.S. guideline for NAFLD (proposed by the American Association for the Study of Liver Diseases (AASLD), American College of Gastroenterology (ACG), and American Gastroenterological Association NAFLD guideline) as ongoing or recent alcohol consumption of >21 drinks per week in men and >14 drinks per week in women(4).

NAFLD is an umbrella term that covers a spectrum of histopathological conditions ranging from nonalcoholic fatty liver (NAFL) or simply “fatty liver” to nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis with its complications(5). While NAFL is known to be benign and often has a relatively favorable clinical course, NASH carries greater potential to develop hepatic cirrhosis(6). Histologically, NAFL is defined as hepatic steatosis without evidence of significant inflammation(7) and NASH defined by the presence of steatosis, inflammation, and hepatocyte injury (ballooning) with or without fibrosis(8, 9). Patients with NAFLD may have reduced life expectancy compared to the general population due to high risk of cardiovascular events and cancer, whereas patients with NASH may have a reduced survival also due to progression to

cirrhosis and end-stage complications, hepatocellular carcinoma (HCC) and liver transplantation (LT)(2).

The principal predictors of disease outcomes in NAFLD are the development and progression of liver fibrosis(4, 10). Therefore, early detection of hepatic fibrosis has become the main priority for its prognostic implications(11).

Epidemiology

Since first described in 1980 as "unnamed disease," NAFLD has been investigated extensively(12). Over the last four decades, the rate of NAFLD has grown dramatically. This rise coexists with the overall trend of increasing obesity and obesity-related complications, such as diabetes and metabolic syndrome (MetS), particularly in developed countries(6). Currently, NAFLD is considered the most common cause of chronic liver disease and cryptogenic cirrhosis worldwide and its progressive form NASH is now recognized as the second most frequent indication for LT in the North America after hepatitis C-related cirrhosis(13). A recent study showed that the number of NASH patients awaiting LT in the USA almost quadrupled between 2002-2012(14). Due to concomitant rapidly growing population of chronic hepatitis C patients achieving sustained virological responses with direct-acting antivirals, it is expected that NASH will become the leading indication for LT in the next 10 years(15). More importantly, NASH is already the main indication for LT in women(16).

The epidemiology and demographic characteristics of NAFLD varies significantly depending on the population studied, geographic area targeted, and the definition being applied(17). It is estimated that the global prevalence of the disease is 25.24%, highly prevalent across all continents, with highest figures reported from the Middle East and South America, 31% and 30%

respectively, followed by Asia (27%), USA (24%) and Europe (23%). The lowest prevalence is reported in Africa, with only 14% [Fig. 1](18). Based on the fact that patients with NAFLD are typically asymptomatic until they develop decompensated cirrhosis, these figures may be underestimating the true prevalence of the disease(6).

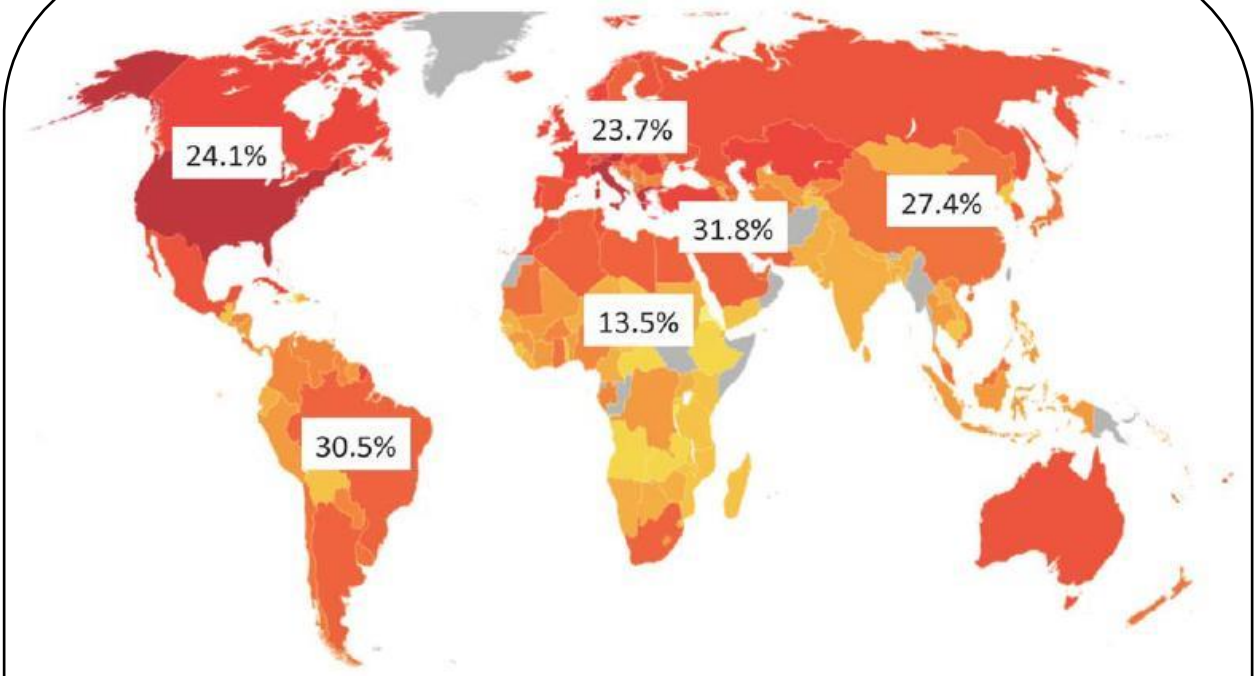

Figure. 1 worldwide estimated prevalence of nonalcoholic fatty liver disease.

Adapted from reference 19

Obesity, in the form of excessive BMI and central adiposity, is a well-known risk factor for NAFLD. Morbidly obese patients who underwent bariatric surgery have a prevalence of NAFLD reaching 90%. High frequency was also reported in individuals with type 2 diabetes mellitus (T2DM). In a study using ultrasonography (US) in patients with T2DM, the prevalence of NAFLD was 69%(19). Another study on 204 diabetic patients showed that 127 had ultrasonographic finding of NAFLD. Of these, 87% underwent biopsy that further confirmed the presence of fatty

infiltration. Among patients with dyslipidemia the prevalence of NAFLD was estimated to be 50%(20).

Age, biological sex and ethnicity are also common risk factors associated with a differential prevalence for NAFLD. It is well documented that disease progression to advanced fibrosis or mortality rises in older individuals. Additionally, older patients undoubtedly have higher chance to have NAFLD risk factors, such as hypertension, obesity, diabetes and dyslipidemia, than their younger counterparts(21). Regarding biological sex, many recent studies reported that men are at greater risk than women for NAFLD. Fertile women are less predisposed to develop NAFLD in comparison with men, while women at menopause have almost a similar risk as men. The reason behind sex differences is not completely clear. However, some theories support the notion that premenopausal women are at less risk due to the metabolic and hepatic protective effects of estrogen(22). In terms of ethnicity, some ethnic groups showed greater tendency to develop NAFLD than others. In a large study, Browning *et al*. used Magnetic Resonance Spectroscopy (MRS) to compare the prevalence of NAFLD among whites, blacks, and Hispanics. Figures varied significantly among ethnicities: Hispanics had by far a higher prevalence than other groups (45%). Obesity and IR were the main associated risk factors. In whites, sex difference has also been observed, with men having as twice the prevalence of hepatic steatosis as women(23).

In the last two decades, NAFLD burden had an alarming trend in South Asian countries, such as India, Pakistan, Sri Lanka, Bangladesh, Nepal, Bhutan, Burma, and Maldives. Its prevalence reached 30%, which correlates with the epidemic of obesity and MetS among youngsters due to inactive lifestyle, poor health awareness, economic thrive, and westernization of diet. Similar to other ethnic groups, obesity, IR, and MetS are the culprit risk factors. However, lean South Asians

tend to have an increased risk of developing NAFLD and NASH as they are considered to have more metabolic abnormalities, compared with other ethnic groups(24).

Recently, there is expanding evidence that NAFLD is a multisystem disease, affecting a variety of organs and metabolic regulatory pathways, rather than an isolated liver disease. Even though NAFLD affects primarily the liver parenchyma leading to fibrosis and cirrhosis, which are responsible for liver-related morbidity and mortality, CVD is the main cause of death in NAFLD patients. NAFLD extrahepatic associations include CVD, T2DM, chronic kidney disease (CKD), hypothyroidism, osteoporosis, sleep apnea, cancers, human immunodeficiency virus (HIV), inflammatory bowel disease, and polycystic ovary syndrome (PCOS) (7, 25-27) [Fig. 2].

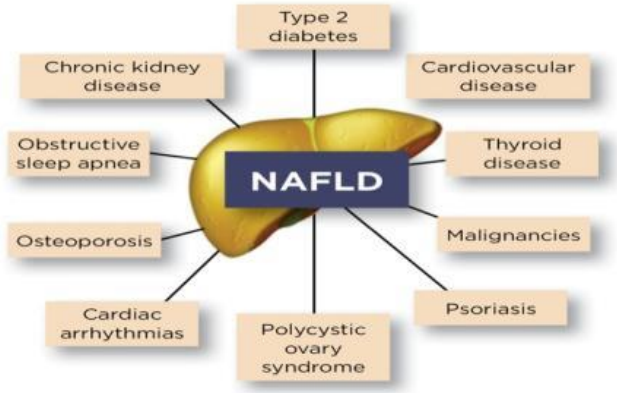

Figure. 2 Extrahepatic manifestation of NAFLD. Adapted from reference 7

Natural history & pathogenesis

The natural history of NAFLD is dichotomous between NAFL, the benign form, and NASH, the progressive one. It is generally agreed that patients with simple steatosis have very slow, if any, histological progression, while patients with NASH can exhibit faster histological progression pace to cirrhotic-stage disease. However, the whole histopathological process is incompletely

understood(28). As the disease affects almost a quarter of the world population, up to 25% of all NAFLD cases may progress to NASH and 5-10% will develop advanced liver disease over 10-20-year time span(28-30) [Fig. 3].

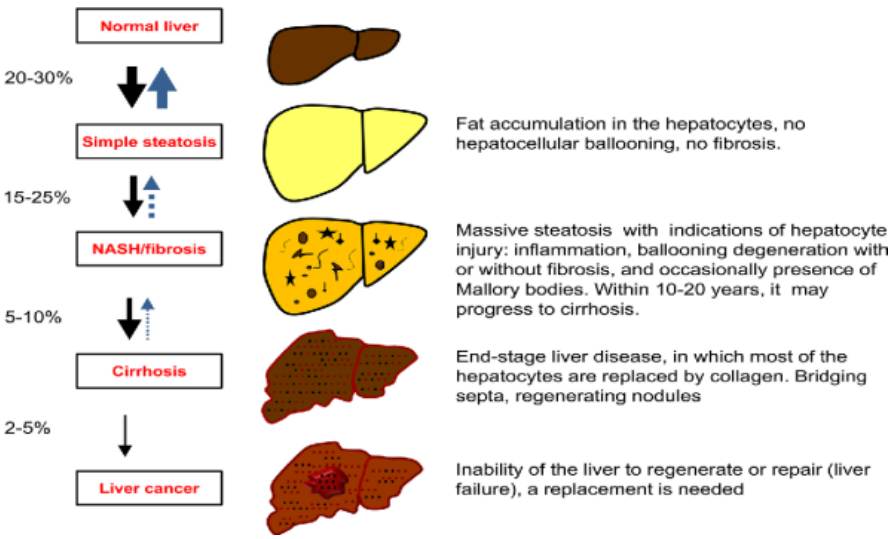

Figure. 3 Natural history of NAFLD.

Adapted from reference 31

Many theories for NAFLD pathogenesis have been proposed, leading initially to the traditional "two-hit" hypothesis that was first described by Day and James(31). In this theory, IR represents the "first-hit" that leads to hepatic steatosis. The state of IR acts on the liver by impairing suppression of gluconeogenesis, which leads to an increase in intrahepatic glucose that act as substrate for *de novo* lipogenesis and thus increased production of free fatty acids (FFA); and on adipose tissue by abolishing lipolysis inhibition, which leads to efflux excessive amount of FFA to the blood stream that will eventually circulate to the liver. FFA within the liver can be utilized in 3 different pathways; β-oxidation, production and release of very low-density lipoproteins

(VLDL), and triglycerides synthesis. Overaccumulation of triglycerides within liver hepatocytes forms fat droplets (steatosis) that sensitizes the liver for the subsequent hit. In the "second hit", oxidative stress derived from dysfunctional mitochondrial FFA oxidation triggers proinflammatory cytokines and release free radicals, which lead to inflammation and cell death "apoptosis" that ultimately evolve into NASH/fibrosis(32-34). More recently, this theory has been criticized since it simplifies a very sophisticated histopathological process, as well as ignores many important factors that play major roles in NAFLD pathogenesis(34, 35). Thus, "multiple-hit" and "distinct hit" hypotheses were suggested. The former adopts the same assumption as the "two-hit" hypothesis, in which the model of progression is linear, starts with steatosis as a consequence of IR followed by multiple coexistent hits that mediate progression to NASH/fibrosis(36, 37) [Fig. 4]. Such multiple pathogenic factors include dysregulated inflammatory cytokines and adipokines, gut-derived endotoxins, and genetic predisposition. Cytokines such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), and interleukin-1 (IL-1) in addition to adiponectin, leptin, are thought to be responsible for the inflammatory process(36, 38). Moreover, altered composition of gut flora "dysbiosis" stimulates fatty acids production in the intestines, induces lipogenesis, and enhances small bowel permeability resulting in increased serum fatty acids and bacterial toxins that trigger inflammatory pathways and release of proinflammatory cytokines(39). Finally, many studies showed that genetic predisposition plays a role towards development of NAFLD. The patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene, especially the PNPLA3 I148M variant, has been shown to be associated with severe steatosis and the presence of NASH(40). Finally, the "distinct hit" hypothesis postulates that hepatic steatosis and NASH are distinct entities rather a continuum process ranging from less to high degree of hepatocyte damage. In other words, NASH is not necessarily preceded by steatosis. Even though this theory proposes

that fibrosis and cirrhosis develop via separate pathologic pathways, IR is still the main driving factor for both processes.

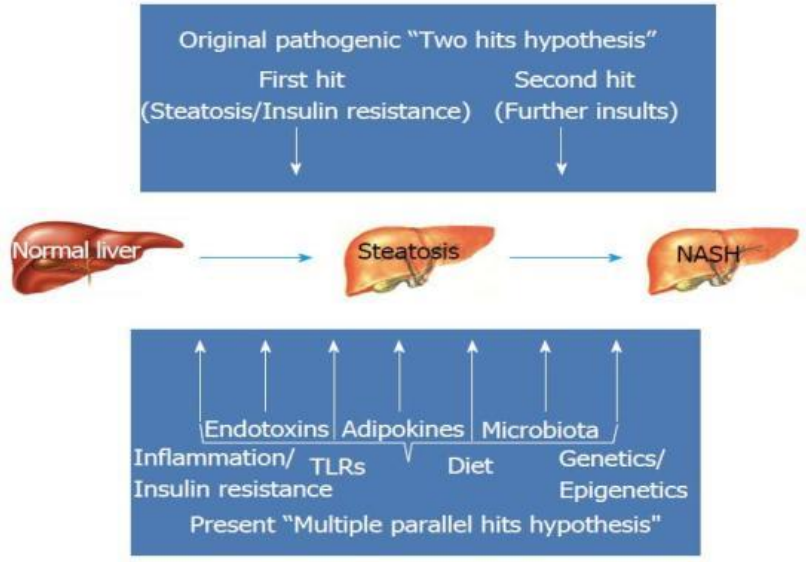

Figure. 4 NAFLD pathogenesis "Two-hit & Multiple-hit" hypotheses.

Adapted from reference 33

Diagnosis & screening

As NAFLD encompasses a range of histopathological conditions which are best assessed through direct evaluation of liver tissue, liver histology remains the gold standard for the diagnosis and staging of NAFLD as well as assessment of liver fibrosis. However, the procedure has many limitations associated with its cost, invasiveness, complications, inter/intra-observer variability, and sampling error that occur when fat deposition is unevenly distributed(2, 11, 41). Due to the fact that NAFLD is often asymptomatic and diagnosed accidentally on thoracic or abdominal imaging for reasons other than liver symptoms, it is not feasible to perform liver biopsy as a screening tool for such a prevalent disease in clinical practice and for assessing response to

therapeutic interventions. Therefore, there is an urgent need for optimization of noninvasive testing(42).

Elevated serum aminotransferases are the most common laboratory finding in NAFLD patients. Nevertheless, aminotransferases are normal in a majority of patients, some of them having even advanced fibrosis. Hence, its application to rule out NAFLD-related advanced liver disease is inaccurate(21, 43). Another serum biomarker that has been recently investigated is cytokeratin 18 (CK–18) fragments. CK-18 is proposed as an apoptosis biomarker that distinguishes NASH from NAFL(42, 44). In a study by Wieckowska *et al.*(45) high serum CK-18 fragments level were indicative for NASH, with adequate diagnostic accuracy. However, this result was questionable due to lack of power (only 39 subjects), and subsequent validation study that included larger sample size (139 participants) showed less favorable results(46). Furthermore, none of the available NASH biomarkers display superiority to clinical prediction models at identifying or ruling out steatohepatitis, making the latter more suitable option due to its cost effectiveness(47, 48).

Clinical prediction models are scoring systems that rely on clinical measurements and routine lab results in the prediction of NASH/fibrosis(2). Several predictive models have been developed and validated to predict hepatic steatosis, including hepatic steatosis index (HSI), fatty liver index (FLI), and lipid accumulation product (LAP). On the other hand, NAFLD fibrosis score (NFS), fibrosis–4 (FIB – 4) index, aspartate aminotransferase (AST) to platelet ratio index (APRI) are used to predict fibrosis. These indices are suitable for community healthcare settings that aimed to estimate NAFLD prevalence, but as yet have been applied only to preselected populations(40).

Various imaging methods have been utilized to evaluate patients with NAFLD including US, computed tomography (CT) scan, magnetic resonance imaging (MRI), MRS, and TE/CAP. US is

the most commonly used imaging study for diagnosing NAFLD in clinical settings since the procedure is inexpensive and widely available. US sensitivity and specificity are estimated to be 60%-94% and 66%-97%, respectively. Nevertheless, its sensitivity declines dramatically if the liver has mild (<30%) steatosis(7, 49). Non-enhanced computerized tomography is superior to contrast-enhanced CT scan at detecting steatosis(50). Its accuracy is comparable to US at diagnosing moderate to severe NAFLD, with advantage of detecting focal steatosis(49). CT use in assessing NAFLD has been limited because of the risk of radiation exposure and lack of sensitivity at identifying mild degree of steatosis as well(50). MRI and MRS are the most precise imaging tools. They can detect as low as 3% of fat accumulation in the liver parenchyma(49). Although MRI studies demonstrated very accurate sensitivity compared to other imaging techniques, they are not feasible as they are resource intensive and have restricted availability(49, 51). Therefore, cheap, safe, accurate, and more available tools are needed. These restrictions may be overcome by CAP, which is an US-based method that quantifies hepatic steatosis during the measurement of liver stiffness (LSM) via TE(41). CAP is based on the notion that fat affects US propagation. Thus, the more the fat is in the liver, the faster the attenuation of the ultrasound waves, the higher the reading. The procedure has many advantages; 1. CAP measurement is not affected by LSM, this allows evaluation of steatosis and fibrosis simultaneously; 2. It is a 5 minutes easy bedside procedure to detect and quantify steatosis; 3. The liver volume that TE/CAP probes assess is much larger than liver biopsy, hence it is less prone to sampling error(52). Several liver-biopsy based studies have validated TE/CAP as noninvasive tool for diagnosing NAFLD(42, 53, 54). Despite the fact that imaging studies are considered the best noninvasive surrogate to liver histology, they do not reliably differentiate between NAFL and NASH.

1.2 Polycystic ovary syndrome

Definition & clinical features

PCOS is the commonest endocrinopathy in women of childbearing age, its prevalence in premenopausal women ranging between 6% (when applying the older, more conservative criteria) and 20% (when using the current, more liberal definitions)(55-57). Establishing a diagnosis of PCOS is a challenging task as it is a heterogeneous disorder without pathognomonic features(58). Its various characteristics has led to multiple proposed diagnostic criteria. In 1990 conference, the National Institute of Child Health and Human Development of National Institute of Health (NIH) agreed that PCOS criteria should include HA and menstrual dysfunction(59). However, this definition depicts only the most severe phenotype of PCOS spectrum. Later in 2003, the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine in Rotterdam amended the consensus criteria to include a third diagnostic marker, which is polycystic ovarian morphology (PCOM)[1]. Based on Rotterdam criteria, patients are deemed eligible if at least two of the following features are present; 1. HA either clinically (i.e. hirsutism, acne/seborrhea, and/or alopecia) or biochemically (i.e. hyperandrogenaemia); 2. ovulatory dysfunction (oligo-ovulation/anovulation); and/or 3. PCOM(60). Since Rotterdam definition has been applied, some researchers raised a controversy regarding whether or not women who present with ovulatory dysfunction and PCOM, but do not exhibit any signs of either clinical or biochemical androgen excess, have actually PCOS(61, 62). Despite this ongoing dispute,

[1] PCOM: ultrasonographic findings of ≥12 follicles per whole ovary measuring 2-9 mm in diameter or increased ovarian volume (>10 cm^3). 58. Dewailly D. Diagnostic criteria for PCOS: Is there a need for a rethink? Best Pract Res Clin Obstet Gynaecol. 2016;37:5-11.

Rotterdam criteria is still the most widely accepted classification and currently supported by most scientific communities(63). In 2006, the Androgen Excess and PCOS Society (AES) formed a task force to review all available data in order to recommend an evidence-based definition for PCOS. Their final statement advocates for making HA criterion as mandatory prerequisite accompanied by the evidence of either menstrual dysfunction or PCOM. Of note, all three definitions took into account HA as an essential element(64, 65). However, they necessitate other specific diagnoses of androgen excess to be ruled out(59, 60, 64, 65). After all, to date, there are no clear data which favors a definition over the others.

In addition to the previously mentioned main features, metabolically most PCOS patients have IR with compensatory hyperinsulinemia as an intrinsic feature(66, 67). Notwithstanding that obesity, which is present in up to 80% of PCOS patients, can also cause and aggravate the pre-existent state of IR(67). The underlying metabolic abnormality in PCOS can affects women's health with long-term metabolic consequences including hypertension, impaired glucose tolerance, diabetes mellitus, dyslipidemia, and MetS. Also, due to shared risk factors such as IR and obesity, PCOS is frequently linked with higher prevalence of NAFLD(68). Moreover, altered endothelial function and vascular morphology, along with the previously mentioned disorders, make PCOS patients at greater risk for CVD(69). From a clinical point of view, it is important to understand the natural history as well as the long-term outcomes. PCOS clinical features usually appear during adolescence and may improve with patient getting older. On the contrary, associated underlying metabolic disturbances often worsen with advanced age. Therefore, screening for potential metabolic dysfunction could help early diagnosis and initiation of interventions to avoid catastrophic metabolic sequalae(70).

Putative mechanisms linking NAFLD to PCOS

Over the past decade there is growing body of evidence demonstrating that NAFLD is multisystemic disease and it has strong clinical associations with many metabolic conditions. One of these is PCOS(7, 25-27). The etiology of NAFLD in PCOS patients remains largely unknown, but it is plausible to assume that the mechanisms underlying the association between NAFLD and PCOS are multifactorial, involve both genetic and acquired factors [Fig. 5].

Regarding genetic involvement, several studies have displayed disturbances in the function of some genes that may be related to NAFLD in PCOS patients(71). Brower *et al.* identified at least four PCOS susceptibility loci in common genes in both Chinese and European PCOS women (i.e., all functionally involved in androgen synthesis, insulin action, and secretion). Nevertheless, further studies to provide more understanding of the genetic role in NAFLD pathogenesis are needed(72). Further supporting the role of genetic factors in PCOS, Plaksej *et al.* proposes that polymorphism of the cannabinoid receptor 1 gene may be responsible for individual susceptibility to obesity and subsequently NAFLD. He demonstrated that the frequency of the G allele of rs806381, especially GG genotype of rs10485170 and GT genotype of rs6454674, was significantly higher in women with PCOS and NAFLD than in PCOS women without NAFLD(73).

IR, which has been shown to be an essential feature of the syndrome affecting both obese and lean patients, seems to play a principal role in NAFLD pathogenesis in PCOS patients. Since the first documented biopsy-proven NASH case in a woman with PCOS by Brown *et al.* in 2005 (74), many reports have described a high prevalence of NAFLD among PCOS population. Gambarin-Gelwan *et al.* found that the presence of hepatic steatosis was associated with a higher BMI and homeostatic model assessment for insulin resistance (HOMA-IR), reflecting that obesity and IR are main predictors of NAFLD in PCOS patients(74). Interestingly enough, non-obese PCOS

patients have been found to have higher IR scores than their non-PCOS counterparts after accounting for age and BMI(74-76). Thus, it appears that for a given BMI, PCOS patients have a more severe IR which likely contributes to a higher prevalence of NAFLD.

HA has also been shown to contribute to NAFLD development in women with PCOS either independently or synergistically with IR(75). Many reports have failed to confirm significant differences in circulating total androgens between NAFLD and non NAFLD patients with PCOS (77, 78). However, lower sex hormone binding globulin (SHBG) levels indicating higher circulating biologically active free androgens has been frequently identified and linked to NAFLD pathogenesis(69, 79). Insulin regulates hepatic production of SHBG, and its low levels could be a marker for IR. Moreover, higher androgen level is an additional factor that decreases SHBG secretion in PCOS patients with NAFLD, this explains the even lower SHBG levels found in PCOS patients with hepatic steatosis compared with controls with hepatic steatosis, after controlling for obesity and IR(69).

