ORIGINAL RESEARCH

Comparative Effects of Metformin and GLP-1 Agonists on Polyendocrine Metabolic Ovary Syndrome and Gallbladder Disease

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Background

Polyendocrine metabolic ovarian syndrome (PMOS) is a common endocrine disorder affecting 5% to 15% of reproductive-aged individuals and is frequently accompanied by hyperinsulinemia and obesity. These metabolic disturbances contribute to gallbladder dysmotility and increased cholesterol synthesis, elevating the risk of cholelithiasis, cholecystitis, and cholangitis. Metformin is widely used to manage dyslipidemia, type 2 diabetes mellitus, and obesity in patients with PMOS, but emerging therapies such as glucagon-like peptide-1 (GLP-1) receptor agonists (RAs) may offer additional benefit.

Methods

This study used the TriNetX network to compare the incidence of gallbladder disease between patients with PMOS treated with metformin and those treated with GLP-1 RAs. After 1:1 propensity score matching on age, type 2 diabetes mellitus, obesity, body mass index, and lipid-lowering therapy, two cohorts of 53,258 patients were analyzed.

Results

Glucagon-like peptide-1 RAs were associated with a lower incidence of cholelithiasis and cholecystitis than metformin, whereas cholangitis rates were similar.

Conclusion

These findings suggest that GLP-1 RAs may confer a protective effect through weight reduction and improved insulin sensitivity. Further study is warranted to confirm the effect of GLP-1 RAs on cholelithiasis and cholecystitis.

INTRODUCTION

Polyendocrine metabolic ovarian syndrome (PMOS) arises from genetic and environmental factors that disrupt neuroendocrine function, leading to hyperandrogenism and hyperinsulinemia. Increased pulsatile secretion of gonadotropin-releasing hormone, elevated luteinizing hormone, and ovarian thecal androgen production occur alongside relatively suppressed follicle-stimulating hormone, producing anovulation and, clinically, ovulatory dysfunction and infertility. Polyendocrine metabolic ovarian syndrome is

frequently accompanied by metabolic syndrome, insulin resistance, and gallbladder disease. Chronic hyperinsulinemia promotes lipogenesis and inhibits lipolysis, contributing to visceral adiposity and gallstone risk. Insulin resistance also produces dyslipidemia, including elevated triglycerides, low high-density lipoprotein cholesterol level, and elevated very-low-density lipoprotein, which favors cholesterol-rich bile and impairs bile acid synthesis, encouraging gallstone formation.[1] This mechanism supports the reported 3-fold increase in common gallbladder pathologies, such as cholelithiasis, cholecystitis, and cholangitis, and explains their association with risk factors including metabolic syndrome, obesity, and type 2 diabetes mellitus (T2DM).[2-5] Cholelithiasis denotes the formation of gallstones within the gallbladder; cholecystitis refers to inflammation of the gallbladder wall, most often precipitated by cystic duct obstruction by a stone; and cholangitis is inflammation of the biliary tree, typically caused by bacterial infection superimposed on obstruction of the common bile duct.

Metformin, derived from galegine, a natural compound found in *Galega officinalis*, has a complex mechanism of action involving multiple sites.[6] It decreases hepatic glucose production both directly and indirectly, promotes intestinal glucose utilization, increases glucagon-like peptide-1 (GLP-1) secretion, and alters the gut microbiome.[6,7] Among its major long-term effects is enhancement of hepatic insulin sensitivity, likely mediated through AMP-activated protein kinase.[6] Clinically, metformin is a first-line treatment for T2DM because of its antihyperglycemic properties, and it is also used to manage insulin resistance and hyperinsulinemia in individuals with PMOS, a population predisposed to long-term metabolic complications, including T2DM.[7] Metformin's direct effects on the gallbladder are less well characterized than its glycemic actions; by raising endogenous GLP-1 and modifying bile acid handling, it may influence gallbladder motility and bile composition, although direct evidence linking metformin to gallbladder disease risk in PMOS remains limited.[6,7]

Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) are another class of medication used to treat T2DM and obesity. These agents stimulate insulin secretion in response to oral glucose intake by augmenting the incretin effect.[8] The incretin effect is the normal physiological phenomenon whereby insulin secretion is greater after oral glucose than after an equivalent intravenous glucose load, owing to the postprandial release of gut-derived incretin hormones; this response is impaired, though not abolished, in T2DM.[9] Glucagon-like peptide-1 RAs activate cellular pathways that enhance calcium-dependent insulin release, improve glucose sensitivity, and modulate gene expression related to insulin regulation.[10] Although the incretin effect is diminished in T2DM, the insulinotropic and glucagonostatic actions of GLP-1 are sufficiently preserved that pharmacological GLP-1 receptor stimulation lowers blood glucose and improves glycemic control.[9] Additional

actions include delayed gastric emptying and suppression of glucagon secretion when blood glucose is elevated.[8] Glucagon-like peptide-1 also acts beyond the gut on adipose tissue, bone, skeletal muscle, liver, and the neuroendocrine and cardiovascular systems. Whether the incretin effect is similarly impaired in PMOS has not been directly established; because the insulin resistance and hyperinsulinemia that characterize PMOS overlap mechanistically with T2DM, GLP-1 RAs are expected to exert comparable insulinotropic and weight-reducing effects in this population.

