

# Liver Enzyme Biomarkers Before or in Early Pregnancy as Predictors for Gestational Diabetes Mellitus: A Systematic Review and Meta-Analysis

Samuel Pratama,<sup>1,2</sup> Rizka Dwi Aulia,<sup>1</sup> Indi Jazilah,<sup>1</sup> Brigitta Cindy Lauren<sup>2</sup>

<sup>1</sup>Department of Internal Medicine, Dr. Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia

<sup>2</sup>Faculty of Medicine, University of Indonesia, Jakarta, Indonesia

## Abstract

**Background.** Liver enzymes may reflect early metabolic disturbances and insulin resistance preceding gestational diabetes mellitus (GDM). This systematic review and meta-analysis evaluated whether liver enzyme biomarkers measured before or in early pregnancy are associated with subsequent development of GDM.

**Methodology.** PubMed, Cochrane, EBSCOHost, and SCOPUS databases were searched through May 2025 for observational studies or trials assessing pre- or early pregnancy liver enzymes in relation to GDM development. Pooled mean differences (MD) and odds ratios (OR) with 95% confidence intervals (CI) were calculated using a random-effects model. Risk of bias was assessed using RoB 2.0 and ROBINS-E; certainty of evidence was evaluated using GRADE.

**Results.** Twenty-seven studies were included in the analyses. GDM was associated with higher AST (MD 0.97 U/L; OR 1.42), ALT (MD 2.38 U/L; OR 1.69), GGT (MD 3.77 U/L; OR 2.57), and hepatic steatosis index (HSI) (MD 2.82; OR 2.19). ALP showed no significant mean difference but an elevated GDM risk (OR 1.47). Substantial heterogeneity was observed with very low certainty of evidence across outcomes.

**Conclusion.** Elevated liver enzymes, especially GGT and HSI, are associated with increased GDM risk at a population level. However, high heterogeneity and very low certainty of evidence limit current clinical applicability, warranting further prospective validation.

**Key words:** liver enzymes, gestational diabetes mellitus, pregnancy, meta-analysis, odds ratio

## INTRODUCTION

Gestational diabetes mellitus (GDM) refers to glucose intolerance diagnosed for the first time during pregnancy.<sup>1</sup> It is one of the most common metabolic disturbances during pregnancy. Around 16.7% of live births are complicated by diabetes during pregnancy, and of these, 84% are diagnosed with GDM, bringing notable health risks for both the mother and fetus.<sup>2</sup> Gestational diabetes mellitus is shown to have an association with preeclampsia, macrosomia, and long-term risk of type 2 diabetes mellitus for both mother and offspring.<sup>3</sup> Early diagnosis of GDM in pregnancy is necessary for opportune intervention to improve pregnancy outcomes and decrease the risk of further metabolic complications.<sup>4</sup>

The diagnostic criteria for GDM have grown significantly in recent decades and vary internationally. In 2010, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) suggested new diagnostic points based

on data from the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study, which revealed a constant association between mother glucose levels and unfavorable perinatal outcomes. These recommendations were later adopted by the World Health Organization (WHO) in 2013 and have since been implemented in various countries.<sup>5</sup> In Indonesia, the Indonesian Endocrinology Society advocated these WHO/IADPSG criteria in their national guidelines, suggesting a one-step 75-gram oral glucose tolerance test (OGTT) examined at 24–28 weeks of gestation. According to these standards, GDM is diagnosed when one or more of the following blood glucose thresholds are met or exceeded: fasting  $\geq 92$  mg/dL, 1-hour  $\geq 180$  mg/dL, or 2-hour  $\geq 153$  mg/dL.<sup>6</sup> There are also other guidelines, such as the American Diabetes Association (ADA) and the American College of Obstetricians and Gynecologists (ACOG), which differ in screening approach, glucose load, number of abnormal values required for diagnosis, and diagnostic thresholds. Nonetheless, diagnosing GDM at this stage decreases the possibility of early preventive steps. Identifying steadfast

biomarkers in the preconception phase or early pregnancy could ameliorate risk assessment and encourage an earlier implementation of lifestyle or therapeutic interventions.<sup>7</sup>

Liver enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT), have been acknowledged as indicators of hepatic function and insulin resistance. Increased levels of these indicators have been linked to metabolic syndrome, type 2 diabetes mellitus (T2DM), and metabolic dysfunction-associated fatty liver disease (MAFLD) in the general population.<sup>8</sup> In line with type 2 diabetes mellitus, the core mechanism of GDM is insulin resistance. Therefore, liver enzyme levels may serve as early markers of metabolic disturbances that precede the onset of the condition.<sup>9</sup>

Several studies have analyzed whether liver enzyme levels measured prior to or early in pregnancy are associated with the risk of developing GDM, but the findings have been conflicting. Therefore, this systematic review and meta-analysis seeks to assess and quantitatively analyze the significance of liver enzymes as biomarkers to predict the occurrence of GDM.

**METHODOLOGY**

**Search strategy**

A systematic search of four databases (i.e., PubMed, Cochrane, EBSCOHost, and SCOPUS) was conducted to gather available literature concerning the predictive value of liver enzymes measured before or in early pregnancy towards GDM. The keywords listed in Table 1 were utilized for the search. There were no restrictions on language or on publication time frame. Additional references were manually searched to locate more literature. Duplicates in the initial results were subsequently eliminated, and then the titles and abstracts were screened. Comprehensive reviews were performed to further identify suitable studies for inclusion in the data analysis. The process of searching and screening was carried out separately by

four investigators with any disagreements resolved by discussion. This systematic review has been registered in PROSPERO with identification code CRD420251077120.

**Study eligibility criteria**

The inclusion criteria for a study to be included in the analysis were the following: 1) Clinical trials, cohort, or case control studies; 2) Human female subjects aged 18 years old or above as the subjects; 3) No history of Type 2 Diabetes Mellitus (T2DM) or known liver diseases among the subjects; and 4) The liver enzymes were measured before pregnancy or during early pregnancy (before 24 weeks of gestation). A study was excluded if there were any of these following criteria: 1) Other types of articles (e.g., reviews, commentary, etc.); 2) Single arm study (with no control group); 3) The liver enzymes were measured at the time of GDM testing; 4) Subjects with history of T2DM or liver diseases; and 5) No full text available.

**Data extraction and risk of bias assessment**

The data extraction was done independently by four reviewers. The following data were extracted from each included study: 1) First author and publication year; 2) Study characteristics, including study location, study design, time of marker measurement, time of GDM diagnosis, and GDM diagnosis criteria used; 3) Characteristics of GDM and non-GDM group, including sample size, age, pre-gravid body mass index (BMI), and the percentage of GDM subjects for cohort study; and 4) Study outcomes, including Mean Differences (MDs) of measured liver enzymes and odd ratio for increased markers between GDM and non-GDM group. The liver enzymes of interest included serum AST, ALT, GGT, and ALP level. Additional data of AST/ALT ratio and Hepatic Steatosis Index (HSI) were also extracted and analysed if reported. HSI is typically used to assess fatty liver disease and can be calculated by using AST, ALT, and BMI of the subject.<sup>10</sup> The odds ratio was calculated using the number of events and non-events in subjects with high and normal liver enzymes. Definitions

**Table 1.** Keywords used in each database

Database	Keywords
<b>Pubmed</b>	("Liver Function Tests"[Mesh] OR "liver enzymes"[tiab] OR "ALT"[tiab] OR "AST"[tiab] OR "GGT"[tiab] OR "alanine aminotransferase"[tiab] OR "aspartate aminotransferase"[tiab] OR "gamma-glutamyl transferase"[tiab]) AND ("Diabetes, Gestational"[Mesh] OR "gestational diabetes"[tiab] OR "GDM"[tiab] OR "pregnancy-induced diabetes"[tiab]) AND ("clinical trial"[Publication Type] OR "observational study"[tiab] OR "systematic review"[tiab] OR "meta-analysis"[Publication Type])
<b>Cochrane Library</b>	#1 MeSH descriptor: [Diabetes, Gestational] explode all trees #2 "Liver enzyme" OR "Liver Biomarker" #3 "ALT" OR "Alanine transaminase" OR "SGPT" OR "Serum Glutamic Pyruvic Transaminase" #4 "AST" OR "Aspartate transaminase" OR "SGOT" OR "Serum Glutamic Oxaloacetic Transaminase" #5 "ALP" OR "Alkaline Phosphatase" #6 "GGT" OR "Gamma-Glutamyl Transferase" #7 #1 AND (#2 OR #3 OR #4 OR #5 OR #6)
<b>EBSCOHost</b>	("Gestasional diabetes mellitus" OR "GDM" OR "Gestasional diabetes") AND (("Liver enzyme" OR "Liver Biomarker") OR ("ALT" OR "Alanine transaminase" OR "SGPT" OR "Serum Glutamic Pyruvic Transaminase") OR ("AST" OR "Aspartate transaminase" OR "SGOT" OR "Serum Glutamic Oxaloacetic Transaminase") OR ("GGT" OR "Gamma-Glutamyl Transferase") OR ("ALP" OR "Alkaline Phosphatase"))
<b>SCOPUS</b>	("Gestasional diabetes mellitus" OR "GDM" OR "Gestasional diabetes") AND (("Liver enzyme" OR "Liver Biomarker") OR ("ALT" OR "Alanine transaminase" OR "SGPT" OR "Serum Glutamic Pyruvic Transaminase") OR ("AST" OR "Aspartate transaminase" OR "SGOT" OR "Serum Glutamic Oxaloacetic Transaminase") OR ("GGT" OR "Gamma-Glutamyl Transferase") OR ("ALP" OR "Alkaline Phosphatase"))

of elevated liver enzyme levels varied across studies. Most studies categorized liver enzyme concentrations using relative thresholds within each cohort, commonly defining “high” levels as values in the upper quartile or highest percentile category. Because absolute laboratory cut-offs were not standardized across populations or pregnancy stages, relative categorization was used to enable within-study risk comparisons. These comparisons therefore represent relative exposure contrasts rather than pathological liver dysfunction. Subjects with liver enzymes included in the upper fourth quartile among other subjects, were considered as high or elevated.

The risk of bias for each study was assessed using the appropriate tools. Cohort and case control studies were assessed using the Cochrane risk of bias in non-randomized follow-up studies of exposure effects (ROBINS-E) tool, whereas randomized trials were assessed using the Cochrane Risk of Bias 2.0 (RoB 2.0) tool.<sup>11,12</sup> The risk of bias assessment was done independently, with any discrepancies among the investigators were resolved by discussion. The summary of the assessment is presented as plots generated using the Robvis tool.<sup>13</sup>

### Data synthesis and statistical analysis

The extracted data was compiled into tables and summarized descriptively. The variations in enzyme levels and the occurrences of GDM for both the exposed and unexposed groups were computed for quantitative analysis. The evaluation was conducted utilizing Review Manager 5.4 (Cochrane). The extracted dichotomous data were examined using the Mantel-Haenszel statistical method with a random effects analysis model. For continuous outcomes, pooled MDs with 95% confidence intervals were calculated using the inverse-variance method under a random-effects model. The odds ratio (OR) was computed to assess the comparative impact of the intervention against the control. A p-value of less than 0.05 was deemed statistically significant, 95% confidence intervals were used to estimate precision, and heterogeneity analysis computed  $I^2$  as the measure. An  $I^2$  of <50% signified no notable heterogeneity, 50-70% signified substantial heterogeneity, 70-90% signified high heterogeneity, and >90% signified very high heterogeneity. The pooled results were converted into forest plots for enhanced data visualization. Publication bias was assessed using funnel plots for each outcome of interest.

