Review Article

Circulating HMGB1 as a Potential Biomarker of Inflammation in Type 2 Diabetes Mellitus and Its Complications: A Systematic Review and Meta-Analysis

Usha Kiran Pothu[1], Roshan Kumar Mahat[1*]

[1]*Department of Biochemistry, Teerthanker Mahaveer Medical College & Research Centre, Teerthanker Mahaveer University, Moradabad, India*

Article Info

[*]***Corresponding Author:***
Roshan Kumar Mahat, MSc (Med), PhD (Med), MBA (HHM)
Professor of Biochemistry
Teerthanker Mahaveer Medical College & Research Centre, Teerthanker Mahaveer University, Moradabad, India
E-mail: mahatroshan79@gmail.com
ORCID: 0000-0003-1202-6227

Keywords

HMGB1, type 2 diabetes mellitus, inflammation, biomarker, meta-analysis, diabetic complications

Abstract

Background: Chronic low-grade inflammation plays a pivotal role in the pathogenesis of type 2 diabetes mellitus (T2DM) and its complications. High mobility group box-1 (HMGB1), a damage-associated molecular pattern molecule, has been implicated in insulin resistance, metabolic dysregulation, and vascular injury. However, findings on circulating HMGB1 levels in T2DM are inconsistent. This systematic review and meta-analysis evaluated the association between circulating HMGB1 levels and T2DM, including its complications.

Methods: PubMed/MEDLINE, Web of Science, and Scopus were systematically searched from inception to 14 February 2026. Observational studies reporting circulating HMGB1 levels in adults with T2DM were included. A random-effects meta-analysis was performed to calculate pooled standardized mean differences (SMDs) with 95% confidence intervals (CIs). Heterogeneity was assessed using Cochran's Q, I^2, and prediction intervals. Subgroup analyses and meta-regression were conducted to explore potential sources of heterogeneity. Publication bias was evaluated using funnel plots, Egger's and Begg's tests, Doi plots with Luis Furuya-Kanamori (LFK) index, and trim-and-fill analysis.

Results: Nineteen studies were included, with sixteen studies in the primary analysis. HMGB1 levels were significantly higher in T2DM patients compared to controls (SMD = 1.39, 95% CI 0.89–1.90; $p < 0.0001$), with substantial heterogeneity ($I^2 = 96.3\%$). However, this association became non-significant after trim-and-fill adjustment. Patients with diabetic complications had significantly higher HMGB1 levels than those without (SMD = 0.52; $p < 0.0001$), particularly in microvascular complications.

Conclusion: Circulating HMGB1 levels appear to be elevated in patients with T2DM and associated complications; however, considerable heterogeneity, a prediction interval spanning the null value, and evidence of publication bias limit the certainty of this association.

Further well-designed prospective studies employing standardized methodologies are needed before HMGB1 can be considered a reliable clinical biomarker in T2DM.

Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder characterized by insulin resistance, progressive pancreatic β-cell dysfunction, and chronic low-grade inflammation. The global prevalence of T2DM continues to increase, contributing substantially to morbidity, mortality, and healthcare burden worldwide [1]. Persistent hyperglycaemia and metabolic dysregulation promote the development of microvascular complications, including diabetic nephropathy, retinopathy, and neuropathy, as well as macrovascular complications such as coronary artery disease and peripheral vascular disease [2]. Increasing evidence suggests that inflammatory mechanisms play a central role in linking metabolic disturbances with vascular injury and tissue damage in diabetes [3]. Genetic susceptibility and environmental factors further influence disease progression and complication risk [4].

High mobility group box-1 (HMGB1) is a highly conserved non-histone nuclear protein that functions as a chromatin-binding factor regulating transcription under physiological conditions [5]. During cellular stress, injury, or immune activation, HMGB1 is released extracellularly and functions as a damage-associated molecular pattern (DAMP), activating innate immune responses [6]. Extracellular HMGB1 interacts with pattern-recognition receptors such as the receptor for advanced glycation end products (RAGE) and toll-like receptors, triggering intracellular signalling cascades including nuclear factor kappa B (NF-κB) activation and inflammatory cytokine production [7]. These inflammatory pathways are implicated in metabolic dysfunction, endothelial injury, and insulin resistance [8, 9].

Emerging evidence suggests that HMGB1 is a key mediator linking hyperglycaemia-induced cellular stress with chronic inflammation and the development of diabetic complications. Experimental studies have demonstrated increased HMGB1 expression in adipose tissue, pancreatic islets, and vascular tissues under hyperglycaemic conditions, where it contributes to impaired insulin signalling, endothelial dysfunction, oxidative stress, and tissue injury [9-12]. HMGB1 has also been shown to regulate autophagy and mitochondrial function, further connecting cellular stress responses with metabolic dysregulation [13, 14]. In addition to its role in diabetes pathogenesis, elevated circulating HMGB1 levels have been associated with diabetic nephropathy through inflammatory and fibrotic mechanisms [12], retinal inflammation and angiogenesis in diabetic retinopathy [15], neuropathic changes associated with diabetic neuropathy [16], and vascular inflammation contributing to atherosclerosis and other macrovascular complications [17, 18]. These findings suggest that HMGB1 may serve not only as a mediator of chronic inflammation but also as a potential biomarker reflecting disease progression and vascular complications in T2DM.

Despite growing interest in HMGB1 as a biomarker of metabolic inflammation, findings from individual studies remain inconsistent due to variations in study design, population characteristics, and laboratory measurement methods. Therefore, systematic synthesis of available evidence is necessary to clarify the relationship between circulating HMGB1 levels and T2DM. The present systematic review and meta-analysis aimed to evaluate circulating HMGB1 levels in patients with T2DM compared with controls and to determine whether HMGB1 levels differ between T2DM patients with and without complications. Additionally, subgroup analyses were conducted to explore potential sources of heterogeneity based on geographical region, biological sample type, study design, sample size, and type of diabetic complications. Furthermore, meta-regression analyses were performed using study-level moderators, including mean age, body mass index (BMI), glycated hemoglobin (HbA1c), and diabetes duration, to investigate their potential contribution to between-study heterogeneity.

Materials and Methods

Study Design and Reporting Guidelines

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [19]. The protocol for this review was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO) under registration number CRD420261297845 (available at: https://www.crd.york.ac.uk/PROSPERO/view/CRD420261297845).

Literature Search Strategy

A comprehensive literature search was performed in the following electronic databases: PubMed/MEDLINE, Web of Science and Scopus from database inception to 14th February 2026. The search strategy combined keywords and Medical Subject Headings (MeSH) related to HMGB1 and type 2 diabetes mellitus. The following terms were used: ("Type 2 diabetes" OR "type 2 diabetes mellitus" OR "diabetes mellitus" OR "T2D" OR "T2DM") AND ("HMGB1 protein" OR "HMGB1" OR "HMG1 Protein" OR "HMG1" OR "amphoterin" OR "heparin-binding protein p30" OR "high-mobility group box 1" OR "high mobility group box protein 1"). The complete database search strategy is outlined in Supplementary Table 1. Only studies published in the English language were considered eligible for inclusion in this review. Additionally, reference lists of relevant articles and reviews were manually screened to identify potentially eligible studies.

Eligibility Criteria

Studies were included according to the PECOS framework.

Criterion	Definition
Population (P)	Adults diagnosed with type 2 diabetes mellitus.
Exposure (E)	Measurement of circulating HMGB1 levels in serum or plasma.
Comparator (C)	Healthy non-diabetic individuals or control subjects without diabetes, or normal glucose tolerant subjects.
Outcomes (O)	Primary outcome: circulating HMGB1 levels in patients with T2DM compared with controls. Secondary outcome: HMGB1 levels in T2DM with complications vs uncomplicated T2DM.
Study Design (S)	Observational studies including case–control studies and cross-sectional studies.

For studies including multiple diabetes subtypes, only data corresponding to type 2 diabetes mellitus were extracted to ensure population homogeneity.

Exclusion Criteria

Studies were excluded if they:

- Included type 1 diabetes mellitus or gestational diabetes mellitus
- Were animal or in vitro studies
- Were reviews, editorials, case reports, or conference abstracts
- Did not report quantitative HMGB1 data
- Included mixed diabetes populations without separate T2DM data
- Had no appropriate control group

Study Selection

Two reviewers independently screened the titles and abstracts of all retrieved records to identify potentially eligible studies. The full texts of relevant articles were then obtained and assessed according to the predefined inclusion criteria. Any disagreements between the two reviewers were resolved through discussion until consensus was reached.

Data Extraction

Data were independently extracted by two investigators using a standardized data extraction form. The following information was collected: first author, year of publication, country of study, study design, study population, diagnostic criteria for type 2 diabetes mellitus, HMGB1 measurement method, mean HMGB1 levels with corresponding standard deviations, type of biological sample (serum or plasma), and other relevant information. Information regarding whether HMGB1 concentrations represented crude or adjusted estimates, together with details of covariate adjustment where available, was also extracted (Supplementary Table 2). When data were presented graphically, numerical values were extracted using WebPlotDigitizer (version 5.2). Details of the studies requiring graphical data extraction are provided in Supplementary Table 3. Any discrepancies in the extracted data were resolved through discussion between the two reviewers.