A systematic review of 26 randomized controlled trials reported that the GLP-1 RAs semaglutide and liraglutide achieved weight loss of up to 13.9% at 68 weeks and up to 5.8% at 26 weeks, respectively.[11] Tirzepatide, a dual agonist of the glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 receptors, was excluded from the present analysis to isolate the effect of GLP-1 RAs. Although GLP-1 RAs are approved primarily for T2DM and obesity, liraglutide has been shown to be more effective than metformin in reducing body weight, improving menstrual regularity, and lowering androgen levels in PMOS.[12]

Prior studies have reported an increased risk of gallbladder and biliary disease with GLP-1 RA use, particularly at higher doses and longer durations.[13] In a meta-analysis, GLP-1 RAs were associated with higher odds of gallbladder disease, predominantly in T2DM and obese populations treated at weight-loss doses.[13] Two mechanisms are thought to contribute: rapid, pronounced weight loss raises biliary cholesterol saturation and promotes gallstone formation, and GLP-1 receptor activation may reduce gallbladder motility and delay emptying, favoring bile stasis. Whether this risk extends to the PMOS population, in which GLP-1 RAs are typically used off-label at lower doses, is unknown. This study addresses that gap using a large propensity score–matched cohort.

MATERIALS AND METHODS

This retrospective cohort study was conducted using the TriNetX Global Collaborative Network, a federated electronic health record database composed of de-identified patient data from participating healthcare organizations. At the time of analysis, the network included 142 healthcare organizations, with contributing institutions varying according to individual query response rates. Data within TriNetX are compliant with the Health Insurance Portability and Accountability Act, and only aggregate, de-identified data are accessible to investigators.

Patient Population

Two cohorts of patients with PMOS were identified using International Classification of Diseases, 10th Revision (ICD-10) code E28.2. Cohort 1 comprised patients with PMOS who were treated with metformin

(Anatomical Therapeutic Chemical [ATC] code A10BA02), and cohort 2 comprised patients with PMOS who were treated with GLP-1 RAs (ATC code A10BJ), which includes liraglutide, semaglutide, dulaglutide, and exenatide. Tirzepatide, a dual GIP/GLP-1 RA, was excluded. The metformin cohort query yielded 149,229 eligible patients from 101 responding healthcare organizations, and the GLP-1 RA cohort yielded 54,938 eligible patients from 89 responding healthcare organizations. Patients in either cohort were not restricted from concurrent use of the comparator medication, reflecting real-world prescribing practice. Because concurrent exposure was permitted, some patients may have received both medications during follow-up, and the potential effect of this overlap is addressed in the Limitations subsection.

Index Event and Time Window

The index event was defined as the first documented occurrence of PMOS with concurrent exposure to either metformin or a GLP-1 RA. Outcomes were assessed from the date of the index event forward, and patients were censored after their last recorded encounter within the electronic health record. Patients whose index event occurred more than 20 years before the analysis dates were excluded automatically by the TriNetX platform; no patients met this exclusion criterion.

Matching and Covariate Adjustment

To reduce baseline confounding, 1:1 propensity score matching was performed using nearest-neighbor matching without replacement. Matching variables included age at index, female sex, T2DM (ICD-10 E11), unspecified obesity (ICD-10 E66.9), body mass index (ICD-10 Z68), fenofibrate use (ATC code C10AB05), and statin use (ATC code C10AA). Before matching, the metformin cohort was younger (mean age, 31.4 vs 36.6 years; standardized mean difference [SMD] 0.51), with lower rates of diabetes (11.3% vs 23.0%; SMD 0.31) and obesity (19.7% vs 39.5%; SMD, 0.44). After matching, all covariates were well balanced: mean age, 36.2 ± 10.4 vs 36.2 ± 10.1 years (SMD, 0.004); diabetes, 23.1% vs 21.8% (SMD, 0.03); obesity, 37.4% vs 38.1% (SMD, 0.014); White, 64.0% vs 63.8% (SMD, 0.004); Black or African American, 14.3% vs 14.3% (SMD, 0.001); Hispanic or Latino, 11.9% vs 11.8% (SMD, 0.004); Asian, 3.3% vs 3.4% (SMD, 0.006); and fenofibrate, 0.5% vs 0.4% (SMD, 0.007), with all statin agents showing SMD <0.02. All SMDs after propensity score matching were below 0.10, indicating adequate covariate balance. Following matching, each cohort contained 53,258 patients (**Figure 1**).