The results are presented according to the PRISMA 2020 guideline for systematic review and meta-analysis.<sup>14</sup> The certainty of evidence for each outcome was assessed using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) approach. The GRADE domains include risk of bias, inconsistency, indirectness, imprecision, and publication bias.<sup>15</sup> Each outcome was rated as having high, moderate, low, or very low certainty based on these domains. The assessments were performed independently by four reviewers, and discrepancies were resolved through discussion.

### Ethical approval

Due to the nature and design of this study, no ethical approval was needed.

## RESULTS

### Study selection

The study selection process was done according to the PRISMA guideline and is reported as the PRISMA flow chart in Figure 1. The initial search was done on May 15, 2025, across four databases and resulted in 2,916 hits. Duplicates were removed and the remaining records underwent title and abstract screening. The full text review was performed to 50 records, with another 3 records added from manual searching. A total of 26 records were excluded due to reasons stated in Figure 1. Findings are presented using quantitative meta-analytic methods. One study was excluded from the quantitative synthesis due to exclusively reported data as median (interquartile range) without sufficient information for the mean-difference meta-analyses. Other results are described narratively where meta-analysis was not feasible.

### Study characteristics and risk of bias

There are 27 studies included in the analyses with characteristics summarized in Table 2.<sup>9,16-41</sup> They were published between 2014 and 2024, mostly from Asian countries, particularly China. The included studies consist of 17 prospective cohort studies, 6 retrospective cohort studies, 3 case control studies, and 1 pilot RCT study. Pre-pregnancy marker measurements were done in 5 studies, while the others measured the liver marker during 4-20 weeks of pregnancy. The GDM diagnosis was mostly done during the 24-28 weeks of gestation period using various diagnosis criteria, but the majority were using the IADPSG criteria. The total subjects included in the GDM group are 61,858 subjects and 1,018,947 subjects in the non-GDM group. The pilot RCT and prospective studies reported a wide range of GDM incidence among their cohorts, ranging from 5.9-56.3%. The incidence was lower in studies that only included insulin-treated GDM, ranging from 0.6-2.74%. In addition, almost all studies reported that subjects in the GDM group were older and had higher pre-gravid BMI when compared with the non-GDM group. Other information regarding each study (e.g., funding, ethnicity, socioeconomic status) were highly variable and are not discussed in this manuscript.

The risk of bias assessment was done using the RoB 2.0 tool for 1 pilot RCT study by Maitland et al., showing some concerns within the study (Figure 2). The remaining 26 cohort or case-control studies were assessed using the ROBINS-E tool, showing that 17 out of 26 studies also had some concerns regarding the risk of bias (Figure 3A and Figure 3B).

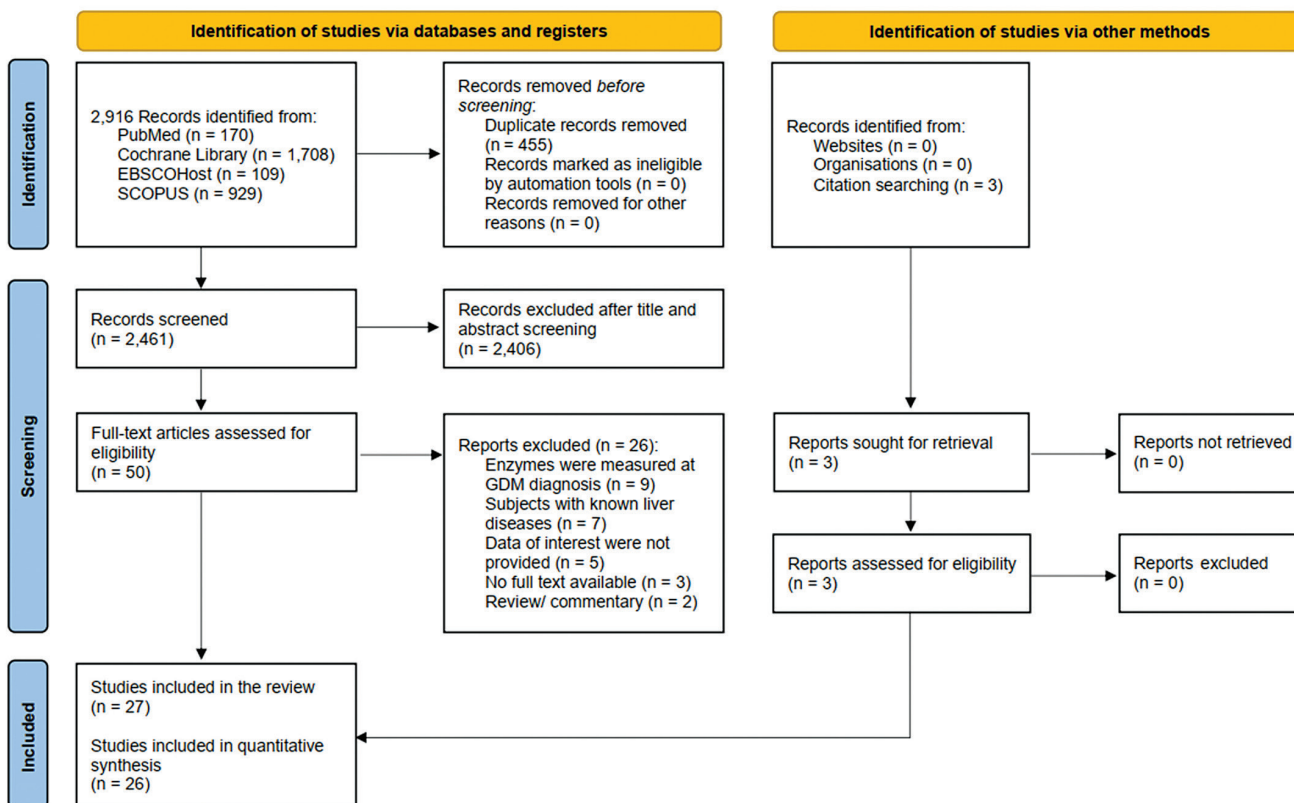


Figure 1. PRISMA flow diagram of this systematic review.

		Risk of bias domains					
		D1	D2	D3	D4	D5	Overall
Study	Maitland (2014)	+	-	-	+	-	-

Domains:  
 D1: Bias arising from the randomization process.  
 D2: Bias due to deviations from intended intervention.  
 D3: Bias due to missing outcome data.  
 D4: Bias in measurement of the outcome.  
 D5: Bias in selection of the reported result.

Judgement  
 - Some concerns  
 + Low

Figure 2. The risk of bias assessment using the RoB 2.0 tool.

**Study outcomes**

The study outcomes from the included studies are extracted and summarized in Table 3. Data of interest were collected from the included studies to be analyzed quantitatively. It is noteworthy that most of the studies compared the event of GDM between subjects who had elevated liver markers (i.e., upper 4<sup>th</sup> quartile) to those with lower liver markers (i.e., lower 1<sup>st</sup> quartile) to calculate their odds ratio.

**Serum AST level**

There are 15 studies that compare the AST level between GDM and non-GDM groups (Figure 4A). The quantitative analysis showed that subjects in the GDM group had higher levels of AST prior to the diagnosis compared with subjects without GDM, with a mean difference of 0.97 U/L (95% CI 0.29-1.64; *p* < 0.005). The result analysis from 5 studies showed that subjects with higher AST levels had

significantly higher odds of developing GDM (OR 1.42, 95% CI 1.24-1.62; *p* < 0.0001), as shown in Figure 4B. The heterogeneity analysis showed substantial heterogeneities among these studies. The funnel plots for both the mean difference and odds ratio showed a symmetric distribution of studies, suggesting a low risk of publication bias (Supplementary Figure S1A-B).

**Serum ALT level**

The analysis on serum ALT levels can be seen in Figure 5A and 5B. The level of pre-gravid or early pregnancy serum ALT was also shown to be higher in the GDM group compared to the non-GDM group, with a mean difference of 2.38 U/L (95% CI 0.97-3.79; *p* = 0.001). The increased serum ALT level was also shown to increase the odds of GDM occurrence in pregnant subjects (OR 1.69, 95% CI 1.17-2.45; *p* = 0.005). A substantial heterogeneity was also shown in the analysis, suggesting notable variability among

**Table 2.** The characteristics of included studies

Author	Year published	Study location	Study design	Marker measurement timing (wog)	Definition of elevated liver enzyme <sup>a</sup>	GDM diagnosis timing (wog)
<i>Maitland et al</i>	2014	UK	Pilot RCT	16-18	Study-defined percentile categories	27-28
<i>Sridhar et al</i>	2014	USA	Case Control	Pre-pregnancy	Highest (Q4) vs lowest (Q1) quartile	27-28
<i>Zhao et al</i>	2016	China	Prospective Cohort	8-12	Not dichotomized	24-28
<i>White et al</i>	2016	UK	Prospective Cohort	15-18	Not dichotomized	24-33
<i>Leng et al</i>	2016	China	Prospective Cohort	4-12	Highest (Q4) vs lowest (Q1) quartile	24-28
<i>Yarrington et al</i>	2016	USA	Prospective Cohort	Mean: 10.4	ALT <19 U/L vs ≥19 U/L	24
<i>Kong et al</i>	2018	China	Prospective Cohort	14-18	GGT <26.9 U/L vs ≥26.9 U/L	24-28
<i>Zhu et al</i>	2018	USA	Prospective Cohort	10-13	Highest (Q4) vs lowest (Q1) quartile	24-28
<i>Xiong et al</i>	2019	China	Prospective Cohort	<20	Highest (Q4) vs lowest (Q1) quartile	24-28
<i>Correa et al</i>	2019	Chile	Case Control	<14	Not dichotomized	24-28
<i>Lee et al</i>	2019	South Korea	Prospective Cohort	10-14	High-risk HIS (>36) vs ≤36	24-28
<i>Gao et al</i>	2020	China	Prospective Cohort	<12	Study-defined percentile categories	24-28
<i>Lee et al</i>	2020	South Korea	Retrospective Cohort	4-20	ALT >95 <sup>th</sup> percentile vs ≤95 <sup>th</sup> percentile	24-28
<i>Park et al<sup>d</sup></i>	2021	South Korea	Retrospective Cohort	Pre-pregnancy	Highest (Q4) vs lowest (Q1) quartile	24-28
<i>Zhao et al</i>	2021	China	Prospective Cohort	8-12	Study-defined percentile categories	24-28
<i>Kim et al<sup>d</sup></i>	2021	South Korea	Retrospective Cohort	Pre-pregnancy	Highest (Q4) vs lowest (Q1) quartile	24-28
<i>Sang et al<sup>d</sup></i>	2021	South Korea	Retrospective Cohort	Pre-pregnancy	Highest (Q4) vs lowest (Q1) quartile	24-28
<i>Wang et al</i>	2021	China	Case Control	12	Highest (Q4) vs lowest (Q1) quartile	24-28
<i>Shuoning et al</i>	2021	China	Prospective Cohort	6-12	Highest (Q4) vs lowest (Q1) quartile	24-28
<i>Shuoning et al</i>	2022	China	Prospective Cohort	6-12	Highest (Q4) vs lowest (Q1) quartile	24-28
<i>Rongjing et al</i>	2022	China	Prospective Cohort	10-14	Study-defined percentile categories	24-28
<i>Quotah et al</i>	2022	UK	Prospective Cohort	15-18	Not dichotomized	23-30
<i>Duo et al</i>	2023	China	Prospective Cohort	6-12	AST/ALT ratio ≥0.825 vs <0.825	24-28
<i>Wu et al</i>	2023	China	Prospective Cohort	6-15	Highest (Q4) vs lowest (Q1) quartile	24-28
<i>Lee et al<sup>d</sup></i>	2023	South Korea	Retrospective Cohort	Pre-pregnancy	Serum GGT ≥20 vs <10 U/L	24-28
<i>Zhen et al</i>	2024	China	Prospective Cohort	<14	Highest (Q4) vs lowest (Q1) quartile	24-28
<i>Liu et al</i>	2024	China	Retrospective Cohort	8-14	Highest (Q4) vs lowest (Q1) quartile	24-28

Abbreviations: GDM, Gestational Diabetes Mellitus; WOG, Weeks of Gestations; BMI, Body Mass Index; RCT, Randomized Controlled Trial; IADPSG, International Association of the Diabetes Pregnancy Study Groups; ACOG, American College of Obstetricians and Gynecologist; ADA, American Diabetes Association; UK, United Kingdom; USA, United States of America; N/A, Not Applicable.