Quality assessment

The methodological quality of the included studies was assessed according to study design. Case–control studies were evaluated using the Newcastle–Ottawa Scale (NOS) [20], whereas cross-sectional studies were appraised using the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Analytical Cross-Sectional Studies [21]. For studies assessed using the NOS, a maximum score of nine points could be assigned across the domains of selection, comparability, and exposure/outcome assessment. Studies scoring ≤4 points were considered low quality (high risk of bias), 5–6 points moderate quality (moderate risk of bias), and ≥7 points high quality (low risk of bias). For studies evaluated using the JBI checklist, methodological quality was determined based on the proportion of affirmative (“yes”) responses. Studies with ≤49% “yes” responses were classified as having a high risk of bias (low quality), those with 50–69% as moderate risk of bias (moderate quality), and those with ≥70% as low risk of bias (high quality).

Statistical Analysis

We performed meta-analyses using a random-effects model to account for between-study variability. The standardized mean difference (SMD) with a 95% confidence interval (CI) was calculated to compare circulating HMGB1 levels between: (1) patients with type 2 diabetes mellitus (T2DM) and controls, and (2) patients with T2DM with complications and those without complications. Effect estimates were weighted using the inverse-variance method, and between-study variance was estimated using the DerSimonian–Laird approach. For studies reporting HMGB1 levels as medians with interquartile ranges or ranges, corresponding means and standard deviations were estimated using established methods [22-24]. Statistical heterogeneity was assessed using the Cochran Q test and quantified with the I^2 statistic, with $p < 0.10$ and $I^2 > 50\%$ indicating substantial heterogeneity. To characterise the dispersion of true effects, 95% prediction intervals were calculated. Subgroup analyses were conducted to explore potential sources of heterogeneity based on geographical region (China vs. other countries), sample size (<100 vs.

≥100), biological sample type (serum vs. plasma), and study design (cross-sectional vs. case–control). Differences between subgroups were assessed using the chi-square test for subgroup differences, with a significance threshold of $p < 0.05$. To further elucidate potential sources of heterogeneity, meta-regression analyses were conducted utilizing mixed-effects models, incorporating mean age, body mass index (BMI), glycated hemoglobin (HbA1c), and diabetes duration as moderators. The robustness of pooled estimates was examined through leave-one-out sensitivity analyses. Small-study effects and publication bias were assessed by visual inspection of funnel plots, alongside Egger's regression test and Begg's rank correlation test. Additionally, Doi plots were constructed, and asymmetry was quantified using the Luis Furuya-Kanamori (LFK) index [25], with values between −1 and +1 indicating symmetry, ±1 to ±2 indicating minor asymmetry, and values beyond ±2 indicating major asymmetry. To evaluate the potential impact of reporting bias, the trim-and-fill method was applied to estimate missing studies and provide bias-adjusted effect estimates. P-values <0.05 were considered statistically significant, unless otherwise specified.

Results

Characteristics of included studies

Following the predefined search strategy, a total of 1,328 records were initially identified. After removal of duplicates, 730 unique articles remained for screening. Title and abstract screening yielded 34 potentially eligible studies, which were subsequently assessed through full-text review. Ultimately, 19 studies met the inclusion criteria and were incorporated into the systematic review and meta-analysis [10, 26-43] (Figure 1). The key characteristics of the included studies are summarized in Table 1. Of the 19 included studies, 16 compared circulating HMGB1 levels between patients with T2DM and controls; these were therefore incorporated into the primary meta-analysis assessing HMGB1 levels in T2DM versus controls [10, 26, 28, 30-37, 39-43]. Five of these studies also reported HMGB1 levels in T2DM patients with and without diabetic complications [30, 31, 33, 40, 42]. Furthermore, three studies [27, 29, 38] exclusively compared T2DM patients with and without complications and did not include a healthy control group. Consequently, these three studies were excluded from the primary meta-analysis of T2DM versus controls but were included in the secondary meta-analysis comparing T2DM patients with and without complications. In total, eight studies contributed to the meta-analysis evaluating HMGB1 levels based on the presence of diabetic complications [27, 29-31, 33, 38, 40, 42].

Figure 1: PRISMA flow diagram for the study selection process.

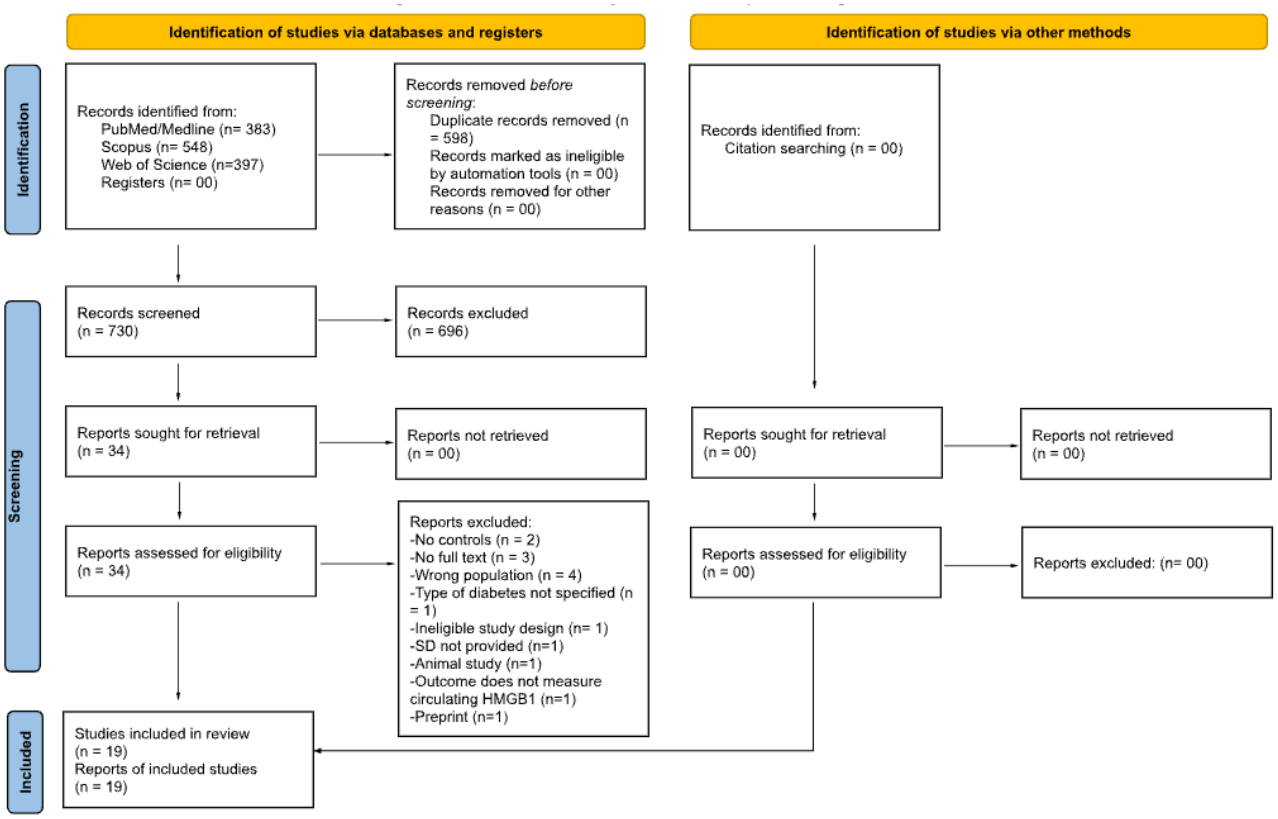


Table 1: Baseline characteristics of studies included in meta-analysis.