Outcomes and Analysis

The primary outcomes were cholelithiasis (ICD-10 K80), cholecystitis (ICD-10 K81), and cholangitis (ICD-10 K83.0). Analyses used an all-comers design that retained patients with prior gallbladder disease in both cohorts,

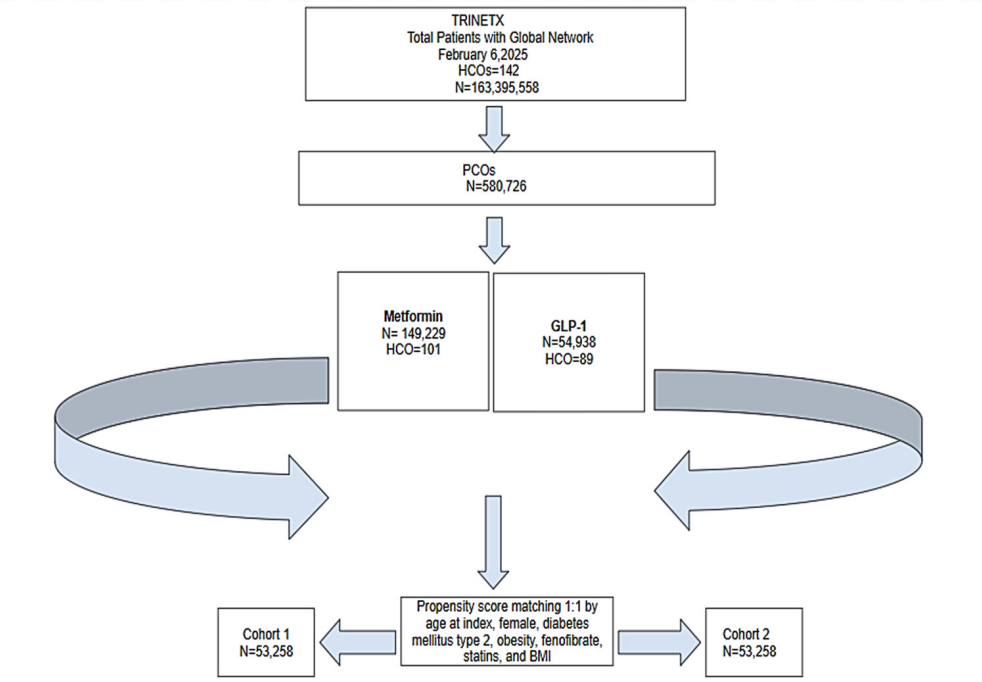


Figure 1. Matching of the cohorts using the TriNetX database and polyendocrine metabolic ovarian syndrome (PMOS) population, concurrent metformin or glucagon-like peptide-1 (GLP-1) receptor agonist use, and propensity score matching based on age at index, sex, type 2 diabetes mellitus diagnosis, obesity, body mass index (BMI), fenofibrate, and/or statin usage. HCO indicates healthcare organization.

because the study was designed to evaluate overall gallbladder disease burden in a population at elevated metabolic risk rather than strictly incident outcomes. Gallbladder disease burden was operationalized as the documented occurrence of any of these 3 coded diagnoses during the observation window, encompassing both prevalent and incident events under the all-comers design. Because both cohorts were balanced after matching across the major gallbladder risk factors, pre-existing disease burden is unlikely to explain the observed differences. Comparative analyses were performed using the TriNetX analytics platform. Measures-of-association analyses were used to calculate risk differences, risk ratios, odds ratios (ORs), and corresponding 95% confidence intervals (CIs). Kaplan-Meier survival analyses were performed for time-to-event outcomes, with comparisons assessed using log-rank testing and hazard ratios (HRs). The *P* values reported alongside HRs in the TriNetX output reflect the Schoenfeld residual test of the proportional hazards assumption and do not indicate HR significance; HR significance was established by the 95% CI and the log-rank *P* value. Statistical significance was defined as a 2-sided $P < .05$.

RESULTS

Among patients with PMOS treated with metformin vs GLP-1 RAs, we observed significant differences in gallbladder outcomes. The risk of cholelithiasis was higher with metformin, at an incidence of 4.78% compared with 3.60% among GLP-1 RA users (**Figure 2**).