Data for continuous variables with normal distribution are presented as mean (standard deviation) or as median (interquartile range) if the distribution is abnormal.

<sup>a</sup> Elevated liver enzyme levels for dichotomized analyses were defined using study-specific relative thresholds due to heterogeneity in laboratory reference ranges and timing of measurement during pregnancy.

<sup>b</sup> Only applicable for RCT or cohort studies.

<sup>c</sup> Statistically significant in difference from the GDM group ( $p < 0.05$ ).

<sup>d</sup> Data from the insulin treated group was used

studies. The shape of the funnel plots for ALT analysis appeared symmetrical, indicating no significant evidence of publication bias (Supplementary Figure S2A–B).

### Serum GGT level

Based on the findings from 10 studies, the meta-analysis revealed a significantly higher mean GGT level in the GDM group compared to the non-GDM group, with a mean difference of 3.77 U/L (95% CI 1.97-5.58;  $p < 0.0001$ ). Furthermore, the results from 8 studies indicate that women with high GGT levels had 2.57 times higher odds of developing GDM compared to those with lower GGT levels

(OR 2.57, 95% CI 2.07-3.20;  $p < 0.0001$ ). Heterogeneity was also found to be substantial in these studies. The forest plot can be observed in Figure 6A and 6B. Funnel plots for GGT were visually symmetrical, reflecting minimal small-study effects or reporting bias (Supplementary Figure S3A–B).

### Serum ALP level

The forest plots based on analyses of serum ALP level can be seen in Figure 7A and 7B. There was no significant difference in mean ALP levels between women with and without GDM (mean difference 0.86 U/L, 95% CI -1.22-2.95;  $p = 0.42$ ) and also moderate heterogeneity was present

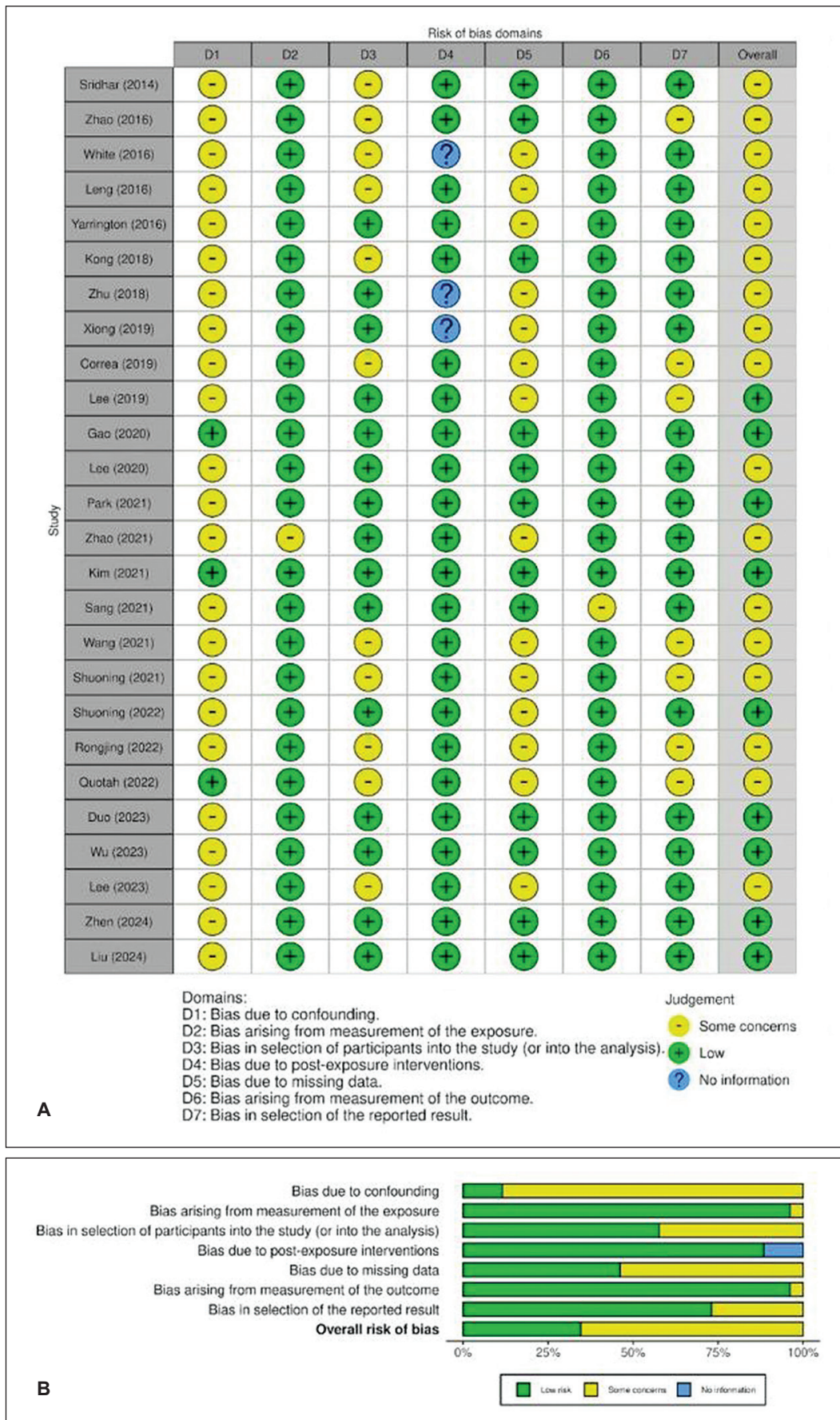
GDM diagnosis criteria	GDM group			% of gdm <sup>b</sup>	Non-GDM group		
	Sample size	Age (Years)	Pre-gravid BMI		Sample size	Age (Years)	Pre-gravid BMI
IADPSG	29	34 (31-36)	35.27 (3.60)	27.4	77	31 (26-34) <sup>c</sup>	36.11 (4.95)
ACOG	256	28.2 (5.5)	26.0 (6.5)	N/A	497	28.4 (5.2)	23.7 (4.6) <sup>c</sup>
ADA	725	32 (29-35)	21.7 (19.9-24.0)	56.3	935	31 (28-34) <sup>c</sup>	20.7 (19.2-22.6) <sup>c</sup>
IADPSG	337	32 (4.9)	36.2 (33.1-39.9)	25.85	966	30.3 (5.5) <sup>c</sup>	34.7 (32.7-38.1) <sup>c</sup>
IADPSG	1,332	29.5 (3.1)	24.1 (3.9)	7.67	16,027	28.4 (2.8) <sup>c</sup>	22.1 (3.3) <sup>c</sup>
Carpenter-Coustan Criteria	83	34 (5)	29.7 (6.6)	25.15	247	32 (6) <sup>c</sup>	29.2 (6.6)
IADPSG	122	29.4 (3.4)	21.8 (3)	8.1	1,309	27.9 (3.1) <sup>c</sup>	20.7 (2.5) <sup>c</sup>
Carpenter-Coustan Criteria	117	Age range 18-24: 5 (4.3%) 25-29: 24 (20.5%) 30-34: 56 (47.9%) ≥35: 32 (27.4%)	BMI range <18.5: 1 (0.9%) 18.5-24.9: 20 (17.1%) 25.0-29.9: 36 (30.8%) ≥30.0: 32 (27.4%)	33.52	232	Age range 18-24: 16 (6.9%) 25-29: 51 (22%) 30-34: 114 (49.1%) ≥35: 51 (22%)	BMI range <sup>c</sup> <18.5: 6 (2.6%) 18.5-24.9: 100 (43.1%) 25.0-29.9: 56 (24.1%) ≥30.0: 70 (30.2%)
IADPSG	169	Categorized according to serum ALP quartiles		8.15	1,904	Categorized according to serum ALP quartiles	
IADPSG	16	32.63 (6.36)	26.55 (6.29)	N/A	80	32.63 (6.36)	24.9 (4.2)
ADA	36	33.0 (31.0-34.0)	26.4 (23.0-29.0)	5.9	572	33.0 (30.0-34.0)	21.7 (19.8-23.8) <sup>c</sup>
IADPSG	1,485	29.6 (3.2)	24.2 (3.9)	7.68	17,846	28.4 (2.9) <sup>c</sup>	22.1 (3.3) <sup>c</sup>
IADPSG	160	Categorized according to serum ALT level		6.8	2,162	Categorized according to serum ALT level	
ACOG	119	33.04 (3.60)	23.41 (4.28)	2.74	3,860	31.22 (3.65) <sup>c</sup>	21.18 (2.8) <sup>c</sup>
IADPSG	49,611	29.33 (4.88)	20.8 (19.1-23.0)	26.47	137,821	28.34 (4.45) <sup>c</sup>	20.1 (18.7-22.0) <sup>c</sup>
ACOG	2,614	33.13 (4.19)	23.79 (4.13)	1.1	219,937	30.28 (3.83) <sup>c</sup>	21.05 (2.86) <sup>c</sup>
Insulin prescription	1,984	Categorized according to the fatty liver index score		0.6	306,111	Categorized according to the fatty liver index score	
IADPSG	202	31 (28-34)	23.7 (30.4-26.4)	N/A	516	30 (28-33) <sup>c</sup>	22.7 (20.5-24.5) <sup>c</sup>
IADPSG	239	31.40 (3.92)	23.13 (3.53)	22.09	843	30.08 (3.93) <sup>c</sup>	21.68 (2.81) <sup>c</sup>
IADPSG	249	31.43 (3.91)	22.8 (20.4-25.5)	22.07	879	30.11 (3.93) <sup>c</sup>	21.5 (19.7-23.2) <sup>c</sup>
IADPSG	94	31.93 (4.69)	22.00 (2.82)	14.1	572	29.15 (3.92) <sup>c</sup>	20.44 (2.50) <sup>c</sup>
IADPSG	119	32.6 (4.3)	36.7 (33.8-40.4)	7.6	112	33.6 (5.3)	36.9 (34.0-41.3)
IADPSG	272	32 (29-34)	22.9 (21.3-25.0)	21.1	1,017	30 (28-32) <sup>c</sup>	21 (19.5-22.5) <sup>c</sup>
IADPSG	492	28 (25-31)	21.8 (19.9-24.1)	7.2	6,368	26 (24-29) <sup>c</sup>	20.4 (18.9-22.3) <sup>c</sup>
Insulin prescription	2,024	Age range <25: 29 (1.4%) 25-29: 447 (22.1%) 30-34: 946 (46.7%) ≥35: 602 (29.7%)	BMI range <18.5: 124 (6.1%) 18.5-22.9: 952 (47.0%) 23-24.9: 355 (17.5%) 25-29.9: 443 (21.9%) ≥30: 150 (7.4%)	0.69	290,024	Age range <sup>c</sup> <25: 14,395 (5%) 25-29: 115,476 (39.8%) 30-34: 126,465 (43.6%) ≥35: 33,688 (11.6%)	BMI range <sup>c</sup> <18.5: 45,073 (15.5%) 18.5-22.9: 190,175 (65.6%) 23-24.9: 30,109 (10.4%) 25-29.9: 21,056 (7.3%) ≥30: 3611 (1.3%)
ACOG	37	Categorized according to the ALT/HDL-C ratio		6.27	553	Categorized according to the ALT/HDL-C ratio	
IADPSG	1,668	30 (27-34)	21.9 (19.9-24.5)	18.2	7,480	28 (26-31) <sup>c</sup>	20.8 (19.2-22.8) <sup>c</sup>

( $I^2 = 69\%$ ) among the studies. However, the odds ratio analysis showed that women with high ALP levels had 47% higher odds of developing GDM (OR 1.47, 95% CI 1.26-1.72;  $p < 0.00001$ ) with acceptable consistency between studies. Although some dispersion was observed, the overall distribution remained balanced, with no substantial asymmetry (Supplementary Figure S4A-B).