It is believed that the occurrence and progression of metabolic disorders in PCOS patients are closely related to the chronic low-grade inflammation of intra-abdominal adipose tissue with the liver being both a target organ for the systemic inflammation and the origin of various pro-inflammatory cytokines, such as IL-1 and IL-6(80-84). These cytokines which are produced by triggered immune cells act on adipose tissue causing disturbance in its secretory profile (i.e. decrease adiponectin and increase leptin) and thus promoting development of NAFLD(71). Moreover, many studies outlined that IR and androgen excess may directly and indirectly play roles in cytokines and adipokines regulation(85-87). Finally, activation of these inflammatory mediators may further magnify both the metabolic/hormonal/inflammatory derangements as well as the vascular endothelial injury, which is observed in many patients with PCOS(43).

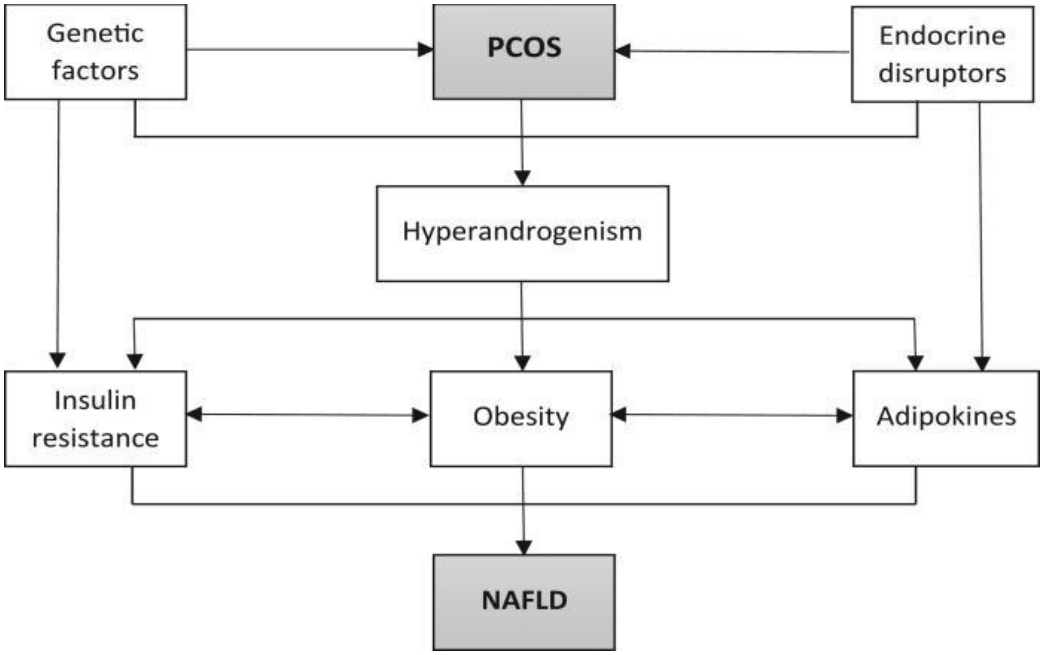

Figure. 5 Factors involved in NAFLD pathogenesis in PCOS and the proposed mechanism.

As the process of NAFLD development in PCOS patients is quite complicated, we are demonstrating a simple schematic representation of the involved factors and the proposed mechanism. Women with PCOS are genetically predisposed to have HA, IR, and other endocrinological disruptions as predominant features. HA and IR will act synergistically alongside with central obesity and cytokines/adipokines dysregulation leading to development of NAFLD.

2 HYPOTHESIS & RATIONALE

NAFLD has been linked to many extrahepatic conditions. Recent evidence showed that T2DM and obesity carry greater risk for NAFLD(88-92). As a result, some liver and diabetes organizations advocate for NAFLD screening in these population to avoid detrimental consequences of progressive NAFLD. Identification of high-risk groups is critical and cost-effective in management of NAFLD, especially in those who are more susceptible to develop severe disease (NASH).

Based on literature, we hypothesized that NAFLD is highly frequent in women with PCOS due to the fact that both conditions share a number of metabolic dysfunctions, such as IR. Therefore, we researched the prevalence of NAFLD among adult PCOS patients through a cross-sectional study using TE with CAP in South Asian PCOS women and a systematic review and meta-analysis in PCOS population in general, aiming to identify the magnitude of this emerging global health issue in a relatively young at-risk population.

3 Manuscripts

Manuscript A

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Observational Study

Prevalence and predictors of nonalcoholic fatty liver disease in South Asian women with polycystic ovary syndrome

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Ghali P, Deschenes M and Wong P contributed to data and interpretation of data; Sebastiani G contributed to conception, study design, data and interpretation of the data, statistical analysis and first draft of the manuscript. All approved the final manuscript.

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Abstract

BACKGROUND

Polycystic ovary disease (PCOS) may be a risk factor for nonalcoholic fatty liver disease (NAFLD) due to common pathogenetic pathways, including insulin resistance and obesity. Both PCOS and NAFLD are more severe in South Asian women. Data on NAFLD in South Asian women with PCOS are lacking.

AIM

To investigate prevalence and predictors of NAFLD and liver fibrosis in PCOS patients from South Asia.

METHODS

We conducted an observational routine screening program by means of transient elastography (TE) with associated controlled attenuation parameter (CAP). NAFLD was defined as CAP ≥288 decibels per meter. Significant liver fibrosis (stage 2 and higher out of 4) was defined as TE measurement ≥8.0 kilopascals. Elevated alanine transaminase (ALT) was defined as ALT >24 IU/L, as per upper limit of normal reported in South Asian women. Hyperandrogenism was defined as free androgen index >5. Predictors of NAFLD were determined by logistic regression analysis.

RESULT

101 PCOS patients (mean age 36.3 years) with no significant alcohol intake or viral hepatitis were included. Prevalence of NAFLD and significant liver fibrosis was 39.6% and 6.9%, respectively. Elevated ALT was observed in 40.0% and 11.5% of patients with and without NAFLD,

respectively. After adjusting for duration of PCOS and insulin resistance measured by homeostasis model for assessment of insulin resistance, independent predictors of NAFLD were higher body mass index [adjusted odds ratio (aOR) 1.30, 95% confidence interval (CI): 1.13-1.52], hyperandrogenism (aOR 5.32, 95% CI: 1.56-18.17) and elevated ALT (aOR 3.54, 95% CI: 1.10-11.47). Lifetime cardiovascular risk was higher in patients with NAFLD compared to those without NAFLD (0.31 ± 0.11 *vs* 0.26 ± 0.13).

CONCLUSION

Despite their young age, NAFLD diagnosed by TE with CAP is a frequent comorbidity in South Asian women with PCOS and is strongly associated with higher body mass index and hyperandrogenism. Noninvasive screening strategies could help early diagnosis and initiation of interventions, including counselling on weight loss, cardiovascular risk stratification and linkage to hepatology care where appropriate.

Keywords: Body mass index; Transient elastography; Controlled attenuation parameter; Hyperandrogenism; Alanine transaminase; Lifetime cardiovascular risk

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Core Tip: This is the first cohort study using transient elastography with controlled association parameter to investigate nonalcoholic fatty liver disease in patients with polycystic very syndrome.

Despite their young age, South Asian women with polycystic ovary disease have high frequency of nonalcoholic fatty liver disease at 36.7%, which could also result in liver fibrosis. Noninvasive screening strategies could help early diagnosis and initiation of interventions, including weight loss, correction of dyslipidemia and cardiovascular risk stratification to initiate statin.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease, affecting 25% of the general adult population globally(1, 2). Nonalcoholic steatohepatitis (NASH), the progressive form of NAFLD leading to liver fibrosis and cirrhosis, currently represents the second indication for liver transplantation, with projections to become the leading indication in the next 10 years(3). Importantly, NASH is already the leading indication for liver transplantation in women, with ethnical differences(4). This alarming ascent would call for identification of higher risk groups, where screening strategies could be targeted more effectively, as recommended by several guidelines(2, 5, 6). NAFLD is often associated with common extra-hepatic conditions, particularly cardiovascular disease which drives most of the mortality(7).

The prevalence of NAFLD may be higher in women with polycystic ovary syndrome (PCOS)(8). PCOS represents the most frequent endocrinopathy in women of reproductive age. PCOS seems more frequent and severe in South Asian women(9). Moreover, NAFLD is a major health issue in South Asian women, which is even greater if they emigrate to Western countries(10). Some studies have observed an overlap between NAFLD and PCOS: In both conditions, metabolic comorbidities are relevant pathogenetic drivers(2). In the context of PCOS, a more complex pathogenesis may account for a relationship between the two diseases, particularly hyperandrogenism(11). Despite these considerations, the prevalence of NAFLD in PCOS varies largely between 5.5% and 73.3% across studies(12). This discrepancy may be attributed to retrospective study design leading to selection bias and to varying diagnostic methods and definitions adopted for NAFLD. The majority of studies employed ultrasonography as diagnostic tool for NAFLD, which presents with intrinsic limitations including relatively low accuracy, inter-observer variation and inability of detecting hepatic steatosis involving less than 20%-30% of liver

parenchyma(13). Furthermore, there are limited data on the prevalence of significant liver fibrosis, which mirrors the spectrum of liver disease severity and provides a proxy for NASH prevalence.

Liver biopsy is still considered the gold standard for the diagnosis of NAFLD and associated liver fibrosis, but it is costly, invasive and with an intrinsic risk of sampling error, making it impracticable as screening tool(14). Transient elastography (TE) is an ultrasonography-based noninvasive method using liver stiffness as a surrogate for histologic liver fibrosis(15). The controlled attenuation parameter (CAP) measures the degree of hepatic attenuation by hepatic fat and is measured simultaneously with liver stiffness measurement (LSM). As such, CAP measurement is a surrogate for hepatic steatosis(16). In various clinical settings, TE with CAP presents with a good performance compared to liver histology for the detection of hepatic fibrosis and steatosis(16-19). Thus far, there has been no study employing TE with CAP to screen for NAFLD and associated liver fibrosis in a PCOS population.

We employed TE with CAP in consecutive PCOS patients from South Asia as a part of a routine screening program with the following aims: (1) To assess prevalence and associated predictors of NAFLD; (2) To determine prevalence of significant liver fibrosis. Secondary aims included evaluation of lifetime cardiovascular risk and of other comorbidities associated with NAFLD.

MATERIALS AND METHODS

Study design and population

We performed a cross-sectional cohort study at the Department of Obstetrics and Gynecology of McGill University Health Centre (MUHC), which follows about 1000 active PCOS patients. At MUHC, there is a large population of South Asian women with PCOS. Between October 2018 and July 2019, consecutive South Asian adult patients with PCOS were invited to

participate in the study by undergoing a TE examination with CAP as part of a screening program for liver disease. We included patients with PCOS defined by the modified Rotterdam criteria, after excluding other endocrine disorders. All patients met at least two criteria among clinical (hirsutism and/or other signs and symptoms of hyperandrogenism, *i.e.* acne/seborrhea and alopecia) and/or biochemical hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology(20). Exclusion criteria were the following: (1) Positivity for hepatitis C virus antibody or hepatitis B virus (HBV) surface antigen; (2) History of pre-existing liver disease or new diagnosis at the screening visit (auto-immune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, hemochromatosis, Wilson's disease, alpha-1 anti-trypsin); (3) History of hepatocellular carcinoma, liver transplantation or decompensated liver disease (ascites, hepatorenal syndrome, spontaneous bacterial peritonitis, hepatic encephalopathy, variceal hemorrhage); (4) Hazardous alcohol intake, as estimated by an Alcohol Use Disorders Identification Test (AUDIT-C) score ≥7(21); (5) Pregnancy at time of recruitment; and (6) Failure of TE examination or unreliable measurement. All patients provided written informed consent for participation into the study. In order to validate the TE examination with CAP measurement in our cohort, we also reported the CAP values from another routine screening program for liver fibrosis running at MUHC. As part of routine assessment at our center, patients with chronic HBV undergo CAP quantification during TE examination for LSM. We included only female patients aged <50 years old with chronic HBV, for an appropriate comparator with our PCOS population. We chose this validation group as young patients with chronic HBV have been reported to have low prevalence of NAFLD(22, 23). The Research Ethic Board of the Research Institute of the MUHC approved the study (study code 2019-4584), which was conducted according to the Declaration of Helsinki.

Outcome measures

The primary outcomes of the study were: (1) prevalence and associated predictors of NAFLD; (2) prevalence of significant liver fibrosis. Any grade NAFLD (>5% of hepatocytes) was defined as CAP ≥288 decibels per meter (dB/m)(19), and significant liver fibrosis (stage ≥F2 out of 4) as TE measurement ≥8.0 kilopascals(24-26). We also explored the use of the recently proposed cut-off of 302 dB/m to diagnose any grade NAFLD(18).

Secondary outcomes were evaluation of the lifetime cardiovascular risk through the atherosclerotic cardiovascular risk equation, according to American College of Cardiology/American Heart Association guidelines(27) and of extra-hepatic diseases linked to NAFLD. Sleep apnea and hypothyroidism were diagnosed on the basis of clinical history. Chronic kidney disease (CKD) was defined as estimated glomerular filtration rate <60 mL/min/1.73 m^2, calculated using the CKD-Epi formula, as per KDIGO guidelines(28, 29).

TE examination

TE examination was performed on a 4-h fasting patient by two experienced operators. The standard M probe was first used in all patients. The XL probe was performed in case of failure with M probe. Examinations were considered valid if the operator was able to obtain at least 10 validated measures and the interquartile range of those measures was <30% of the median(17, 30). Given recent data on the lack of effect of probe type and steatosis on LSM, we did not use adjusted cut-off values(18).

Serum biomarkers

The simple biomarker hepatic steatosis index (HSI) was calculated and the standard cut-off value of 36 was used to diagnose NAFLD(31, 32). The simple fibrosis biomarkers fibrosis-4 (FIB-4), aspartate aminotransferase-to-Platelets Ratio Index (APRI) and NAFLD fibrosis score were computed, as previously described(33-35).

Clinical and biological parameters

Anthropometric, clinical, and biochemical measurements and data were collected at recruitment. Family history of liver and cardiovascular diseases was also recorded. Regular physical exercise was defined as at least 150 min of moderate aerobic exercise(5). The diagnosis of diabetes was based on treatment with antidiabetic drugs or the International Diabetes Federation definition(36). Any alcohol intake was defined as a score $\geq$5 by the questionnaire AUDIT-C. Biological parameters, collected at time of recruitment, included: AST, Elevated alanine transaminase (ALT), gamma-glutamyl transferase, platelets, bilirubin, albumin, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, insulin and glycosylated hemoglobin, C-reactive protein. All patients were screened for pre-existing liver disease with the following: HBV and hepatitis C virus serologies, anti-nuclear antibody, anti-mitochondrial antibody, anti-smooth muscle antibody, ferritin, ceruloplasmin, alpha-1-antitrypsin. Elevated ALT was defined as ALT > upper limit of normal (ULN) of 24 IU/L, as previously described for South Asian women(37). Patients were classified into four groups according to their measured body mass index values, and cut-off values from Asian guidelines were used for this categorization; lean <23 kg/m^2, overweight 23–25 kg/m^2, obese >25 kg/m^2. Waist circumference values exceeding 80 cm was used as the cut-off value for central obesity(38). Insulin was used to compute the homeostasis model for assessment of insulin resistance (HOMA-IR) index (fasting

insulin (mIU/L) X fasting glucose (mmol/L)/22.5)(39). HOMA-IR >1.9 was considered indicative of insulin resistance. A patient was defined as metabolically abnormal in presence of diabetes, hypertension or hyperlipidemia (triglycerides ≥ 1.7 mmol/L and/or high-density lipoprotein <1.3 mmol/L), while the absence of all three conditions defined a metabolically normal patient. The following hormonal parameters were evaluated for the diagnosis of biochemical hyperandrogenism: total testosterone, bioavailable testosterone and sex hormone-binding globulin. Free androgen index (FAI) was calculated as the ratio of total testosterone levels in nmol/L to sex hormone-binding globulin levels in nmol/L × 100 (%)(40). A FAI >5 was considered indicative of hyperandrogenism.

Statistical analysis

We compared characteristics of study subjects by NAFLD status using Student's *t*-test for continuous variables and Pearson's χ^2 or Fisher's exact test for categorical variables. Multivariable logistic regression modelling was employed to identify factors predictive of NAFLD. Results were reported as adjusted odds ratio (aOR) with 95% confidence interval (CI). Covariates were included *a priori* based on their clinical relevance or on their significance in univariate analysis (*P* <0.10). Final models were adjusted for duration of PCOS based on self-reporting, defined as the period from the year of diagnosis until the date of TE/CAP exam, body mass index, HOMA-IR, FAI >5 and ALT >24 IU/L. The corrected Akaike information criteria (AIC) and the Bayesian information criteria (BIC) were calculated and compared among the models to determine which one had the best goodness-of-fit measure. A lower AIC and/or BIC was indicative of a better fit. The performance of body mass index, ALT and FAI to predict NAFLD was measured as area under the receiver operating characteristic curve (AUC). Standard errors of AUC were calculated

by DeLong method. A concordance analysis between CAP and HSI was carried out using the kappa score, with results interpreted as follows: less than 0, less than chance agreement; 0.01–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement and 0.81–0.99, almost perfect agreement(41). Pairwise correlation was employed to test association of serum fibrosis biomarkers (FIB-4, APRI and NAFLD fibrosis score) with LSM. All tests were two-tailed and with a significance level of $\alpha = 0.05$. Statistical analyses were performed using STATA 13.1 (STATA Corp. LP, College Station, TX, United States).

RESULTS

After applying exclusion criteria (Figure A.1), 101 patients were included into the present study. The XL probe was employed in 19 (18.8%) cases, while the standard M probe was used in all other patients. The failure rate of TE examination (1%) was similar to previous studies(17). The characteristics of the study population are reported in Table 1. Only 2 out of 101 included patients reported any alcohol intake. Twelve (11.9%) patients were overweight [Body mass index (BMI): 23–25 kg/m^2], and 72 (71.3%) were obese (BMI >25 kg/m^2). Central obesity was present in 97 (96%) cases. Elevated ALT was observed in 23 (22.8%) patients.

Prevalence of NAFLD and significant liver fibrosis

The mean CAP value in the study population was 266.9 dB/m (standard deviation 63.0). In our validation group of 125 female patients with chronic HBV aged < 50 years, we found a much lower mean CAP value of 214 dB/m (standard deviation 55.5). Prevalence of NAFLD was 39.6% in the study population of PCOS women, while only 8% in the validation group of female patients

with chronic HBV. By employing the cut-off of 302 dB/m, the prevalence of NAFLD in PCOS women was 29.7%. Table A.1 depicts the characteristics of patients with and without NAFLD, with relative univariate analysis. All patients with NAFLD were metabolically abnormal (Figure A.2). By HSI, prevalence of NAFLD was 39.6%. The number of observed agreements between HSI and CAP was 66 (65.3%) for the 288 dB/m and 60 (59.4%) for the 302 dB/m cut-off, respectively. The kappa-value was 0.34 (standard error: 0.08; 95% CI: 0.17-0.50) and 0.25 (standard error: 0.08; 95% CI: 0.10–0.40), compatible with a "fair" strength of agreement. Prevalence of significant liver fibrosis in the cohort was 6.9%. In patients with NAFLD, the prevalence of significant liver fibrosis was 15%, compared to only 1.6% among those without NAFLD. Table A.2 depicts the main characteristics of patients with significant liver fibrosis. The prevalence of NAFLD and significant liver fibrosis was higher in obese patients compared to those overweight or lean (Figure A.3a). As showed in Figure A.3b, the prevalence of NAFLD was significantly higher in patients with hyperandrogenism ($P = 0.007$), insulin resistance ($P < 0.001$) and elevated ALT ($P = 0.001$). Given the known association between false positive results of LSM and elevated ALT, we conducted a sensitivity analysis by excluding patients with elevated ALT(42). First, no patient had ALT >10 times the ULN. Second, among the 9 patients with ALT >2 times the ULN, 3 had significant liver fibrosis. If we would exclude these patients from the analysis, the prevalence of significant liver fibrosis would be 4.3%. Among the serum fibrosis biomarkers, APRI was the only one showing a significant correlation with LSM (Figure A.4).

Predictors of NAFLD by multivariate analysis

Table A.3 illustrates the multivariate analyses for predictors of NAFLD by CAP cut-offs of 288 and 302 dB/m. After adjustments, independent predictors of NAFLD were higher BMI (aOR: 1.30, 95% CI: 1.13-1.52; P <0.001), hyperandrogenism (aOR: 5.32, 95% CI: 1.56-18.17; P = 0.008) and elevated ALT (aOR: 3.54, 95% CI: 1.10-11.47; P = 0.035). When the cut-off of 302 dB/m was applied, higher BMI (aOR: 1.33, 95% CI: 1.14-1.55; P <0.001) and hyperandrogenism (aOR: 3.54, 95% CI: 1.00-12.57; P = 0.049) were independently associated with NAFLD. These models had lower AIC and BIC values than others, hence providing support for their use. The performance of BMI, FAI and ALT to predict NAFLD is reported in Figure A.5. There was no difference in performance among the three predictors: AUC was 0.808 (standard error: 0.045; 95% CI: 0.719-0.897) for BMI, 0.761 (standard error: 0.049; 95% CI: 0.665-0.858) for FAI, and 0.722 (standard error: 0.054; 95% CI: 0.615-0.828) for ALT.

Cardiovascular risk and other extra-hepatic complications

The atherosclerotic cardiovascular risk was higher in patients with NAFLD (Table A.1). Only 12.5% of patients with NAFLD were on statin treatment. There was no difference in prevalence of hypothyroidism among patients with NAFLD (25%) and those without NAFLD (31.1%). There was one case of sleep apnea (1.6%) and one case of CKD (1.6%) among patients with NAFLD.

DISCUSSION

This study, performed in a cohort of consecutive South Asian women with PCOS undergoing a routine screening program for liver disease, showed that NAFLD is a frequent comorbidity. To our knowledge, this is the first study to adopt TE with CAP to investigate NAFLD in PCOS women. TE with CAP is already commonly used in other at-risk populations(43-45). We also

showed that, despite their young age, women with PCOS and NAFLD could have significant liver fibrosis, possibly indicating the coexistence of NASH, the progressive counterpart of NAFLD. Finally, PCOS patients with NAFLD had higher cardiovascular risk score, which should be taken into account for overall risk stratification.

NAFLD affects one quarter of the general population globally(1, 2). NASH is now the second indication for liver transplantation in North America, predicted to become the leading indication within the next 10 years(46). This will soon impact on the physiognomy of liver transplant waiting lists and on organ supply(47). As such, there is an urgent need for diagnostic and treatment strategies. The prevalence of NAFLD increases in populations at risk, including those with type 2 diabetes and obesity(2). NAFLD is often a clinically silent disease until end-stage complications arise. Early identification and risk stratification for those at higher risk for fibrosis progression could help institute interventions to prevent NAFLD progression, and ultimately reduce liver-related morbidity and mortality.

NAFLD is frequent in women with PCOS. Patients with PCOS may be at higher risk for NAFLD due shared pathophysiological features with NAFLD, including insulin resistance, chronic inflammation, dyslipidemia(48). Moreover, hyperandrogenism likely represents a unique and independent risk factor for NAFLD in this population(11). Finally, alteration in gut microbiota has been linked to disease severity in both PCOS and NAFLD, thus acting as an additional potential pathogenic bridge between the two conditions(49, 50). In our routine screening program for liver disease, we reported a prevalence of NAFLD at 39.6% and such diagnosis was confirmed in many cases by another noninvasive method, namely the biomarker HSI. These figures are higher than those reported for the general population, where the prevalence of NAFLD is 25%(51, 52). Previous estimates of NAFLD prevalence among PCOS patients ranged widely, between

5.5% and 73.3% across studies(12). In the present study, we have included a homogeneous population of South Asian women, as both PCOS and NAFLD prevalence vary across ethnicities(53). South Asian women have been reported to have more severe PCOS symptoms at younger age, with greater insulin resistance than Caucasians(54). Moreover, NAFLD seems a major health issue in South Asian women, with high rates of advanced liver fibrosis, particularly if they emigrate to Western countries(10). Previous studies were either of retrospective nature or have employed less accurate diagnostic tools, such as ultrasound or simple serum biomarkers(13, 32, 55). In the present study, we employed TE with CAP to investigate the prevalence of both NAFLD and significant liver fibrosis. We have adopted a cut-off value reported as optimal to detect any grade steatosis(19) and we have also applied a recently reported higher cut-off(18). Significant liver fibrosis affected 6.9% of our cohort, which suggests the coexistence of a progressive disease, namely NASH(2). Of note, there was a poor correlation between LSM and NAFLD fibrosis score or FIB-4, likely because these two biomarkers incorporate age in their formula, while our study population was young. Conversely, APRI, which does not include age in its formula, had a significant correlation with LSM. Our data suggest that the simple fibrosis biomarker APRI may be preferable to FIB-4 or NAFLD fibrosis score in young PCOS patients.

We found that BMI, hyperandrogenism and ALT were independent predictors of NAFLD. Among them, BMI had the highest AUC to predict NAFLD. This finding underlines the relevance of obesity and associated metabolic conditions. Indeed, in our study population all patients with NAFLD were metabolically abnormal. South Asians have a higher proportion of visceral fat distribution and are more likely to have dyslipidemia than Western patients(56). However, South Asian patients with NAFLD have an overall lower BMI compared to Caucasians(57). Other factors contributing to NAFLD in this ethnic group may include genetic variants of the patatin-like

phospholipase domain-containing 3 protein, physical inactivity, reduced disease awareness, late diagnosis, as well as sociocultural factors in comparison with Western patient populations(56). Indeed, in our cohort of young women, only 19.8% were practicing regular physical exercise. Hyperandrogenism measured by FAI was also an independent predictor of NAFLD. Our finding confirms previous data that high FAI correlates with liver disease markers and is a PCOS-specific feature that further increases the risk of NAFLD(12). Elevated ALT was also an independent predictor of NAFLD on multivariable analysis. Although only 22.8% of patients had elevated ALT, this finding indicates that liver enzyme abnormalities in patients with PCOS and no known pre-existing liver disease should prompt further investigations, including tests for etiologies of chronic liver disease and subsequent referral for TE examination to evaluate the degree of liver fibrosis. Indeed, 21.7% of patients with elevated ALT had significant liver fibrosis on TE examination, compared to only 2.6% of patients with normal ALT. On the other hand, 60% of the patients with NAFLD had normal ALT. These figures are in line with data from the general population and suggests the development of NAFLD may be occult(58, 59). This finding emphasizes the need for sensitive diagnostic tools in this at-risk population. Currently, guidelines recommend routine screening strategies for NAFLD in at-risk individuals, such as those with type 2 diabetes and metabolic comorbidities, particularly in case of elevated ALT(5, 6). It is further recommended that at-risk populations should be looked for liver fibrosis using noninvasive markers (serology based or TE) to quantify the risk of progression to liver cirrhosis(2). A similar strategy may be applicable in patients with PCOS, whereby those with obesity, elevated ALT or hyperandrogenism should undergo liver fibrosis assessment.