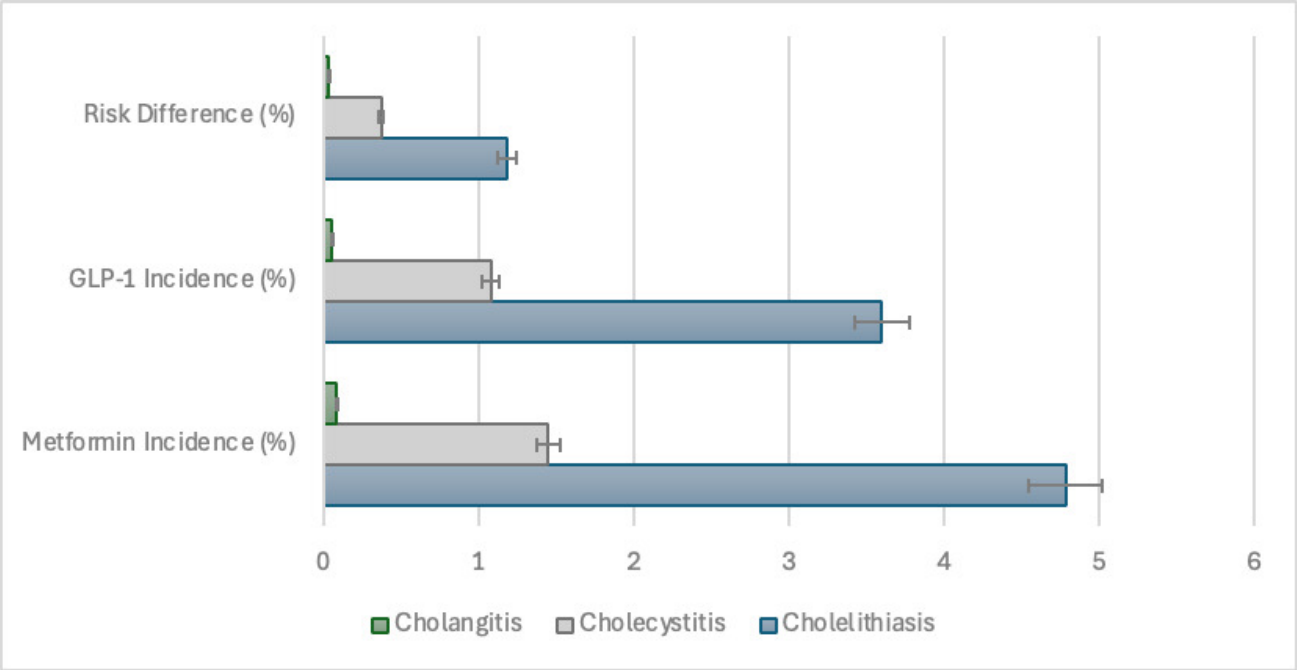


Figure 2. Comparison of the risk and incidence of cholelithiasis, cholecystitis, and cholangitis in individuals with polyendocrine metabolic ovarian syndrome treated with metformin versus glucagon-like peptide-1 (GLP-1) receptor agonists, with a 5% margin of error.

Table 1. Association between GLP-1 RA vs metformin exposure and gallbladder disease outcomes in patients with PMOS after 1:1 propensity score matching (n = 53,258 per cohort).*

Outcome	Metformin, %	GLP-1 RA, %	Risk Difference (95% CI)	Risk Ratio (95% CI)	Odds Ratio (95% CI)	Hazard Ratio (95% CI)	*P* Value
Cholelithiasis	4.78	3.60	1.18 (0.94 to 1.42)	1.33 (1.25-1.41)	1.34 (1.27-1.43)	0.84 (0.79-0.89)	<.001
Cholecystitis	1.45	1.08	0.37 (0.24 to 0.50)	1.34 (1.21-1.49)	1.348 (1.21-1.50)	0.82 (0.73-0.91)	<.001
Cholangitis	0.09	0.06	0.03 (−0.002 to 0.06)	1.53 (0.97-2.43)	1.534 (0.97-2.43)	—	.07

Abbreviations: CI, confidence interval; GLP-1 RA, glucagon-like peptide-1 receptor agonist; PMOS, polyendocrine metabolic ovarian syndrome.
Incidence values are the proportion of each cohort with the coded outcome during follow-up. Risk differences, risk ratios, and odds ratios are expressed for metformin relative to GLP-1 RA exposure. Hazard ratios are expressed for GLP-1 RA relative to metformin exposure (values <1 favor GLP-1 RAs) and are derived from Kaplan-Meier analysis with log-rank testing; the hazard ratio for cholangitis was not reported. P *values correspond to the measures-of-association analysis.

As shown in **Table 1**, the risk difference was 1.18% (95% CI, 0.94%-1.42%), with a risk ratio of 1.33 (95% CI, 1.25-1.41) and an OR of 1.34 (95% CI, 1.27-1.43), all statistically significant ($P < .001$). Kaplan-Meier analysis confirmed a higher event-free survival probability for the GLP-1 RA cohort (85.6%) than for the metformin cohort (83.8%), with an HR of 0.84 (95% CI, 0.79-0.89; log-rank $P < .001$).