#### AST/ALT ratio

The analyses on AST/ALT ratio are visualized in Figure 8A and 8B. There was no meaningful difference in AST/ALT ratio between the two groups on average (mean difference

0.01, 95% CI -0.16-0.17;  $p = 0.94$ ). The heterogeneity was found to be substantial among these studies. However, the odds ratio analysis showed that individuals with a higher AST/ALT ratio had 26% lower odds of having GDM compared to those with a lower AST/ALT ratio. This association was statistically significant, even though only one study provided data for this analysis (OR 0.74, 95% CI 0.64-0.84;  $p < 0.00001$ ). The funnel plots showed consistent symmetry, suggesting that the results are unlikely to be influenced by publication bias (Supplementary Figure S5A-B).



**Figure 3. (A)** The risk of bias assessment using the ROBINS-E tool for each study. **(B)** The summary for risk of bias assessment using the ROBINS-E tool.

**Table 3.** The outcomes of included studies

Study (year published)	Reported outcomes, GDM vs Non GDM group					
	Serum AST, U/L	Serum ALT, U/L	Serum GGT, U/L	Serum ALP, U/L	AST/ALT ratio	HSI
<i>Maitland et al (2014)</i>	30.63 (1.53) vs 25.07 (1.41); $p = 0.11$ OR (95% CI) 1.17 (0.96-1.43); $p = 0.11$	21.41 (1.79) vs 19.00 (1.57); $p = 0.42$ OR (95% CI) 1.12 (0.84-1.50); $p = 0.42$	ND	ND	ND	ND
<i>Sridhar et al (2014)</i>	13.9 (25.3) vs 11.8 (6.6); $p = 0.18$ Crude OR (95% CI) 0.84 (0.48-1.45)	8.5 (9.5) vs 6.7 (3.8); $p < 0.001$ Crude OR (95% CI) 1.55 (0.92-2.59)	28.0 (21.7) vs 22.4 (16.6); $p < 0.001$ Crude OR (95% CI) 2.91 (1.77-4.77)	ND	ND	ND
<i>Zhao et al (2016)</i>	Median (IQR) 41 (26-43) vs 41 (23-43); $p = 0.588$	Median (IQR) 18 (12-30) vs 16 (11-26); $p = 0.004$	ND	ND	ND	ND
<i>White et al (2016)</i>	4.5 (0.5) vs 4.5 (0.5); $p = 0.10$	4.2 (0.7) vs 4.1 (0.8); $p = 0.32$	4.0 (1.0) vs 3.6 (1.0); $p < 0.001$	ND	ND	ND
<i>Leng et al (2016)</i>	ND	18 (13-26) vs 15 (11-21); $p < 0.001$ Univariate OR (95% CI) 2.03 (1.60-2.56); $p < 0.001$ Multivariate OR (95% CI) 1.62 (1.31-2); $p < 0.001$	ND	ND	ND	ND
<i>Yarrington et al (2016)</i>	ND	15 (12-19) vs 13 (11-18); $p = 0.07$ Crude OR (95% CI) 1.50 (0.88-2.55) Adjusted OR (95% CI) 1.37 (0.73-2.59)	ND	ND	ND	ND
<i>Kong et al (2018)</i>	27.3 (24.1) vs 25.3 (23.0); $p = 0.229$	23.7 (16.2) vs 22.9 (14.0); $p = 0.082$	18.7 (13.0) vs 14.5 (7.0); $p < 0.001$ Crude RR (95% CI) 5.81 (3.72-9.08)	ND	ND	ND
<i>Zhu et al (2018)</i>	ND	15.5 (1.0) vs 13.5 (0.8); $p = 0.04$ Crude OR (95% CI) 2.05 (1.06-3.99); $p < 0.035$ (for trend)	17 (1.5) vs 12 (1.0); $p < 0.001$ Crude OR (95% CI) 3.78 (1.93-7.39); $p < 0.001$ (for trend)	ND	ND	ND
<i>Xiong et al (2019)</i>	ND	ND	ND	Mean ALP level for all cohort = 44.87 (9.66) Crude OR (95% CI) 2.62 (1.63-4.21); $p < 0.001$ (for trend)	ND	ND
<i>Correa et al (2019)</i>	19.5 (16.5-22.5) vs 20 (18.25-21.75); $p = 0.22$	29.5 (20.25-38.75) vs 26.5 (21.75-31.25); $p = 0.437$	ND	71.5 (60.5-82.5) vs 66.5 (55-78); $p = 0.371$	ND	ND
<i>Lee et al (2019)</i>	18 (15-22) vs 16 (15-22); $p = 0.047$	16 (10-27) vs 11 (8-14); $p = 0.001$	17 (11-24) vs 12 (10-15); $p = 0.001$	ND	ND	35.5 (32.0-39.5) vs 29.0 (26.8-32.5); $p < 0.001$ Crude OR (95% CI) 1.23 (1.16, 1.31)
<i>Gao et al (2020)</i>	ND	Log Transferred 1.29 (0.23) vs 1.22 (0.21); $p < 0.001$ OR (95% CI) 1.73 (1.10-2.71) $p = 0.024$	ND	ND	ND	ND
<i>Lee et al (2020)</i>	ND	Adjusted OR 1.795 (1.002-3.217); $p < 0.05$	ND	ND	ND	ND
<i>Park et al (2021)</i>	19.69 (18.49-20.98) vs 18.52 (18.35-18.68); $p = 0.019$ Crude OR (95% CI) 1.349 (0.677-2.691)	17.33 (15.79-19.02) vs 13.67 (13.47-13.87); $p < 0.001$ Crude OR (95% CI) 2.714 (1.308-5.633)	18.16 (16.62-19.83) vs 14.59 (14.39-14.8); $p < 0.001$ Crude OR (95% CI) 2.126 (1.098-4.114)	ND	ND	ND
<i>Zhao et al (2021)</i>	16.20 (14-20) vs 16.10 (14-20); $p = 0.282$ Crude OR (95% CI) 1.04 (1.01-1.07)	14 (10.90-20) vs 14 (10.70-19.50); $p < 0.001$ Crude OR (95% CI) 1.06 (1.04-1.08)	ND	ND	ND	ND

**Table 3.** The outcomes of included studies (*continued*)

Study (year published)	Reported outcomes, GDM vs Non GDM group					
	Serum AST, U/L	Serum ALT, U/L	Serum GGT, U/L	Serum ALP, U/L	AST/ALT ratio	HSI
<b>Kim et al (2021)</b>	20 (19.71-20.29) vs 18.22 (18.19-18.24); $p < 0.001$  Crude OR (95% CI) 1.294 (1.137-1.473)	17.95 (17.55-18.36) vs 13.34 (13.32-13.36); $p < 0.001$  Crude OR (95% CI) 2.205 (1.913-2.541)	19.21(18.79-19.63) vs 14.12(14.1-14.15); $p < 0.001$  Crude OR (95% CI) 2.748 (2.337-3.232)	ND	ND	ND
<b>Sang et al (2021)</b>	ND	ND	Adjusted OR (95% CI); 2.78 (2.54-3.04)	ND	ND	ND
<b>Wang et al (2021)</b>	18.67 (6.57) vs 18.97 (8.70); $p > 0.05$	20.39 (13.33) vs 17.69 (13.07); $p > 0.05$  Crude OR (95% CI) 1.02 (1.01 - 1.03); $p < 0.05$	ND	ND	ND	ND
<b>Shuoning et al (2021)</b>	16.17 (14.00-19.00) vs 16 (14-18.8); $p = 0.406$	15.28 (11-20.8) vs 12.56 (10-17.7); $p < 0.001$	ND	ND	ND	30.67 (27.2 - 35.1) vs 27.98 (25.7-30.82); $p < 0.001$  Crude OR (95% CI) 3.166 (2.069-4.845)
<b>Shuoning et al (2022)</b>	ND	ND	ND	ND	0.92 (0.75-1.18) vs 0.80 (0.65-1.02); $p < 0.001$  Crude OR (95% CI) 2.143 (1.500 - 3.061); $p < 0.001$	ND
<b>Rongjing et al (2022)</b>	15.88 (17.9 - 21.9) vs 17.7 (14.1 - 20.1); $p = 0.054$	18 (13.1 - 25.93) vs 14.3 (10.3 - 18.6); $p < 0.001$	ND	ND	0.96 (0.79 - 1.21) vs 1.18 (1.02 - 1.49); $p < 0.001$  RR (95% CI) 0.228 (0.107-0.488); $p < 0.001$	ND
<b>Quotah et al (2022)</b>	Median (IQR) 23.7 (19.2 -29.2) vs 21.8 (17.8 - 27.9); $p$ value not reported	Median (IQR) 16.9 (12.5 - 24.2) vs 15.7 (11.9-22.6); $p$ value not reported	21.7 (17.73) vs 16.2 (12.19); $p$ value not reported	ND	ND	ND
<b>Duo et al (2023)</b>	16 (14-18.1) vs 16 (14-18.2); $p = 0.634$	15 (11-19.6) vs 12.1 (9.8-16.4); $p < 0.001$	ND	ND	0.9 (0.8-1.2) vs 0.8 (0.6-0.9); $p < 0.001$  Univariate OR (95% CI) 6.310 (3.968-10.036); $p < 0.001$  Multivariate OR (95% CI) 3.345 (1.969-5.683); $p < 0.001$	ND
<b>Wu et al (2023)</b>	19 (16-23) vs 18 (16-22); $p < 0.001$  Crude OR (95% CI) 1.75 (1.36-2.25); $p < 0.001$	19 (14-23) vs 16 (12-24); $p < 0.001$  Crude OR (95% CI) 2.39 (1.84-3.11); $p < 0.001$	16 (12-24) vs 13 (10-18); $p < 0.001$  Crude OR (95% CI) 2.15 (1.66-2.79); $p < 0.001$	49 (31-58) vs 46 (40-54); $p < 0.001$  Crude OR (95% CI) 1.65 (1.28-2.13); $p < 0.001$	ND	32.3 (29-36) vs 29.9 (27.4-33.2); $p < 0.001$  Crude OR (95% CI) 3.45 (2.59-4.59); $p < 0.001$
<b>Lee et al (2023)</b>	ND	ND	Univariate OR (95% CI) 4.24 (3.50-5.14)  Multivariate OR (95% CI) 2.17 (1.78-2.65)	ND	ND	ND
<b>Zhen et al (2024)</b>	Median for all cohort: 16 (14-20)  OR (95% CI) 1.02 (0.99-1.05); $p = 0.138$	Median for all cohort: 11.00 (8-15)  OR (95% CI) 1.04 (1.01-1.06); $p = 0.002$	Median for all cohort: 12 (10-15)  OR (95% CI) 1.04 (1.01-1.07); $p = 0.002$	ND	ND	ND
<b>Liu et al (2024)</b>	17 (15-20) vs 17 (14-19); $p > 0.05$  Adjusted OR (95% CI) 1.25 (1.06-1.48)	14 (11-21) vs 13 (10-19); $p < 0.001$  Adjusted OR (95% CI) 1.29 (1.11-1.51)	12 (10-17) vs 11 (9-15); $p < 0.001$  Adjusted OR (95% CI) 1.33 (1.13-1.56)	51 (44-59) vs 49 (43-57); $p < 0.001$  Adjusted OR (95% CI) 1.39 (1.19-1.63)	1.1 (0.9-1.4) vs 1.2 (0.9-1.5); $p < 0.001$  Adjusted OR (95% CI) 0.72 (0.61-0.84)	31.3 (28.3-35.4) vs 29.7 (27.3-32.8); $p < 0.001$  Adjusted OR (95% CI) 1.91 (1.55-2.36)

Abbreviations: GDM, Gestational Diabetes Mellitus; AST, Aspartate Transaminase; ALT, Alanine Transaminase; GGT, Gamma-Glutamyl Transferase; ALP, Alkaline Phosphatase; HSI, Hepatic Steatosis Index; OR, Odd Ratio; RR, Relative Risk; CI, Confidence Interval; ND, No Data.