We found that young South Asian patients with PCOS and NAFLD have an increased lifetime risk of atherosclerotic cardiovascular risk. Emerging data support the concept that NAFLD is a

multisystem disease affecting a variety of extra-hepatic organ systems. Recent evidences indicate an increased risk of all-cause mortality and a strong link between NAFLD and extra-hepatic disease, such as cardiovascular disease, hypothyroidism and sleep apnea(60). Cardiovascular disease risk prediction in younger female patients has been more challenging than in older or male patients. Decisions to implement primary prevention measures are often consequently hindered in this patient population. Our study helps shed new insights in the understanding of cardiovascular risk profile in young female population from the NAFLD perspective. Our findings should be taken into consideration for risk stratification, especially after transition of women with PCOS to menopause, and for consideration of statin therapy.

Our study presents with several strengths, including the well-characterized homogeneous population and the use of a validated and accurate diagnostic method. The enrollment of consecutive patients minimizes the risk of selection bias. Some limitations of our study should be acknowledged. First, the cross-sectional study design did not allow us to capture the dynamics and associated factors of the disease in a longitudinal fashion. Second, the unavailability of genetic variants of the patatin-like phospholipase domain-containing 3 and other polymorphisms linked to hepatic steatosis prevented us from understanding their contribution to the pathogenesis of NAFLD in PCOS. Third, we included only South Asian women, so we cannot speculate on applicability of our findings to other ethnicities. Fourth, we did not include a group of age-matched patients without PCOS to act as control group. Finally, our study was carried out at a tertiary care center, which may limit generalizability of our findings.

CONCLUSION

In conclusion, NAFLD diagnosed by TE with CAP is a frequent comorbidity in young South Asian women with PCOS without known liver disease. Obesity and hyperandrogenism seem the main associated factors. NAFLD can also progress to significant liver fibrosis, pointing towards the coexistence of NASH. Considering the young age of this population, these data suggest that monitoring for liver disease should be proposed in South Asian women with PCOS in case of obesity, elevated ALT, or hyperandrogenism. Early diagnosis of NAFLD *via* noninvasive screening tools may help prompt initiation of interventions, including life-style modification, hepatology specialized care and cardiovascular risk stratification. Future longitudinal studies should assess the effect of early diagnosis and interventions on long-term outcomes.

ARTICLE HIGHLIGHTS

Research background

Nonalcoholic fatty liver disease (NAFLD) is the most frequent liver disease worldwide. It is essential to identify higher risk groups, where screening strategies could be targeted. Women with polycystic ovary syndrome (PCOS) may be at higher risk for NAFLD.

Research motivation

To date, no study has employed transient elastography (TE) with associated controlled attenuation parameter (CAP) to screen women with PCOS for NAFLD.

Research objectives

This work aims to determine prevalence and associated predictors of NAFLD and prevalence of significant liver fibrosis in South Asian women with PCOS.

Research methods

A routine screening program through TE with CAP was conducted at a single center. NAFLD was defined as CAP ≥288 decibels per meter. Significant liver fibrosis was defined as TE measurement ≥8.0 kilopascals. Predictors of NAFLD were determined by logistic regression analysis.

Research results

Prevalence of NAFLD and significant liver fibrosis was 39.6% and 6.9%, respectively. Independent predictors of NAFLD were higher body mass index, hyperandrogenism and elevated alanine aminotransferase.

Research conclusions

NAFLD diagnosed by TE with CAP is a frequent comorbidity in South Asian women with PCOS, who can also develop liver fibrosis despite their young age.

Research perspectives

To reduce the burden and complications of NAFLD, noninvasive screening strategies should be considered in South Asian women with PCOS.

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Part of this work has been presented at the Liver Meeting of the American Association for the Study of Liver Diseases 2019 (Boston, America).

Footnotes

Institutional review board statement: The study was approved by the Research Ethic Board of the Research Institute of the MUHC (study code 2019-4584).

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: Ghali P has acted as consultant for Merck and Gilead. Deschenes M has served as an advisory board member for Merck, Janssen, Gilead; Wong P has acted as consultant for BMS, Gilead, Merck, Novartis; Sebastiani G has acted as speaker for Merck, Gilead, Abbvie, Novonordisk, Novartis, Pfizer, served as an advisory board member for Merck, Intercept, Novartis, Gilead, Allergan and has received research funding from Merck and Theratec Inc. Shengir M, Krishnamurthy S and Chen T have no conflicts of interest to declare.

Data sharing statement: According to stipulations of the patient consent form signed by all study participants, ethical restrictions imposed by our Institutional Ethics review boards (Institutional Ethics Review Board Biomedical B Research Ethics Board of the McGill University Health Centre), and legal restrictions imposed by Canadian law regarding clinical trials, anonymized data are available upon reasonable request. Please send data access requests to Sheldon Levy, Biomedical B (BMB) Research Ethics Board (REB) Coordinator Centre for Applied Ethics, 5100, boul. de Maisonneuve Ouest, 5th floor, Office 576, Montréal, Québec, H4A 3T2, Canada.

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FIGURE LEGENDES

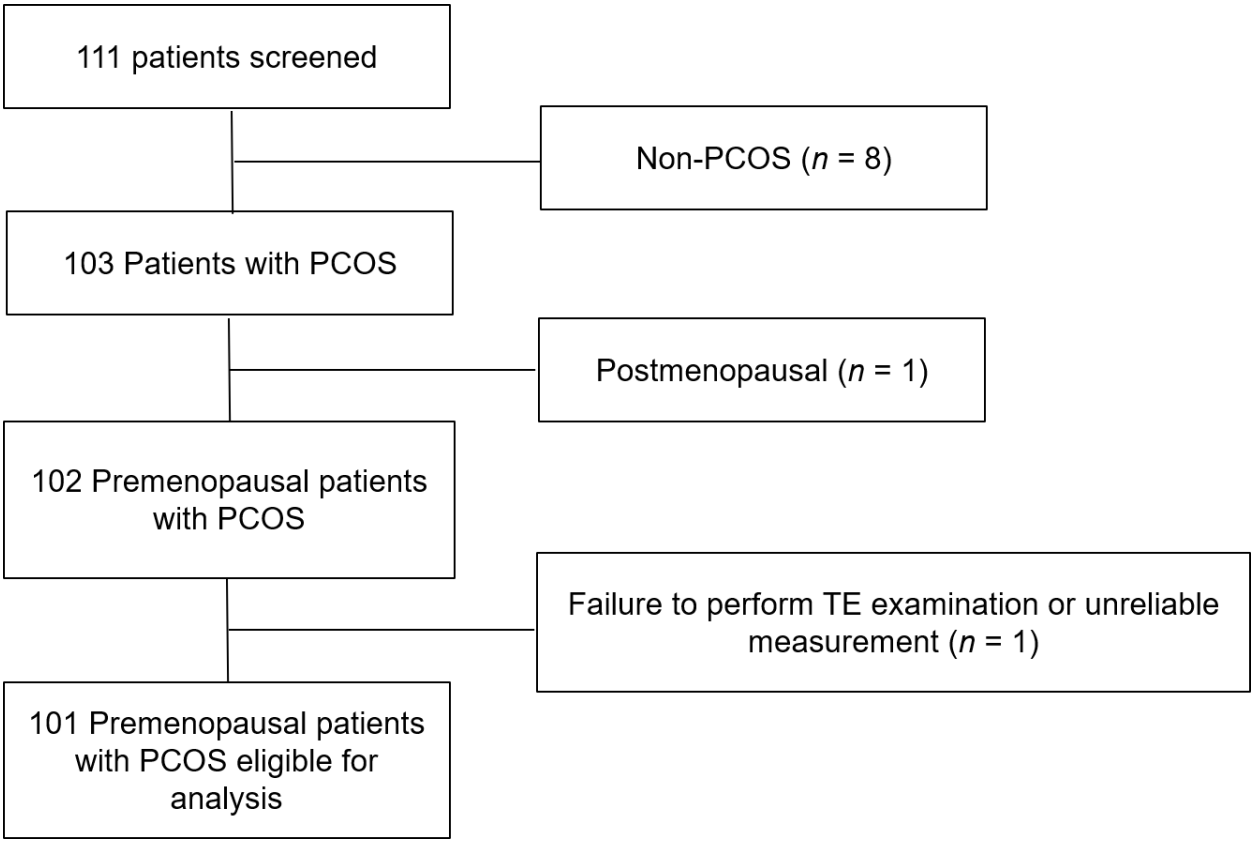

Figure. A. 1 Flow chart displaying the selection of participants in the study cohort.

PCOS: Polycystic ovary syndrome.

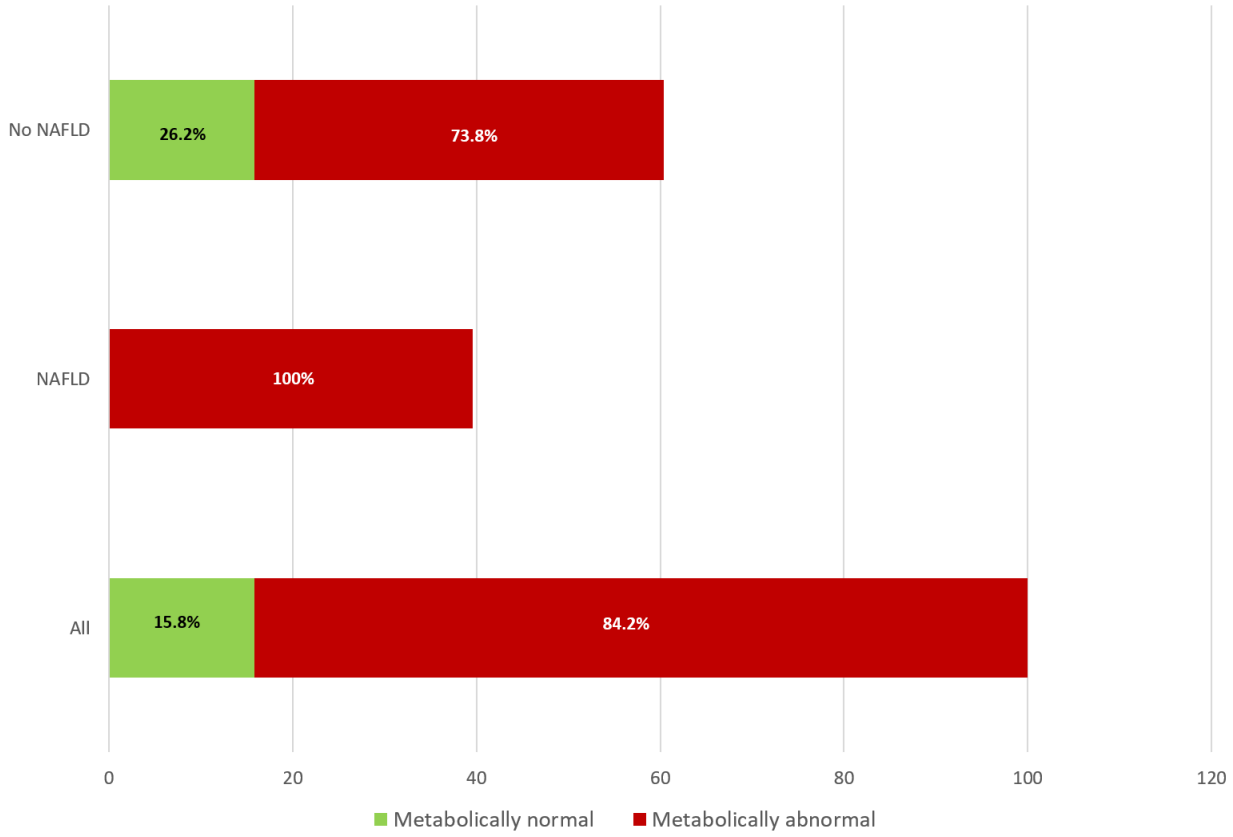

Figure. A. 2 Distribution of metabolically normal and abnormal patients by nonalcoholic fatty liver disease category. NAFLD: Nonalcoholic fatty liver disease.

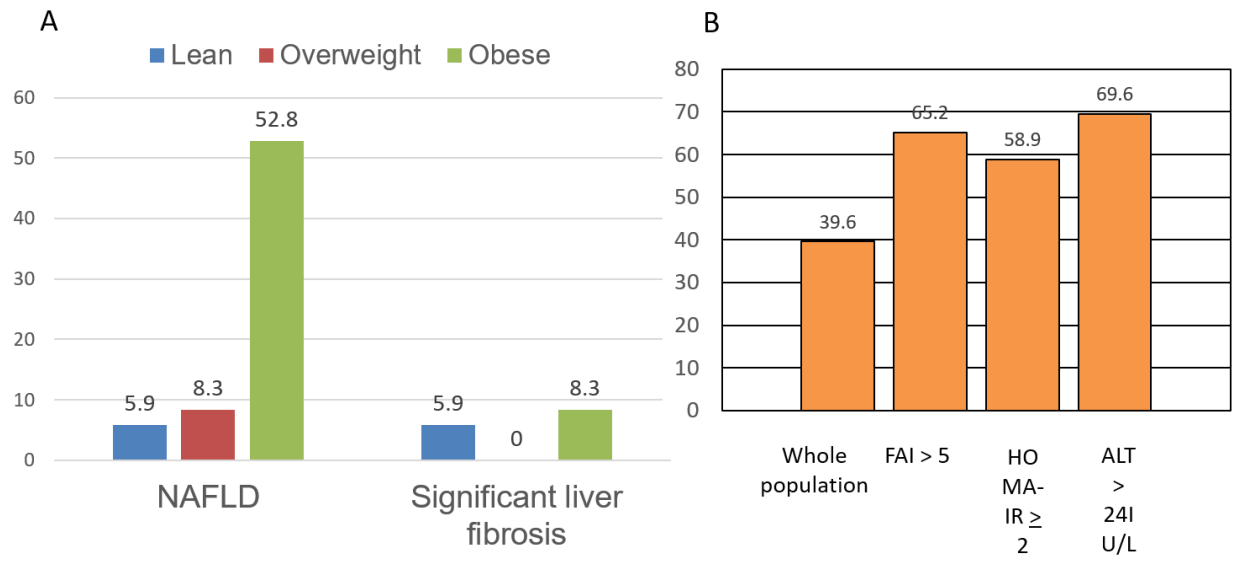

Figure. A. 3 Prevalence of nonalcoholic fatty liver disease and significant liver fibrosis.

A: Prevalence of nonalcoholic fatty liver disease (NAFLD), severe NAFLD and significant liver fibrosis according to body mass index category; and B: Prevalence of NAFLD according to patients' characteristics. NAFLD: Nonalcoholic fatty liver disease; ALT: Alanine transaminase; HOMA-IR: Homeostasis model for assessment of insulin resistance.

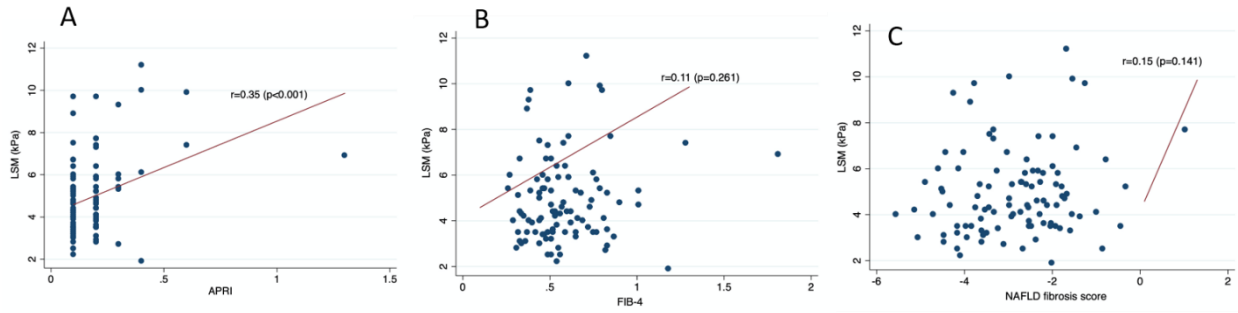

Figure. A. 4 Scatterplot depicting the correlation between liver stiffness measurement.

A: Aspartate aminotransferase-to-Platelets Ratio Index; B: Fibrosis-4; and C: Nonalcoholic fatty liver disease fibrosis score. NAFLD: Nonalcoholic fatty liver disease; APRI: aminotransferase-to-Platelets Ratio Index; FIB-4: Fibrosis-4.

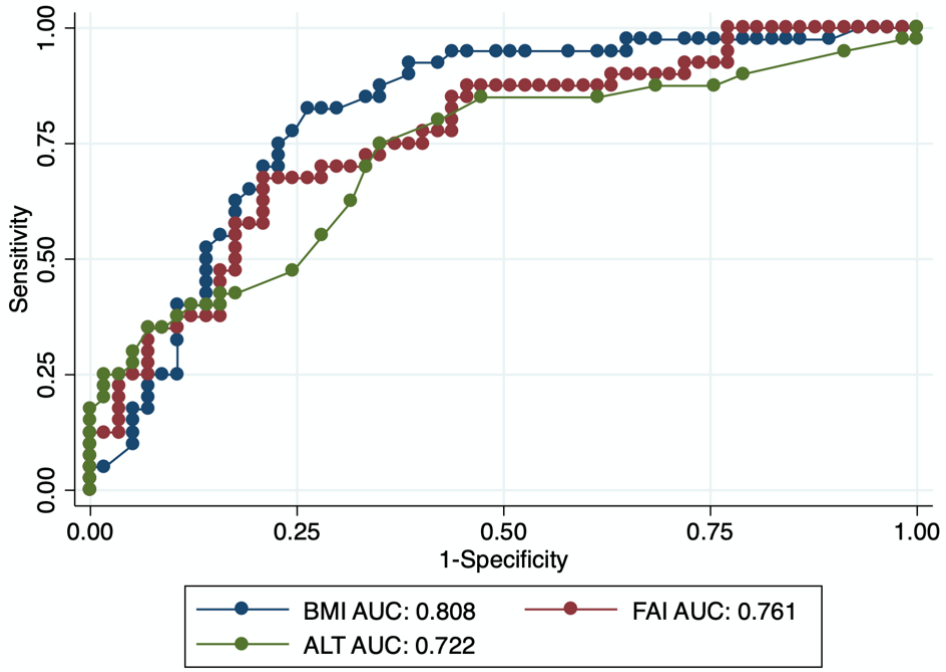

Figure. A. 5 Area under the curve of body mass index, free androgen index and alanine aminotransferase for prediction of nonalcoholic fatty liver disease.

BMI: Body mass index; ALT: Alanine transaminase; AUC: Area under curve; FAI: Free androgen index.

TABLES

Table. A. 1 Demographic, clinical, biochemical, histologic and pharmacological characteristics of the study population (n = 101) and univariable analyses by outcome status, that is presence of nonalcoholic fatty liver disease

Variable	Total cohort (*n* = 101)	NAFLD (*n* = 40)	No NAFLD (*n* = 61)
Age (yr)	36.3 (4.8)	36.1 (5.6)	36.4 (4.3)
PCOS duration (yr)	7.0 (4.1)	7.4 (4.4)	6.8 (3.9)
Regular physical exercise (%)	20 (19.8)	8 (20.0)	12 (19.7)
ASCVD risk (lifetime)	0.28 (0.12)	0.31 (0.11)[a]	0.26 (0.13)[a]
Metabolic factors			
Diabetes (%)	18 (17.8)	12 (30.0)[a]	6 (9.8)[a]
Hypertension (%)	6 (5.9)	1 (2.5)	5 (8.2)
Waist circumference (cm)	101.1 (12.3)	107.8 (11.1)[b]	96.7 (11.1)[b]
BMI (Kg/m^2)	27.6 (5.0)	30.6 (4.5)[b]	25.7 (4.4)[b]
Medications			
Metformin (%)	32 (31.7)	20 (50.0)[a]	12 (19.7)[a]
Steroids contraceptive (%)	5 (4.9)	2 (5.0)	3 (4.9)
Statin (%)	5 (4.9)	5 (12.5)	0
Biochemical parameters			
Platelet count (10^9/L)	271.9 (59.5)	271.9 (54.7)	271.9 (62.9)
AST (IU/L)	18.6 (11.8)	23.5 (17.2)[b]	15.3 (3.9)[b]

ALT (IU/L)	21.7 (18.7)	30.9 (25.7)[b]	15.7 (8.0)[b]
GGT (IU/L)	21.4 (19.1)	24.8 (16.8)	19.3 (20.4)
Total bilirubin (µmol/L)	9 (2.9)	9.8 (3.6)[a]	8.5 (2.2)[a]
Albumin (mg/L)	43.0 (2.9)	42.9 (3.0)	43.0 (2.8)
HOMA-IR	3.2 (2.9)	4.5 (3.3)[b]	2.4 (2.2)[b]
HbA1c (%)	6.4 (1.8)	6.9 (2.1)[b]	5.6 (0.6)[b]
Total cholesterol (mmol/L)	4.5 (1.0)	4.5 (1.0)	4.5 (0.9)
HDL cholesterol (mmol/L)	1.1 (0.3)	1.1 (0.3)[a]	1.2 (0.3)[a]
LDL cholesterol (mmol/L)	2.7 (0.8)	2.6 (0.9)	2.7 (0.7)
Triglycerides (mmol/L)	1.5 (1.2)	1.8 (1.0)	1.4 (1.3)
Creatinine (mmol/L)	56.8 (10.1)	55.2 (8.9)	57.9 (10.8)
TSH	2.6 (2.7)	2.6 (2.5)	2.5 (2.8)
Total testosterone (nmol/L)	1.6 (0.7)	1.8 (0.8)	1.6 (0.6)
SHBG (nmol/L)	32.2 (20.6)	22.4 (9.7)[b]	39.1 (23.3)[b]
FAI	3.6 (3.7)	5.4 (4.6)[b]	2.4 (2.1)[b]
CRP (mg/L)	5.3 (4.9)	6.9 (6.2)[a]	4.3 (3.5)[a]
Noninvasive tests for NAFLD and liver fibrosis			
CAP (dB/m)	266.9 (63.0)	326.9 (30.5)	227.5 (45.1)
LSM (kPa)	4.9 (1.9)	5.7 (2.2)[b]	4.4 (1.4)[b]
APRI	0.18 (0.15)	0.23 (0.21)[a]	0.15 (0.07)[a]

FIB-4	0.6 (0.2)	0.60 (0.3)	0.6 (0.2)
NAFLD Fibrosis Score	-2.9 (1.2)	-2.5 (1.3)[a]	-3.1 (1.1)[a]
HSI	38.3 (5.7)	40.8 (6.7)[b]	36.6 (4.2)[b]

Continuous variables are expressed as mean (standard deviation) and categorical variables as numbers (%). [a]$P < 0.05$; [b]$P < 0.001$. The P values refer to t test or χ^2 test between patients with the outcome (nonalcoholic fatty liver disease or significant liver fibrosis) and those without the outcome. ALT: Alanine aminotransferase; APRI: Aspartate aminotransferase-to-platelet ratio index; ASCVD: Atherosclerotic cardiovascular disease; AST: Aspartate aminotransferase; BMI: Body mass index; dB/m: Decibels per meter; CAP: Controlled association parameter; CRP: C-reactive protein; FAI: Free androgen index; FIB-4: Fibrosis-4 score; GGT: Gamma-glutamyl transpeptidase; HbA1c: Hemoglobin glycosylated; HDL: High-density lipoprotein; HOMA-IR: Homeostasis model for assessment of insulin resistance; HSI: Hepatic steatosis index; IU: International unit; LDL: Low-density lipoprotein; LSM: Liver stiffness measurement; NAFLD: Nonalcoholic fatty liver disease; TSH: Thyroid-stimulating hormone.

Table. A. 2 Demographic, clinical, biochemical and pharmacological characteristics of patients with significant liver fibrosis (n = 7)

	PCOS duration (yr)	**HOMA-IR**	**BMI (Kg/m^2)**	**ALT (IU/L)**	**Triglycerides (mmol/L)**	**FAI**	**CAP (dB/m)**
Patient 1	10	3.2	31.8	62	0.93	3.1	317
Patient 2	4	1.4	26.2	12	0.91	3.0	186
Patient 3	6	10.9	30.1	88	1.36	5.9	372
Patient 4	13	2.8	28.2	20	0.91	6.3	298
Patient 5	6	5.9	31.2	78	1.66	8.0	346
Patient 6	8	7.9	36.4	101	1.37	3.1	386
Patient 7	13	5.6	20.2	31	2.4	13.9	325

ALT: Alanine aminotransferase; BMI: Body mass index; dB/m: Decibels per meter; CAP: Controlled association parameter; FAI: Free androgen index; HOMA-IR: Homeostasis model for assessment of insulin resistance; PCOS: Polycystic ovary syndrome.