For cholecystitis, metformin users had a higher incidence (1.4%) than GLP-1 RA users (1.1%), with a risk difference of 0.37% (95% CI, 0.24%-0.50%), a risk ratio of 1.34 (95% CI, 1.21-1.49), and an OR of 1.35 (95% CI, 1.21-1.50; $P < .001$). Kaplan-Meier analysis showed event-free survival of 95.2% in the metformin cohort vs 94.5% in the GLP-1 RA cohort (log-rank $\chi^2 = 12.7$; $P < .001$; HR, 0.82; 95% CI, 0.73-0.91). The lower end-of-window survival probability in the GLP-1 RA group, despite its lower event rate, may reflect differential follow-up time between cohorts. In contrast, cholangitis risk did not differ significantly, with incidence rates of 0.09% in the metformin group and 0.06% in the GLP-1 RA group. The risk difference was 0.03% (95% CI, −0.002%-0.06%), and although the risk ratio (1.53; 95% CI, 0.97-2.43) and OR (1.53; 95% CI, 0.97-2.43) suggested a possible trend, the difference did not reach statistical significance ($P = .07$).

DISCUSSION

Glucagon-like peptide-1 RAs have emerged as a promising therapy for individuals with PMOS, a syndrome closely linked to obesity, insulin resistance, and T2DM, in which these metabolic disturbances drive both reproductive dysfunction and gallbladder disease. Using the TriNetX network, we analyzed 2 propensity score–matched cohorts of 53,258 patients, matched on age at index, T2DM, obesity, fenofibrate and statin use, and body mass index. Analysis of the data revealed that, compared with metformin, GLP-1 RA use was associated with a lower risk of developing cholecystitis and cholelithiasis in individuals with PMOS, though no significant difference was observed for cholangitis. Framed against the metabolic profile of PMOS, these findings suggest that a therapy which improves insulin sensitivity and reduces weight may also modify the biliary consequences of the syndrome.

These findings contrast with prior literature reporting an increased risk of gallbladder disease with GLP-1 RAs (OR, 1.27; 95% CI, 1.05-1.54) in predominantly T2DM and obese populations treated at higher weight-loss doses for longer durations.[13] Several factors may account for the discrepancy. The PMOS population studied here was younger, with a mean post-match age of 36 years, and likely received GLP-1 RAs at lower doses and for shorter durations, reflecting their off-label use in PMOS. High-dose GLP-1 RA therapy produces rapid weight loss, which independently raises gallstone risk through biliary cholesterol supersaturation; this mechanism is less likely to operate at the doses and durations typical of the PMOS cohort. The metabolic context also differs. Whereas gallbladder disease in T2DM is driven substantially by hyperglycemia and rapid weight change, PMOS is characterized by hyperinsulinemia-driven gallbladder dysmotility, and the improvement in insulin sensitivity afforded by GLP-1 RAs may offset this susceptibility to gallstone formation. Prospective studies with standardized

dosing data and longer follow-up are needed to determine whether these findings reflect a true protective effect or differences in exposure, dosing, and disease phenotype between the PMOS and T2DM populations.

The mechanistic link between PMOS and gallbladder disease centers on the effect of insulin resistance on gallbladder motility. Hyperinsulinemia and insulin resistance reduce the gallbladder's sensitivity to cholecystokinin, the principal hormonal stimulus for gallbladder contraction, resulting in slower and less complete emptying and higher residual volume.[14] Insulin additionally promotes hepatic cholesterol synthesis and its secretion into bile, yielding more lithogenic bile, and alters autonomic control of the biliary tract, further reducing gallbladder wall contraction. These changes are amplified by obesity, which is strongly associated with increased gallbladder size. Fasting gallbladder volume measured by sonography ranges from 17 to 18 mL in nonobese individuals but reaches 30 to 47 mL in obese individuals, and the resulting impairment in emptying favors gallstone formation.[15,16] Because PMOS aggregates hyperinsulinemia, insulin resistance, and obesity, these pathways are directly relevant to the elevated gallbladder disease risk observed in this population, and they identify the metabolic targets through which GLP-1 RAs may act.

Glucagon-like peptide-1 RAs may reduce gallstone risk in part through effects on lipid metabolism. Glucagon-like peptide-1 inhibits lipogenesis, promotes lipolysis, enhances cholesterol metabolism, and stimulates browning of white adipose tissue, actions that could lower biliary cholesterol saturation and complement the improvement in insulin sensitivity.[17] Although the present study did not perform a separate analysis of lipid outcomes, these documented metabolic effects offer a plausible mechanism by which GLP-1 RAs, relative to metformin, might attenuate cholelithiasis and cholecystitis risk in PMOS.