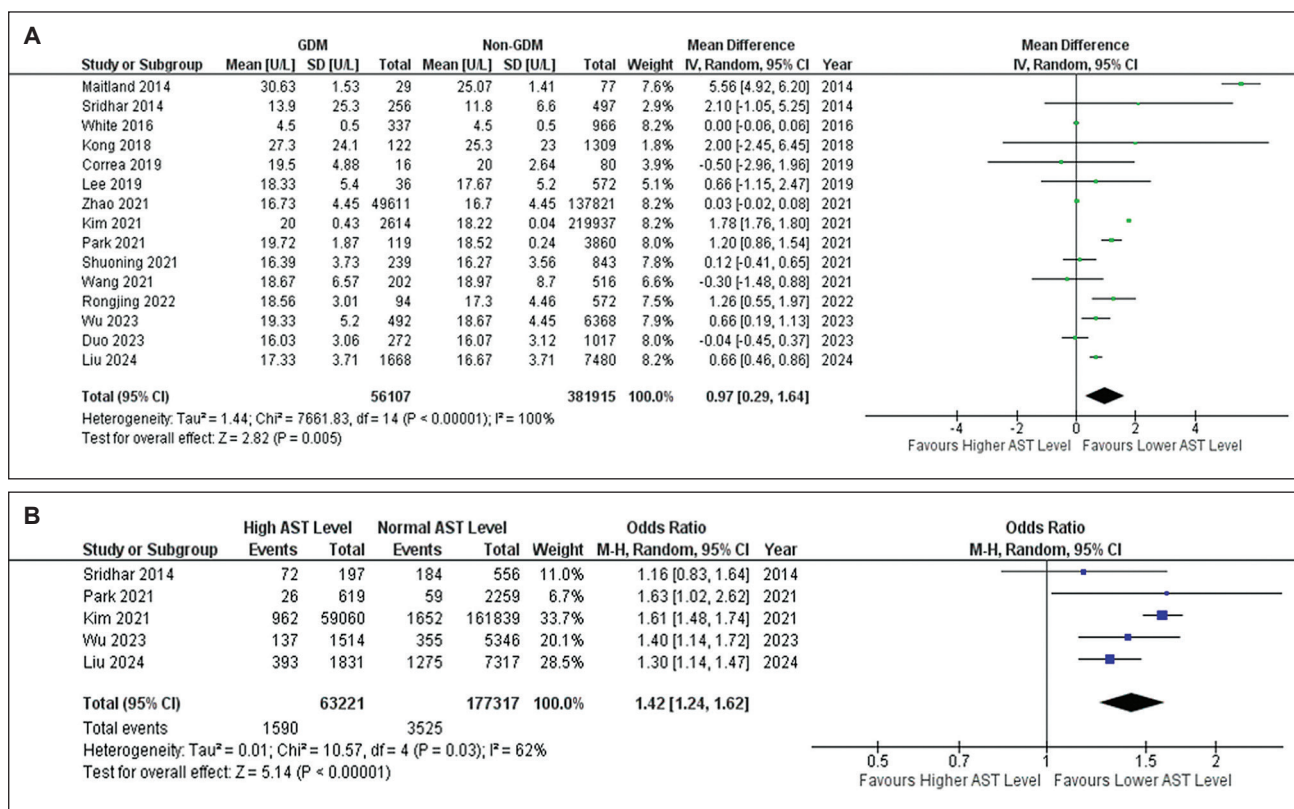
Data for continuous variables with normal distribution are presented as mean (standard deviation) or as median (interquartile range) if the distribution is abnormal.

**HSI**

On average, GDM patients had significantly higher HSI compared to non-GDM counterparts (mean difference of 2.82, 95% CI 1.89-3.76;  $p < 0.00001$ ), suggesting a stronger degree of hepatic lipid accumulation or metabolic dysfunction (Figure 9A). The odds ratio analysis showed that the odds of developing GDM were significantly increased in patients with high HSI before or in early pregnancy (OR 2.19, 95% CI 2.00-2.40;  $p < 0.00001$ ). Even though the heterogeneity was high in the mean difference analysis, it was minimal in the odds ratio analysis (Figure 9B). The funnel plots were generally symmetrical, further supporting the robustness of the findings (Supplementary Figure S6A–B).

**Certainty of evidence**

Using the GRADE framework, the certainty of evidence for all outcomes was rated as very low. As most included studies were observational, the initial certainty of evidence was low. Further downgrading was applied due to serious inconsistency, reflected by substantial heterogeneity across studies, and concerns regarding risk of bias. Consequently, the overall certainty of evidence was judged to be very low for all liver enzyme outcomes. A summary of the GRADE ratings is presented in Table 4.

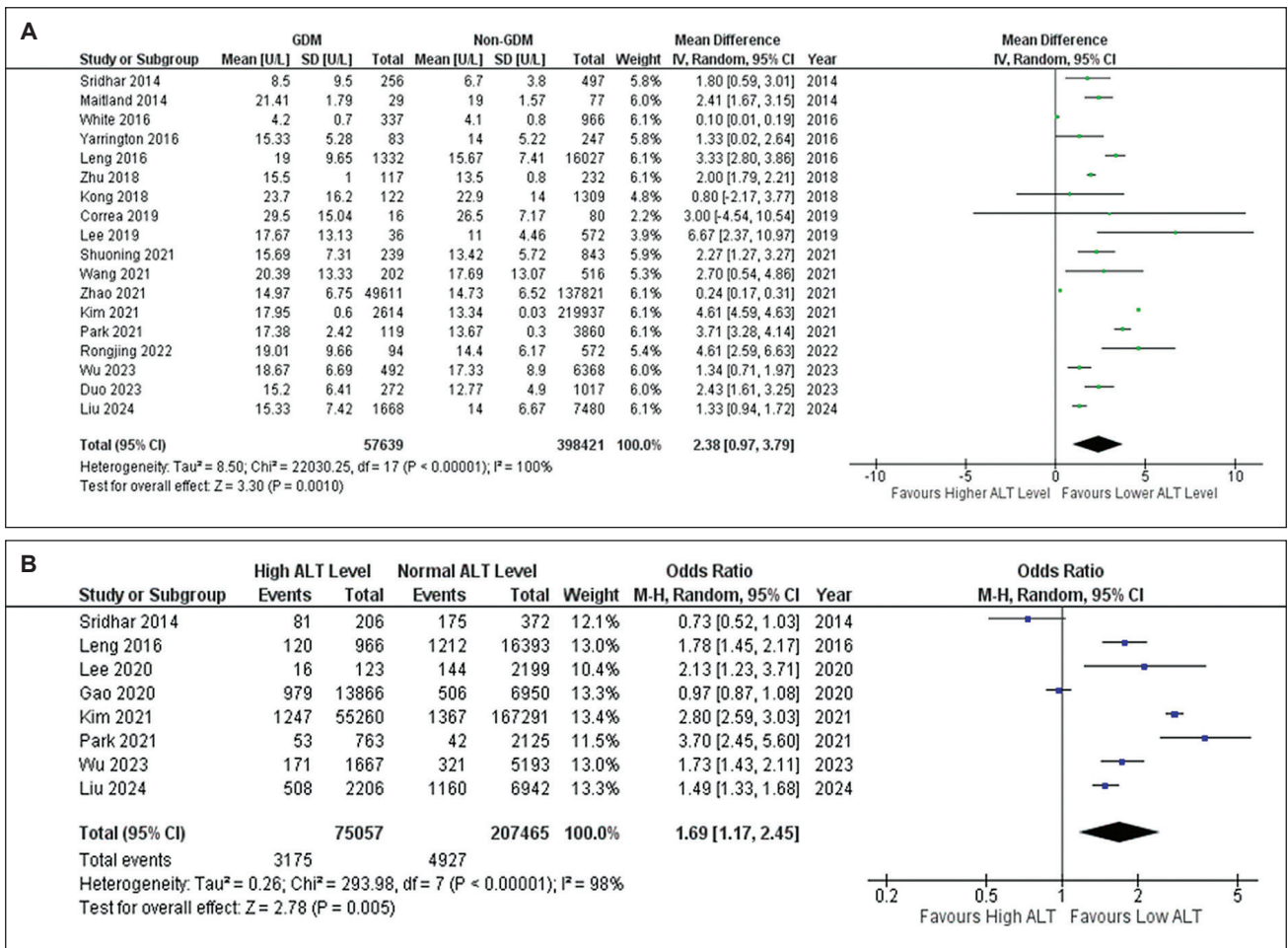


**Figure 4. (A)** The mean difference in pre- or early pregnancy serum AST level between GDM and non-GDM groups. **(B)** The odds ratio of GDM in subjects with higher AST level.

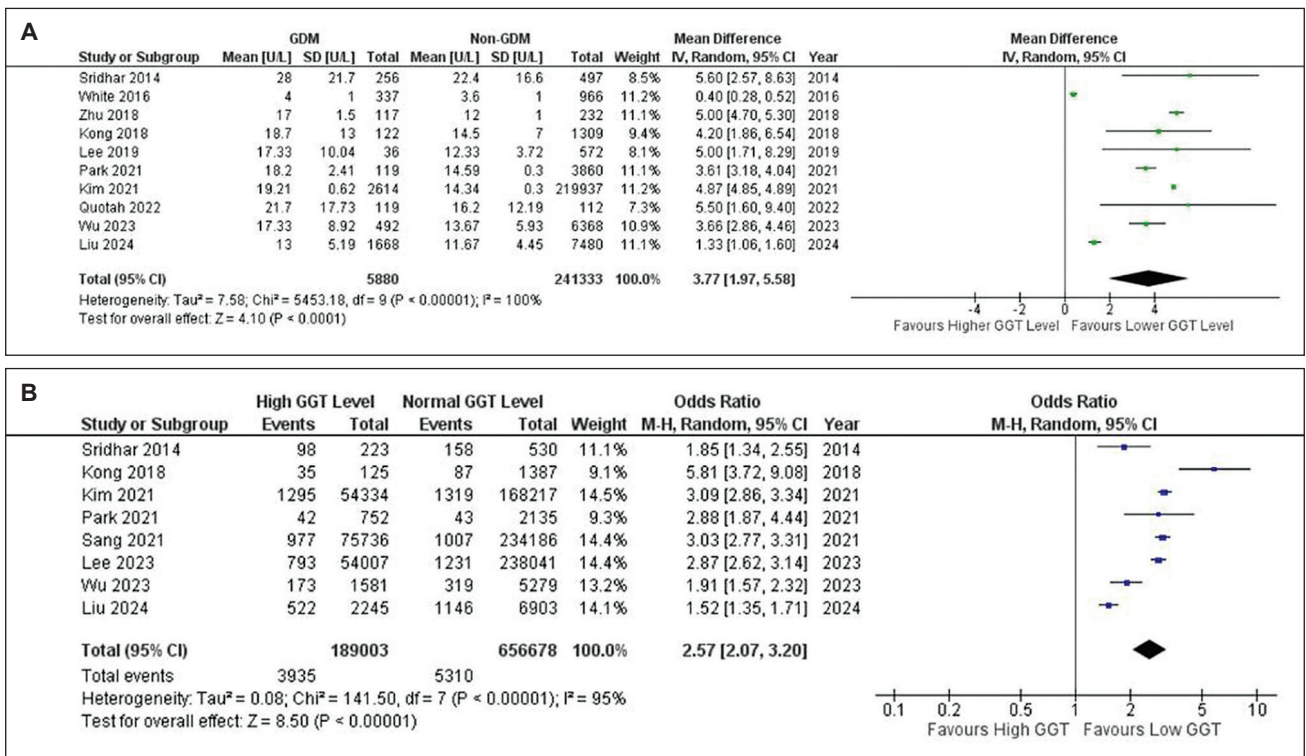
**Table 4.** The Summary of Findings using GRADE approach