Table. A. 3 Multivariable analysis of factors associated with nonalcoholic fatty liver disease

CAP cut-off 288 dB/m		
Variable	Unadjusted OR	aOR
PCOS duration (per yr)	1.03 (0.94-1.14)	1.04 (0.92-1.17)
BMI (per Kg/m^2)	1.31 (1.16-1.48)[b]	1.31 (1.13-1.52)[b]
HOMA-IR (per unit)	1.42 (1.14-1.78)[a]	1.13 (0.90-1.41)
Hyperandrogenism (yes *vs* no)	3.68 (1.37-9.83)[a]	5.32 (1.56-18.17)[a]
Elevated ALT (yes *vs* no)	5.14 (1.87-14.12)[a]	3.54 (1.10-11.47)[a]
CAP cut-off 302 dB/m		
Variable	Unadjusted OR	aOR
PCOS duration (per yr)	0.81 (0.33-2.00)	0.94 (0.83-1.07)
BMI (per Kg/m^2)	1.12 (1.06-1.18)[b]	1.33 (1.14-1.55)[b]
HOMA-IR (per unit)	1.39 (1.14-1.70)[a]	1.18 (0.95-1.46)
Hyperandrogenism (yes *vs* no)	1.28 (1.10-1.48)[a]	3.54 (1.00-12.57)[a]
Elevated ALT (yes *vs* no)	1.93 (1.32-2.84)[a]	2.55 (0.80-8.14)

Odds ratios and 95% confidence intervals are shown for each variable analyzed in univariable and multivariable logistic regression analysis. [a]$P < 0.05$; [b]$P < 0.001$. CAP: Controlled attenuation parameter; FAI: Free androgen index; HOMA-IR: Homeostasis model for assessment of insulin resistance; IU: International unit; aOR: Adjusted odds ratio; NAFLD: Nonalcoholic fatty liver disease; PCOS: Polycystic ovary syndrome.

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Manuscript B

Nonalcoholic fatty liver disease in premenopausal women with polycystic ovary syndrome: systematic review & meta-analysis

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Disclosure statement

PG has acted as consultant for Merck and Gilead. MD has served as an advisory board member for Merck, Janssen, Gilead. PW has acted as consultant for BMS, Gilead, Merck, Novartis. GS has acted as speaker for Merck, Novonordisk, Novartis, Pfizer, Gilead, and AbbVie, served as an advisory board member for Merck, Gilead, Intercept, Pfizer, Allergan and Novartis, and has received research funding from Merck, Theratec and Pfizer. All other authors have no conflicts of interest to declare.

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Authors contributions

MS was involved in study concept and design, acquisition of data, interpretation of data, analysis and drafting of manuscript. TC was involved in study concept, acquisition and interpretation of data, critical revision of manuscript and overall study supervision. EG has done the search strategy and was involved in the revision of manuscript. RA performed the meta-analysis and was involved in the revision of manuscript. PG, MD, PW, and SK were involved in study concept and critical revision of manuscript. GS was involved in study concept and design, acquisition of data, interpretation of data, analysis and drafting of manuscript, critical revision of manuscript and

overall study supervision. All authors declare that they have participated in the preparation of the manuscript and have seen and approved the final version.

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Abstract

BACKGROUND & AIMS

Nonalcoholic fatty liver disease (NAFLD) and polycystic ovary syndrome (PCOS) are prevalent conditions sharing common pathogenic factors. We performed a systematic literature review and meta-analysis aiming to investigate the association between NAFLD and PCOS among premenopausal PCOS patients.

METHODS

Relevant studies were systematically identified through scientific databases until 2019. We calculated pooled odds ratio (OR) using a random-effect model, and heterogeneity was addressed through I^2. Subgroup analysis stratified by geographic region, study design, applied PCOS criteria, and diagnostic tool for NAFLD was performed. Frequently reported cofactors were evaluated through meta-regression.

RESULTS

Of the 1833 studies retrieved in the initial search, 23 studies with 7148 participants from 4 different geographic regions qualified for quantitative synthesis. The pooled result showed that women with PCOS had a 2.5-fold increase in the risk of NAFLD compared to controls (pooled OR 2.49, 95% confidence interval [CI] 2.20-2.82; I^2=55.2%, p=0.001). South American/Middle East populations with PCOS had a greater risk of NAFLD than those without PCOS from the same region (OR 3.55, 95% CI 2.27-5.55), compared to their counterpart from Europe (OR 2.22, 95% CI 1.85-2.67) and Asia (OR 2.63, 95% CI 2.20-3.15). Study quality and body mass index (BMI) were the only

covariates that showed a relationship with the outcome in the meta-regression, with a regression coefficient of -2.219 (95% CI -3.927 – -0.511) and -1.929 (95% CI -3.776- -0.0826), respectively.

CONCLUSION

This meta-analysis indicates that premenopausal PCOS is associated with a 2.5 risk of NAFLD, and BMI seems the main cofactor.

Keywords: NAFLD, pooled odds ratio, geographic region, study quality, BMI.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a growing global health problem, affecting almost a quarter of the world's population and currently recognized as the most common cause of chronic liver disease globally(1, 2). NAFLD is defined as detection of >5% fat accumulation within the liver, either by imaging or histology, in the absence of other identifiable causes of hepatic steatosis, in particular excessive alcohol consumption(3). The disease encompasses a spectrum of conditions ranging from simple steatosis through nonalcoholic steatohepatitis (NASH) to fibrosis, cirrhosis, and eventually hepatocellular carcinoma(4). NAFLD estimated global prevalence ranges from 6.3 to 33%, with a median of 20%(5). However, its risk is considerably higher in some populations such as obese and type 2 diabetic patients, where the prevalence reaches 69.4%(6). NASH, the progressive form of NAFLD, is presently the second indication for liver transplantation and projected to become the leading indication in the coming decade(7). Furthermore, NASH is already the most frequent indication for liver transplantation in women(8). Parallel to its liver-related outcomes, there is growing body of evidence supporting that NAFLD is a multisystemic disease, and it has strong clinical associations with many extra-hepatic conditions(9). Insulin resistance (IR), which is considered the gameplayer in NAFLD pathogenesis, seems the culprit risk factor for most of these associations(10).

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women at reproductive period, with a prevalence of up to 20%(11). It is characterized by oligo-amenorrhea, clinical or biochemical hyperandrogenism, and/or polycystic ovary morphology on ultrasonography(12). In addition to these main features, metabolically, most PCOS patients have IR with compensatory hyperinsulinemia as an intrinsic feature. Notwithstanding that obesity, which is frequently associated with PCOS, can also cause IR(13). Several studies demonstrated

that non-obese PCOS patients have higher IR in comparison with non-PCOS women(14-16). The association between PCOS and IR may come with a high prevalence of NAFLD among women with PCOS. NAFLD and PCOS are considered the hepatic and ovarian manifestation of the metabolic syndrome (MetS), respectively(17-23). However, while some studies reported a higher prevalence of NAFLD in PCOS patients compared to controls, others were inconclusive. One meta-analysis reported a significant association between PCOS and NAFLD, although independent of obesity and geographic region(24).

In this study, we aim to conduct a systematic review & meta-analysis to estimate the strength of association between NAFLD and PCOS in premenopausal women with PCOS, as well as identifying cofactors associated with NAFLD.

MATERIALS & METHODS

Search strategy

This systematic literature review was conducted following a designated protocol and Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines(25)(Suppl. 1 Appendix 1). The protocol was submitted to the international prospective register of systematic reviews (PROSPERO)(26), registration # CRD42020154363. The following databases were searched from inception until June 1, 2018 and then updated on February 1, 2020 with inputs from a medical librarian (EM), which ran the former search, and study investigators (MS, TC, GS). To identify articles that addressed the association between NAFLD and PCOS, the following databases were searched: Africa-Wide Information (Ebsco), Biosis (Ovid & Clarivate Analytics), Cochrane (Wiley), Embase (Ovid), Global Health (Ovid), Global Index Medicus (WHO), Medline (Ovid) and Web of Science (Clarivate Analytics). We used the following variations in text words

found in the title, abstract or keyword fields, and relevant subject headings; fatty liver OR hepatic steatosis OR nonalcoholic fatty liver disease OR NAFLD OR nonalcoholic steatohepatitis OR NASH OR liver fibrosis OR cirrhosis AND polycystic ovary syndrome OR PCOS. The search was neither limited to defined geographic area nor specific language. Appendix 2 reports the full search strategy.

Eligibility criteria

Eligible studies were selected according to the following criteria; (i) Original observational (cohort, case-control, or cross-sectional) studies; (ii) Conducted on premenopausal women ≥18 years old; (iii) Holding the diagnosis of PCOS according to one of the following criteria: Rotterdam criteria, National Institute of Health (NIH) criteria, or Androgen excess and PCOS Society (AES) criteria; (iv) NAFLD diagnosis determined by either imaging studies or noninvasive biomarkers; (v) Reporting the measure of association (odds ratio [OR]) or providing sufficient data to be calculated.

Data extraction

All retrieved articles in the initial search were read independently by two reviewers (MS and TC), starting with titles and abstracts screening, followed by the full-text reading, and concluded by data extraction(27). Any disagreements were resolved by mutual discussion or by a third independent reviewer (GS) if necessary. The following data were retrieved from the full text of the selected articles: geographic region, first author, year of publication, country, age, body mass index (BMI), number of participants, enrollment period, PCOS criteria, NAFLD diagnosis

modality, prevalence of NAFLD, prevalence of MetS, cofactors of NAFLD, and ORs with confidence intervals (CIs). Data were extracted from each article into customized tables.

Quality assessment

Evaluation of risk of bias for each paper was performed by two independent reviewers (MS, TC) using the Newcastle-Ottawa Scale (NOS)(28) for non-randomized studies. The scale judges three broad perspectives: the selection of participants, the comparability of the groups, and the ascertainment of either the exposure or outcome of interest for case-control or cohort studies, respectively. Since there is no specific scale available for cross-sectional studies from the original source, an adjusted NOS(29) has been adapted. Further modifications have been applied based on the purpose of this review (Suppl. 5 "coding manuals"). In this scale, each study is given an overall quality score; this score is the sum of sub-scores assigned for each domain that was used to categorize overall study quality(30). The selection of participant domain sub-score was amended (using ≥2-star points instead of ≥3 for fair to good threshold) to account for all study designs. For interpretation of overall scores, modified dichotomous limits (good vs. poor) were applied for simplification purposes. The original description of overall scores was as follows: good (>7), fair (5-7), and poor (<5); however, we replaced it with the following thresholds: fair to good (>5) and poor (<5) (Table B.1,2).

Outcome measures

The primary outcome was to study the association between NAFLD and PCOS among premenopausal women with PCOS. The secondary outcome was to determine cofactors of NAFLD.

Statistical analysis

In the meta-analysis, forest plots were provided to illustrate pooled ORs, and corresponding 95% CIs using random-effect model(31). As only 7 studies have reported adjusted odds ratios (aOR), we calculated crude OR for all reviews in order to have a standardized measure of association. Statistical heterogeneity was assessed using the inconsistency (I^2) index. Sensitivity analyses were performed by excluding: (i) poor quality studies, defined as total NOS score <5 and/or sub-score thresholds <2 in selection of participants and ascertainment of exposure and outcome and <1 in comparability domain; (ii) studies weighed <5%. Publication bias was examined visually via funnel plot, which is represented as a scatterplot of degree of association of NAFLD in women with PCOS against sample size(32). Subgroup analyses were performed by: (i) geographic region; (ii) study design; (iii) NAFLD identification tool; (iv) PCOS diagnostic criteria; (v) presence of IR; (vi) presence of MetS. Finally, we evaluated the effect of frequently reported cofactors on the desired outcome (NAFLD) through a meta-regression. Statistical analyses were performed using STATA 14.2 (STATA Corp. LP, College Station, Texas, USA) & funnel plots using R 3.5.1 (The R Foundation for Statistical Computing).

RESULTS

Study selection

The PRISMA flowchart is shown in Figure B.1. The search strategy retrieved 1833 records after excluding duplicates. Upon applying our eligibility criteria using Rayyan web application(27), titles and abstracts screening have resulted in an elimination of 1781 citations for different reasons; the remaining 52 studies have met the criteria for full-text reading. Ultimately, 29 articles published between 2007 – 2019 were found to be qualified for the systematic review. Of these, 23

studies were eligible for quantitative synthesis. Six studies did not enter the analysis: five were excluded due to lack of sufficient data, and one article because the number of events was zero in both PCOS and control groups.

Study characteristics

We placed our inputs from 29 studies into two tables. Table B.3 shows articles selected for the systematic review. These were divided according to their geographic areas; 1-12 Europe, 13-21 Asia, 22-25 South America, 26-28 the Middle East, 29 North America. The majority of these articles (25 studies) have used qualitative ultrasonography (alone or combined with additional method) as a diagnostic tool for NAFLD. We also noticed that Rotterdam criteria were by far the predominant standards utilized (23 studies) to identify patients with PCOS. All except four studies were prospective studies. Cofactors of NAFLD determined through multivariate logistic regression analyses were reported only in twelve studies. The three most frequently stated ones were IR measured by homeostatic model assessment (HOMA), BMI, and free androgen index (FAI), in descending order. Table B.4 depicts the quantitative data such as the number of participants, age, BMI, NAFLD proportion, prevalence of MetS and ORs with 95% CIs in both arms, PCOS and controls.

Meta-analysis

Across 23 studies with 4164 PCOS cases and 2984 matched controls that entered the meta-analysis, pooled OR using the random-effect model, was estimated to be 2.49 (95% CI 2.20-2.82), an almost 2.5-fold increase in the risk of NAFLD in PCOS compared to controls (Figure 2). The results were significant, with moderate heterogeneity (I^2=55.2%, p=0.001). Publication bias was

addressed using the trim-and-fill method developed by Duval and Tweedie(33, 34). The adjusted result from the random-effect model, after accounting for the missing studies (Figure 3), was an OR of 2.56 (95% CI 2.07-3.17), which indicates that the result of the present meta-analysis is reliable. Furthermore, sensitivity analyses excluding poor-quality studies and studies weighed <5% both displayed similar results, with OR of 2.38 (95% CI, 2.09-2.71) and 2.36 (95% CI, 2.05-2.70), respectively. Subgroup analyses were conducted to explore potential sources of heterogeneity (Figure B.4). The pooled ORs of most subgroups were not markedly changed by the study characteristics. However, stratification by geographic location revealed that South American/Middle East populations with PCOS had a greater risk of NAFLD than those without PCOS (OR 3.55, 95% CI 2.27-5.55), compared to their European (OR 2.22, 95% CI 1.85-2.67) and Asian (OR 2.63, 95% CI 2.20-3.15) counterparts. Additional stratification based on IR and presence of MetS showed that PCOS patients had a significantly higher risk of NAFLD, compared to controls (OR 1.97, 95% CI 1.44–2.71 and OR 3.39, 95% CI 2.42-4.76, respectively). We also noticed that the risk of NAFLD was less with the NIH/AES criteria (OR 1.96, 95% CI 1.31-2.91) compared to the Rotterdam criteria (OR 2.56, 95% CI 2.25-2.91). Significant heterogeneity was observed in subgroup analyses for study design (cross-sectional, I^2=62.2%, p=<0.001; case-control, I^2=0%, p=0.418), PCOS diagnostic criteria (Rotterdam, I^2=56.8%, p=0.001; NIH/AES, I^2=48.4%, p=0.121) and geographic area (Asia, I^2=67.4%, p=0.003; Europe, I^2=61%, p=0.009; Middle East & South America, I^2=0%, p=0.721). For all groups of NAFLD diagnostic modality (qualitative ultrasonography, I^2=54.6%, p=0.002; imaging/noninvasive biomarkers, I^2=67.5%, p=0.026), IR (I^2=86.2%, p<0.001), and MetS (**I^2**=55.4, p=0.022), heterogeneity reached statistical significance. Nonetheless, the degree of heterogeneity remained not altered from the main result (Table B.5). Of note, only three among the included studies looked for the association between

PCOS and severity of NAFLD, evaluated via ultrasound. There was no significant difference in NAFLD severity between PCOS patients and controls, possibly to the relative limited patient populations(35, 36). One of these studies reported the prevalence of significant liver fibrosis in PCOS patients at 4.7%, as determined by transient elastography(36). No study reported on the effect of PCOS on clinical outcomes of NAFLD.

Meta-regression

General study characteristics, such as the presence of matching and aOR, as well as study design and NAFLD diagnostic modalities, revealed no significant association except for study quality. Study quality was defined as; (i) fair to good, when the total NOS score was >5 given that the sub-score for the selection of participants and ascertainment of exposure and outcome domains was ≥2 and for comparability domain was ≥1; (ii) poor, when the total NOS score was <5 given that the sub-score for the selection of participants and ascertainment of exposure and outcome domains was <2 and for comparability domain was <1 (regression coefficient -2.219, CI -3.927 – -0.511) (Suppl. 1). Moreover, among most frequently reported risk factors, only BMI showed an elevated risk with the desired outcome (regression coefficient -1.929, 95% CI -3.776 – -0.082) (Suppl. 2).

DISCUSSION

As NAFLD is becoming the most common cause of chronic liver disease globally, health care authorities and liver organizations are currently advocating for NAFLD screening in high-risk groups, such as people with metabolic syndrome and type 2 diabetes mellitus(37-40). Therefore, identifying populations at risk is the first step toward implementing an effective screening strategy that might help alleviating the burden of NAFLD-related outcomes, including cirrhosis,

hepatocellular carcinoma, and liver transplantation. Over the past decade, there was an increasing interest in researching NAFLD in women with PCOS since the relationship seems very relevant in clinical settings: both conditions are common, and their coexistence may synergistically increase the risk for catastrophic consequences of progressive NAFLD, especially in a relatively young PCOS population. Moreover, menstrual and reproductive factors, as well as the use of exogenous hormones, have been associated with the risk of NAFLD in females(41). Finally, NASH already represents the first indication for liver transplant in women(8). So far, some studies have found a positive relationship between PCOS and NAFLD when compared to non-PCOS counterparts(17-19, 22, 23, 35, 36, 42-52). At the same time, others could not determine this association, either because there were no differences between both groups and/or because the prevalence in the PCOS group was less than general population(21, 53-55). To date, data regarding this topic are inconsistent and still evolving.

When we reviewed the literature, we found three previous meta-analyses that investigated the relationship between NAFLD and PCOS. First, a report that included seven studies found that NAFLD was markedly prevalent among PCOS patients presumably due to shared risk factors such as obesity and IR(56). A subsequent meta-analysis including 17 studies confirmed NAFLD as a frequent occurrence in the PCOS group. Additionally, the report shed a light on hyperandrogenemia as an additional risk factor contributing to the development of NAFLD in the PCOS population(57). Finally, another meta-analysis that included 17 studies showed results that were also in agreement with previous findings. However, the authors were also aiming to identify if these higher NAFLD figures were related to the presence of PCOS itself or were rather due to common risk factors(24). Indeed, determining culprit factor(s) contributing to a higher prevalence of NAFLD, especially in PCOS, is a challenging task since this raises the argument of PCOS

defining criteria. AES and NIH definitions mandate the presence of hyperandrogenemia to establish the diagnosis of PCOS. Therefore, PCOS and hyperandrogenemia are relatively synonymous in this context. The only criteria that include a subset of PCOS patients without exhibiting any signs of either clinical or biochemical androgen excess are the Rotterdam criteria. Discussing etiology of NAFLD in PCOS patients is beyond the scope of this study. Although all aforementioned meta-analyses suggested NAFLD as a frequent comorbidity in PCOS patients, each of them has its limitation in terms of generalizability. One meta-analysis has a small sample size(56), the other two meta-analyses have searched only two databases each, with restriction of study selection to only English articles(24, 57). To have larger, more representative sample and add robustness to the argument that PCOS patients are in fact at higher risk for NAFLD, we carried out a systematic literature review searching eight scientific databases and meta-analysis of studies reporting the prevalence of NAFLD in PCOS patients up to 2020, without any restrictions in the search strategy. Our summary result indicates that PCOS patients are at higher risk for NAFLD (OR 2.49, 95% CI 2.20-2.82), a 2.5-fold increase compared to controls. Although this result is in line with previous meta-analyses(24, 56, 57), the present study confirms, updates, and adds more strength to the previous findings because it includes more reviews, a total of 29 publication and thus larger sample size of 7148 participants compared to 17 studies with 5334 participants in the most recent meta-analysis(24) and 7 studies with 1185 participants in the oldest one(56), a total increase of 12 and 22 studies, respectively. The characteristics of included studies are variable in terms of study design, PCOS definition, quality appraisal and results. However, our subgroup and sensitivity analyses suggest that all these factors did not impact the overall results.

Epidemiological studies found different prevalence for NAFLD in the general population across continents. The highest figures were reported from South American and the Middle East, with a

prevalence of around 30% for each region(58). Thus, when we stratified according to geographic locations, we combined these two areas based on the similarity that both ethnicities exhibited. Our results depict that NAFLD risk was also considerably elevated in PCOS patients from these geographic regions. Factors associated with higher risk were increased IR(21, 22, 42, 45, 49), worse metabolic profile(21, 49), and hyperandrogenemia(35). In this meta-analysis, we investigated the association between IR and NAFLD in PCOS, as well as determined the prevalence of metabolic syndrome in this population. To our knowledge, this is the first meta-analysis researching IR and MetS in PCOS setting. On top of the aforementioned factors, genetic predisposition seems to be an undisputed contributing factor to the high prevalence of NAFLD in South Americans, where the rs738409 G allele of the patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene is highly prevalent, despite much lower daily caloric intake than in North America and Western Europe(58).

Our meta-regression identified an association between study quality and the prevalence of NAFLD in women with PCOS. Conversely, other general study characteristics such as matching, study design, adjusted OR, and NAFLD diagnostic tool did not show any significant results. Among most frequently reported risk factors including age, HOMA-IR, and FAI, only BMI indicated an elevated risk for the desired outcome.

This study has several strengths. We broadened our literature search to include eight databases without search strategy restrictions in order to provide a representative sample size that can reflect the PCOS population in general. Additionally, studies that have used aminotransferase as a method to diagnose NAFLD were excluded to be consistent with NAFLD definition. Although our study allows for a clinically meaningful expansion of the literature, it is not without limitations. First, the included studies were all observational, which might be biased due to unmeasured confounders.

Second, our summary result was based on crude ORs since only 7 reviews reported adjusted ORs. Although some studies were adjusted for main confounders, other modifiable factors were not accounted for in all these studies, such as family history, dietary habits, and/or exercise. The presence of adjusted ORs for all studies and taking into account all possible relevant confounders may have influenced the overall result. Third, ultrasonography was the predominant method for diagnosing NAFLD rather than the gold standard liver biopsy. This can be justified by the difficulty in applying such an invasive procedure for research purposes. Ultrasonography is a readily available, cheap, noninvasive technique with discrete sensitivity and specificity for epidemiological studies.

In conclusion, our findings indicate that PCOS patients are at higher risk for NAFLD, and BMI seems to be the main driving factor. This risk is increased in women from South America and the Middle East. Therefore, early detection and initiation of intervention plans, including counselling on weight loss and linkage to hepatology care, will be crucial and can reduce the possibility of disease progression as these women can develop NAFLD at a relatively young age. Future research efforts should target the association between PCOS and severity of NAFLD, including liver fibrosis and clinical outcomes.

FIGURE LEGENDS

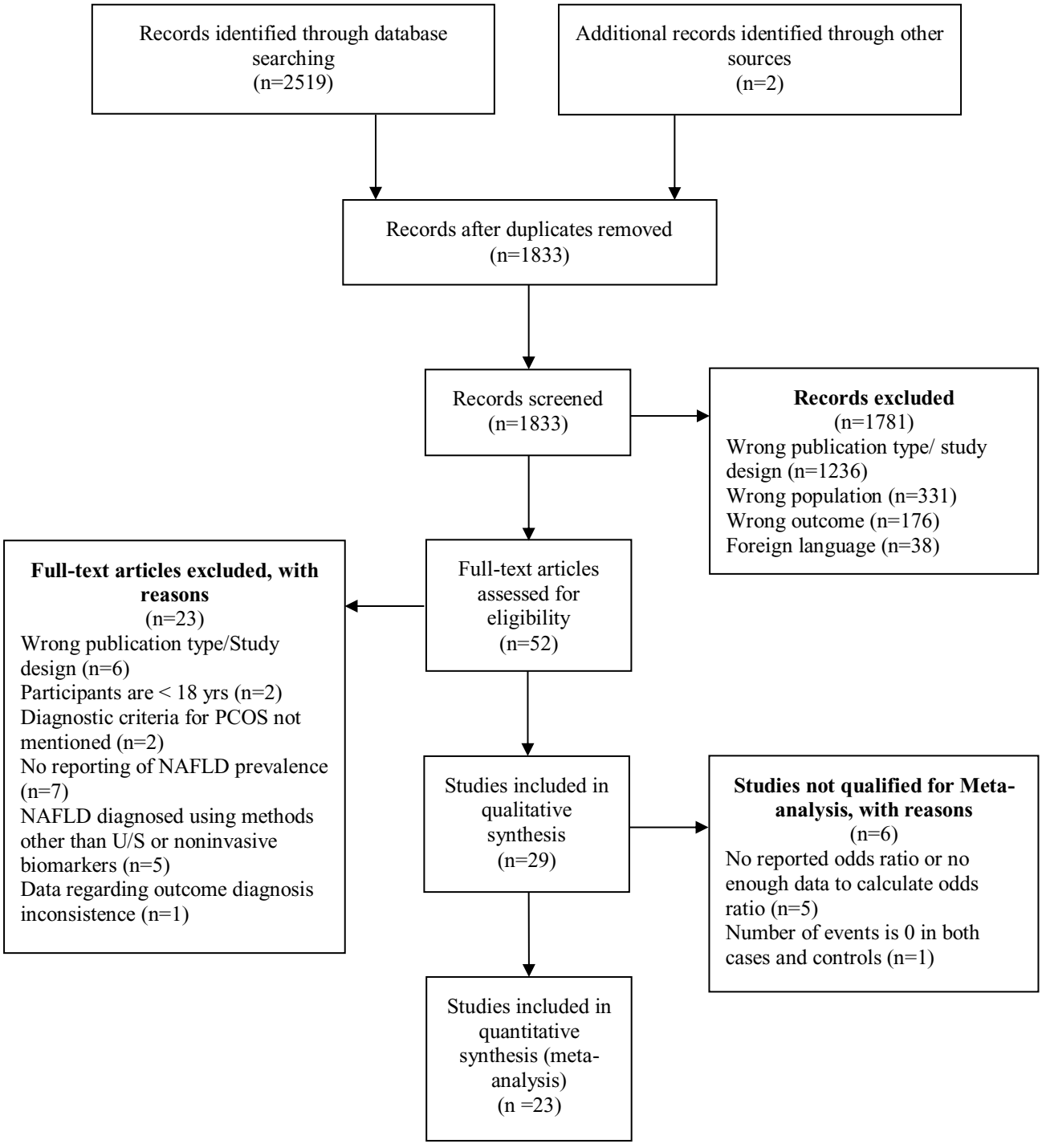


Figure. B. 1 Prisma flow chart.