In contract, cholangitis arises from mechanical obstruction of the common bile duct with subsequent bacterial ascension, a process that GLP-1 RAs would not be expected to prevent directly. The absence of a significant difference in cholangitis risk is therefore consistent with mechanism: by modulating cholesterol metabolism and reducing gallstone formation, GLP-1 RAs may indirectly lower the likelihood of the obstruction that precipitates cholangitis, but they do not act on the obstructive event itself.

These observations carry implications for treatment selection in PMOS. Because rapid weight loss and reduced gallbladder motility are dose- and duration-dependent, longer courses of GLP-1 RA therapy at higher, weight-loss doses are expected to increase gallbladder disease risk, whereas the lower doses and shorter durations common in PMOS may confer a more favorable biliary profile.[13] For a patient with PMOS in whom both agents are otherwise appropriate, a lower baseline risk of cholelithiasis and cholecystitis may favor a GLP-1 RA, particularly when weight reduction and improved

insulin sensitivity are therapeutic priorities. Conversely, in patients with established gallbladder disease or those anticipated to undergo rapid weight loss, closer monitoring and attention to exposure duration and dose remain warranted. These findings should inform, rather than dictate, individualized therapy pending prospective confirmation.

The insulin resistance central to these mechanisms is itself sustained by chronic low-grade inflammation. In PMOS and obesity, enlarged adipocytes and infiltrating immune cells release proinflammatory cytokines, principally tumor necrosis factor-α and interleukin-6, that impair insulin signaling. Tumor necrosis factor-α disrupts insulin receptor substrate-1 and lowers adiponectin, and interleukin-6 induces suppressor of cytokine signaling 3, which blocks insulin receptor signaling.[18] This inflammatory contribution to insulin resistance links the reproductive and biliary manifestations of PMOS and reinforces the rationale for therapies that improve insulin sensitivity.

Management of PMOS remains focused on symptom control and fertility, as no curative therapy exists. Combined oral contraceptives are the mainstay for menstrual irregularity and hyperandrogenism, although they can aggravate insulin resistance. In a cross-sectional study of 2,272 participants, combined oral contraceptive users had higher lipid levels and greater odds of abnormal total cholesterol than nonusers (OR, 1.29; 95% CI, 1.05-1.58).[19,20] Alternative hormonal options include cyclic or continuous progestin therapy and progestin-releasing intrauterine devices. Nonhormonal agents targeting the metabolic comorbidities of PMOS include metformin, thiazolidinediones, dipeptidyl peptidase-4 inhibitors, statins, and sodium-glucose cotransporter-2 inhibitors.[19] Metformin remains the preferred first-line agent for insulin resistance but is limited by gastrointestinal adverse effects in approximately 25% of users, with discontinuation in roughly 5%, and by a rare risk of lactic acidosis in patients with reduced kidney function.[6] Against this backdrop, the biliary profile of GLP-1 RAs observed herein adds a consideration relevant to therapy selection in PMOS.

Limitations

As a retrospective analysis of TriNetX data, the findings depend on the accuracy and completeness of clinical coding and documentation and are subject to misclassification bias. Propensity score matching mitigates measured confounding but not confounding from unmeasured variables such as patient behavior and social determinants of health. Other unmeasured confounders that may influence gallbladder disease risk, including oral contraceptive use, obstetric history, treatment dose and duration, and medication adherence, were not evaluated.

Prescribing bias is also possible; clinicians may preferentially prescribe metformin rather than a GLP-1 RA to patients at elevated gallbladder risk, which could produce channeling bias toward a false protective signal in the

GLP-1 RA cohort. Because patients were not restricted from concurrent use of the comparator medication, some individuals may have received both metformin and a GLP-1 RA during follow-up. Such overlap would tend to make the cohorts more similar and bias the comparison toward the null, so the observed differences may underestimate the true contrast between therapies. A sensitivity analysis excluding patients with overlapping exposure, or adjustment for concomitant therapy, would help quantify this effect and is warranted in future work.

This study used an all-comers design in which patients with prior gallbladder disease were retained, because the TriNetX platform was configured to include outcomes occurring before the time window. Although both cohorts were subject to this design, and SMDs after propensity score matching confirm balance across key risk factors, this approach captures real-world burden rather than strictly incident outcomes. We did not adjust for clustering across the 89 to 101 contributing healthcare organizations, and site-level differences in prescribing or imaging frequency may influence the results. Association does not imply causation, even with propensity score matching in observational data. Given the complex nature of gallbladder disease and PMOS, unmeasured comorbidities, such as inflammatory bowel disease, may also contribute to gallbladder disease risk. Finally, although the reported risk differences reached statistical significance, their magnitude was modest and their clinical applicability may be limited.