Author	Year	Country	Study design	Study subjects	Diagnostic criteria of type 2 DM	Sample	Main findings
Al-Hakeim HK et al. [26]	2022	Iraq	Cross-sectional study	Adults with T2DM with (n=34) and without hypertension (n=76) and healthy controls (n=51)	WHO	Serum	Serum HMGB1 levels were significantly higher in T2DM patients compared with controls and were associated with increased glucose toxicity, reduced β-cell function, and higher atherogenic indices.
Biscetti F et al. [27]	2021	Italy	Case–control study	T2DM patients with internal carotid artery stenosis (ICAS) vs T2DM patients without ICAS. Total = 873 T2DM patients (ICAS = 347, WICAS = 526)	Type 2 diabetes mellitus was defined as a fasting plasma glucose ≥126 mg/dL and/or HbA1c ≥6.5% or as medical history for the presence of diabetes plus treatment with diabetes medication.	Serum	Serum HMGB1 levels were significantly higher in T2DM patients with ICAS compared with those without ICAS, and HMGB1 was independently associated with carotid plaque vulnerability in diabetic patients.
Chen Y et al. [28]	2015	China	Case–control study	Patients with type 2 diabetes mellitus (n=50) and healthy controls (n=49)	WHO	Serum	Serum HMGB1 levels were significantly higher in T2DM patients compared with controls. HMGB1 levels were positively correlated with inflammatory cytokines IL-6 and TNF-α.
Dasu MR et al. [10]	2010	USA	Case–control study	Type 2 diabetes patients (n=23) and healthy controls (n=23)	NR	Serum	Serum HMGB1 levels were significantly higher in patients with type 2 diabetes compared with healthy controls, indicating that HMGB1 is elevated in T2DM and may contribute to the pro-inflammatory state associated with the disease.
Giovannini S. et al. [29]	2017	Italy	Case–control study	Type 2 diabetes patients with peripheral arterial disease (PAD) (n=569) vs T2DM without PAD (n=824)	HbA1c >5.8%, fasting glucose >126 mg/dL, or treatment with insulin/oral antidiabetic drugs.	Serum	Serum HMGB1 levels were significantly higher in T2DM patients with PAD compared with T2DM without PAD; HMGB1 was independently associated with PAD occurrence and severity.
Huang D et al. [30]	2020	China	Cross-sectional study	Patients with diabetes mellitus with diabetic peripheral neuropathy (DPN) (n=43), diabetes without neuropathy (n=34), incipient diabetes (n=26), and healthy controls (n=30)	WHO	Serum	Serum HMGB1 levels were significantly higher in diabetes patients compared with healthy controls and were highest in patients with diabetic peripheral neuropathy. HMGB1 levels were positively correlated with fasting glucose, postprandial glucose, HbA1c, and LDL levels.
Hui Y et al. [31]	2019	China	Cross-sectional study	Patients with type 2 diabetes mellitus with proliferative diabetic retinopathy (PDR) (n=36), non-proliferative diabetic retinopathy (NPDR) (n=43), T2DM without retinopathy (n=40), and healthy controls (n=30)	WHO	Serum	Serum HMGB1 levels were significantly elevated in patients with proliferative diabetic retinopathy compared with NPDR, T2DM without retinopathy, and healthy controls. HMGB1 levels were associated with duration of diabetes, HbA1c, and inflammatory markers.
Liu S et al. [32]	2022	China	Cross-sectional study	Newly diagnosed T2DM patients (n=40) and normal glucose tolerant (NGT) controls (n=40), further divided by obesity status	WHO	Serum	Serum HMGB1 levels were significantly higher in T2DM patients compared with normal glucose tolerant subjects. HMGB1 levels were positively correlated with BMI, fasting plasma glucose, and triglyceride levels, and negatively correlated with HDL.
Liu T et al. [33]	2024	China	Cross-sectional study	Patients with T2DM with diabetic kidney disease (DKD) (n=109), T2DM without DKD (n=87), and healthy controls (n=60)	ADA	Serum	Serum and urine HMGB1 levels were significantly higher in DKD patients compared with T2DM patients without DKD and healthy controls. Serum HMGB1 showed strong correlation with kidney injury markers (TNFR-1 and KIM-1) and renal function indicators. Elevated HMGB1 was an independent predictor of DKD progression, and levels ≥27 ng/mL were associated with a higher risk of renal function decline.
Majid H et al. (1) [34]	2025	India	Case–control study	Patients with type 2 diabetes mellitus (n=100) compared with healthy controls (n=100)	WHO and ADA diagnostic criteria	Serum	Serum HMGB1 levels were significantly higher in patients with T2DM compared with healthy controls. Increased HMGB1 levels were associated with neuroinflammatory pathways and cognitive impairment in T2DM patients.
Majid H et al. (2) [35]	2025	India	Case–control study	T2DM patients on SGLT2 inhibitors (n=57), sulfonylureas (n=54), and healthy controls (n=55)	WHO and ADA diagnostic criteria	Serum	HMGB1 and other neuroinflammatory markers were significantly elevated in T2DM patients compared to controls. SGLT2 inhibitors showed lower HMGB1 levels and better neuroinflammatory and cognitive outcomes compared to sulfonylureas.
Shen Y et al. [36]	2020	China	Cross-sectional study	Patients with proliferative diabetic retinopathy (n=32) and non-diabetic controls (n=46)	ADA	Serum	Serum and vitreous HMGB1 levels were significantly higher in diabetic retinopathy and correlated with inflammatory cytokines and angiogenic factors.
Škrha J et al. [37]	2012	Czech Republic	Case–control study	Patients with type 2 diabetes (n=66), and healthy controls (n=43)	NR	Serum	HMGB1 and other RAGE ligands were elevated in diabetic patients and associated with endothelial dysfunction markers.
Stempkowska A. et al. [38]	2020	Poland	Case–control study	T2DM patients with cardiovascular disease (n=34) vs T2DM patients without cardiovascular disease (n=60)	NR	Serum	Serum HMGB1 levels showed no significant difference between T2DM patients with cardiovascular disease and those without cardiovascular disease.
Wang H et al. [39]	2015	China	Cross-sectional study	Adults with normal glucose tolerance (n=75) and newly diagnosed T2DM (n=80)	WHO	Plasma	Plasma HMGB1 levels were significantly higher in T2DM compared with NGT and positively correlated with insulin resistance, waist-hip ratio, triglycerides, IL-6, and HOMA-IR.
Wang LJ et al. [40]	2011	China	Cross-sectional study	T2DM with HF (n=125), T2DM without HF (n=79), non-diabetic with HF (n=222), non-diabetic controls (n=220)	ADA	Serum	HMGB1 levels were significantly higher in HF patients (with and without T2DM) compared to controls.
Wei H et al. [41]	2016	China	Cross-sectional study	Subjects with normal glucose tolerance (n=62), and newly diagnosed T2DM (n=56)	ADA	Plasma	Plasma HMGB1 levels were higher in T2DM and were independently associated with insulin resistance and inflammatory markers such as IL-6.

Author	Year	Country	Study design	Study subjects	Diagnostic criteria of type 2 DM	Sample	Main findings
Yan XX et al. [42]	2009	China	Cross-sectional study	Non-diabetic without CAD (n=132), non-diabetic with CAD (n=149), T2DM without CAD (n=80), and T2DM with CAD (n=151)	Two fasting plasma glucose levels ≥7.0 mmol/L or two 2-h postprandial plasma glucose ≥11.1 mmol/L after a glucose load of 75 g or two casual glucose readings ≥11.1 mmol/L, or taking oral hypoglycemic drugs or parenteral insulin	Serum	Serum HMGB1 levels were significantly higher in patients with CAD. Among diabetic patients, HMGB1 levels were significantly higher in those with CAD compared to those without CAD (P < 0.001), indicating an association between elevated HMGB1 and diabetic coronary artery disease.
Yang X et al. [43]	2020	China	Cross-sectional study	Type 2 diabetes mellitus (n=90), and age-matched healthy controls (n=81)	WHO	Plasma	HMGB1 levels were significantly higher in T2DM patients compared with healthy controls. HMGB1 levels were associated with cognitive impairment and inflammatory processes in diabetic patients.

T2DM: Type 2 Diabetes Mellitus; HMGB1: High Mobility Group Box 1; WHO: World Health Organization; ADA: American Diabetes Association; NR: Not Reported; ICAS: Internal Carotid Artery Stenosis; WICAS: Without Internal Carotid Artery Stenosis; PAD: Peripheral Arterial Disease; DPN: Diabetic Peripheral Neuropathy; DKD: Diabetic Kidney Disease; PDR: Proliferative Diabetic Retinopathy; NPDR: Non-Proliferative Diabetic Retinopathy; CAD: Coronary Artery Disease; HF: Heart Failure; HbA1c: Glycated Hemoglobin; IL-6: Interleukin-6; TNF-α: Tumor Necrosis Factor Alpha; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein; BMI: Body Mass Index; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; TNFR-1: Tumor Necrosis Factor Receptor 1; KIM-1: Kidney Injury Molecule-1; NGT: Normal Glucose Tolerance. Three studies (Biscetti F et al. [27], Giovannini S et al. [29], and Stempkowska A et al. [38]) were excluded from the primary meta-analysis of T2DM versus healthy controls because they did not include a healthy control group and only compared T2DM patients with and without diabetic complications.

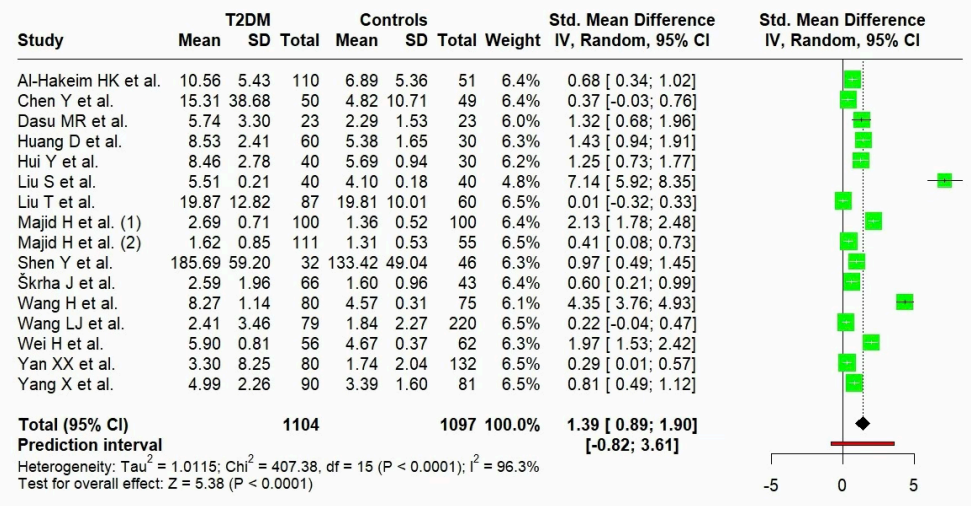
These studies were published between 2009 and 2025 and were geographically diverse, with the majority conducted in China (n = 11) [28, 30-33, 36, 39-43], followed by Italy (n = 2) [27, 29] and India (n = 2) [34, 35], and one study each from Iraq [26], the United States [10], the Czech Republic [37], and Poland [38]. In terms of study design, 11 studies were cross-sectional [26, 30-33, 36, 39-43], while 8 employed a case–control design [10, 27-29, 34, 35, 37, 38]. Regarding sample type, 16 studies measured HMGB1 in serum [10, 26-38, 40, 42] and 3 in plasma [39, 41, 43]. HMGB1 levels were assessed using enzyme-linked immunosorbent assay (ELISA) in all studies except one [28], in which the detection method was not specified. Detailed information on the ELISA kits used across studies is provided in the Supplementary Table 4. Study quality assessments are presented in Supplementary Tables 5 and 6.