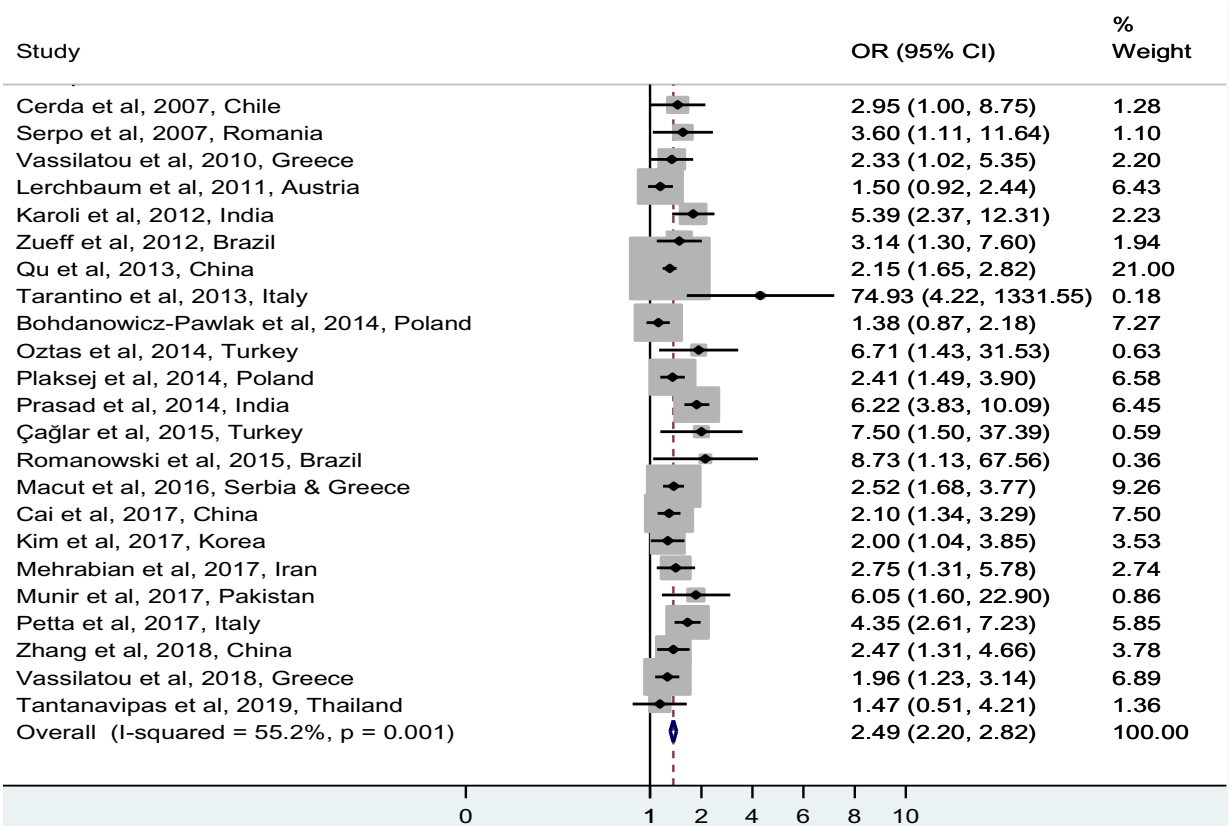


Figure. B. 2 Forest plot of studies investigated the association of NAFLD and PCOS.

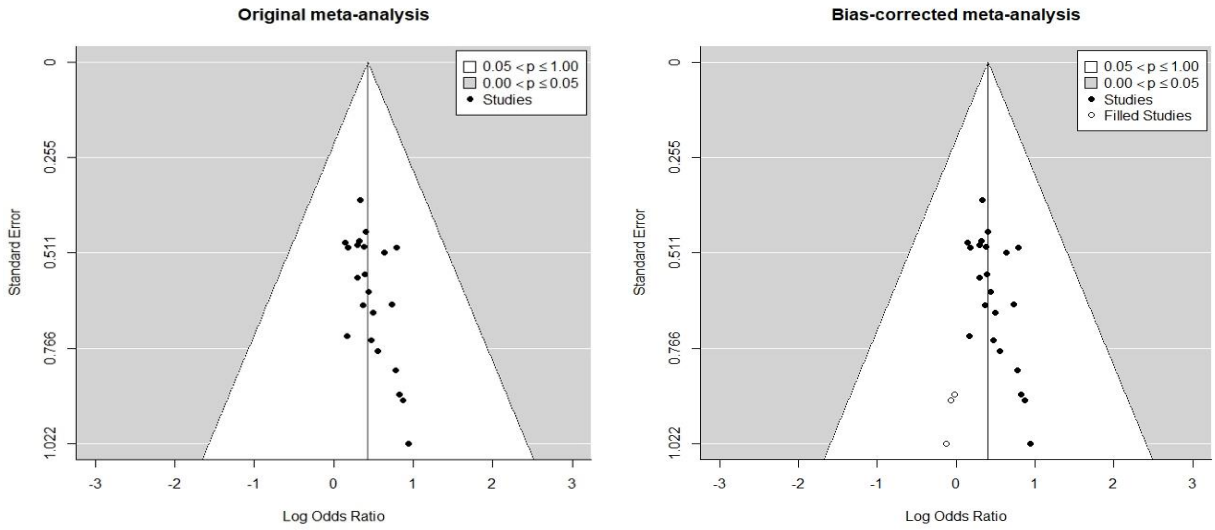
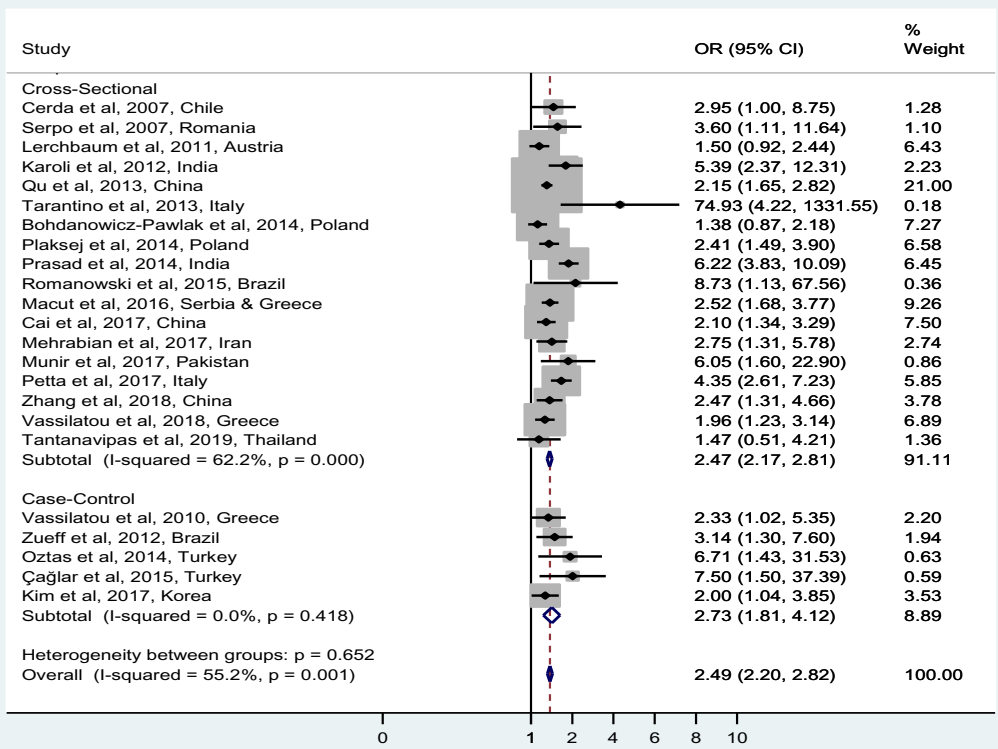


Figure. B. 3 Funnel plots of the meta-analysis.

before (panel A) and after (panel B) applying the trim-and-fill method. The closed dots indicate the observed studies, and the open dots indicate the missing studies imputed by the trim-and-fill method. The dashed lines that create a triangular area indicate the 95% confidence limits, and the vertical solid line represents the overall effect size.


Study
OR (95% CI)
% Weight
Cross-Sectional
Cerda et al, 2007, Chile 2.95 (1.00, 8.75) 1.28
Serpo et al, 2007, Romania 3.60 (1.11, 11.64) 1.10
Lerchbaum et al, 2011, Austria 1.50 (0.92, 2.44) 6.43
Karoli et al, 2012, India 5.39 (2.37, 12.31) 2.23
Qu et al, 2013, China 2.15 (1.65, 2.82) 21.00
Tarantino et al, 2013, Italy 74.93 (4.22, 1331.55) 0.18
Bohdanowicz-Pawlak et al, 2014, Poland 1.38 (0.87, 2.18) 7.27
Plaksej et al, 2014, Poland 2.41 (1.49, 3.90) 6.58
Prasad et al, 2014, India 6.22 (3.83, 10.09) 6.45
Romanowski et al, 2015, Brazil 8.73 (1.13, 67.56) 0.36
Macut et al, 2016, Serbia & Greece 2.52 (1.68, 3.77) 9.26
Cai et al, 2017, China 2.10 (1.34, 3.29) 7.50
Mehrabian et al, 2017, Iran 2.75 (1.31, 5.78) 2.74
Munir et al, 2017, Pakistan 6.05 (1.60, 22.90) 0.86
Petta et al, 2017, Italy 4.35 (2.61, 7.23) 5.85
Zhang et al, 2018, China 2.47 (1.31, 4.66) 3.78
Vassilatou et al, 2018, Greece 1.96 (1.23, 3.14) 6.89
Tantanavipas et al, 2019, Thailand 1.47 (0.51, 4.21) 1.36
Subtotal (I-squared = 62.2%, p = 0.000) 2.47 (2.17, 2.81) 91.11
Case-Control
Vassilatou et al, 2010, Greece 2.33 (1.02, 5.35) 2.20
Zueff et al, 2012, Brazil 3.14 (1.30, 7.60) 1.94
Oztas et al, 2014, Turkey 6.71 (1.43, 31.53) 0.63
Çağlar et al, 2015, Turkey 7.50 (1.50, 37.39) 0.59
Kim et al, 2017, Korea 2.00 (1.04, 3.85) 3.53
Subtotal (I-squared = 0.0%, p = 0.418) 2.73 (1.81, 4.12) 8.89
Heterogeneity between groups: p = 0.652
Overall (I-squared = 55.2%, p = 0.001) 2.49 (2.20, 2.82) 100.00
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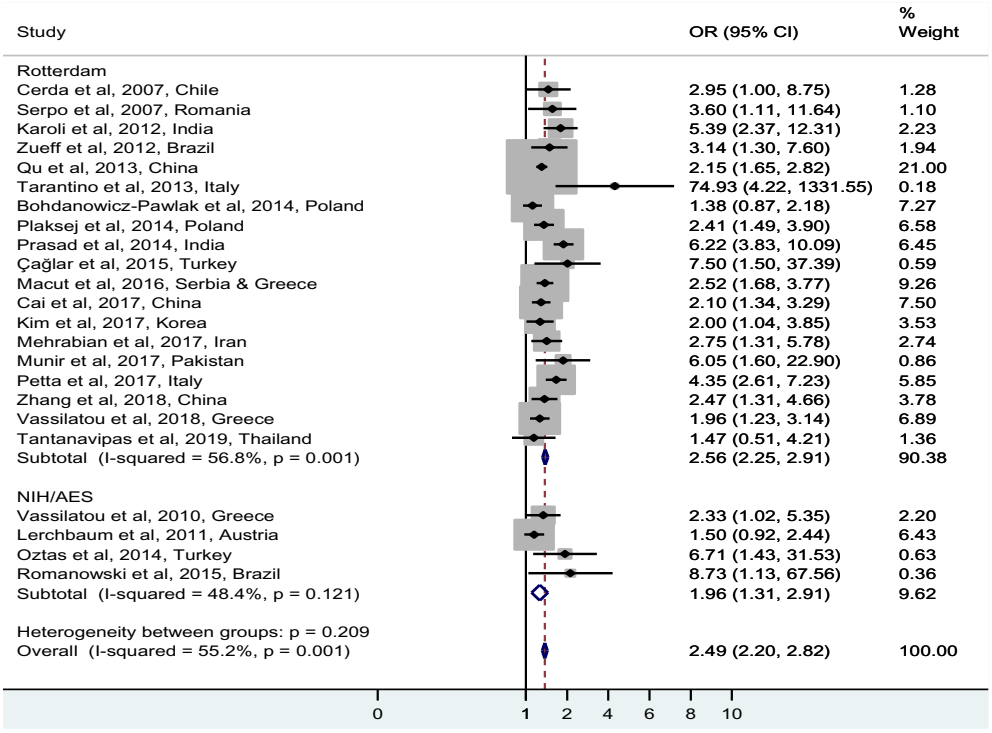
A


Study
OR (95% CI)
% Weight
Rotterdam
Cerda et al, 2007, Chile 2.95 (1.00, 8.75) 1.28
Serpo et al, 2007, Romania 3.60 (1.11, 11.64) 1.10
Karoli et al, 2012, India 5.39 (2.37, 12.31) 2.23
Zueff et al, 2012, Brazil 3.14 (1.30, 7.60) 1.94
Qu et al, 2013, China 2.15 (1.65, 2.82) 21.00
Tarantino et al, 2013, Italy 74.93 (4.22, 1331.55) 0.18
Bohdanowicz-Pawlak et al, 2014, Poland 1.38 (0.87, 2.18) 7.27
Plaksej et al, 2014, Poland 2.41 (1.49, 3.90) 6.58
Prasad et al, 2014, India 6.22 (3.83, 10.09) 6.45
Çağlar et al, 2015, Turkey 7.50 (1.50, 37.39) 0.59
Macut et al, 2016, Serbia & Greece 2.52 (1.68, 3.77) 9.26
Cai et al, 2017, China 2.10 (1.34, 3.29) 7.50
Kim et al, 2017, Korea 2.00 (1.04, 3.85) 3.53
Mehrabian et al, 2017, Iran 2.75 (1.31, 5.78) 2.74
Munir et al, 2017, Pakistan 6.05 (1.60, 22.90) 0.86
Petta et al, 2017, Italy 4.35 (2.61, 7.23) 5.85
Zhang et al, 2018, China 2.47 (1.31, 4.66) 3.78
Vassilatou et al, 2018, Greece 1.96 (1.23, 3.14) 6.89
Tantanavipas et al, 2019, Thailand 1.47 (0.51, 4.21) 1.36
Subtotal (I-squared = 56.8%, p = 0.001) 2.56 (2.25, 2.91) 90.38
NIH/AES
Vassilatou et al, 2010, Greece 2.33 (1.02, 5.35) 2.20
Lerchbaum et al, 2011, Austria 1.50 (0.92, 2.44) 6.43
Oztas et al, 2014, Turkey 6.71 (1.43, 31.53) 0.63
Romanowski et al, 2015, Brazil 8.73 (1.13, 67.56) 0.36
Subtotal (I-squared = 48.4%, p = 0.121) 1.96 (1.31, 2.91) 9.62
Heterogeneity between groups: p = 0.209
Overall (I-squared = 55.2%, p = 0.001) 2.49 (2.20, 2.82) 100.00
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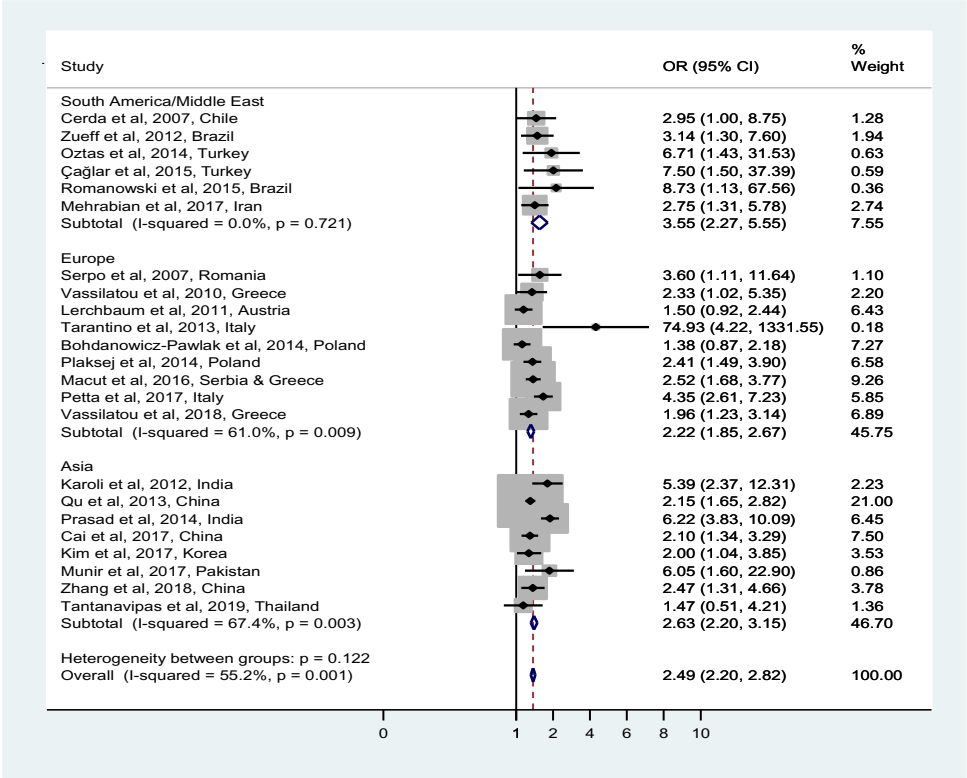
B


Study
OR (95% CI)
% Weight
South America/Middle East
Cerda et al, 2007, Chile 2.95 (1.00, 8.75) 1.28
Zueff et al, 2012, Brazil 3.14 (1.30, 7.60) 1.94
Oztas et al, 2014, Turkey 6.71 (1.43, 31.53) 0.63
Çağlar et al, 2015, Turkey 7.50 (1.50, 37.39) 0.59
Romanowski et al, 2015, Brazil 8.73 (1.13, 67.56) 0.36
Mehrabian et al, 2017, Iran 2.75 (1.31, 5.78) 2.74
Subtotal (I-squared = 0.0%, p = 0.721) 3.55 (2.27, 5.55) 7.55
Europe
Serpo et al, 2007, Romania 3.60 (1.11, 11.64) 1.10
Vassilatou et al, 2010, Greece 2.33 (1.02, 5.35) 2.20
Lerchbaum et al, 2011, Austria 1.50 (0.92, 2.44) 6.43
Tarantino et al, 2013, Italy 74.93 (4.22, 1331.55) 0.18
Bohdanowicz-Pawlak et al, 2014, Poland 1.38 (0.87, 2.18) 7.27
Plaksej et al, 2014, Poland 2.41 (1.49, 3.90) 6.58
Macut et al, 2016, Serbia & Greece 2.52 (1.68, 3.77) 9.26
Petta et al, 2017, Italy 4.35 (2.61, 7.23) 5.85
Vassilatou et al, 2018, Greece 1.96 (1.23, 3.14) 6.89
Subtotal (I-squared = 61.0%, p = 0.009) 2.22 (1.85, 2.67) 45.75
Asia
Karoli et al, 2012, India 5.39 (2.37, 12.31) 2.23
Qu et al, 2013, China 2.15 (1.65, 2.82) 21.00
Prasad et al, 2014, India 6.22 (3.83, 10.09) 6.45
Cai et al, 2017, China 2.10 (1.34, 3.29) 7.50
Kim et al, 2017, Korea 2.00 (1.04, 3.85) 3.53
Munir et al, 2017, Pakistan 6.05 (1.60, 22.90) 0.86
Zhang et al, 2018, China 2.47 (1.31, 4.66) 3.78
Tantanavipas et al, 2019, Thailand 1.47 (0.51, 4.21) 1.36
Subtotal (I-squared = 67.4%, p = 0.003) 2.63 (2.20, 3.15) 46.70
Heterogeneity between groups: p = 0.122
Overall (I-squared = 55.2%, p = 0.001) 2.49 (2.20, 2.82) 100.00
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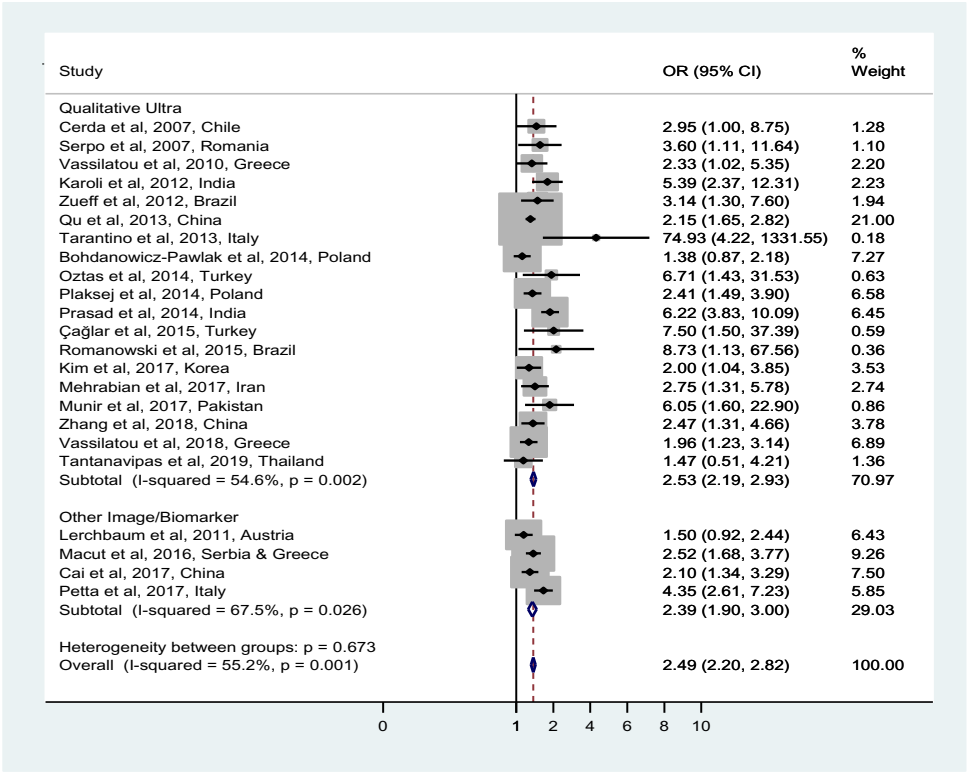
C


Study
OR (95% CI)
% Weight
Qualitative Ultra
Cerda et al, 2007, Chile 2.95 (1.00, 8.75) 1.28
Serpo et al, 2007, Romania 3.60 (1.11, 11.64) 1.10
Vassilatou et al, 2010, Greece 2.33 (1.02, 5.35) 2.20
Karoli et al, 2012, India 5.39 (2.37, 12.31) 2.23
Zueff et al, 2012, Brazil 3.14 (1.30, 7.60) 1.94
Qu et al, 2013, China 2.15 (1.65, 2.82) 21.00
Tarantino et al, 2013, Italy 74.93 (4.22, 1331.55) 0.18
Bohdanowicz-Pawlak et al, 2014, Poland 1.38 (0.87, 2.18) 7.27
Oztas et al, 2014, Turkey 6.71 (1.43, 31.53) 0.63
Plaksej et al, 2014, Poland 2.41 (1.49, 3.90) 6.58
Prasad et al, 2014, India 6.22 (3.83, 10.09) 6.45
Çağlar et al, 2015, Turkey 7.50 (1.50, 37.39) 0.59
Romanowski et al, 2015, Brazil 8.73 (1.13, 67.56) 0.36
Kim et al, 2017, Korea 2.00 (1.04, 3.85) 3.53
Mehrabian et al, 2017, Iran 2.75 (1.31, 5.78) 2.74
Munir et al, 2017, Pakistan 6.05 (1.60, 22.90) 0.86
Zhang et al, 2018, China 2.47 (1.31, 4.66) 3.78
Vassilatou et al, 2018, Greece 1.96 (1.23, 3.14) 6.89
Tantanavipas et al, 2019, Thailand 1.47 (0.51, 4.21) 1.36
Subtotal (I-squared = 54.6%, p = 0.002) 2.53 (2.19, 2.93) 70.97
Other Image/Biomarker
Lerchbaum et al, 2011, Austria 1.50 (0.92, 2.44) 6.43
Macut et al, 2016, Serbia & Greece 2.52 (1.68, 3.77) 9.26
Cai et al, 2017, China 2.10 (1.34, 3.29) 7.50
Petta et al, 2017, Italy 4.35 (2.61, 7.23) 5.85
Subtotal (I-squared = 67.5%, p = 0.026) 2.39 (1.90, 3.00) 29.03
Heterogeneity between groups: p = 0.673
Overall (I-squared = 55.2%, p = 0.001) 2.49 (2.20, 2.82) 100.00
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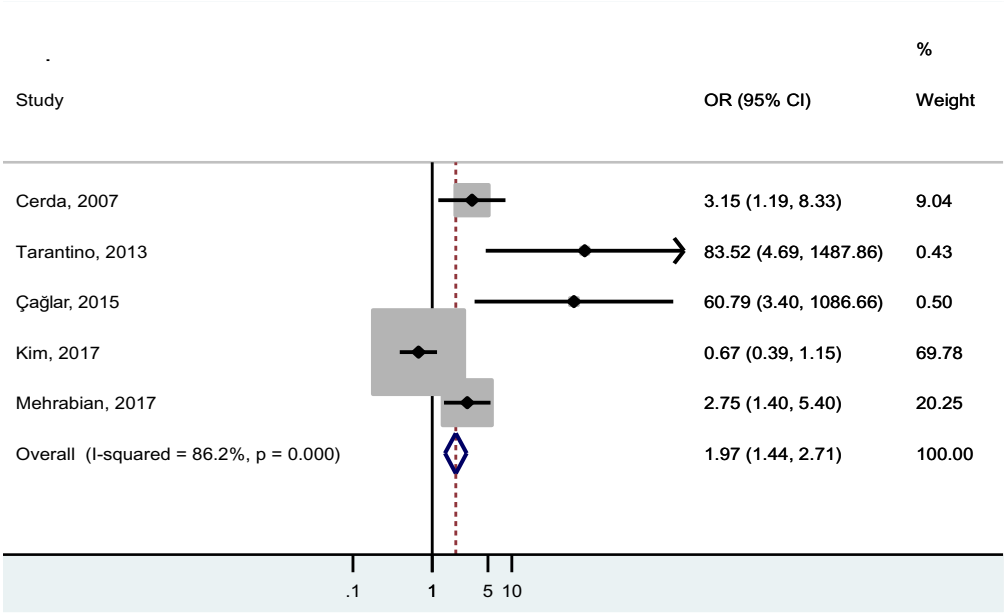
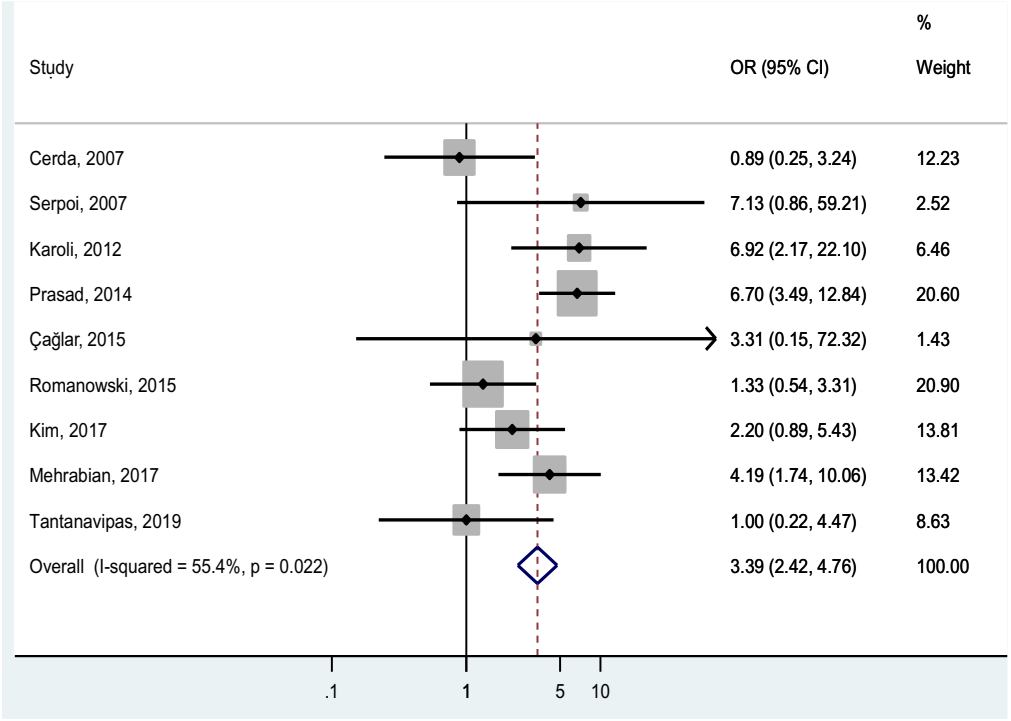
D



E



F

Figure. B. 4 Subgroup analyses.