CONCLUSION

In this propensity score–matched retrospective cohort study, GLP-1 RA use was associated with a significantly lower incidence of cholelithiasis and cholecystitis than metformin among patients with PMOS, whereas cholangitis risk was comparable between cohorts. These findings contrast with prior reports of increased biliary disease in T2DM and obese populations and are best explained by population-specific differences in age, exposure duration, dosing, and the metabolic phenotype of PMOS, as detailed herein. Prospective studies with longer follow-up, standardized dosing data, and exclusion of pre-existing gallbladder disease are needed to establish the direction and clinical significance of this association.

Ethics Statement

This study was reviewed by the Howard University Institutional Review Board and granted exempt status under 45 CFR §46.104(d)(4). Because only de-identified data were accessed, the requirement for informed consent was waived.

Consent Statement

Written informed consent from the participants was not required to participate in this study in accordance with national legislation and institutional requirements.

Conflict of Interest Statement

The authors declared no potential conflicts of interest with respect to the research, authorship, or publication of this article.

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Peer Review

Abstract

Minor comments

- Line 25: T2DM is the standard abbreviation, please correct TDM2 to T2DM throughout the manuscript.
- Line 37: Currently says GLP-1, please replace GLP-1 with GLP-1 receptor agonists for clarity.

Introduction

Major comments:

- Lines 78-89: The introduction is good at giving an in-depth background of each drug that is being compared, but only the GLP-1 agonist is discussed in detail about how it affects the activity of the gallbladder. In order to give readers a full picture, the authors should note effects of metformin on the gallbladder.

Minor comments:

- Lines 41-42: The sentence in lines 41-42 "Cholelithiasis, cholecystitis, and cholangitis are common..." may be better suited to be written after the introduction of PMOS, as the first two lines are not directly related and cause some confusion. It may be better to be placed after the sentence in line 54. A suggested rewrite could be "This mechanism supports the reported threefold increase in common gallbladder pathologies, such as cholelithiasis, cholecystitis, and cholangitis, and explains their association with risk factors including metabolic syndrome, obesity, and T2DM." It may also be helpful to define the differences between all three diseases.
- Line 45: Consider adding a schematic figure illustrating the metabolic pathways described. Such a figure could also demonstrate where metformin and GLP-1 agonists act.
- Lines 60-64: The sentences starting with "One of metformin's major long-term effects..." are more related to the mechanism of action of metformin and may be better placed after line 56, which talks about its MOA. The next sentences can be what it is prescribed/used for.

- Line 66: The sentence of additional GLP-1 agonist indications doesn't appear to be directly relevant to the study. Consider removing this to maintain the focus of the current study.
- Lines 68 and 74: The incretin effect is discussed but not defined. Throughout the paper it is used as an important marker for insulin regulation and a way to compare GLP-1 to metformin. Therefore, it is important that is defined.
- Line 68: The way it is currently written, the description of the incretin effect suggests it is unique to individuals with T2DM. However, the incretin effect is a normal physiological response that is impaired in patients with T2DM. Consider clarifying this distinction when the incretin effect is first introduced rather than waiting until Line 74.
- Line 69: Consider specifying that insulin secretion is stimulated in response to oral glucose intake which would more accurately describe the incretin effect.
- Line 74: Discusses the incretin effect in TD2M, but are there studies regarding this effect or something similar in patients with PMOS? If not, it would be helpful to state how this effect in TD2M patients is comparable to how we would expect these drugs to work in patients with PMOS.
- Line 80: Identify semiglutide and liraglutide as GLP-1 agonists before introducing these statistics so that it is easier for the reader to follow. Consider specifying that semaglutide and liraglutide are GLP-1 agonists when introduced for readers who may be less familiar with these medications.
- Line 82: Tirzepatide is briefly introduced as a dual GIP/GLP-1 receptor agonist but is not discussed further. There is no information about what a GIP is or its mechanism of action, which would help with clarity and completeness; otherwise, consider either briefly explaining its relevance or removing this if it does not contribute to the manuscript's objectives.
- Lines 86-89: Describe how an increased risk in gallbladder disease is associated with GLP-1 use, but there is no explanation of why, this would be helpful to describe what those studies found regarding this.

- Line 88: In line 88, "One study found that GLP-1 RAs..." the acronym RA was not defined earlier as receptor agonist. GLP-1 RAs is used as an abbreviation without being previously defined. Please define the abbreviation at first use.

Materials & Methods

Major comments:

- Line 112: Because patients were not restricted from concurrent use of the comparator medication, treatment exposure could have overlapped between groups, potentially blurring the distinction between cohorts and introducing a confounding variable that may affect the manuscript's interpretation. Please address the extent of treatment crossover and discuss its potential impact on the reported results. If possible, consider adjusting for concomitant therapies or excluding patients with overlapping exposure.