HMGB1 levels between T2DM and controls

Meta-analysis

A total of 16 studies [10, 26, 28, 30-37, 39-43] including 1,104 patients with T2DM and 1,097 controls were analysed. The pooled results from the random-effects model demonstrated that circulating HMGB1 levels were significantly higher in patients with T2DM compared to controls (SMD = 1.39, 95% CI: 0.89 to 1.90, $p < 0.0001$). There was considerable between-study heterogeneity ($I^2 = 96.3\%$, $\tau^2 = 1.0115$, $p < 0.0001$), indicating substantial variability in effect sizes across studies. The prediction interval ranged from −0.82 to 3.61, suggesting that while the overall effect favors higher HMGB1 levels in T2DM, the magnitude of association may vary widely across different populations and settings. The pooled effect estimates are shown in the forest plot (Figure 2), while the distribution of statistical significance is depicted in the drapery plot (Supplementary Figure 1).

Figure 2: Forest plot showing comparison of circulating HMGB1 levels between T2DM and controls.



Subgroup analysis

When stratified by geographical region, studies conducted in China demonstrated a higher pooled effect size (SMD = 1.59, 95% CI: 0.89–2.29; I^2 = 97.1%) compared to those from other countries (SMD = 1.02, 95% CI: 0.34–1.69; I^2 = 93.5%); however, the difference between subgroups was not statistically significant (p = 0.2464). Similarly, studies with smaller sample sizes (<100 participants) showed larger effect estimates (SMD = 2.08, 95% CI: 0.77–3.39; I^2 = 96.3%) compared to larger studies (≥100 participants; SMD = 1.15, 95% CI: 0.58–1.72; I^2 = 96.6%), although subgroup differences were not statistically significant (p = 0.2025), suggesting possible small-study effects. Analysis based on biological sample type revealed higher HMGB1 levels in plasma-based studies (SMD = 2.36, 95% CI: 0.49–4.24; I^2 = 98.2%) compared to serum-based studies (SMD = 1.14, 95% CI: 0.66–1.61; I^2 = 94.9%); however, this difference was not statistically significant (p = 0.2151). Finally, subgroup analysis by study design showed that cross-sectional studies reported higher effect sizes (SMD = 1.62, 95% CI: 0.93–2.30; I^2 = 97%) compared to case–control studies (SMD = 0.96, 95% CI: 0.23–1.69; I^2 = 94.1%), although no significant subgroup difference was observed (p = 0.1967). The forest plots for these analyses are shown in Supplementary Figures 2-5.

Meta-regression

Meta-regression was conducted to ascertain whether study-level characteristics, specifically mean age, BMI, HbA1c, and diabetes duration, accounted for the observed between-study heterogeneity. None of the covariates assessed demonstrated a statistically significant influence on the pooled effect size. Mean age exhibited a non-significant inverse relationship with the effect size (β = −0.0540, 95% CI: −0.1242 to 0.0161, p = 0.1310). Similarly, BMI did not show a significant association with the pooled effect size (β = 0.0660, 95% CI: −0.1684 to 0.3004, p = 0.5811). HbA1c similarly failed to demonstrate a significant moderating effect (β = −0.4620, 95% CI: −1.0460 to 0.1221, p = 0.1211), and diabetes duration was not significantly associated with the pooled effect size (β = −0.0579, 95% CI: −0.1906 to 0.0748, p = 0.3924). Furthermore, none of the investigated moderators explained a substantial proportion of the between-study heterogeneity (R^2 = 0.0% for all models). Significant residual heterogeneity persisted across all analyses, evidenced by I^2 values ranging from 81.82% to 96.89%, thereby indicating that the variability among studies could not be adequately explained by the covariates under examination. The outcomes of the meta-regression analyses are presented in Table 2, with their corresponding graphical representations, the bubble plots, available in Supplementary Figures 6-9.

Table 2: Meta-regression analyses exploring sources of heterogeneity.

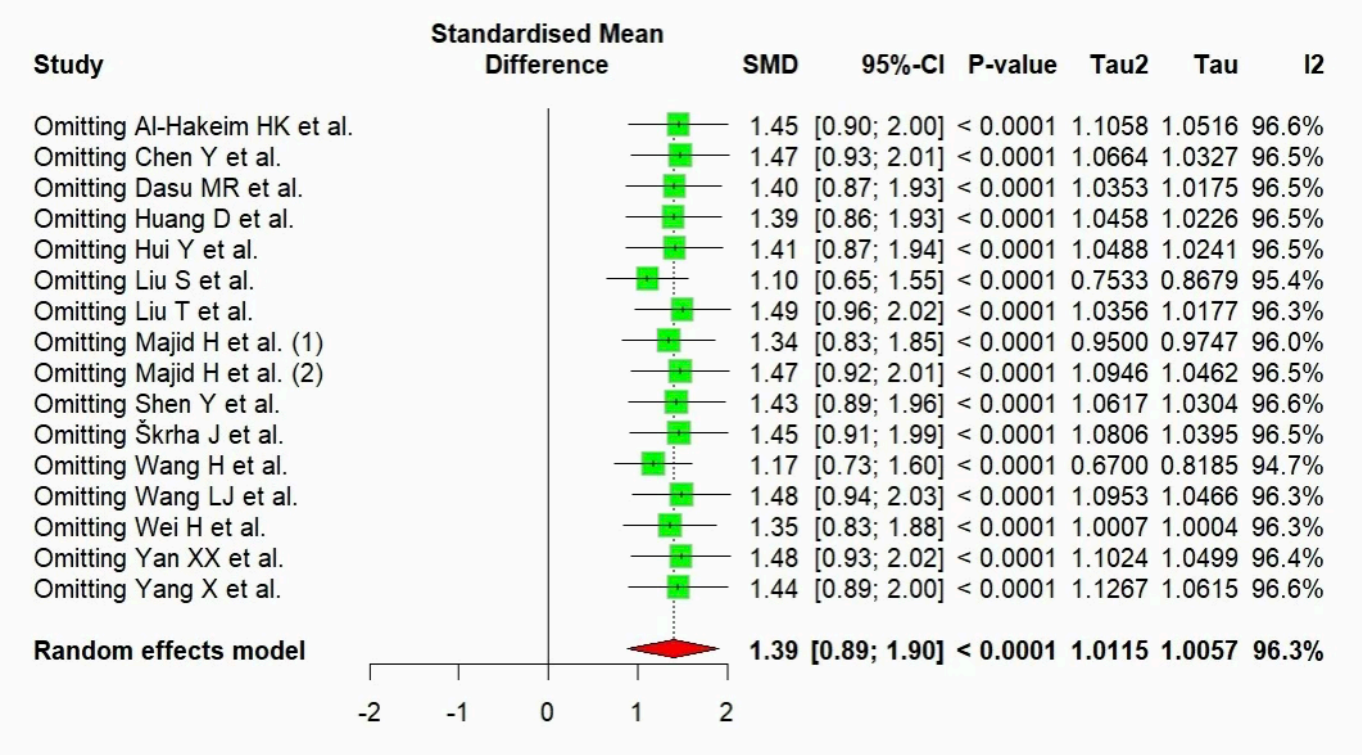
Moderator	k	β-Coefficient	SE	95% CI	QM	P-value	R^2 (%)	τ^2	I^2 (%)
Mean age	16	-0.0540	0.0358	-0.1242 to 0.0161	2.2805	0.1310	0.00	1.0718	96.42
Mean BMI	13	0.0660	0.1196	-0.1684 to 0.3004	0.3045	0.5811	0.00	1.3550	96.89
Mean HbA1c	15	-0.4620	0.2980	-1.0460 to 0.1221	2.4036	0.1211	0.00	0.7927	95.67
Diabetes duration	7	-0.0579	0.0677	-0.1906 to 0.0748	0.7314	0.3924	0.00	0.1820	81.82

β, regression coefficient; SE, standard error; CI, confidence interval; QM, test of moderators; R^2, proportion of between-study heterogeneity explained by the moderator; τ^2, residual between-study variance; I^2, residual heterogeneity; k, number of studies included in the meta-regression.

Sensitivity analysis

Leave-one-out sensitivity analysis demonstrated that the pooled effect size remained robust and statistically significant after sequential exclusion of individual studies. The pooled SMD ranged from 1.10 to 1.49, with all analyses retaining statistical significance ($p < 0.0001$). No single study disproportionately influenced the overall effect estimate (Figure 3). These findings confirm the stability and reliability of the pooled results.

Figure 3: Results of leave-one-out method in sensitivity analysis.