Outcome	Number of studies	Participants	Effect estimate (MD)	Effect estimate (OR)	Certainty of evidence	Reasons for downgrade
<b>AST</b>	15 (MD), 5 (OR)	MD: 438,022 OR: 240,538	0.97 U/L (95% CI 0.29–1.64)	1.42 (95% CI 1.24–1.62)	Very low	Inconsistency and risk of bias
<b>ALT</b>	18 (MD), 8 (OR)	MD: 456,060 OR: 282,522	2.38 U/L (95% CI 0.97–3.79)	1.69 (95% CI 1.17–2.45)	Very low	Inconsistency and risk of bias
<b>GGT</b>	10 (MD), 8 (OR)	MD: 247,213 OR: 845,681	3.77 U/L (95% CI 1.97–5.58)	2.57 (95% CI 2.07–3.20)	Very low	Inconsistency and risk of bias
<b>ALP</b>	5 (MD), 3 (OR)	MD: 16,104 OR: 18,081	0.86 U/L (95% CI -1.22–2.95)	1.47 (95% CI 1.26–1.72)	Very low	Imprecision and inconsistency
<b>AST/ALT Ratio</b>	3 (MD), 1 (OR)	MD: 12,231 OR: 9,148	0.01 (95% CI -0.16–0.17)	0.74 (95% CI 0.64–0.84)	Very low	Limited data and imprecision
<b>HSI</b>	6 (MD), 4 (OR)	MD: 17,698 OR: 17,090	2.82 (95% CI 1.89–3.76)	2.19 (95% CI 2.00–2.40)	Very low	Inconsistency and risk of bias

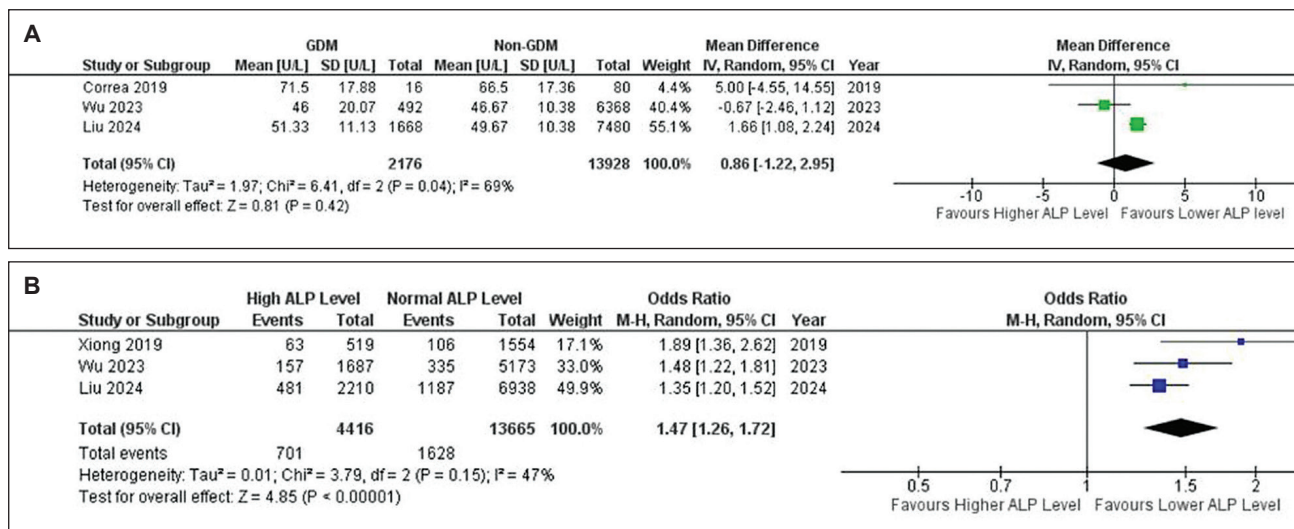
Glutamyl Transferase; ALP, Alkaline Phosphatase; HSI, Hepatic Steatosis Index; MD, Mean Difference; OR, Odd Ratio; CI, Confidence Interval.



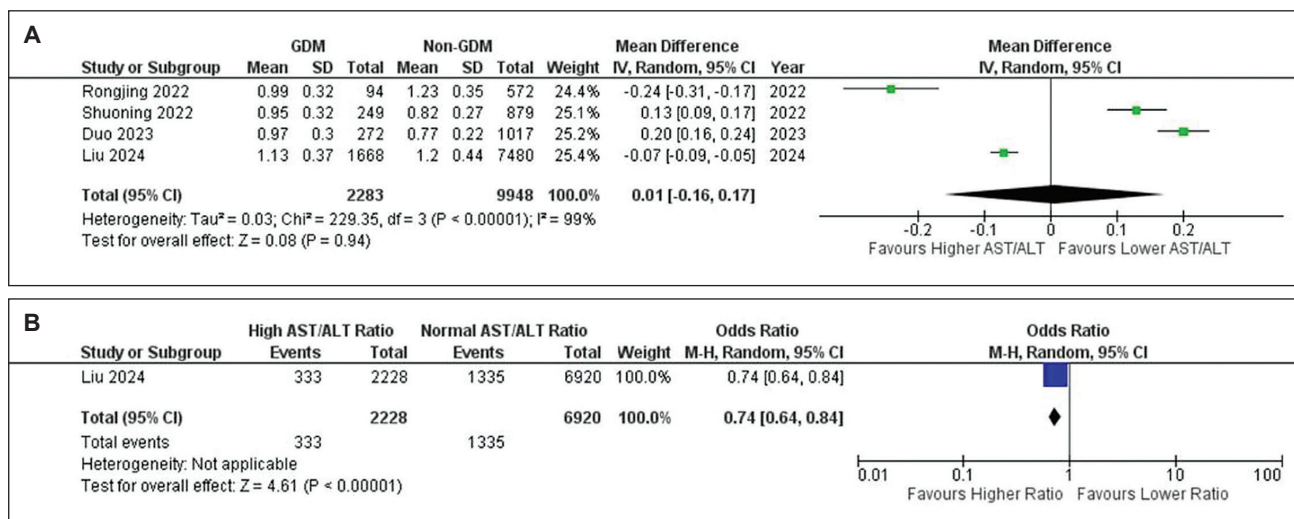
**Figure 5. (A)** The mean difference in pre- or early pregnancy serum ALT level between GDM and non-GDM groups. **(B)** The odds ratio of GDM in subjects with higher ALT level.



**Figure 6. (A)** The mean difference in pre- or early pregnancy serum GGT level between GDM and non-GDM groups. **(B)** The odds ratio of GDM in subjects with higher GGT level.



**Figure 7. (A)** The mean difference in pre- or early pregnancy serum ALP level between GDM and non-GDM groups. **(B)** The odds ratio of GDM in subjects with higher ALP level.



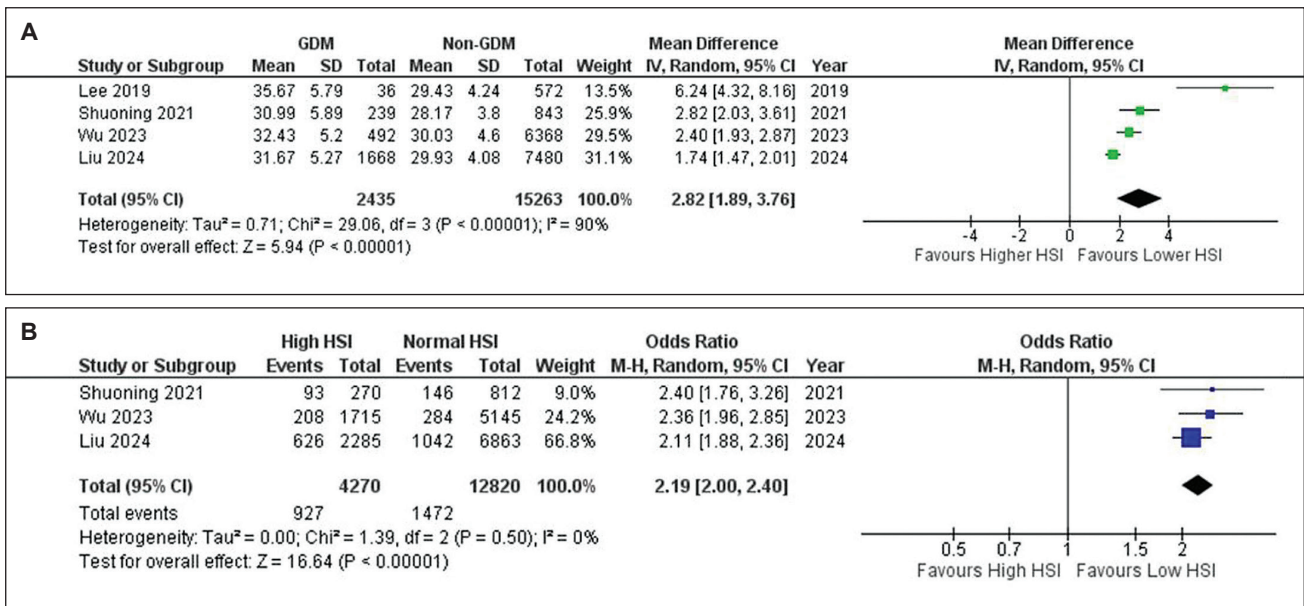
**Figure 8. (A)** The mean difference in pre- or early pregnancy AST/ALT ratio between GDM and non-GDM groups. **(B)** The odds ratio of GDM in subjects with higher ALP level.

**DISCUSSION**

The meta-analysis aimed to determine the correlation between various liver function markers and the risk of developing GDM. In a review of 27 studies, our main findings indicate that higher levels of liver enzymes, especially AST, ALT, and GGT, along with HSI, are significantly linked to a greater likelihood of GDM occurring during pregnancy. Among these factors, GGT exhibited the most significant correlation, with an OR value of 2.57. Similarly, the HSI was also associated with higher odds of GDM (OR 2.19). A significant correlation between ALT and AST was also found to increase the risk of GDM, with a slightly smaller effect size (OR 1.69 and 1.42, respectively). On the other hand, although no significant mean difference in serum ALP levels was observed between groups, the odds ratio of GDM was still marginally higher in individuals with high ALP levels, with an OR of 1.47. These findings imply

that while average ALP levels may not differ dramatically, elevated ALP may still serve as a risk indicator for GDM when elevated above the normal level. AST/ALT ratio findings were inconsistent, with limited evidence supporting its utility as a predictive marker. These findings highlight the potential utility of liver enzyme biomarkers as early screening tools for GDM risk.