A, study design; B, PCOS criteria; C, geographic region; D, NAFLD diagnostic tool; E, insulin resistance; F, metabolic syndrome.

TABLES

Table. B. 1 Quality assessment for cross-sectional studies

Author, year	Selection				Comparability		Outcome		Total (9*)
	Representative of sample (*)	Sample size (*)	Non-respondents (*)	Ascertainment of the exposure (*)	Control for weight and/or IR (*)	Control for additional factors (*)	Assessment of the outcome (**)	Statistical test (*)	
Cerda, 2007	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	–	6
Serpo, 2007	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	–	6
Lerchbaum, 2011	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	*(a)	7
Karoli, 2012	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	*(a)	7
Qu, 2013	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	*(a)	7
Tarantino, 2013	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	–	6
Pawlak, 2014	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	*(a)	7
Plaksej, 2014	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	*(a)	7
Prasad, 2014	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	*(a)	7
Romanowski, 2015	*(b)	–	–	*(a)	–	–	**(a)	–	4
Macut, 2016	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	*(a)	7
Cai, 2017	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	*(a)	7
Mehrabian, 2017	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	*(a)	7
Munir, 2017	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	–	6
Petta, 2017	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	*(a)	7
Zhang, 2018	*(b)	–	–	*(a)	*(a)	*(b)	*(b)	*(a)	6
Vassilatou, 2018	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	*(a)	7
Tantanavipas, 2019	*(b)	–	–	*(a)	*(a)	*(b)	**(a)	*(a)	7

The interpretation of total scores: fair to good (>5) and poor (<5). IR, insulin resistance.

Table. B. 2 Quality assessment for case control studies

Author, year	Selection				Comparability	Exposure			Total (9*)
	Case definition (*)	Representative ness of cases (*)	Selection of controls (*)	Definition of controls (*)	(**)	Ascertainment of exposure (*)	Same method of ascertainment for cases/controls (*)	Non-response rate (*)	
Vassilatou, 2010	*(a)	*(a)	−(b)	*(a)	**(a,b)	*(a)	*(a)	−(C)	7
Zueff, 2012	*(a)	*(a)	−(c)	*(a)	−−(c)	*(a)	*(a)	−(C)	5
Oztasl, 2014	*(a)	*(a)	−(b)	*(a)	−−(c)	*(a)	*(a)	−(C)	5
Çağlar, 2015	*(a)	*(a)	−(c)	*(a)	−−(c)	*(a)	*(a)	−(C)	5
Kim, 2017	*(a)	*(a)	*(a)	*(a)	**(a,b)	*(a)	*(a)	−(C)	8

Interpretation of total scores: fair to good (>5) and poor (<5).

Table. B. 3 Studies included in qualitative synthesis

ID	Region	Author	Year	Country	Study design	Enrollment period	PCOS criteria	NAFLD diagnosis	Cofactors of NAFLD
1	Europe	Serpoi	2007	Romania	Prospective	-	Rotterdam	Ultrasound	-
2		Markou	2010	Greece	Prospective	-	Rotterdam	Ultrasound + CT	-
3		Vassilatou	2010	Greece	Prospective	2006 - 2008	AES	Ultrasound + Transaminases	FAI, HOMA-IR
4		Ciotta	2011	Italy	Prospective	2010 - 2011	NIH	Ultrasound	-
5		Lerchbaum	2011	Austria	Prospective	2006 - 2010	NIH	FLI + APRI + FIB-4	-
6		Tarantino	2013	Italy	Prospective	2009 - 2011	Rotterdam	Ultrasound	-
7		Pawlak	2014	Poland	Prospective	-	Rotterdam	Ultrasound	ALT, BMI, estradiol/ testosterone ratio, fasting blood sugar
8		Kozakowski	2014	Poland	Prospective	-	Rotterdam	Ultrasound	-
9		Plaksej	2014	Poland	Prospective	-	Rotterdam	Ultrasound	-
10		Macut	2016	Serbia & Greece	Prospective	2008 - 2013	Rotterdam	NAFLD-liver fat score	HOMA-IR, lipid accumulation product
11		Petta	2017	Italy	Prospective	2005 - 2015	Rotterdam	HSI, FIB-4	FAI, WC
12		Vassilatou	2018	Greece	Prospective	2007 - 2010	Rotterdam	Ultrasound	BMI
13	Asia	Ma	2011	China	Prospective	-	Rotterdam	Ultrasound	-
14		Karoli	2012	India	Prospective	2008 - 2010	Rotterdam	Ultrasound	HDL, HOMA-IR
15		Qu	2013	China	Prospective	2008 - 2010	Rotterdam	Ultrasound	BMI, HOMA-IR, triglycerides, waist-hip ratio
16		Prasad	2014	India	Prospective	2013 - 2014	Rotterdam	Ultrasound	HDL, HOMA-IR
17		Cai	2017	China	Prospective	2013 - 2016	Rotterdam	Quantitative Ultrasound	BMI, FAI, HOMA-IR, CRP, liver fat content
18		Munir	2017	Pakistan	Prospective	2016	Rotterdam	Ultrasound	-
19		Kim	2017	Korea	Prospective	2004 - 2014	Rotterdam	Ultrasound	FAI, free testosterone
20		Zhang	2018	China	Prospective	2014 - 2015	Rotterdam	Ultrasound	BMI, HOMA-IR, triglycerides
21		Tantanavipas	2019	Thailand	Prospective	2017 - 2018	Rotterdam	Ultrasound	WC
22	South America	Cerda	2007	Chile	Retrospective	2005 - 2006	Rotterdam	Ultrasound	-
23		Zueff	2012	Brazil	Prospective	2009 -2010	Rotterdam	Ultrasound	-
24		Tock	2014	Brazil	Prospective	-	Rotterdam	Ultrasound	-
25		Romanowski	2015	Brazil	Retrospective	2008 - 2009	AES	Ultrasound	-
26	Middle East	Oztas	2014	Turkey	Prospective	2009 - 2011	AES	Ultrasound	anti-Müllerian hormone, sd-LDL
27		Çağlar	2015	Turkey	Prospective	-	Rotterdam	Ultrasound	ALT, VLDL
28		Mehrabian	2017	Iran	Retrospective	2013 - 2014	Rotterdam	Ultrasound	ALT, BMI, IR
29	North American	Gambarin-Gelwan	2007	USA	Retrospective	2004	NIH	Ultrasound	-

AES, androgen excess and PCOS society; ALT, alanine aminotransferase; BMI, body mass index; CRP, C-reactive protein; CT, computerized tomography; FAI, free androgen index; FIB-4, fibrosis 4; FLI, fatty liver index; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; HSI, hepatic steatosis index; IR, insulin resistance; NIH, national institutes of health; sd-LDL, small density low density lipoprotein; VLDL, very low density lipoprotein; WC, waist circumference.

Table. B. 4 Studies included in quantitative analysis

ID	Author	PCOS					Controls					OR (95% CI)
		n	Age (years)	BMI (kg/m^2)	MetS	NAFLD %, (n)	n	Age (years)	BMI (kg/m^2)	MetS	NAFLD (%), n	
1	Cerda, 2007	41	24.6 ± 7	30.3 ± 7	14.6%	42% (17)	31	27.9 ± 7	29.3 ± 5	16.1%	19% (6)	2.95 (0.99-8.74)
2	Serpoi, 2007	44	29.3 ± 7	27.3 ± 6	27%	55% (24)	20	31.8 ± 6	26.9 ±	5%	25% (5)	3.6 (1.11-11.63)
3	Vassilatou, 2010	57	27 ± 8	28.3 ± 8	14%	37% (21)	60	27.6 ± 7	27 ± 8	6.6%	20% (21)	2.33 (1.02-5.35)
4	Lerchbaum, 2011	611	27 (23-31)‡	24.5 (21.4-29.4)‡	12.1%	23% (43)	139	30 (26–37)‡	24.1 (20.9–28.8)‡	4.9%	17% (23)	1.49 (0.92-2.43)
5	Karoli, 2012	54	28.5 ± 6	27.2 ± 5	35%	67% (35)	55	27.8 ± 8	26.8 ± 7	7%	25% (14)	5.39 (2.36-12.30)
6	Zueff, 2012	45	31.6 ± 4	34.7 ± 3	-	73% (33)	45	31.7 ± 4	34.5 ± 3	-	47% (21)	3.14 (1.29-7.59)
7	Qu, 2013	602	28.7 ± 4	29.1 ± 3	-	33% (198)	588	28.1 ± 4	23 ± 3	-	19% (109)	2.15 (1.64-2.81)
8	Tarantino, 2013	40	27.7 ± 6	28.1 ± 7	-	65% (26)	20	26.2 ± 4	22.1 ± 2	-	0	61.08 (3.52-1057.84)
9	Pawlak, 2014	184	-	-	-	58% (106)	125	-	-	-	50% (62)	1.38 (0.87-2.17)
10	Oztas, 2014	58	24.4 ± 3	21.9 ± 2	-	41% (24)	21	24.5 ± 3	21.8 ± 1	-	10% (2)	6.70 (1.42-31.52)
11	Plaksej, 2014	172	25.3 ± 6	28.7 ± 7	-	53% (92)	125	27.7 ± 6	26.4 ± 6	-	32% (40)	2.41 (1.49-
12	Prasad, 2014	162	27.6 ± 7	27.6 ± 6	36%	66% (106)	165	27.9 ± 8	26.9 ± 7	8%	23% (38)	6.21 (3.82 -10.09)
13	Çağlar, 2015	34	26 ± 3	22 ± 1	5.8%	44% (15)	21	26 ± 3	22.1 ± 2	0%	10% (2)	7.5 (1.50-37.39)
14	Romanowski, 2015	101	26.8 ± 5	28.5 ± 6	32.7%	24% (24)	30	33.7 ± 7	26.1 ± 4	26.6%	3% (1)	8.72 (1.12-67.56)
15	Macut, 2016	600	25.6 (25.1-26.1)†	30.7 (30.1–31.3)†	35.7%	51% (366)	125	31.4 (30.4-32.4)†	29.4 (28.1–30.7)†	29.4%	34% (42)	2.51 (1.67-3.76)
16	Cai, 2017	400	25.8 ± 5	25.6 ± 5	-	56% (225)	100	26.8 ± 6	24.7 ± 5	-	38% (38)	2.09 (1.33-3.28)
17	Kim, 2017	275	30.4 ± 5	20.3 ± 2	2.9%	6% (15)	892	35.1 ± 4	19.9 ± 2	1.3%	3% (25)	2 (1.03-3.85)
18	Mehrabian, 2017	75	-	24.7 ± 2	33.3%	39% (29)	75	-	24.6 ± 2	10.7%	19% (14)	2.74 (1.30-5.77
19	Munir, 2017	30	25.8 ± 6	31.9 ± 6	-	73% (22)	16	30.6 ± 7	29.2 ± 6	-	31% (5)	6.05 (1.59-22.90)
20	Petta, 2017	202	33.2 ± 6	25.7 ± 3	-	69% (139)	101	34.9 ± 8	23.9 ± 3	-	33% (34)	4.34 (2.61-7.23)
21	Zhang, 2018	188	27.1 ± 5	25.1 ± 3	-	45% (84)	65	26.9 ± 5	24.2 ± 3	-	25% (16)	2.47 (1.31-4.66)
22	Vassilatou, 2018	145	27.5 ± 7	31.8 ± 7	-	54% (78)	145	32.1 ± 8	30.5 ± 7	-	37% (54)	1.96 (1.22-3.13)
23	Tantanavipas, 2019	42	27.7 ± 5	27 ± 6	14.2%	52% (22)	21	31.4 ± 6	25.7 ± 5	14.2%	43% (9)	1.46 (0.51-4.21)

Data expressed in mean ± SD or percentage, unless otherwise indicated.

† mean & 95% CI.

‡ median with interquartile range.

BMI, body mass index; CI, confidence interval; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; PCOS, polycystic ovary syndrome.

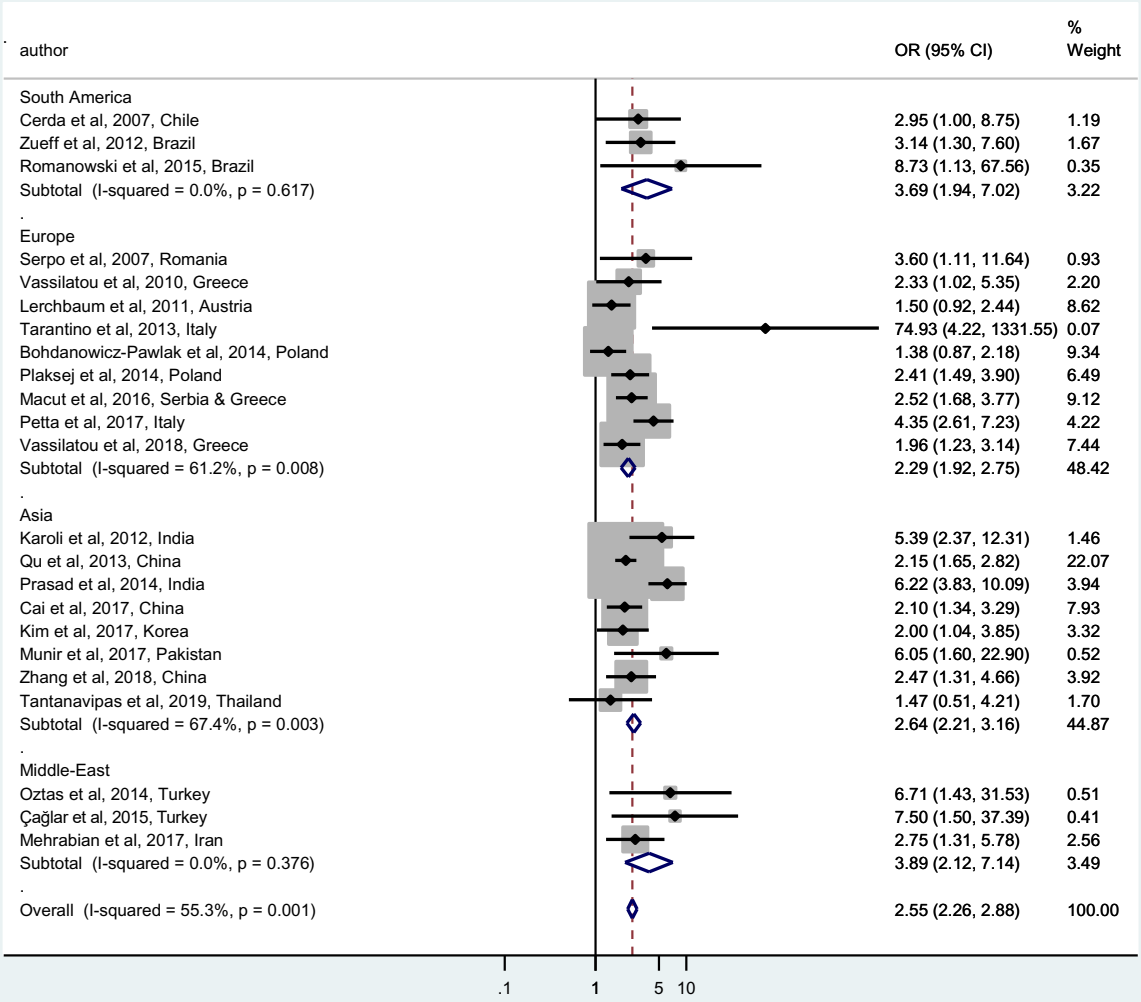
Table. B. 5 Subgroup analyses for risk of NAFLD in PCOS patients

Stratification	Subgroup	Number of studies	OR (95% CI)	I^2 (%)	p-value
		23	2.49 (2.20-2.82)	55.2	0.001
Study design	Cross-sectional	18	2.47 (2.17-2.81)	62.2	<0.001
	Case-control	5	2.73 (1.81-4.12)	0	0.418
PCOS criteria	Rotterdam	19	2.56 (2.25-2.91)	56.8	0.001
	AES/NIH	4	1.96 (1.31-2.91)	48.4	0.121
Geographic region	South America/Middle East	6	3.55 (2.27-5.55)	0	0.721
	Europe	9	2.22 (1.85-2.67)	61	0.009
	Asia	8	2.63 (2.20-3.15)	67.4	0.003
NAFLD diagnosis	Ultrasound	19	2.53 (2.19-2.93)	54.6	0.002
	Other imaging/noninvasive biomarkers	4	2.39 (1.90-3.00)	67.5	0.026
Insulin resistance	PCOS vs. Controls	5	1.97 (1.44-2.71)	86.2	<0.001
Metabolic syndrome	PCOS vs. Controls	9	3.39 (2.42-4.76)	55.4	0.022

AES, androgen excess and PCOS society; HOMA-IR, homeostatic model assessment of insulin resistance; NAFLD, nonalcoholic fatty liver disease; NIH, national institutes of health; PCOS, polycystic ovary syndrome.

SUPPLEMENTARY MATERIALS

Supplemental. 1 Subgroup analysis based on geographic location: South American and Middle East population separated.



Supplemental. 2 Meta-regression of study characteristics.

Variable	Regression coefficient (95% CI)	p-value
Study with matching controls	-0.386 (-1.047, 0.274)	0.238
Studies with aOR	-0.408 (-2.262, 1.445)	0.652
Study design	0.877 (-1.323, 3.079)	0.416
NAFLD diagnostic modality	-1.127 (-3.439, 1.184)	0.322
Study quality	-2.219 (-3.927,-0.511)	0.013

aOR, adjusted odds ratio; CI, confidence interval; NAFLD, nonalcoholic fatty liver disease.

Supplemental. 3 Meta-regression of cofactors of NAFLD.

Variable	Number of studies	Regression coefficient (95% CI)	p-value
Age	23	-0.106 (-0.511, 0.298)	0.588
HOMA-IR	7	-0.335 (-2.308, 1.636)	0.727
BMI	6	-1.929 (-3.776, -0.082)	0.041
FAI	4	-1.021 (-3.420, 1.377)	0.385

BMI, body mass index; CI, confidence interval; FAI, free androgen index; HOMA-IR, homeostatic model assessment of insulin resistance.

Supplemental. 4 Appx 1. PRISMA Checklist

Section/topic	#	Checklist item
TITLE		
Title	1	Identify the report as a systematic review, meta-analysis, or both.
ABSTRACT		
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.
INTRODUCTION		
Rationale	3	Describe the rationale for the review in the context of what is already known.
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).
METHODS		
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.

RESULTS		
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).
DISCUSSION		
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.
FUNDING		
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097.

For more information, visit: **www.prisma-statement.org**.

Supplemental. 5 Appx 2. Search Strategy

Databases Searched

Africa-Wide Information [EBSCO] (June 1, 2018)

S10	S4 AND S9	11
S9	S5 OR S6 OR S7 OR S8	8,082
S8	TI (((liver* or hepatic*) N2 (index* or eval* or test?))) OR AB (((liver* or hepatic*) N2 (index* or eval* or test?))) OR KW (((liver* or hepatic*) N2 (index* or eval* or test?)))	1,855
S7	TI (((reye* N2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* N1 fever*))) OR AB (((reye* N2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* N1 fever*))) OR KW (((reye* N2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* N1 fever*)))	116
S6	TI ((steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) N3 fibros*))) OR AB ((steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) N3 fibros*))) OR KW ((steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) N3 fibros*)))	5,438
S5	TI ((NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) N2 (liver* or hepatic* or intrahepat* or biliar* or hepato*)))) OR AB ((NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) N2 (liver* or hepatic* or intrahepat* or biliar* or hepato*)))) OR KW ((NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) N2 (liver* or hepatic* or intrahepat* or biliar* or hepato*))))	1,379
S4	S1 OR S2 OR S3	946
S3	TI ((hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*)) OR AB ((hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*)) OR KW ((hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*))	389
S2	TI (((ovar* N1 cystic*) or (multi* N5 (ovar* or follicl*) N5 cyst*))) OR AB (((ovar* N1 cystic*) or (multi* N5 (ovar* or follicl*) N5 cyst*))) OR KW (((ovar* N1 cystic*) or (multi* N5 (ovar* or follicl*) N5 cyst*)))	60
S1	TI ((PCOS or ((ovar* or syndrom*) N2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* N5 leventhal*)))))) OR AB ((PCOS or ((ovar* or syndrom*) N2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* N5 leventhal*)))))) OR KW ((PCOS or ((ovar* or syndrom*) N2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* N5 leventhal*)))))	602

UPDATE Africa-Wide Information [EBSCO] (February 1, 2020) NO NEW RESULTS

S11	S4 AND S9 - Year Published: 2018-2020	0
S10	S4 AND S9	12
S9	S5 OR S6 OR S7 OR S8	8,263
S8	TI (((liver* or hepatic*) N2 (index* or eval* or test?))) OR AB (((liver* or hepatic*) N2 (index* or eval* or test?))) OR KW (((liver* or hepatic*) N2 (index* or eval* or test?)))	1,468
S7	TI (((reye* N2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* N1 fever*))) OR AB (((reye* N2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* N1 fever*))) OR KW (((reye* N2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* N1 fever*)))	114
S6	TI ((steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) N3 fibros*))) OR AB ((steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) N3 fibros*))) OR KW ((steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) N3 fibros*)))	5,832
S5	TI ((NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) N2 (liver* or hepatic* or intrahepat* or biliar* or hepato*)))) OR AB ((NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) N2 (liver* or hepatic* or intrahepat* or biliar* or hepato*)))) OR KW ((NAFLD	1,543

	or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) N2 (liver* or hepatic* or intrahepat* or biliar* or hepato*))))	
S4	S1 OR S2 OR S3	980
S3	TI ((hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgenization* or androgenization*)) OR AB ((hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgenization* or androgenisation*)) OR KW ((hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgenization* or androgenisation*))	396
S2	TI (((ovar* N1 cystic*) or (multi* N5 (ovar* or follicl*) N5 cyst*))) OR AB (((ovar* N1 cystic*) or (multi* N5 (ovar* or follicl*) N5 cyst*))) OR KW (((ovar* N1 cystic*) or (multi* N5 (ovar* or follicl*) N5 cyst*)))	65
S1	TI ((PCOS or ((ovar* or syndrom*) N2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* N5 leventhal*))))) OR AB ((PCOS or ((ovar* or syndrom*) N2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* N5 leventhal*))))) OR KW ((PCOS or ((ovar* or syndrom*) N2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* N5 leventhal*)))))	638

Biosis [Ovid] (June 1, 2018) until 2017

BIOSIS Previews 1969 to 2017 Week 47, BIOSIS Previews Archive 1926 to 1968.