Publication bias

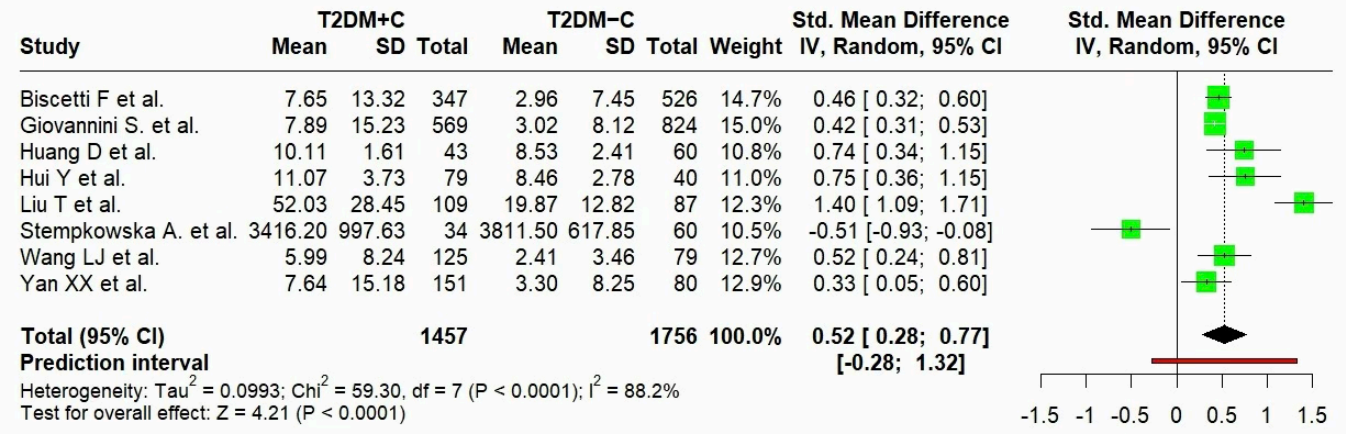
Visual inspection of the funnel plots revealed asymmetry, with an uneven distribution of studies around the pooled effect size, suggesting the presence of potential small-study effects (Supplementary Figure 10). This observation was further supported by formal statistical testing. Egger's regression test demonstrated significant funnel plot asymmetry ($t = 4.15$, $p = 0.0010$), indicating the presence of small-study effects. Similarly, Begg's rank correlation test confirmed significant asymmetry ($z = 2.97$, $p = 0.0030$), reinforcing the likelihood of publication bias. In addition, the Doi plot demonstrated major asymmetry, with an LFK index of 2.55, which is indicative of substantial publication bias (Supplementary Figure 11). To account for potential bias, a trim-and-fill analysis was performed. After adjustment, a total of 6 potentially missing studies were imputed, increasing the number of included studies to 22 (Supplementary Figure 12). The adjusted pooled effect size was attenuated and became non-significant (SMD = 0.51, 95% CI: −0.07 to 1.09; $p = 0.087$), compared to the original significant estimate. Despite adjustment, substantial heterogeneity persisted ($I^2 = 97.7\%$, $p < 0.0001$), suggesting that the observed variability is likely influenced not only by publication bias but also by clinical and methodological differences across studies.

HMGB1 levels between T2DM with complications and without complications

Meta-analysis

A total of 8 studies [27, 29-31, 33, 38, 40, 42] comprising 1,457 patients with type 2 diabetes mellitus with complications (T2DM+C) and 1,756 patients without complications (T2DM−C) were included in the meta-analysis. The pooled analysis using a random-effects model demonstrated that circulating HMGB1 levels were significantly higher in patients with T2DM with complications compared to those without complications (SMD = 0.52, 95% CI: 0.28 to 0.77, $p < 0.0001$). Substantial heterogeneity was observed across studies ($I^2 = 88.2\%$, $\tau^2 = 0.0993$). The prediction interval ranged from −0.28 to 1.32, indicating that although the overall effect favored higher HMGB1 levels in T2DM+C, the true effect may vary across different clinical settings. The results of the meta-analysis are illustrated using a forest plot (Figure 4) and a drapery plot (Supplementary Figure 13).

Figure 4: HMGB1 levels between T2DM with complications (T2DM+C) and without complications (T2DM-C).



Sensitivity analysis

Leave-one-out sensitivity analysis demonstrated that the pooled effect estimate remained statistically significant following the sequential exclusion of each individual study, indicating the robustness of the overall findings. Among the included studies, the omission of Stempkowska et al. [38] resulted in the largest change in the pooled effect estimate, increasing the standardized mean difference from 0.52 (95% CI: 0.28–0.77) to 0.63 (95% CI: 0.42–0.85), concurrently reducing heterogeneity from $I^2 = 88.2\%$ to 84.4%. Nevertheless, the direction and statistical significance of the association remained consistent, indicating that no single study exerted a substantial influence on the overall conclusions (Figure 5).

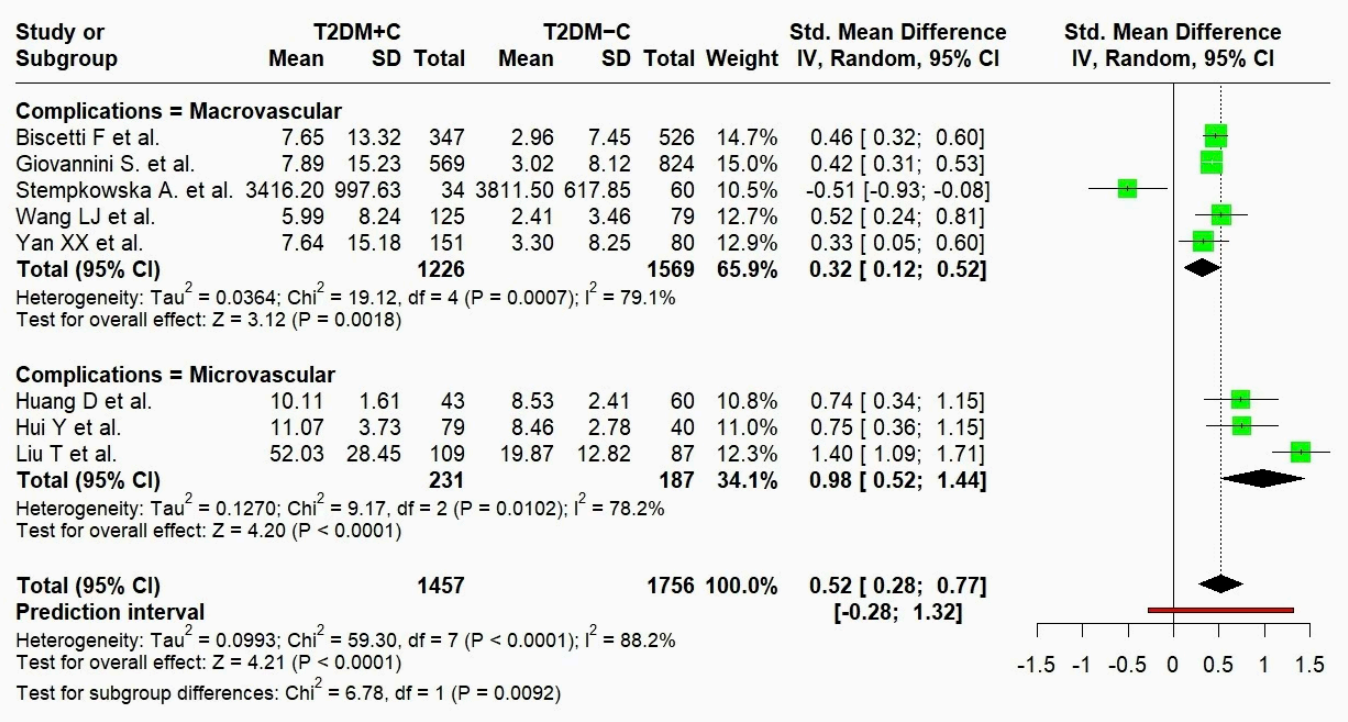
Figure 5: Sensitivity analysis illustrating the influence of individual studies on the pooled estimate for T2DM with complications.