Minor comments:

- Line 149: States "gallbladder disease burden" but did not describe how this burden is determined, and it may be helpful for completeness.

Results

Major comments:

- None

Minor comments:

- Line 167: The manuscript refers to Figure 3, although only two figures are included. Please correct this to reference Figure 3.
- Lines 167, 174, 182: The confidence intervals of risk differences are reported in the text but are not included in Table 1. Since the confidence intervals for the ratios and odds ratios are both reported in the text and Table 1, consider including the confidence intervals for the risk differences in Table 1 for consistency.

Discussion

Major comments:

- Lines 203-205: The claim that "GLP-1 agonist may decrease the risk of developing cholecystitis and cholelithiasis" implies an absolute risk reduction relative to no treatment or baseline. Since the study compares GLP-1 agonists to metformin, the conclusion should be framed as a relative comparison between the two therapies. I suggest revising to: "Analysis of the data revealed that, compared to metformin, GLP-1 agonist use was associated with a lower risk of developing cholecystitis and cholelithiasis in individuals with PMOS, though no significant difference was observed for cholangitis."

Minor comments:

- General: This discussion has many good elements, but it feels disconnected, and the relevant information is hard to piece together. A rewrite focusing on the continuity of information would greatly strengthen this article.
- Lines 203-205: After stating the main results of the paper "Analysis of the data revealed GLP-1 agonists may decrease the risk of developing cholecystitis and cholelithiasis in individuals with PMOS, but not the risk of developing cholangitis." The paper does not continue to expand here or later in the paper on how these findings may impact public health or clinical care. The authors may want to add a brief discussion about how these findings may impact treatment decisions. For example, whether the risk profile for cholecystitis and cholelithiasis should factor into choosing between GLP-1 agonists and metformin in patients with PMOS, and what criteria might guide that choice.
- Lines 214-215: State "PMOS is also characterized by hyperinsulinemia-driven gallbladder dysmotility...," and it may be helpful to include a contrast here to the characteristics of T2DM to better underscore the differences in the findings.
- Lines 220-221: The information in lines 220-221 regarding the definition of cholangitis would be helpful in the introduction, as mentioned above.
- Lines 226-227: Could benefit from some explanation as to why longer duration use of GLP-1 agonist at higher dosing will increase an individual's risk of developing gallbladder disease.

- Lines 228-230: The author begins to discuss a "subset of the population included individuals with PMOS treated with either metformin or GLP-1 agonist for concurrent effects on insulin resistance and cholesterol metabolism." This subset was not mentioned earlier in the paper, nor are its findings adequately discussed. Knowing how these concurrent effects on insulin metabolism and cholesterol metabolism, and what the results showed, would strengthen the paper and also add a new layer of insight.
- Lines 234-247: The paragraph from 234-247 regarding gallbladder size seems a bit off topic as it does not relate to either metformin or GLP-1 drugs or directly to PMOS. Is this paragraph meant to give an alternative explanation for increased risk of gallstones in T2DM? More clarity is needed on why this information is specifically relevant to the topic at hand.
- Line 243: CCK would be better written out as cholecystokinin (CCK) for clarity. CCK is used without being defined, please define this abbreviation at first use.
- Line 248: Consider introducing earlier in the discussion that PMOS is associated with obesity, insulin resistance to more clearly frame the clinical significance of the study.
- Lines 248-258: Similar to what is stated above, the paragraph from 248-258 has valuable information regarding the pathophysiology of PMOS, but it feels disconnected from the rest of the information and out of place. It may benefit from being put earlier in the discussion or more needs to be added as to how this pathophysiology directly relates to the findings with the two medications and gallbladder disease incidence.
- Lines 304-305: The line from 304-305 states "...obesity cohorts and likely reflect population-specific differences in age, medication exposure duration, dosing, and the metabolic phenotype of PMOS." But this matter is not clear from the discussion. See above comments regarding a potential rewrite with a focus on tying the information together instead of laying it all out for the reader to assume.

Subheadings/Tables (if applicable)

Major comments:

- None

Minor comments:

- **Figures:** The figure uses PCOS instead of PMOS, please ensure terminology is consistent. The figure should also change GLP-1 to GLP-1 agonist to match the figure caption and should be kept consistent throughout the manuscript. Lastly, the figure caption uses DM2 diagnosis. Please change this to the standard abbreviation of T2DM.

References

Major comments:

- Reference 17: This reference supports an association between fatty liver and gallstone disease but does not directly address the stated mechanism involving gallbladder sensitivity to CCK. Consider replacing this reference with studies specifically investigating the pathophysiology of how exactly hyperinsulinemia and insulin resistance decrease CCK sensitivity.