The subjects with GDM had a higher mean level of serum AST, ALT, GGT, and higher HIS. However, the pooled mean differences observed for AST, ALT, and GGT were numerically small and may fall within accepted laboratory variability at the individual level. It can be explained by the fact that mean differences represent population-level averages and do not capture risk concentration among individuals with higher enzyme levels. In contrast, dichotomized analyses compared women in the highest exposure categories—typically the upper quartile—against



**Figure 9. (A)** The mean difference in pre- or early pregnancy HSI between GDM and non-GDM groups. **(B)** The odds ratio of GDM in subjects with higher HSI level.

those with lower levels. This approach identifies subgroups with disproportionate metabolic risk with both groups generally remained within clinically normal enzyme ranges. Consequently, small shifts in population means can coexist with significantly increased odds of GDM among women with relatively elevated liver enzyme levels.

The resulting odds ratios should therefore not be interpreted as comparing women with overtly abnormal liver function to those with normal function, but rather as reflecting risk gradients within the normal distribution of liver enzymes. In this context, relatively higher enzyme levels may serve as markers of subclinical metabolic dysfunction or hepatic insulin resistance rather than biochemical liver injury. This distinction explains how small absolute mean differences can coexist with sizable relative odds ratios.

Furthermore, given the small absolute mean differences observed for AST, ALT, and GGT, these biomarkers are unlikely to be clinically useful as standalone indicators for categorizing individual patients into high- or low-risk groups for gestational diabetes mellitus. However, liver enzyme abnormalities may reflect underlying hepatic insulin resistance or metabolic dysfunction and could potentially serve as adjunctive variables within existing multivariable GDM risk prediction models.

Substantial heterogeneity was observed across most pooled analyses, which is not unexpected given the diversity of study designs and populations included. Several factors likely contributed to this heterogeneity. First, population characteristics varied considerably, particularly with respect to ethnicity and pre-pregnancy body mass index, both of which are known to influence baseline liver enzyme levels, insulin resistance, and gestational diabetes risk. Second, the timing of biomarker measurement ranged

from the pre-conception period to as late as 20 weeks of gestation, during which physiological changes in hepatic metabolism and insulin sensitivity may differentially affect enzyme concentrations. Third, diagnostic criteria for gestational diabetes mellitus were not uniform across studies, with the use of IADPSG, ACOG, ADA, and insulin-treatment-based definitions, each capturing different clinical phenotypes and disease severity. Finally, variation in covariate adjustment, particularly for age, BMI, and parity, may have further contributed to between-study variability in effect estimates. Collectively, these methodological and biological differences plausibly explain the high heterogeneity observed and underscore the need for cautious interpretation of pooled estimates.

Liver transaminase values are known to vary across laboratories and populations due to differences in assay methods and reference ranges. In the present review, this variability was mitigated by focusing on within-study comparisons rather than absolute enzyme concentrations. Mean differences compared GDM and non-GDM groups measured within the same laboratory context, while dichotomized analyses relied on study-specific relative thresholds such as quartiles or validated indices. As a result, inter-laboratory differences in normal reference values are unlikely to fully account for the observed associations, although they may have contributed to between-study heterogeneity. Given the heterogeneity of included studies, lack of standardized laboratory reference ranges, and very low certainty of evidence, we do not propose specific liver enzyme cut-off values for clinical use.

In the third trimester, particularly beyond 26 weeks of gestation, the placenta secretes a range of hormones (including estrogen, progesterone, cortisol, and human placental lactogen) that exert anti-insulin effects, thereby

inducing a state of progressive insulin resistance. This physiological adaptation facilitates increased maternal glucose availability to support optimal fetal growth and development. To maintain euglycemia, maternal pancreatic  $\beta$ -cells must compensate through enhanced insulin secretion. GDM develops when this compensatory response is inadequate, resulting in maternal hyperglycemia due to insufficient insulin production or heightened peripheral insulin resistance.<sup>42</sup> In Indonesia, GDM is still a major problem affecting up to 4% of all pregnancies.<sup>43</sup>

Our findings align with prior studies linking liver dysfunction and insulin resistance to metabolic diseases, including GDM. The liver plays a crucial role in maintaining glucose homeostasis, insulin clearance, and the production of inflammatory cytokines. Various liver conditions have been associated with diabetes, which is reflected by alterations in liver parameters. Elevated GGT levels are proposed as markers of oxidative stress and liver dysfunction, contributing to the development of GDM or T2DM.<sup>44</sup> A previous systematic review and meta-analysis by Zhao et al. in 2019 also reported a significant association between GGT levels and risk of GDM (OR 2.10, 95% CI 1.14-3.86), though fewer studies and participants were included.<sup>45</sup> Increased AST and ALT levels are associated with liver inflammation or injury, particularly in MAFLD. The HSI is a composite marker reflecting hepatic fat accumulation in conditions such as MAFLD as well. Insulin resistance has been implicated in the pathogenesis of MAFLD by promoting glucotoxicity and lipotoxicity. Likewise, populations with MAFLD have a twofold higher risk of developing T2DM than those without MAFLD and are often associated with a worse cardiometabolic profile.<sup>46</sup>

Elevated ALP levels can be influenced by liver inflammation, biliary dysfunction, or bone metabolism changes. During pregnancy, ALP levels will progressively increase due to placental production and typically reach their highest concentration in the third trimester. This explained the insignificant mean difference between the groups.<sup>47</sup> Notably, consistent with this meta-analysis result, a prospective cohort study by Xiong et al in 2019 reported a higher risk of GDM with higher early maternal ALP level (OR 2.47, 95% CI 1.47-4.15).<sup>24</sup>

When GDM is not properly managed, it can lead to higher rates of illness and death for both the mother and the baby. Women who have experienced GDM are at a greater long-term risk of developing T2DM. More than 50% of women diagnosed with GDM will progress to T2DM within 20 years postpartum.<sup>48</sup> Recent studies by Peramaki et al, in 2023, reported the risk for T2DM was 11 times higher in women with than without a history of GDM. Alongside an elevated risk of developing T2DM, women with a prior history of GDM also exhibit increased predisposition to metabolic syndrome and cerebrovascular disease.<sup>49</sup> Furthermore, the offspring of mothers with GDM are also at elevated long-term risk for metabolic dysregulation, including impaired glucose tolerance and obesity, as well

as increased susceptibility to endocrine disorders, adverse neurodevelopmental outcomes, and neuropsychiatric morbidities.<sup>48</sup>

The Indonesian Endocrinologist Society has published its guideline for GDM in 2021, encouraging OGTT to be done in populations with moderate or high risk for GDM combined with blood glucose level at the first antenatal visit. The mentioned criteria for risk stratification include obesity, personal history of T2DM or history of T2DM among first-degree relatives, history of macrosomia, presence of glycosuria, and certain high-risk ethnicities. Unfortunately, the screening and diagnosis of GDM are rarely done due to limited resources and a lack of urgency. In addition, the guideline also includes the management of GDM, which generally consists of medical nutrition therapy, physical activity or exercise, self-blood glucose monitoring, weight monitoring, and pharmacological treatment if needed.<sup>6</sup> Given the widespread availability and affordability of liver function tests, integrating them into first-trimester or preconception risk assessments could provide a cost-effective strategy for early intervention among the Indonesian population.

There are several limitations in this study. This study is limited by substantial between-study heterogeneity, which reduced the certainty of pooled estimates and precluded robust subgroup or threshold analyses. Variability in laboratory assays and reference ranges across studies may have contributed to heterogeneity, although the use of within-study comparisons likely reduced systematic bias. The very low certainty of evidence underscores that the observed associations should be interpreted cautiously and viewed as hypothesis-generating rather than confirmatory. Although the funnel plots suggest minimal publication bias, most included studies were observational in nature, which may introduce residual confounding. Subgroup analyses based on diagnostic criteria or population characteristics would be informative. However, inconsistent reporting and limited data availability across studies precluded statistically robust subgroup analyses. Lastly, not all studies adjusted for key confounders such as age, BMI, and parity, limiting the internal validity of pooled estimates.

While we conducted a comprehensive database search and followed rigorous inclusion criteria, gray literature was not included, which may have led to the omission of relevant unpublished studies. Furthermore, the inclusion of studies with heterogeneous methods and reporting styles limited the ability to conduct subgroup and sensitivity analyses, particularly for cut-off thresholds of liver enzyme elevation. That being said, no studies were rated as having high risk of bias, reducing the necessity of excluding studies to test robustness. Future prospective studies should aim to establish standardized thresholds, explore causality, and evaluate whether early lifestyle or pharmacologic interventions based on elevated liver enzymes can reduce GDM incidence and improve maternal-fetal outcomes. Further exploration of composite scores like HSI may offer

enhanced predictive power, particularly in populations with high metabolic risk. Lastly, future models may consider incorporating liver enzymes as continuous predictors alongside established risk factors such as maternal age, pre-pregnancy BMI, ethnicity, family history of diabetes, and prior obstetric outcomes. Importantly, such integration would require prospective validation to determine whether inclusion of liver enzymes meaningfully improves model discrimination, calibration, or clinical decision-making.

## CONCLUSION

Relatively higher liver enzyme biomarkers, particularly GGT, ALT, AST, and the HSI measured before or during early pregnancy are associated with an increased risk of developing GDM. These associations support the potential role of liver enzyme abnormalities as population-level markers of metabolic risk preceding GDM. However, substantial heterogeneity among studies and the very low certainty of evidence limit their immediate clinical applicability as individual screening tools. The observed associations should therefore be interpreted within a population and risk-stratification framework rather than as indicators of clinically meaningful differences in absolute enzyme values for individual patients. Further prospective studies are needed to confirm these associations, establish diagnostic thresholds, and evaluate the impact of early preventive strategies guided by liver enzyme profiles.

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## Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

## CRedit Author Statement

**SP:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft preparation; **RDA:** Formal analysis, Investigation, Writing – original draft preparation; **IJ:** Formal analysis, Investigation, Writing – original draft preparation; **BCL:** Writing – review and editing, Visualization.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Author Disclosure

The authors declared no conflict of interest.

## Funding Source

None.