Subgroup analysis

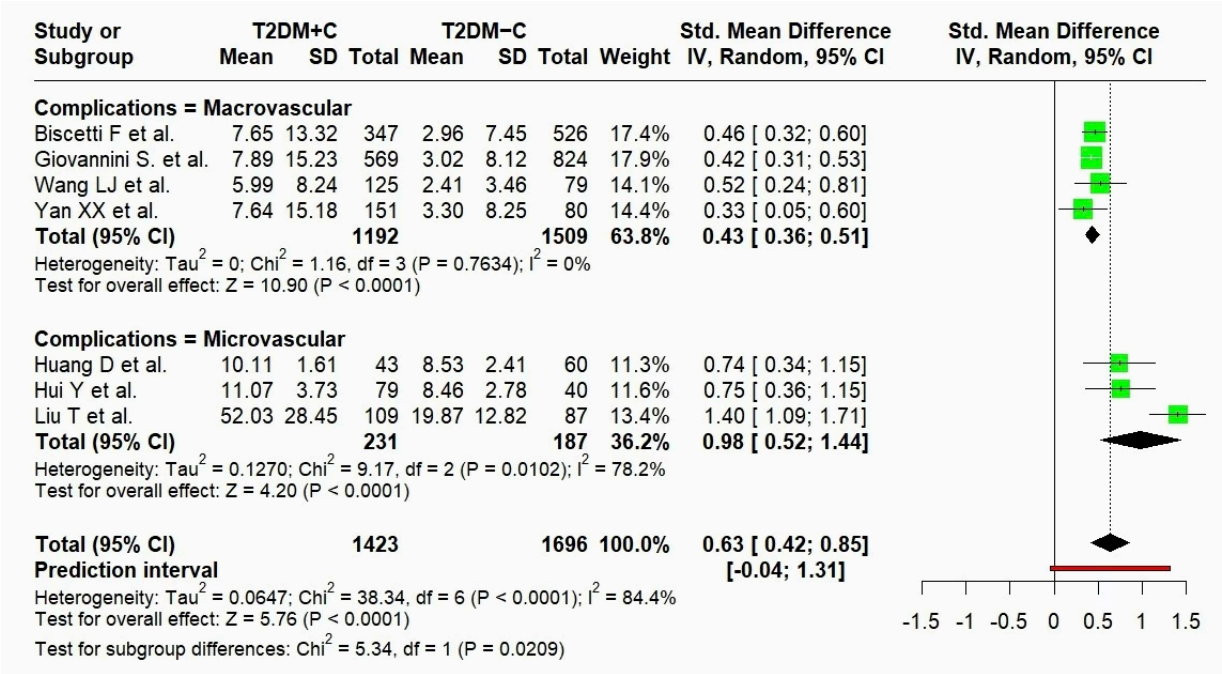
Subgroup analysis according to the type of diabetic complications demonstrated that circulating HMGB1 levels were significantly elevated in both macrovascular and microvascular complications compared with T2DM patients without complications. The pooled effect size was modest for macrovascular complications (SMD = 0.32, 95% CI: 0.12–0.52; p = 0.0018), with substantial heterogeneity (I^2 = 79.1%), whereas a considerably larger effect was observed for microvascular complications (SMD = 0.98, 95% CI: 0.52–1.44; p < 0.0001), also with substantial heterogeneity (I^2 = 78.2%). The difference between the two subgroups was statistically significant (p = 0.0092), indicating that the elevation in circulating HMGB1 levels was more pronounced in microvascular than in macrovascular complications (Figure 6).

Figure 6: Forest plot of subgroup analysis comparing circulating HMGB1 levels in patients with microvascular and macrovascular complications of T2DM versus patients without complications.



As Stempkowska et al. [38] exerted the greatest influence on the pooled estimate in the leave-one-out sensitivity analysis as shown in Figure 5, a sensitivity subgroup analysis was performed after excluding this study from the macrovascular subgroup. Exclusion of Stempkowska et al. [38] increased the pooled effect estimate for macrovascular complications from SMD = 0.32 (95% CI: 0.12–0.52) to 0.43 (95% CI: 0.36–0.51) and reduced heterogeneity from I^2 = 79.1% to 0%. The pooled estimate for microvascular complications remained unchanged, and the difference between the macrovascular and microvascular subgroups remained statistically significant (Figure 7), confirming the robustness of the subgroup findings.

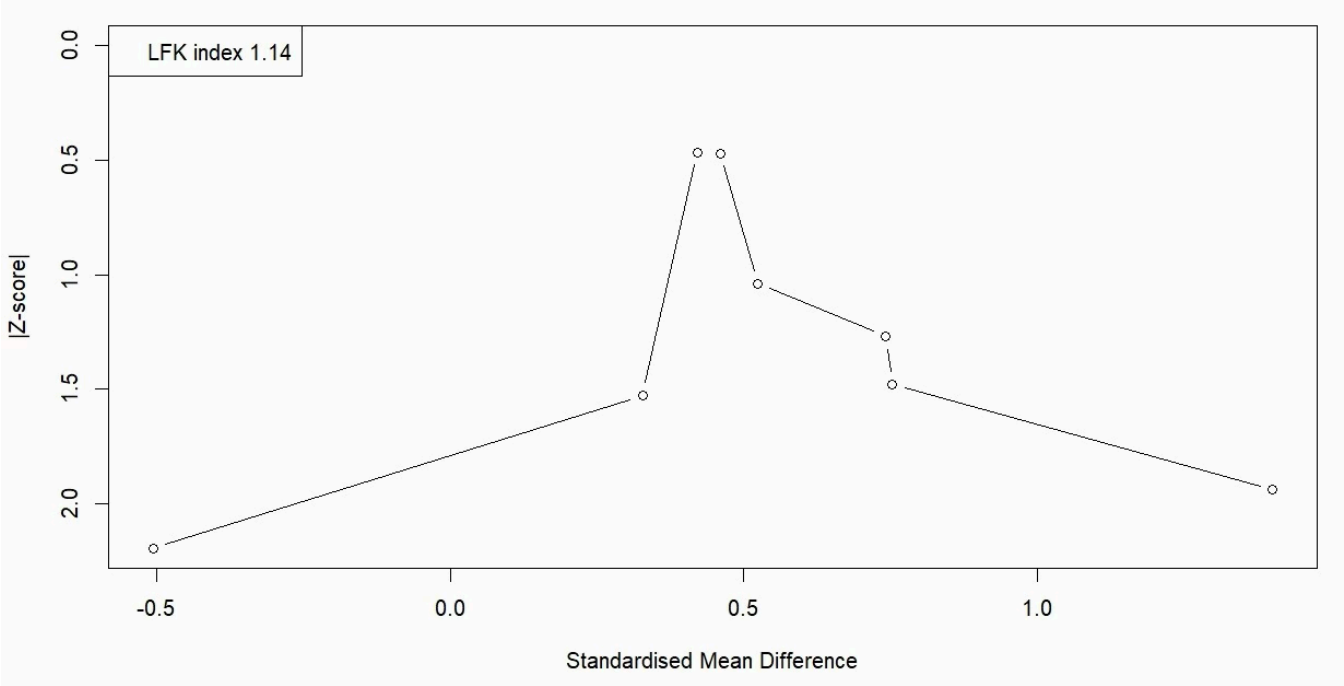
Figure 7: Forest plot of the sensitivity subgroup analysis comparing circulating HMGB1 levels in patients with microvascular and macrovascular complications of type 2 diabetes mellitus after exclusion of the influential study (Stempkowska et al. [38]).



Publication bias

Assessment of publication bias using the Doi plot demonstrated minor asymmetry, with an LFK index of 1.14, indicating low to moderate asymmetry. These findings suggest that publication bias is unlikely to have a major influence on the pooled results, although the presence of small-study effects cannot be entirely excluded (Figure 8).

Figure 8: Doi plot showing potential publication bias in T2DM with complications.



Discussion

This systematic review and meta-analysis found that circulating HMGB1 levels were higher in patients with T2DM than in non-diabetic controls in the primary analysis. These findings are consistent with the concept that chronic low-grade inflammation is a fundamental component of T2DM pathophysiology and contributes to disease progression [17,44]. The observed elevation of HMGB1 supports its potential role in metabolic dysregulation and inflammatory activation; however, the strength of this association should be interpreted cautiously because trim-and-fill analysis attenuated the pooled effect estimate to non-significance.

HMGB1 plays an important role in innate immune signalling and inflammatory amplification. Hyperglycaemia and oxidative stress stimulate active secretion of HMGB1 from immune cells and passive release from damaged tissues, promoting inflammatory signalling cascades that contribute to insulin resistance [45]. Activation of RAGE-dependent pathways further enhances cytokine production and endothelial dysfunction, linking metabolic stress with vascular injury [46]. Experimental studies have demonstrated that HMGB1-mediated activation of inflammatory pathways contributes to impaired insulin signalling and chronic metabolic inflammation [47]. Importantly, the present meta-analysis demonstrated that circulating HMGB1 levels were significantly higher in T2DM patients with complications compared with those without complications, suggesting that HMGB1 may reflect disease severity and progression. Experimental evidence indicates that HMGB1 contributes to renal inflammation and fibrosis in diabetic kidney disease, promotes inflammatory responses and pathological angiogenesis in diabetic retinopathy, and participates in neuroinflammatory pathways associated with diabetic neuropathy [12, 15, 16]. Proteomic studies further support the potential value of HMGB1 as an early biomarker of diabetic nephropathy progression [48]. In addition, HMGB1 has been implicated in macrovascular disease through its ability to promote endothelial activation, vascular smooth muscle cell proliferation, inflammatory cell recruitment, and plaque instability, thereby contributing to vascular inflammation and atherosclerosis [49, 50]. These findings suggest that HMGB1 may represent a common molecular mediator linking metabolic inflammation with vascular pathology.

A particularly noteworthy finding of the present meta-analysis was the significantly stronger association of circulating HMGB1 with microvascular complications than with macrovascular complications. This observation is biologically plausible because HMGB1 is more directly involved in the inflammatory processes underlying capillary endothelial injury. Under hyperglycaemic conditions, extracellular HMGB1 activates receptor for advanced glycation end products (RAGE)- and toll-like receptor (TLR)-mediated signalling pathways, leading to oxidative stress, endothelial dysfunction,

leukocyte recruitment, and sustained cytokine production [6, 17, 51]. These mechanisms promote increased vascular permeability, basement membrane thickening, extracellular matrix accumulation, and tissue fibrosis, which are hallmark features of diabetic nephropathy, retinopathy, and neuropathy [12, 15, 16, 48]. In contrast, although HMGB1 also contributes to macrovascular disease by promoting endothelial activation, vascular smooth muscle cell proliferation, monocyte recruitment, and plaque instability, the development of large-vessel atherosclerosis is influenced by a broader interplay of lipid metabolism, haemodynamic stress, vascular remodelling, and chronic inflammation [6, 17, 18, 49-53]. Consequently, HMGB1 may play a relatively greater role in the progression of diabetic microvascular complications than macrovascular disease, providing a biological explanation for the significantly larger pooled effect observed in the microvascular subgroup. Nevertheless, prospective mechanistic studies are required to determine whether HMGB1 has differential diagnostic or prognostic value across different diabetic vascular complications.