1	(PCOS or ((ovar* or syndrom*) adj2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* adj5 leventhal*))))).ti,ab,mi.	12114
2	((ovar* adj1 cystic*) or (multi* adj5 (ovar* or follicl*) adj5 cyst*)).ti,ab,mi.	1939
3	(hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*).ti,ab,mi.	11849
4	1 or 2 or 3	22246
5	(NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) adj2 (liver* or hepatic* or intrahepat* or biliar* or hepato*))).ti,ab,mi.	40084
6	(steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) adj3 fibros*)).ti,ab,mi.	112430
7	((reye* adj2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* adj1 fever*)).ti,ab,mi.	1518
8	((liver* or hepatic*) adj2 (index* or eval* or test?)).ti,ab,mi.	17558
9	5 or 6 or 7 or 8	155468
10	4 and 9	240
11	(animal* not (animal* and hominidae)).st,tn.	8906143
12	((animal or animals or canine* or cat or cats or dog or dogs or feline or hamster* or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or veterinar*) not (human* or patient*)).ti,mi.	3489126
13	10 not (11 or 12)	217
14	remove duplicates from 13	185

Biosis [via Clarivate Analytics] (2018) VIA WEB OF SCIENCE - 2017-2018

Indexes=BCI

#15	12	#12 NOT #13 *Indexes=BCI Timespan=2017-2018*
#14	116	#12 NOT #13
#13	154	PMID=("21157321" or "26412465" or "21910085" or "16198620" or "29184820" or "21454995" or "24829577" or "28457031" or "29024702" or "4134992" or "27310544" or "450019" or "16311212" or "28285710" or "21832111" or "26761949" or "26589977" or "23721173" or "21251033" or "26634686" or "27251917" or "19576817" or "18000969" or "28339345" or "5565843" or "18344805" or "16503761" or "25554608" or "18830783" or "23798298" or "23386652" or "25479351" or "29264465" or "19461374" or "21301018" or "15987930" or "7577849" or "16191492" or "21495873" or "18417315" or "21366813" or "19215335" or "18230819" or "28487551" or "18497727" or "24648300" or "17320032" or "17624467" or "16767302" or "27176209" or "25211442" or "29750655" or "24994518" or "17560682" or "27068725" or "27919367" or "27107966" or "23980352" or "17089862" or "22173246" or "23612512" or "23185203" or "21926952" or "28942551" or "5832873" or "8088001" or "27804265" or "29264544" or "16011474" or "19622617" or "18400637" or "16532658" or "179515" or "25600030" or "28705172" or "23746214" or "26972165" or "28737293" or "20046314" or "24973357" or "27464726" or "24222096" or "11322868" or "29406938" or "23849162" or "16269843" or "27319232" or "26202291" or "25941433" or "23367497" or "15692481" or "19671519" or "15262299" or "8723563" or "16137470" or "25019404" or "17496361" or "19352053" or "2016368" or "15811215" or "1916463" or "8481214" or "21985780" or "28681988" or "15043244" or "17287148" or "21569072" or "11685939" or "20534754" or "27219496" or "22262974" or "27504851" or "29090431" or "27054776" or "28901968" or "27505055" or "3578443" or "29264552" or "23047930" or "26728862" or

		"17330572" or "21805747" or "26210619" or "21057159" or "26114995" or "19923096" or "23978196" or "19098117" or "12647844" or "8927344" or "10663856" or "11315291" or "26101431" or "21413894" or "25293340" or "17035653" or "7447186" or "21114431" or "10395191" or "23938069" or "23108199" or "20870709" or "12930161" or "28712591" or "21865363" or "21632811" or "20883985" or "11668286" or "657160" or "25198445" or "23777024" or "21897446" or "27428059" or "28222828" or "22837189" or "6460612" or "24256515" or "2955749" or "1830560" or "26968296" or "27132324" or "18389188" or "23869143" or "23627982" or "20001571" or "19622949" or "25339805" or "24962189" or "10767668" or "24434909" or "28370150" or "28883861" or "2884725" or "3952483" or "8256457" or "26976018" or "23029709" or "25136364" or "29590099" or "6448264" or "25905173" or "15649101" or "27351028" or "29432207" or "21937505" or "20590731" or "29128060" or "4356759" or "19347015" or "21190980" or "17709876" or "28694246" or "27076501" or "14669838" or "27424378" or "27792214" or "26494962" or "19171337" or "20301444" or "18386674" or "27256547" or "15645693" or "15670668" or "7632042" or "29114377" or "25994073" or "24034940" or "27594563" or "6828272" or "17058987" or "9709937" or "3169499" or "25755422" or "26405614" or "20961180" or "17561981" or "10075099" or "24923289" or "23158933" or "24641921" or "20456209" or "15767536" or "25644898" or "29201403" or "24183950" or "18185059" or "26979020" or "21283374" or "12527343" or "19809879" or "18505888" or "25069836" or "28776742" or "27838603" or "20371655" or "23857978" or "23155707" or "9935128" or "21257877" or "9235083" or "15040152" or "16912555" or "17934394" or "29161258" or "26203576" or "22908056" or "29170905" or "26436371" or "25402369" or "22082482" or "11443175" or "19284314" or "18565127" or "16203173" or "15115430" or "26517932" or "24639812" or "21029639" or "25598791" or "4822971" or "22829562" or "29083730" or "14749503" or "16489319" or "25707325" or "18422468" or "28612285" or "29209995" or "20227029" or "10438402" or "26039829" or "24019220" or "18220635" or "28455679" or "26913880" or "27456105" or "28699503" or "28291240" or "22096112" or "11388600" or "17986832" or "739279" or "26034812" or "14266624" or "19573754" or "21437093" or "15705403" or "2080856" or "26865583" or "17592439" or "17275449" or "24859638" or "16492691" or "15703460" or "18458706" or "29641459" or "11026085" or "2405587" or "19124394" or "26027246" or "5676174" or "24240117" or "23826984" or "2802082" or "1180424" or "22498245" or "28123936" or "16401000" or "16128250" or "19245356" or "16444867" or "19906783" or "25873949" or "12566753" or "22805002" or "18437728" or "26024975" or "19542757" or "15181020" or "26522459" or "27193136" or "24246334" or "12640725" or "4753429" or "25832152" or "27218012" or "25024594" or "19887498" or "26340970" or "24026869" or "25245380" or "2975629" or "15773753" or "15908053" or "22117616" or "21307133" or "26486483" or "8629139" or "15151470" or "26201622" or "3620420" or "28965028" or "29587769" or "22382613" or "25662524" or "22248781" or "19519467" or "29747678" or "26650609" or "5105082" or "25923022" or "15356308" or "16771946" or "29725371" or "18683746" or "26502288" or "21898634" or "4321497")
# 12	246	#10 NOT #11
# 11	2,613,418	TI=((animal or animals or canine* or cat or cats or dog or dogs or feline or hamster* or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or veterinar*) not (human* or patient*))
# 10	259	#9 AND #4
# 9	173,373	#8 OR #7 OR #6 OR #5
# 8	19,092	TS=((liver* or hepatic*) NEAR/2 (index* or eval* or test?))
# 7	1,580,	TS=((reye* NEAR/2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* NEAR/1 fever*))
# 6	1236,410	TS=(steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) NEAR/3 fibros*))
# 5	49,588	TS=(NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) NEAR/2 (liver* or hepatic* or intrahepat* or biliar* or hepato*)))
# 4	22,691	#3 OR #2 OR #1
# 3	12,252	TS=(hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*)
# 2	2,171	TS=((ovar* NEAR/1 cystic*) or (multi* NEAR/5 (ovar* or follicl*) NEAR/5 cyst*))
# 1	12,085	TS=(PCOS or ((ovar* or syndrom*) NEAR/2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* NEAR/5 leventhal*))))

UPDATE Biosis [via Clarivate Analytics] (February 1, 2020)

Indexes=BCI

#13	50	#10 NOT #11 *imespan=2018-2020*
# 12	298	#10 NOT #11
# 11	2,703,186	TI=((animal or animals or canine* or cat or cats or dog or dogs or feline or hamster* or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or veterinar*) not (human* or patient*))
# 10	313	#9 AND #4
# 9	192,190	#8 OR #7 OR #6 OR #5
# 8	20,741	TS=((liver* or hepatic*) NEAR/2 (index* or eval* or test?))
# 7	1,592	TS=((reye* NEAR/2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* NEAR/1 fever*))
# 6	139,640	TS=(steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) NEAR/3 fibros*))
# 5	58,528	TS=(NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) NEAR/2 (liver* or hepatic* or intrahepat* or biliar* or hepato*)))
# 4	24,255	#3 OR #2 OR #1

# 3	12,764	TS=(hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*)
# 2	2,236	TS=((ovar* NEAR/1 cystic*) or (multi* NEAR/5 (ovar* or follicl*) NEAR/5 cyst*))
# 1	13,345	TS=(PCOS or ((ovar* or syndrom*) NEAR/2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* NEAR/5 leventhal*))))

Cochrane [Wiley] (June 1, 2018)

1	(PCOS or ((ovar* or syndrom*) NEAR/2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* NEAR/5 leventhal*)))):ti,ab,kw	2801
2	((ovar* NEAR/1 cystic*) or (multi* NEAR/5 (ovar* or follicl*) NEAR/5 cyst*)):ti,ab,kw	46
3	(hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*):ti,ab,kw	999
4	#1 or #2 or #3	3285
5	(NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) NEAR/2 (liver* or hepatic* or intrahepat* or biliar* or hepato*))):ti,ab,kw	2684
6	(steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) NEAR/3 fibros*)):ti,ab,kw	9705
7	((reye* NEAR/2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* NEAR/1 fever*)):ti,ab,kw	10
8	((liver* or hepatic*) NEAR/2 (index* or eval* or test?)):ti,ab,kw	2822
9	#5 or #6 or #7 or #8	13326
10	#4 and #9	43

UPDATE Cochrane [Wiley] (February 1, 2020)

1	(PCOS or ((ovar* or syndrom*) NEAR/2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* NEAR/5 leventhal*)))):ti,ab,kw	4041
2	((ovar* NEAR/1 cystic*) or (multi* NEAR/5 (ovar* or follicl*) NEAR/5 cyst*)):ti,ab,kw	146
3	(hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*):ti,ab,kw	1493
4	#1 or #2 or #3	4687
5	(NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) NEAR/2 (liver* or hepatic* or intrahepat* or biliar* or hepato*))):ti,ab,kw	4414
6	(steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) NEAR/3 fibros*)):ti,ab,kw	12566
7	((reye* NEAR/2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* NEAR/1 fever*)):ti,ab,kw	18
8	((liver* or hepatic*) NEAR/2 (index* or eval* or test?)):ti,ab,kw	5118
9	#5 or #6 or #7 or #8	19138
10	#4 and #9	100
11	#4 and #9 with Cochrane Library publication date from May 2018 to Feb 2020	58

Embase [Ovid] (June 1, 2018)

Embase Classic+Embase 1947 to 2018 May 31

1	ovary polycystic disease/	24251
2	(PCOS or ((ovar* or syndrom*) adj2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* adj5 leventhal*))))).tw,kw.	23683
3	((ovar* adj1 cystic*) or (multi* adj5 (ovar* or follicl*) adj5 cyst*)).tw,kw.	2155
4	hyperandrogenism/	6600
5	gonadal disease/	2198
6	hirsutism/	12253
7	(hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*).tw,kw.	19600
8	or/1-7	49572

9	exp fatty liver/	64322
10	(NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) adj2 (liver* or hepatic* or intrahepat* or biliar* or hepato*))).tw,kw.	59521
11	exp liver cirrhosis/	158862
12	(steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) adj3 fibros*)).tw,kw.	195288
13	((reye* adj2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* adj1 fever*)).tw,kw.	2208
14	((liver* or hepatic*) adj2 (index* or eval* or test?)).tw,kw.	40622
15	or/9-14	307929
16	8 and 15	1126
17	limit 16 to (conference abstract or conference paper or conference proceeding or "conference review")	241
18	((animal or animals or canine* or cat or cats or dog or dogs or feline or hamster* or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep*) not (human* or patient*)).ti,kw.	2495329
19	17 not 18	233
20	16 not 17	885
21	(exp animal/ or exp juvenile animal/ or adult animal/ or animal cell/ or animal experiment/ or animal model/ or animal tissue/ or nonhuman/) not human/	6895396
22	20 not (18 or 21)	856
23	19 or 22	1089
24	remove duplicates from 23	1063
25	("21157321" or "26412465" or "21910085" or "16198620" or "29184820" or "21454995" or "24829577" or "28457031" or "29024702" or "4134992" or "27310544" or "450019" or "16311212" or "28285710" or "21832111" or "26761949" or "26589977" or "23721173" or "21251033" or "26634686" or "27251917" or "19576817" or "18000969" or "28339345" or "5565843" or "18344805" or "16503761" or "25554608" or "18830783" or "23798298" or "23386652" or "25479351" or "29264465" or "19461374" or "21301018" or "15987930" or "7577849" or "16191492" or "21495873" or "18417315" or "21366813" or "19215335" or "18230819" or "28487551" or "18497727" or "24648300" or "17320032" or "17624467" or "16767302" or "27176209" or "25211442" or "29750655" or "24994518" or "17560682" or "27068725" or "27919367" or "27107966" or "23980352" or "17089862" or "22173246" or "23612512" or "23185203" or "21926952" or "28942551" or "5832873" or "8088001" or "27804265" or "29264544" or "16011474" or "19622617" or "18400637" or "16532658" or "179515" or "25600030" or "28705172" or "23746214" or "26972165" or "28737293" or "20046314" or "24973357" or "27464726" or "24222096" or "11322868" or "29406938" or "23849162" or "16269843" or "27319232" or "26202291" or "25941433" or "23367497" or "15692481" or "19671519" or "15262299" or "8723563" or "16137470" or "25019404" or "17496361" or "19352053" or "2016368" or "15811215" or "1916463" or "8481214" or "21985780" or "28681988" or "15043244" or "17287148" or "21569072" or "11685939" or "20534754" or "27219496" or "22262974" or "27504851" or "29090431" or "27054776" or "28901968" or "27505055" or "3578443" or "29264552" or "23047930" or "26728862" or "17330572" or "21805747" or "26210619" or "21057159" or "26114995" or "19923096" or "23978196" or "19098117" or "12647844" or "8927344" or "10663856" or "11315291" or "26101431" or "21413894" or "25293340" or "17035653" or "7447186" or "21114431" or "10395191" or "23938069" or "23108199" or "20870709" or "12930161" or "28712591" or "21865363" or "21632811" or "20883985" or "11668286" or "657160" or "25198445" or "23777024" or "21897446" or "27428059" or "28222828" or "22837189" or "6460612" or "24256515" or "2955749" or "1830560" or "26968296" or "27132324" or "18389188" or "23869143" or "23627982" or "20001571" or "19622949" or "25339805" or "24962189" or "10767668" or "24434909" or "28370150" or "28883861" or "2884725" or "3952483" or "8256457" or "26976018" or "23029709" or "25136364" or "29590099" or "6448264" or "25905173" or "15649101" or "27351028" or "29432207" or "21937505" or "20590731" or "29128060" or "4356759" or "19347015" or "21190980" or "17709876" or "28694246" or "27076501" or "14669838" or "27424378" or "27792214" or "26494962" or "19171337" or "20301444" or "18386674" or "27256547" or "15645693" or "15670668" or "7632042" or "29114377" or "25994073" or "24034940" or "27594563" or "6828272" or "17058987" or "9709937" or "3169499" or "25755422" or "26405614" or "20961180" or "17561981" or "10075099" or "24923289" or "23158933" or "24641921" or "20456209" or "15767536" or "25644898" or "29201403" or "24183950" or "18185059" or "26979020" or "21283374" or "12527343" or "19809879" or "18505888" or "25069836" or "28776742" or "27838603" or "20371655" or "23857978" or "23155707" or "9935128" or "21257877" or "9235083" or "15040152" or "16912555" or "17934394" or "29161258" or "26203576" or "22908056" or "29170905" or "26436371" or "25402369" or "22082482" or "11443175" or "19284314" or "18565127" or "16203173" or "15115430" or "26517932" or "24639812" or "21029639" or "25598791" or "4822971" or "22829562" or "29083730" or "14749503" or "16489319" or "25707325" or "18422468" or "28612285" or "29209995" or "20227029" or "10438402" or "26039829" or "24019220" or "18220635" or "28455679" or "26913880" or "27456105" or "28699503" or "28291240" or "22096112" or "11388600" or "17986832" or "739279" or "26034812" or "14266624" or "19573754" or "21437093" or "15705403" or "2080856" or "26865583" or "17592439" or "17275449" or "24859638" or "16492691" or "15703460" or "18458706" or "29641459" or "11026085" or "2405587" or "19124394" or "26027246" or "5676174" or "24240117" or "23826984" or "2802082" or "1180424" or "22498245" or "28123936" or "16401000" or "16128250" or "19245356" or "16444867" or "19906783" or "25873949" or "12566753" or "22805002" or "18437728" or "26024975" or "19542757" or "15181020" or "26522459" or "27193136" or "24246334" or "12640725" or "4753429" or "25832152" or "27218012" or "25024594" or "19887498" or "26340970" or "24026869" or "25245380" or "2975629" or "15773753" or "15908053" or "22117616" or "21307133" or "26486483" or "8629139" or "15151470" or "26201622" or "3620420" or "28965028" or "29587769" or "22382613" or "25662524" or "22248781" or "19519467" or "29747678" or "26650609" or "5105082" or "25923022" or "15356308" or "16771946" or "29725371" or "18683746" or "26502288" or "21898634" or "4321497").pm.	301
26	24 not 25	788

UPDATE Embase [Ovid] (February1, 2020)

Embase Classic+Embase 1947 to 2020 January 31

1	ovary polycystic disease/	27452
2	(PCOS or ((ovar* or syndrom*) adj2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* adj5 leventhal*))))).tw,kw.	26858
3	((ovar* adj1 cystic*) or (multi* adj5 (ovar* or follicl*) adj5 cyst*)).tw,kw.	2312
4	hyperandrogenism/	7329
5	gonadal disease/	2263
6	hirsutism/	12995
7	(hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*).tw,kw.	21174
8	or/1-7	54381
9	exp fatty liver/	76351
10	(NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) adj2 (liver* or hepatic* or intrahepat* or biliar* or hepato*))).tw,kw.	72382

11	exp liver cirrhosis/	175409
12	(steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) adj3 fibros*)).tw,kw.	219732
13	((reye* adj2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* adj1 fever*)).tw,kw.	2241
14	((liver* or hepatic*) adj2 (index* or eval* or test?)).tw,kw.	45352
15	or/9-14	346442
16	8 and 15	1319
17	limit 16 to (conference abstract or conference paper or conference proceeding or "conference review")	292
18	((animal or animals or canine* or cat or cats or dog or dogs or feline or hamster* or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep*) not (human* or patient*)).ti,kw.	2620910
19	17 not 18	280
20	16 not 17	1027
21	(exp animal/ or exp juvenile animal/ or adult animal/ or animal cell/ or animal experiment/ or animal model/ or animal tissue/ or nonhuman/) not human/	7355240
22	20 not (18 or 21)	991
23	19 or 22	1271
24	remove duplicates from 23	1246
25	limit 24 to yr="2018 -Current"	197

Global Health [Ovid] (June 1, 2018)

Global Health 1973 to 2018 Week 21, Global Health Archive 1910 to 1972

1	(PCOS or ((ovar* or syndrom*) adj2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* adj5 leventhal*))))).ti,ab,id.	2330
2	((ovar* adj1 cystic*) or (multi* adj5 (ovar* or follicl*) adj5 cyst*)).ti,ab,id.	83
3	(hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*).ti,ab,id.	938
4	1 or 2 or 3	2731
5	(NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) adj2 (liver* or hepatic* or intrahepat* or biliar* or hepato*))).ti,ab,id.	14427
6	(steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) adj3 fibros*)).ti,ab,id.	31808
7	((reye* adj2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* adj1 fever*)).ti,ab,id.	280
8	((liver* or hepatic*) adj2 (index* or eval* or test?)).ti,ab,id.	5998
9	5 or 6 or 7 or 8	42186
10	4 and 9	83
11	Animals/ not (Animals/ and Humans/)	814056
12	((animal or animals or canine* or cat or cats or dog or dogs or feline or hamster* or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or veterinar*) not (human* or patient*)).ti,id.	344248
13	10 not (11 or 12)	77
14	remove duplicates from 13	77

UPDATE Global Health [Ovid] (February 1, 2020)

Global Health 1973 to 2020 Week 04, Global Health Archive 1910 to 1972

1	(PCOS or ((ovar* or syndrom*) adj2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* adj5 leventhal*))))).ti,ab,id.	2741
2	((ovar* adj1 cystic*) or (multi* adj5 (ovar* or follicl*) adj5 cyst*)).ti,ab,id.	96
3	(hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*).ti,ab,id.	1047
4	1 or 2 or 3	3175
5	(NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) adj2 (liver* or hepatic* or intrahepat* or biliar* or hepato*))).ti,ab,id.	16653
6	(steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) adj3 fibros*)).ti,ab,id.	35889
7	((reye* adj2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* adj1 fever*)).ti,ab,id.	282
8	((liver* or hepatic*) adj2 (index* or eval* or test?)).ti,ab,id.	6634
9	5 or 6 or 7 or 8	47368
10	4 and 9	94
11	Animals/ not (Animals/ and Humans/)	859787
12	((animal or animals or canine* or cat or cats or dog or dogs or feline or hamster* or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or veterinar*) not (human* or patient*)).ti,id.	367480
13	10 not (11 or 12)	87
14	remove duplicates from 13	87
15	limit 14 to yr="2018 -Current"	12

Global Index Medicus [WHO] (May 28, 2018)

2	tw:((tw:(polycystic ovar* OR pcos)) AND (tw:(fat* liver* OR nafld OR nash OR steatohep* OR steatosis OR cirrhos* OR cirrhotic*))) AND (instance:"ghl") AND (db:("LILACS" OR "WPRIM" OR "IMEMR" OR "IMSEAR" OR "AIM"))	222
1	(tw:(polycystic ovar* OR pcos)) AND (tw:(fat* liver* OR nafld OR nash OR steatohep* OR steatosis OR cirrhos* OR cirrhotic*))	6.324

UPDATE Global Index Medicus [WHO] (February 1, 2020)

2	(tw:(polycystic ovar* OR pcos)) AND (tw:(fat* liver* OR nafld OR nash OR steatohep* OR steatosis OR cirrhos* OR cirrhotic*)) AND (year_cluster:[2018 TO 2020])	23
1	(tw:(polycystic ovar* OR pcos)) AND (tw:(fat* liver* OR nafld OR nash OR steatohep* OR steatosis OR cirrhos* OR cirrhotic*))	384

Medline [Ovid] (June 1, 2018)

Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily <1946 to Present>

1	Polycystic Ovary Syndrome/	12756
2	(PCOS or ((ovar* or syndrom*) adj2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* adj5 leventhal*))))).tw,kf.	15917
3	((ovar* adj1 cystic*) or (multi* adj5 (ovar* or follicl*) adj5 cyst*)).tw,kf.	1487
4	Hyperandrogenism/	1783
5	exp Virilism/	5890
6	(hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*).tw,kf.	13050
7	or/1-6	29101
8	exp Fatty Liver/	27452

9	(NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) adj2 (liver* or hepatic* or intrahepat* or biliar* or hepato*))).tw,kf.	36649
10	exp Liver Cirrhosis/	82568
11	(steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) adj3 fibros*)).tw,kf.	121483
12	((reye* adj2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* adj1 fever*)).tw,kf.	1794
13	((liver* or hepatic*) adj2 (index* or eval* or test?)).tw,kf.	25670
14	or/8-13	188661
15	7 and 14	381
16	Animals/ not (Animals/ and Humans/)	4434347
17	((animal or animals or canine* or cat or cats or dog or dogs or feline or hamster* or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or veterinar*) not (human* or patient*)).ti,kf.	2029362
18	15 not (16 or 17)	360
19	remove duplicates from 18	358

UPDATE Medline [Ovid] (February 1, 2020)

Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily 1946 to January 31, 2020

1	Polycystic Ovary Syndrome/	14065
2	(PCOS or ((ovar* or syndrom*) adj2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* adj5 leventhal*)))).tw,kf.	17881
3	((ovar* adj1 cystic*) or (multi* adj5 (ovar* or follicl*) adj5 cyst*)).tw,kf.	1578
4	Hyperandrogenism/	1952
5	exp Virilism/	6018
6	(hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*).tw,kf.	13879
7	or/1-6	31555
8	exp Fatty Liver/	31698
9	(NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) adj2 (liver* or hepatic* or intrahepat* or biliar* or hepato*))).tw,kf.	44019
10	exp Liver Cirrhosis/	87718
11	(steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) adj3 fibros*)).tw,kf.	134114
12	((reye* adj2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* adj1 fever*)).tw,kf.	1824
13	((liver* or hepatic*) adj2 (index* or eval* or test?)).tw,kf.	27751
14	or/8-13	207632
15	7 and 14	451
16	Animals/ not (Animals/ and Humans/)	4636421
17	((animal or animals or canine* or cat or cats or dog or dogs or feline or hamster* or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or veterinar*) not (human* or patient*)).ti,kf.	2122048
18	15 not (16 or 17)	428
19	remove duplicates from 18	426

20	limit 19 to yr="2018 -Current"	79

Web of Science [Clarivate Analytics] (June 1, 2018)

Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH Timespan=All years

#14	284	#12 NOT #13
#13	290	PMID=("21157321" or "26412465" or "21910085" or "16198620" or "29184820" or "21454995" or "24829577" or "28457031" or "29024702" or "4134992" or "27310544" or "450019" or "16311212" or "28285710" or "21832111" or "26761949" or "26589977" or "23721173" or "21251033" or "26634686" or "27251917" or "19576817" or "18000969" or "28339345" or "5565843" or "18344805" or "16503761" or "25554608" or "18830783" or "23798298" or "23386652" or "25479351" or "29264465" or "19461374" or "21301018" or "15987930" or "7577849" or "16191492" or "21495873" or "18417315" or "21366813" or "19215335" or "18230819" or "28487551" or "18497727" or "24648300" or "17320032" or "17624467" or "16767302" or "27176209" or "25211442" or "29750655" or "24994518" or "17560682" or "27068725" or "27919367" or "27107966" or "23980352" or "17089862" or "22173246" or "23612512" or "23185203" or "21926952" or "28942551" or "5832873" or "8088001" or "27804265" or "29264544" or "16011474" or "19622617" or "18400637" or "16532658" or "179515" or "25600030" or "28705172" or "23746214" or "26972165" or "28737293" or "20046314" or "24973357" or "27464726" or "24222096" or "11322868" or "29406938" or "23849162" or "16269843" or "27319232" or "26202291" or "25941433" or "23367497" or "15692481" or "19671519" or "15262299" or "8723563" or "16137470" or "25019404" or "17496361" or "19352053" or "2016368" or "15811215" or "1916463" or "8481214" or "21985780" or "28681988" or "15043244" or "17287148" or "21569072" or "11685939" or "20534754" or "27219496" or "22262974" or "27504851" or "29090431" or "27054776" or "28901968" or "27505055" or "3578443" or "29264552" or "23047930" or "26728862" or "17330572" or "21805747" or "26210619" or "21057159" or "26114995" or "19923096" or "23978196" or "19098117" or "12647844" or "8927344" or "10663856" or "11315291" or "26101431" or "21413894" or "25293340" or "17035653" or "7447186" or "21114431" or "10395191" or "23938069" or "23108199" or "20870709" or "12930161" or "28712591" or "21865363" or "21632811" or "20883985" or "11668286" or "657160" or "25198445" or "23777024" or "21897446" or "27428059" or "28222828" or "22837189" or "6460612" or "24256515" or "2955749" or "1830560" or "26968296" or "27132324" or "18389188" or "23869143" or "23627982" or "20001571" or "19622949" or "25339805" or "24962189" or "10767668" or "24434909" or "28370150" or "28883861" or "2884725" or "3952483" or "8256457" or "26976018" or "23029709" or "25136364" or "29590099" or "6448264" or "25905173" or "15649101" or "27351028" or "29432207" or "21937505" or "20590731" or "29128060" or "4356759" or "19347015" or "21190980" or "17709876" or "28694246" or "27076501" or "14669838" or "27424378" or "27792214" or "26494962" or "19171337" or "20301444" or "18386674" or "27256547" or "15645693" or "15670668" or "7632042" or "29114377" or "25994073" or "24034940" or "27594563" or "6828272" or "17058987" or "9709937" or "3169499" or "25755422" or "26405614" or "20961180" or "17561981" or "10075099" or "24923289" or "23158933" or "24641921" or "20456209" or "15767536" or "25644898" or "29201403" or "24183950" or "18185059" or "26979020" or "21283374" or "12527343" or "19809879" or "18505888" or "25069836" or "28776742" or "27838603" or "20371655" or "23857978" or "23155707" or "9935128" or "21257877" or "9235083" or "15040152" or "16912555" or "17934394" or "29161258" or "26203576" or "22908056" or "29170905" or "26436371" or "25402369" or "22082482" or "11443175" or "19284314" or "18565127" or "16203173" or "15115430" or "26517932" or "24639812" or "21029639" or "25598791" or "4822971" or "22829562" or "29083730" or "14749503" or "16489319" or "25707325" or "18422468" or "28612285" or "29209995" or "20227029" or "10438402" or "26039829" or "24019220" or "18220635" or "28455679" or "26913880" or "27456105" or "28699503" or "28291240" or "22096112" or "11388600" or "17986832" or "739279" or "26034812" or "14266624" or "19573754" or "21437093" or "15705403" or "2080856" or "26865583" or "17592439" or "17275449" or "24859638" or "16492691" or "15703460" or "18458706" or "29641459" or "11026085" or "2405587" or "19124394" or "26027246" or "5676174" or "24240117" or "23826984" or "2802082" or "1180424" or "22498245" or "28123936" or "16401000" or "16128250" or "19245356" or "16444867" or "19906783" or "25873949" or "12566753" or "22805002" or "18437728" or "26024975" or "19542757" or "15181020" or "26522459" or "27193136" or "24246334" or "12640725" or "4753429" or "25832152" or "27218012" or "25024594" or "19887498" or "26340970" or "24026869" or "25245380" or "2975629" or "15773753" or "15908053" or "22117616" or "21307133" or "26486483" or "8629139" or "15151470" or "26201622" or "3620420" or "28965028" or "29587769" or "22382613" or "25662524" or "22248781" or "19519467" or "29747678" or "26650609" or "5105082" or "25923022" or "15356308" or "16771946" or "29725371" or "18683746" or "26502288" or "21898634" or "4321497")
# 12	524	#10 NOT #11
# 11	2,662,535	TI=((animal or animals or canine* or cat or cats or dog or dogs or feline or hamster* or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or veterinar*) not (human* or patient*))
# 10	556	#9 AND #4
# 9	220,735	#8 OR #7 OR #6 OR #5
# 8	22,165	TS=((liver* or hepatic*) NEAR/2 (index* or eval* or test?))
# 7	1,930	TS=((reye* NEAR/2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* NEAR/1 fever*))
# 6	147,175	TS=(steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) NEAR/3 fibros*))
# 5	75,547	TS=(NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) NEAR/2 (liver* or hepatic* or intrahepat* or biliar* or hepato*)))
# 4	31,089	#3 OR #2 OR #1
# 3	13,004	TS=(hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*)
# 2	1,748	TS=((ovar* NEAR/1 cystic*) or (multi* NEAR/5 (ovar* or follicl*) NEAR/5 cyst*))
# 1	21,516	TS=(PCOS or ((ovar* or syndrom*) NEAR/2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* NEAR/5 leventhal*))))

UPDATE Web of Science [Clarivate Analytics] (February 1, 2020)

Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=All years

#13	120	#10 NOT #11 *Timespan=2018-2020*
# 12	575	#10 NOT #11
# 11	2,794,556	TI=((animal or animals or canine* or cat or cats or dog or dogs or feline or hamster* or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or veterinar*) not (human* or patient*))
# 10	609	#9 AND #4
# 9	252,394	#8 OR #7 OR #6 OR #5

# 8	25,216	TS=((liver* or hepatic*) NEAR/2 (index* or eval* or test?))
# 7	1,943	TS=((reye* NEAR/2 (syndrom* or disease* or tumo?r* or tumeur*)) or (jamshedpur* NEAR/1 fever*))
# 6	166,264	TS=(steatos?s or cirrhos* or cirrhotic* or ((liver* or hepatic* or intrahepat* or biliar* or hepato*) NEAR/3 fibros*))
# 5	90,958	TS=(NAFLD or NASH or steatohep* or steato-hep* or ((fat* or adiposit*) NEAR/2 (liver* or hepatic* or intrahepat* or biliar* or hepato*)))
# 4	33,858	#3 OR #2 OR #1
# 3	14,021	TS=(hyperandrogen* or hyper-androgen* or hirsutis* or virilis* or viriliz* or androgeni?ation*)
# 2	1,884	TS=((ovar* NEAR/1 cystic*) or (multi* NEAR/5 (ovar* or follicl*) NEAR/5 cyst*))
# 1	23,716	TS=(PCOS or ((ovar* or syndrom*) NEAR/2 (polycyst* or poly-cyst* or micropolycyst* or sclerocyst* or sclero-cyst* or (stein* NEAR/5 leventhal*))))

Supplemental. 6 Quality appraisal coding manual.

NOS score - Adapted for cross-sectional studies

- **Selection:** (Maximum 4 stars)

1) Representativeness of the sample

a) Truly representative of the average in the target population * (all subjects or random sampling)
b) Somewhat representative of the average in the target population * (non-random sampling)
c) Selected group of users
d) No description of the sampling strategy

2) Sample size

a) Justified and satisfactory *
b) Not justified

3) Non-respondents

a) Comparability between respondents and non-respondents 'characteristics is established, and the response rate is satisfactory *
b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory
c) No description of the response rate or the characteristics of the responders and the non-responders

4) Ascertainment of the exposure (PCOS)

a) Validated measurement tool *
b) Non-validated measurement tool, but the tool is available or described
c) No description of the measurement tool

- **Comparability:** (Maximum 2 stars)

1) The subjects in different outcome groups are comparable, based on the study design or analysis Confounding factors are controlled

a) The study controls for the most important factors (weight and insulin resistance) *
b) The study control for any additional factor *
c) Not reported

- **Outcome:** (Maximum 3 stars)

1) Assessment of the outcome

a) Independent blind assessment **
b) Record linkage
c) Self report
d) No description

2) Statistical test

a) The statistical test used to analyze the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value) *
b) The statistical test is not appropriate, not described or incomplete

NOS score - Case-Control studies

- **Selection:** (Maximum 4 stars)

1) Is the case definition adequate?
a) yes, with independent validation *
b) yes, e.g. record linkage or based on self-reports
c) no description

2) Representativeness of the cases
a) consecutive or obviously representative series of cases *
b) potential for selection biases or not stated

3) Selection of Controls
a) community controls *
b) hospital controls
c) no description

4) Definition of Controls
a) no history of disease (endpoint) *
b) no description of source

- **Comparability:** (Maximum 2 stars)

1) Comparability of cases and controls on the basis of the design or analysis
Confounding factors are controlled
a) study controls for the most important factors (weight and insulin resistance) *
b) study controls for any additional factor *
c) Not reported

- **Exposure:** (Maximum 3 stars)

1) Ascertainment of exposure
a) secure record (e.g. surgical records) *
b) structured interview where blind to case/control status *
c) interview not blinded to case/control status
d) written self-report or medical record only
e) no description

2) Same method of ascertainment for cases and controls
a) yes *
b) no

3) Non-Response rate
a) same rate for both groups *
b) non respondents described
c) rate different and no designation
Quality sub-score thresholds

Quality rating	Selection Domain	Comparability Domain	Outcome Domain
Fair to good	≥2	≥1	≥2

Poor	0-1	0	0-1

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4 DISCUSSION

In the present cross-sectional study and meta-analysis, increased prevalence of hepatic steatosis was observed in PCOS patients of all ethnicities. Premenopausal women from South Asia, South America, and the Middle East are at greater risk. We also noticed that, despite their young age, South Asian women with PCOS and NAFLD can develop significant liver fibrosis, possibly indicating the likely coexistence of NASH, the evolutive counterpart of NAFLD. Finally, PCOS patients from South Asian region showed an increased ASCVD risk, which should be taken into account for the overall risk stratification.

NAFLD is considered the most common cause of chronic liver disease globally. Many natural history studies have shown its potential to cause serious liver damage in the form of cirrhosis and hepatocellular carcinoma, and ultimately increased liver related morbidity and mortality. In the last three decades, the burden of liver cirrhosis in the general population has impressively increased, with significant health and economic consequences. From 1990 to 2010, cirrhosis went from being ranked 14th to 8th in terms of years of life lost in the United States, placing it between diabetes (7th) and Alzheimer's disease (9th) and higher than colorectal cancer (10th), and breast cancer (13th)(59). To date, the only curative treatment for end-stage liver disease due to cirrhosis is LT and the rising trend in NAFLD prevalence will soon show an influence on the physiognomy of LT waiting lists, thus impacting organ supply(60).

Despite the fact that NAFLD and PCOS are extremely relevant in clinical settings, published studies investigated the association between the two conditions are still very scarce and recent. At the same time, data on NAFLD prevalence in this population is inconsistent and ranging widely, between 5.5% and 73.3% across studies(37). Therefore, we carried out two parallel projects to determine the prevalence of NAFLD in PCOS patients. In FLIPCOS study, our screening program

for chronic liver disease on well-defined premenopausal South Asian PCOS women identified a prevalence of NAFLD at 39.6%, which is higher than the community estimates. Notwithstanding that this finding might have been underestimated due to the fact that many participants (31.7%) were on insulin sensitizers (metformin), which reduces the state of IR, the common pathogenic mechanism for NAFLD, thus possibly decreasing the disease frequency. Cerda *et al*. has also determined almost the same prevalence at 41.5%. However, their subjects were not on insulin sensitizers and they were mainly from Hispanic origin, where the highest figures of NAFLD have been reported(42). To our knowledge, our cross-sectional study is the first one to adopt TE with CAP to investigate NAFLD in PCOS women, which is already commonly used in other at-risk populations(61-63). The CAP cut-off value we adopted in this study was reported as optimal to detect any grade steatosis(64). Furthermore, in order to validate CAP measurements in our cohort, we reported CAP values from another routine screening program for liver fibrosis running at same center "MUHC". In this program, patients with chronic HBV undergo CAP quantification during TE examination for LSM were selected as appropriate comparators. To account for age, we included only female patients aged <50 years old. The prevalence of the disease among the validation group was 8%, which is considerably lower than FLIPCOS cohort. In our group of PCOS women, NAFLD diagnosis was further confirmed by a surrogate noninvasive method, namely HSI. Regarding our meta-analysis, it is not the first one to determine the association between NAFLD and PCOS. However, it confirms, updates, and adds more strength to the previous findings because it includes more reviews, a total of 29 publications, and thus larger sample size of 7,148 participants, compared to 17 studies with 5,334 participants in the most recent meta-analysis(24) and 7 studies with 1,185 participants in the oldest one(56). Our figures yielded to a total increase of 12 and 22 studies, respectively. In order to have larger, more representative

sample of PCOS patients and add robustness to the argument that this population is in fact at higher risk for NAFLD, we searched eight scientific databases to identify studies that have reported the prevalence of NAFLD in PCOS patients up to 2020 without any restrictions in the search strategy. In this meta-analysis, we found that the average prevalence of NAFLD in PCOS patients is almost double the controls (49% vs. 24%), with OR 2.49 (95% CI 2.20-2.82), suggesting that PCOS patients have 2.5-fold increase in the risk of the disease. This significant result was supported by applying sensitivity analyses excluding poor-quality studies and studies weighed <5%, both displayed similar figures, OR of 2.38 (95% CI, 2.09-2.71) and 2.36 (95% CI, 2.05-2.70), respectively. Furthermore, we used the trim and fill method, which is a technique that accounts for the missing studies, to overcome the publication bias. Using this technique, the overall summary estimate remained unchanged OR 2.56 (95% CI, 2.07-3.17), indicating a reliable result.

In patients with NAFLD, the estimation of liver fibrosis is essential for risk stratification and prediction of liver-related complications as well as all-cause mortality(65). NAFLD covers a spectrum of pathophysiological conditions includes NAFL or simple steatosis and NASH. The latter stage is commonly associated with elevated ALT and can progress to liver fibrosis. Significant liver fibrosis was identified in 6.9% of our FLIPCOS cohort, more than double what has been reported in the general population(66). Given the known association between false positive results of LSM and elevated ALT, we conducted a sensitivity analysis excluding patients with elevated ALT, which showed a prevalence of significant liver fibrosis of 4.3%. However, the key question is that does this high ALT related to NAFLD or to other conditions? in fact, all excluded patients were diagnosed with NAFLD, which suggests the coexistence of NASH. Therefore, excluding these patients from the analysis will eliminate a very important clinical segment of the disease. Additionally, we assessed the correlation between LSM results and

noninvasive biomarkers namely, NAFLD fibrosis score, FIB-4, and APRI. Of note, there was a poor correlation between LSM and both NAFLD fibrosis score and FIB-4, possibly because these two biomarkers contain age as part of their formula, while our study population was young. Conversely, APRI, which does not include age in its formula, had a significant correlation with LSM. Therefore, our data suggest that the simple fibrosis biomarker APRI may be preferable to FIB-4 or NAFLD fibrosis score in young PCOS patients.

NAFLD pathogenesis is a complex multifaceted process and many factors were reported to be associated with its higher prevalence. Women with PCOS are known to have obesity and IR, which are common determinants for NAFLD, as part of the syndrome(11, 42, 67, 68). Moreover, other reviews identified HA as an additional risk factor for hepatic steatosis in this population(11). In FLIPCOS study, increased central adiposity, BMI, HA, and HOMA-IR were significantly higher in PCOS with NAFLD, compared to those without NAFLD. Other factors that might have contributed to higher NAFLD prevalence in South Asian PCOS patients include genetic variants of the PNPLA3 protein, physical inactivity, reduced disease awareness, late diagnosis, as well as sociocultural factors in comparison with Western populations(69). Nonetheless, the multivariate logistic regression analysis for cofactors of NAFLD in women with PCOS reported BMI, HA, and ALT as independent predictors, and among them, BMI had the highest AUC to predict the outcome.

There are many evidences showing that obesity in PCOS patients is a major determinant in NAFLD pathogenesis(23, 36, 49). Cerda *et al.* who conducted a study on Chilean PCOS women found that those with NAFLD had higher mean BMI compared with the group without NAFLD(42). Petta *et al.* observed the same findings in an Italian PCOS cohort. In addition, the latter group have also detected a proportional correlation between the prevalence of NAFLD and

BMI categories(50). These observations were consistent with ours in FLIPCOS study, which indicate that the higher the BMI, the more the NAFLD and associated liver fibrosis frequency. Although South Asian patients have an overall lower BMI compared to Caucasians(70), they are more liable to have abnormal visceral fat distribution, specifically central obesity and dyslipidemia(69). In this regard, increased WC was found in 94% of our study population. Since we acknowledge that our cohort have higher BMI and WC, and a biased result could be implemented, we also know that overweight and/or obesity are common features in these particular individuals. As a result, we decided to include all PCOS women in order to avoid selection bias by excluding those with higher BMI. Moreover, BMI remained an independent predictor for NAFLD after adjusting for duration of PCOS, BMI, HOMA-IR, FAI>5 and ALT>24 IU/L. Further supporting this observation, our meta-regression showed that BMI is associated with an elevated risk of NAFLD.

Another important factor in NAFLD pathogenesis is IR. In FLIPCOS study, data analysis showed that HOMA-IR did not reach statistical significance, which differ from previous work. However, this finding might have attributed to therapeutic implications. Indeed, administration of metformin to patients with PCOS and NAFLD improves IR and reduces transaminase levels, despite no change in body weight(71, 72). In our cohort, 31.7% of all participants, and 50% of those with NAFLD, were on metformin. Absence of insulin sensitizers may have led to different outcome. On the other hand, in our meta-analysis, when we stratified based on the presence of IR, PCOS patients with IR had considerably higher risk of NAFLD as opposed to those without IR (OR 1.97, 95% CI 1.44–2.71).

NAFLD in PCOS women may occur irrespective of obesity. Increased androgen bioavailability represented by FAI and decreased SHBG were found to be associated with NAFLD and other

metabolic abnormalities(11, 35, 73). This finding suggested that the underlying mechanism connecting PCOS and NAFLD is probably linked to HA. In addition, androgen excess has been reported to favor visceral adiposity and thus contribute to the development of NAFLD(17). Chen *et al.* reported that PCOS patients had a higher prevalence of elevated ALT, which was correlated with androgen levels independent of age, IR, obesity and dyslipidemia(74). Our results showed that HA measured by FAI was also an independent predictor of NAFLD in South Asian PCOS. This is in concordance with the previous data that correlate high FAI with liver disease, particularly NAFLD(57).

Elevated ALT was also an independent predictor of NAFLD on the multivariate analysis of FLIPCOS study. Although only 22.8% of patients had elevated ALT, 70% of them were found to have the outcome. This finding indicates that liver enzyme abnormalities in patients with PCOS without known pre-existing liver disease could be potentially due to NASH and should prompt further investigations, including tests for aetiologies of chronic liver disease and subsequent referral for TE examination to evaluate the degree of liver fibrosis. In a retrospective study by Setji *et al*. 15% of PCOS patients had elevated aminotransferase. Six women had available liver histology; all biopsies showed evidence of NASH with varying degree of fibrosis(75). More importantly, in our FLIPCOS cohort, 21.7% of those with elevated ALT had significant liver fibrosis on TE examination, compared to only 2.6% of patients with normal ALT. On the other hand, 60% of the patients with NAFLD had normal ALT. These figures are also in agreement with data from the general population, suggesting that development of NAFLD may be occult(76, 77). Therefore, this finding emphasizes the need for sensitive diagnostic tools in such at-risk population. Currently, guidelines recommend routine screening strategies for NAFLD in at risk individuals, such as those with T2DM and metabolic comorbidities, particularly in case of elevated

ALT(37, 78). This was based on striking prevalence figures of NAFLD and advanced liver fibrosis, that far exceed those of the general population(66, 79). It is further recommended that potentially at-risk individuals should be screened for liver fibrosis using noninvasive markers (serology based or TE) to quantify the risk of progression to liver cirrhosis(80). A similar strategy may be applicable in patients with PCOS, whereby those with obesity, elevated ALT or HA should undergo fibrosis assessment.

NAFLD prevalence reported in the general population across the globe are vary significantly. The disease is highly prevalent in some ethnic groups and geographic areas than the others(58). In our meta-analysis, when we stratified studies based on geographic regions, we noticed that PCOS patients from South America and the Middle East had a greater risk of NAFLD than those without PCOS (OR 3.55, 95% CI 2.27-5.55), compared to their European (OR 2.22, 95% CI 1.85-2.67) and Asian (OR 2.63, 95% CI 2.20-3.15) counterparts. We also conducted further analysis by breaking down South American and the Middle East population into two separate groups and the result showed that the prevalence of the disease in both populations were almost similar to the original analysis, with OR 3.69 (95% CI 1.94-7.02) and OR 3.89 (95% CI 2.12-7.14), respectively. The difference in prevalence rates across different regions of the world may be related to many factors such as genetic, ethnicity, dietary habit, and lifestyle modifications(81-83).

There are many epidemiological data which support the concept that NAFLD is a multisystem disease affecting a variety of extrahepatic organ systems. Recent evidences indicate an increased risk of all-cause mortality and a strong link between NAFLD and extrahepatic diseases, such as CVD, hypothyroidism, sleep apnea, and MetS(9, 84). CVD risk prediction in younger female patients has been more challenging than in older or male counterparts. Lifetime ASCVD risk assessment based on pooled cohort equation was calculated in our patients according to the

American College of Cardiology and the American Heart Association guidelines on assessment of cardiovascular risk(85). We found that those with NAFLD had increased ASCVD risk compared to those without NAFLD. Decisions to implement primary prevention measures are often consequently hindered in this patient population. Our FLIPCOS study helps shed new insights in the understanding of the cardiovascular risk profile in young female population from the NAFLD perspective. Our findings should be taken into careful consideration for risk stratification, especially after transition of women with PCOS to menopause, and for consideration of statin therapy. Another common extra-hepatic association is MetS. Women with PCOS are more prone to have features of MetS(20, 86-89). In fact, many researchers believed that NAFLD and PCOS are the hepatic and ovarian manifestations of MetS(90). In our FLIPCOS study, 84.2% of all participants and 100% of those who have NAFLD had at least one of the following metabolic abnormalities: diabetes, hypertension, and/or dyslipidemia. Moreover, our meta-analysis indicates that PCOS patients who have MetS had a significantly higher risk of NAFLD, compared to controls (OR 3.39, 95% CI 2.42-4.76). Our findings from both studies seems interesting since women with PCOS are young, and therefore eligible for early detection and treatment of a potentially progressive liver disease. Thus, the first implication of this study is that physicians who provide care for patients with PCOS must be aware of the need to evaluate NAFLD in this population.

Each of these projects has its strengths and limitations. In our cross-sectional study strengths include the well-characterized homogeneous population and the use of a validated and accurate diagnostic method. Moreover, the enrollment of consecutive patients minimizes the risk of selection bias. On the other hand, some limitations of our study should be acknowledged. First, the cross-sectional study design did not allow us to capture the dynamics and associated factors of

the disease in a longitudinal fashion. Second, the unavailability of genetic variants of the PNPLA3 and other polymorphisms linked to hepatic steatosis prevented us from understanding their contribution to the pathogenesis of NAFLD in PCOS. Third, we included only South Asian women, so we cannot speculate on applicability of our findings to other ethnicities. Fourth, we did not include a group of age-matched patients without PCOS to act as control group. Five, due to overweight and/or obesity, standard M probe failed to provide valid results in almost 19% of cases. This has been attributed to the interference with the transmission of shear waves and ultrasound waves through the liver parenchyma by thick subcutaneous adipose tissue. Therefore, we used the XL probe, which has been used and validated in many studies as an alternative for the standard M probe in overweight and obese individuals(91, 92). Likewise, all possible cause that may contribute to unreliable TE measurement, such as acute hepatitis, hepatic congestion, and cholestasis, were ruled out. Finally, our study was carried out at a tertiary care center, which may limit generalizability of our findings.

Regarding the systematic review and meta-analysis, several strengths were present. We broadened the literature search to include eight databases without search strategy restrictions in order to provide a representative sample size that can reflect the PCOS population in general. Additionally, studies that have used aminotransferase as a method to diagnose NAFLD were excluded to be consistent with NAFLD definition. Although our study allows for a clinically meaningful expansion of the literature, it is not without limitations. First, the included studies were all observational, which might be biased due to unmeasured confounders. Second, our summary result was based on crude ORs since only 7 reviews reported aORs. Although some studies were adjusted for main confounders, other modifiable factors were not accounted for in all these studies, such as family history, dietary habits, and/or exercise. The presence of aORs for all studies and

taking into account all possible relevant confounders may have influenced the overall result. Third, US was the predominant method for diagnosing NAFLD rather than the gold standard liver biopsy. This can be justified by the difficulty in applying such an invasive procedure for research purposes. US is a readily available, cheap, noninvasive technique with discrete sensitivity and specificity for epidemiological studies.

5 CONCLUSION

NAFLD is emerging as a highly prevalent condition among women with PCOS, confirming the relevant clinical association. BMI was strongly associated with elevated risk of NAFLD in PCOS of all ethnicities. HA and ALT were also identified as independent predictors for NAFLD in South Asian PCOS patients. Due to the highly reported figures for significant liver fibrosis, South Asian PCOS cohort seems to be at higher risk for liver-related outcomes. Considering the young age of this population, our study suggests that PCOS patients should be assessed for presence of NAFLD, especially those with increased BMI, ALT and HA through a noninvasive screening strategy. This could promote early diagnosis and initiation of intervention plans, including counselling on weight loss, cardiovascular risk stratification and linkage to hepatology care, particularly in obese PCOS patients. Future longitudinal studies should assess the effect of early diagnosis and interventions on long-term outcomes.

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