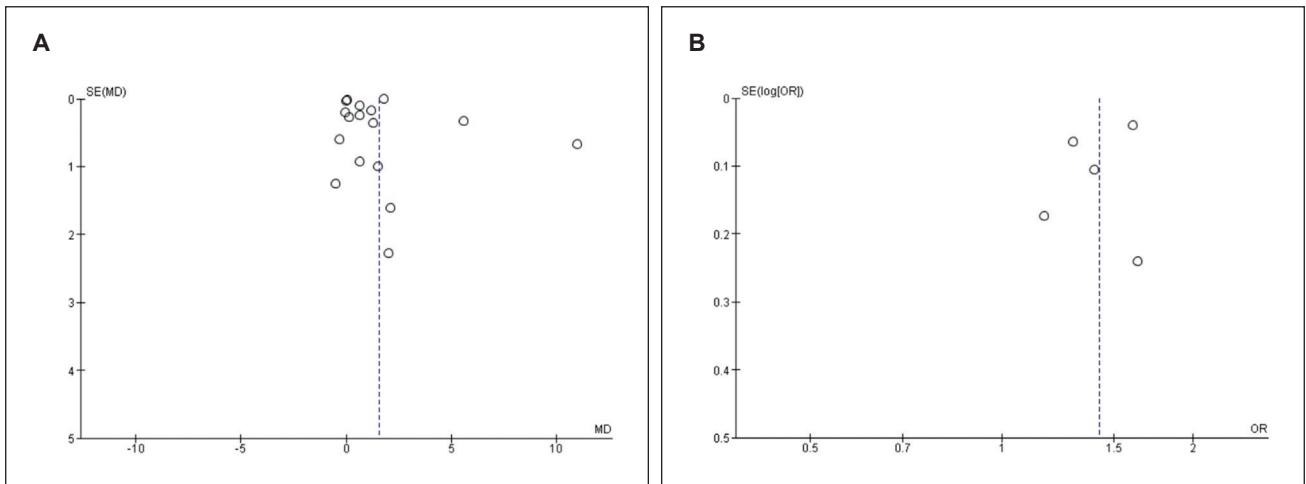
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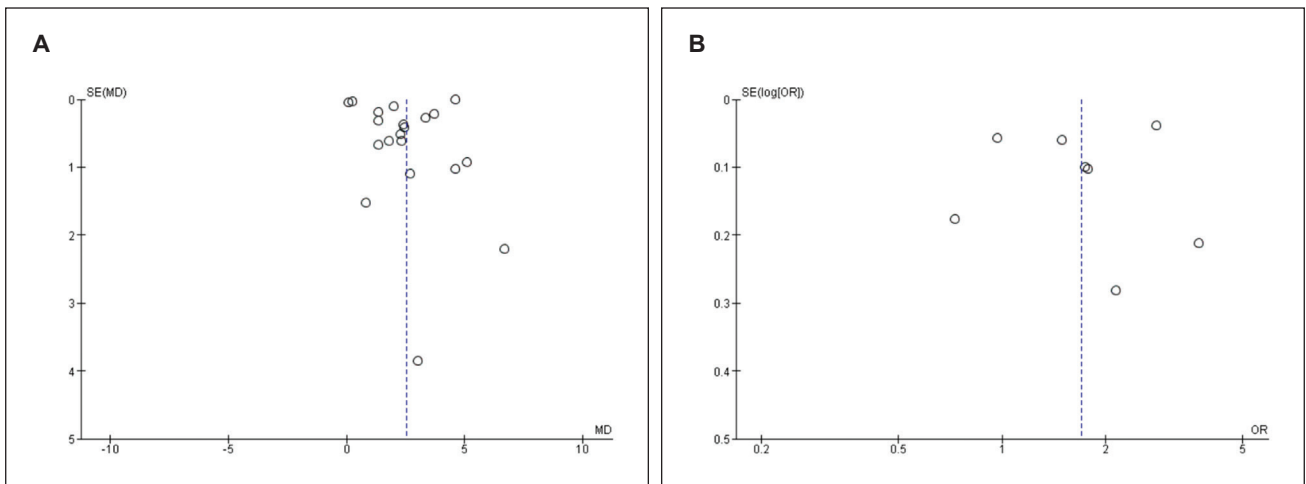
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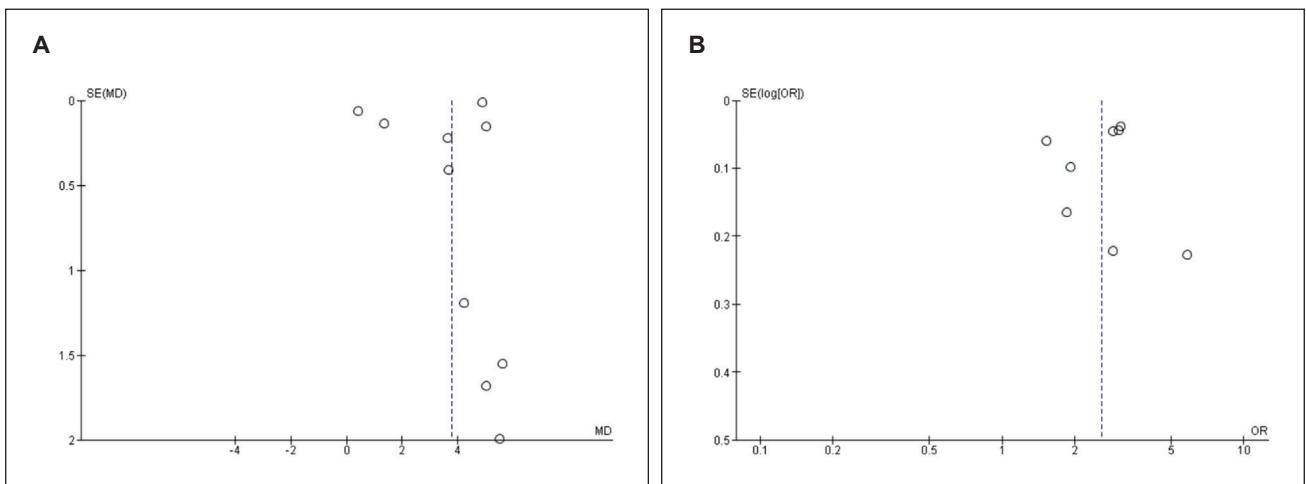
SUPPLEMENTARY FIGURES



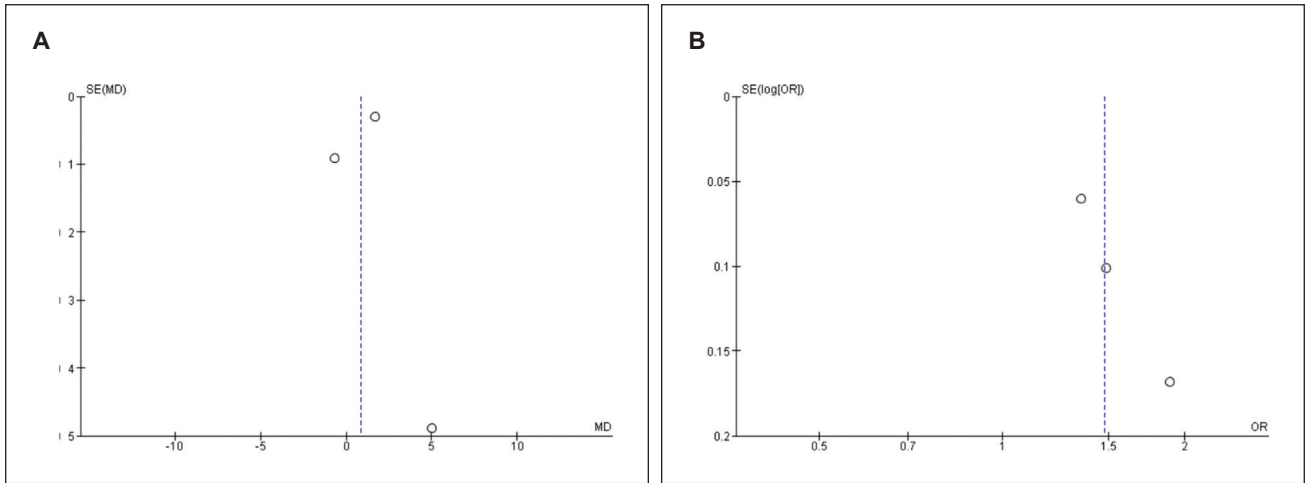
Supplementary Figure S1A-B. The funnel plots for the mean difference (A) and odds ratio (B) for the analysis of AST level.



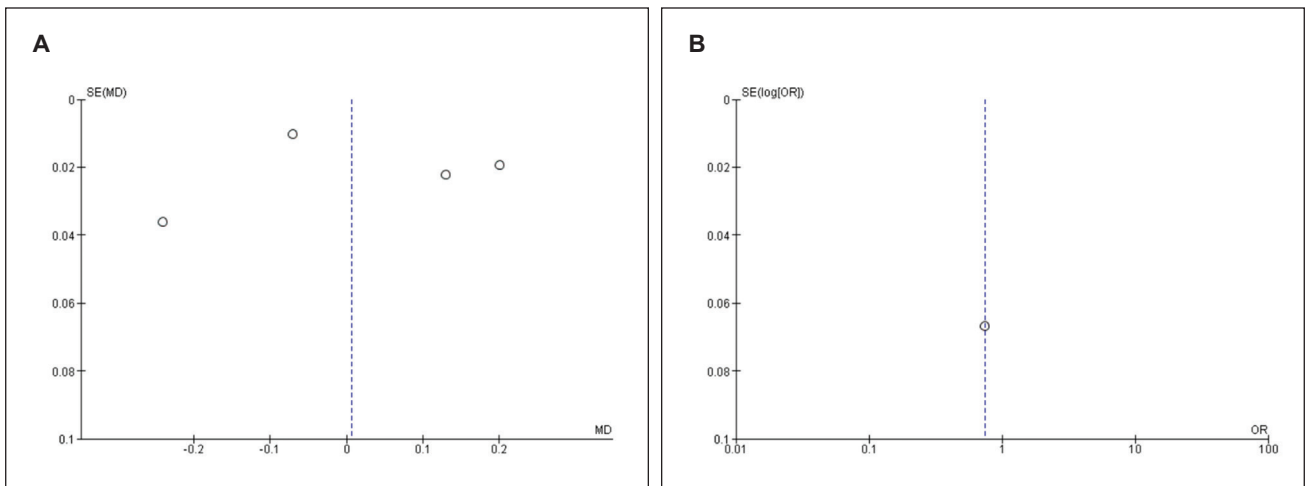
Supplementary Figure S2A-B. The funnel plots for the mean difference (A) and odds ratio (B) for the analysis of ALT level.



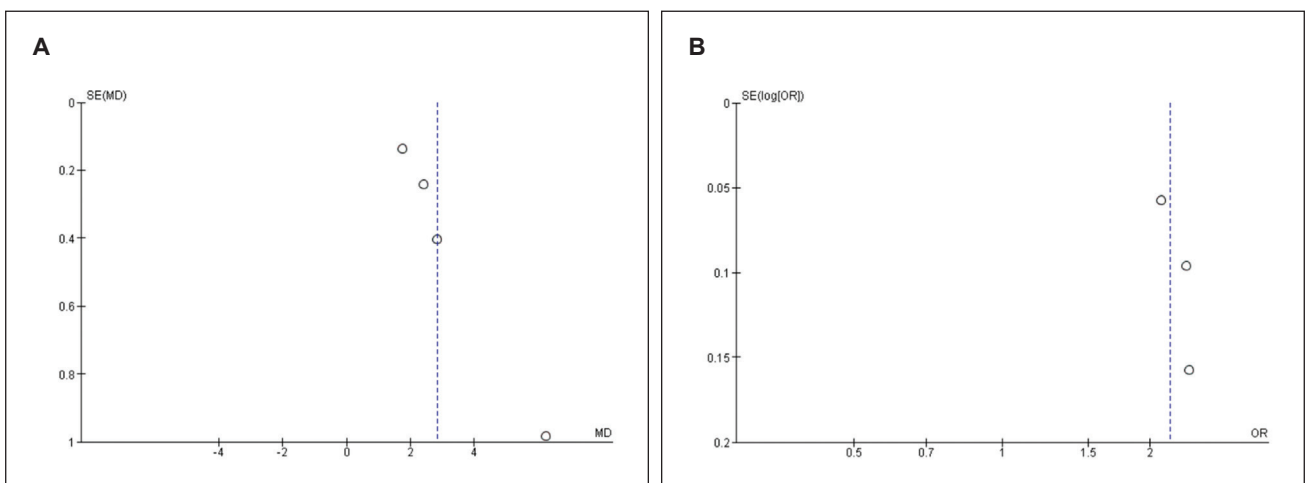
Supplementary Figure S3A-B. The funnel plots for the mean difference (A) and odds ratio (B) for the analysis of GGT level.



**Supplementary Figure S4A-B.** The funnel plots for the mean difference (A) and odds ratio (B) for the analysis of ALP level.



**Supplementary Figure S5A-B.** The funnel plots for the mean difference (A) and odds ratio (B) for the analysis of AST/ALT ratio.



**Supplementary Figure S6A-B.** The funnel plots for the mean difference (A) and odds ratio (B) for the analysis of HSI.