Substantial heterogeneity was observed across the included studies. To explore potential sources of between-study variability, both subgroup and meta-regression analyses were performed. Although studies conducted in China, studies with smaller sample sizes, plasma-based studies, and cross-sectional studies tended to report larger effect sizes than their respective counterparts, none of the subgroup differences reached statistical significance. Similarly, meta-regression analyses demonstrated that mean age, BMI, HbA1c, and diabetes duration were not significant moderators of the pooled effect estimates. These findings suggest that the observed heterogeneity could not be adequately explained by the examined study-level characteristics and may instead reflect the influence of unmeasured clinical and methodological factors. In particular, HMGB1 concentrations were measured using a variety of commercially available ELISA kits from different manufacturers, which may differ with respect to antibody specificity, calibration standards, detection limits, and analytical performance. Although the influence of assay platform could not be formally evaluated because most ELISA kits were represented by only a limited number of studies, assay-related variability should be considered when interpreting the pooled findings. Nevertheless, leave-one-out sensitivity analyses demonstrated that the pooled effect estimates remained stable following the sequential exclusion of individual studies, supporting the robustness of the results despite the presence of substantial heterogeneity.

Publication bias is another important consideration when interpreting the findings of this meta-analysis. While the primary analysis revealed significantly elevated circulating HMGB1 levels in individuals with T2DM, a trim-and-fill analysis imputed six potentially missing studies and attenuated the pooled effect estimate to a non-significant level. This finding suggests that the magnitude of the observed association may have been influenced by small-study effects and selective publication of positive results. Consequently, the current evidence may overestimate the true association between HMGB1 and T2DM. Furthermore, the wide prediction interval encompassing the null value indicates that future studies may observe smaller, null, or even opposite effects depending on the study population and clinical setting. Therefore, although HMGB1 remains a biologically plausible inflammatory biomarker involved in the pathogenesis of T2DM and its complications, the current evidence is insufficient to support its routine clinical application as a diagnostic or prognostic biomarker. Additional large-scale prospective studies using standardized assay methodologies are required to establish the true clinical utility of HMGB1.

From a clinical laboratory perspective, HMGB1 could serve as a complementary inflammatory biomarker, indicative of metabolic dysfunction and vascular injury in T2DM. Unlike established inflammatory biomarkers such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α), HMGB1 is directly implicated in damage-associated molecular pattern (DAMP)-mediated inflammatory signalling via RAGE and toll-like receptor pathways. This offers potential for enhanced mechanistic understanding of diabetes-related tissue injury and vascular complications. Nevertheless, current evidence is insufficient to establish its incremental diagnostic or prognostic value over existing biomarkers in routine clinical practice. Among the included studies, Liu et al. [33] identified a serum HMGB1 concentration of ≥27 ng/mL as a predictor of renal function decline in patients with T2DM, suggesting a potential prognostic role. However, this threshold lacks external validation and therefore cannot be considered a clinically applicable decision limit. Furthermore, the widespread clinical implementation of HMGB1 testing is currently constrained by the absence of standardized assay methodologies, validated reference intervals, and population-specific clinical cutoffs. Consequently, further multicentre prospective studies are warranted to standardize HMGB1 measurement, establish clinically meaningful decision thresholds, and ascertain whether HMGB1 provides incremental value over existing inflammatory biomarkers in risk stratification and disease monitoring. Emerging evidence also suggests that HMGB1 signalling pathways may represent potential therapeutic targets for reducing inflammation and preventing diabetes progression [54].

Several limitations should be acknowledged when interpreting the present meta-analysis. First, the majority of included studies were observational in design, precluding definitive causal inferences. Second, substantial heterogeneity was observed across analyses, which may limit the robustness and generalizability of the pooled estimates. This variability may partly reflect differences in assay methodologies, study populations, and clinical characteristics across studies. Third, evidence of publication bias was identified, and trim-and-fill analysis attenuated the pooled association to a non-significant

level, suggesting that the observed effect size may have been influenced by small-study effects and selective publication of positive findings. Finally, in some studies, summary statistics were not directly reported, necessitating the conversion of medians and interquartile ranges to means and standard deviations, which may introduce additional estimation bias.

Conclusion

In conclusion, circulating HMGB1 levels appear to be elevated in patients with T2DM compared with non-diabetic controls and are further increased in patients with diabetic complications compared with those without complications, particularly in microvascular complications. Nevertheless, significant between-study heterogeneity, a broad prediction interval encompassing the null value, and evidence of publication bias necessitate a cautious interpretation of these findings. Notably, trim-and-fill analysis attenuated the primary association, rendering it statistically non-significant and suggesting that the observed effect size may have been influenced by small-study effects. While HMGB1 remains a biologically plausible marker linking inflammation, metabolic dysfunction, and vascular injury, current evidence is insufficient to support its routine clinical application as a diagnostic or prognostic biomarker. Further large-scale prospective studies employing standardized measurement methodologies are required to elucidate the clinical utility of HMGB1 in T2DM.

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Declaration of Conflict of interests

The authors declare that they have no conflict of interest regarding the publication of this article.

Ethical approval

This study is a systematic review and meta-analysis. No ethical approval is required.

Credit Author Statements

Usha Kiran Pothu: Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration.

Roshan Kumar Mahat: Conceptualization, Methodology, Software, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration.

All authors have read and approved the final manuscript.

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Supplementary Table 1: Search strategies in databases.

Databases	Search terms	No. of articles identified
PubMed/Medline	("Type 2 diabetes"[All Fields] OR "type 2 diabetes mellitus"[All Fields] OR "diabetes mellitus"[All Fields] OR "T2D"[All Fields] OR "T2DM"[All Fields]) AND ("HMGB1 protein"[All Fields] OR "HMGB1"[All Fields] OR "HMG1 Protein"[All Fields] OR "HMG1"[All Fields] OR "amphoterin"[All Fields] OR "heparin-binding protein p30"[All Fields] OR "high-mobility group box 1"[All Fields] OR "high mobility group box protein 1"[All Fields])	383
Scopus	TITLE-ABS-KEY (("Type 2 diabetes" OR "type 2 diabetes mellitus" OR "diabetes mellitus" OR "T2D" OR "T2DM") AND ("HMGB1 protein" OR "HMGB1" OR "HMG1 Protein" OR "HMG1" OR "amphoterin" OR "heparin-binding protein p30" OR "high-mobility group box 1" OR "high mobility group box protein 1"))	548
Web of Science	("Type 2 diabetes" OR "type 2 diabetes mellitus" OR "diabetes mellitus" OR "T2D" OR "T2DM") AND ("HMGB1 protein" OR "HMGB1" OR "HMG1 Protein" OR "HMG1" OR "amphoterin" OR "heparin-binding protein p30" OR "high-mobility group box 1" OR "high mobility group box protein 1")	397

Supplementary Table 2: Nature of HMGB1 estimates reported in the included studies.

Study	Values used	Covariates adjusted
Al-Hakeim HK et al. [26]	Adjusted	Age, sex, BMI, smoking
Biscetti F et al. [27]	Crude	Not applicable
Chen Y et al. [28]	Crude	Not applicable
Dasu MR et al. [10]	Crude	Not applicable
Giovannini S. et al. [29]	Crude	Not applicable
Huang D et al. [30]	Crude	Not applicable
Hui Y et al. [31]	Crude	Not applicable
Liu S et al. [32]	Crude	Not applicable
Liu T et al. [33]	Crude	Not applicable
Majid H et al. (1) [34]	Crude	Not applicable
Majid H et al. (2) [35]	Crude	Not applicable
Shen Y et al. [36]	Crude	Not applicable
Škrha J et al. [37]	Crude	Not applicable
Stempkowska A. et al. [38]	Crude	Not applicable
Wang H et al. [39]	Crude	Not applicable
Wang LJ et al. [40]	Crude	Not applicable
Wei H et al. [41]	Crude	Not applicable
Yan XX et al. [42]	Crude	Not applicable
Yang X et al. [43]	Crude	Not applicable

Covariates adjusted are reported only for studies in which the HMGB1 values used in the meta-analysis were presented as adjusted estimates.

Supplementary Table 3: Studies requiring graphical data extraction using WebPlotDigitizer (version 5.2) and the corresponding values used in the meta-analysis.

Study	Values extracted using WebPlotDigitizer	Values used in meta-analysis (Mean±SD)
Dasu MR et al. [10]	The median, minimum, and maximum HMGB1 concentrations for both the T2DM and control groups were extracted from the published figure. These values were subsequently converted to mean ± SD using established statistical methods.	Controls = 2.29±1.53 T2DM = 5.74±3.3
Hui Y et al. [31]	The mean and SD of HMGB1 concentrations were extracted for patients with proliferative diabetic retinopathy (PDR), non-proliferative diabetic retinopathy (NPDR), T2DM without retinopathy, and healthy controls. For the complications meta-analysis, the PDR and NPDR groups were combined to generate a single T2DM with complications group (T2DM+C).	Controls = 5.69±0.94 T2DM = 8.46±2.78 T2DM+C = 11.07±3.73
Liu S et al. [32]	The mean and SD of HMGB1 concentrations were extracted for the T2DM non-obese, T2DM obese, NGT non-obese, and NGT obese groups. For the primary meta-analysis, the T2DM non-obese and T2DM obese groups were combined to form a single T2DM group, while the NGT non-obese and NGT obese groups were combined to form the control group.	Controls = 4.1±0.18 T2DM = 5.51±0.21

The extracted values were independently verified before inclusion in the quantitative synthesis.

Supplementary Table 4: Details of ELISA kits used for the measurement of HMGB1.

Authors	ELISA kit used
Al-Hakeim HK et al. [26]	Elabscience, Inc. CA, USA
Biscetti F et al. [27]	Shino-Test Corporation, Tokyo
Chen Y et al. [28]	Not reported
Dasu MR et al. [10]	Shino-Test, Japan
Giovannini S. et al. [29]	Shino-Test Corporation, Tokyo
Huang D et al. [30]	Shanghai Hengyuan Biochemical Reagent Co., Ltd., China
Hui Y et al. [31]	Quantikine, XiTang, Inc., Shanghai, China
Liu S et al. [32]	Cloud-clone Corp
Liu T et al. [33]	Elabscience
Majid H et al. (1) [34]	LabReCon, New Delhi, India
Majid H et al. (2) [35]	LabReCon, New Delhi, India
Shen Y et al. [36]	Shanghai XY biotechnology Company, Ltd., China
Škrha J et al. [37]	IBL, Hamburg, Germany
Stempkowska A. et al. [38]	Human HMGB1/HMG-1 Quantikine ELISA Kit, R&D System, Abingdon, UK
Wang H et al. [39]	Uscn Life Science Inc, Wuhan, China
Wang LJ et al. [40]	Shino-Test Corporation, Tokyo, Japan
Wei H et al. [41]	Uscn Life Science Inc., Wuhan, China
Yan XX et al. [42]	Shino-Test Corporation, Tokyo
Yang X et al. [43]	R & D Systems, Minneapolis, USA

Supplementary Table 5: Quality assessment of included case-control studies by New Castle Ottawa Scale (NOS).

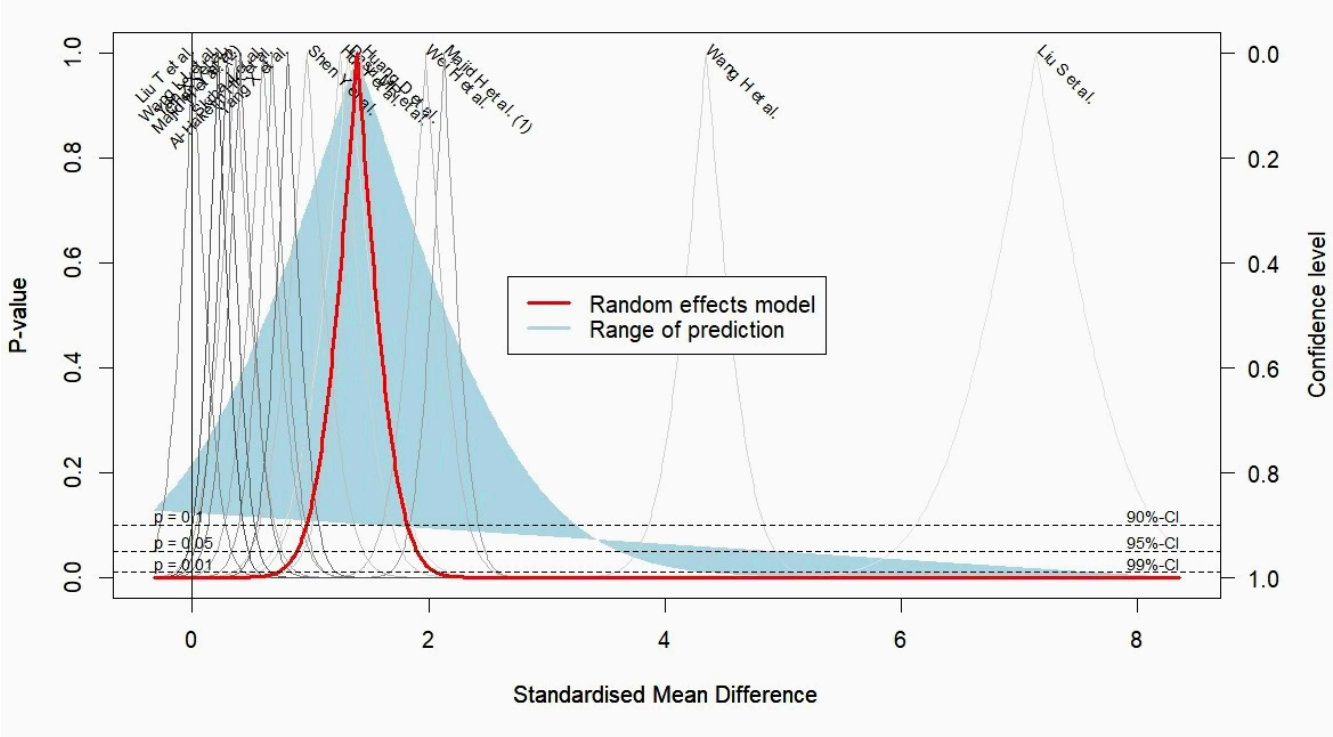
Study	Selection				Comparability		Outcome			Total Score	Risk of bias
	Adequate definition of case	Representativeness of cases	Selection of controls	Definition of controls	Age & sex	Additional factors	Ascertainment of method	Same method for both cases & controls	Non responserate		
Biscetti F et al. [27]	*	*	*	–	*	*	*	*	–	7	Low
Chen Y et al. [28]	*	*	*	*	*	–	–	*	–	6	Moderate
Dasu MR et al. [10]	*	*	–	*	*	–	*	*	–	6	Moderate
Giovannini S et al. [29]	*	*	–	*	*	*	*	*	–	7	Low
Majid H et al. [34]	*	–	–	*	*	–	*	–	*	5	Moderate
Majid H et al. [35]	*	–	–	*	*	*	*	–	–	5	Moderate
Škrha et al. [37]	*	–	–	*	*	–	*	*	–	5	Moderate
Stempkowska A. et al. [38]	*	*	–	–	–	–	*	*	–	4	High

Supplementary Table 6: Quality assessment of included cross-sectional studies by JBI Critical appraisal checklist.

Study	Were the criteria for inclusion in the sample clearly defined?	Were the study subjects and the setting described in detail?	Was the exposure measured in a valid and reliable way?	Were objective, standard criteria used for measurement of the condition?	Were confounding factors identified?	Were strategies to deal with confounding factors stated?	Were outcomes measured in a valid and reliable way?	Was appropriate statistical analysis used?	% Yes	Risk of bias
Al Hakeim HK et al. [26]	Y	Y	Y	Y	N	Y	N	N	63	Moderate
Huang D et al. [30]	N	Y	N	Y	N	N	Y	Y	50	Moderate
Hui Y et al. [31]	Y	Y	Y	Y	N	N	Y	Y	75	Low
Liu S et al. [32]	N	Y	Y	Y	N	N	N	Y	50	Moderate
Liu T et al. [33]	Y	Y	Y	Y	Y	N	Y	Y	88	Low
Shen Y et al. [36]	Y	Y	Y	Y	Y	N	Y	Y	88	Low
Wang H et al. [39]	Y	Y	Y	N	Y	Y	Y	N	75	Low
Wang L J et al. [40]	Y	Y	N	Y	Y	Y	Y	Y	88	Low
Wei H et al. [41]	Y	Y	Y	Y	Y	N	Y	Y	88	Low
Yan XX et al. [42]	Y	Y	Y	Y	N	N	Y	Y	75	Low
Yang X et al. [43]	N	Y	Y	N	N	N	Y	Y	50	Moderate

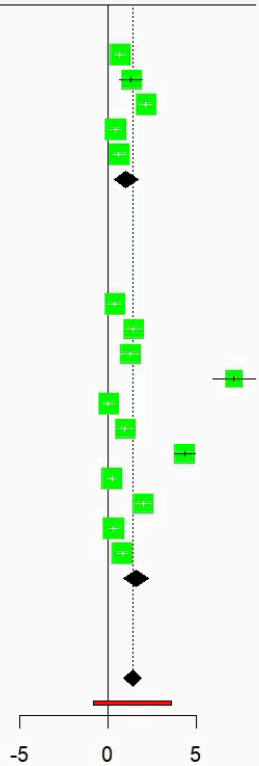
Y= Yes; N= No; Studies with ≤49% "yes" responses were classified as having a high risk of bias (low quality), those with 50–69% as moderate risk of bias (moderate quality), and those with ≥70% as low risk of bias (high quality).

Supplementary Figure 1: Drapery plot depicting the meta-analysis results based on study-specific p-value functions in T2DM.

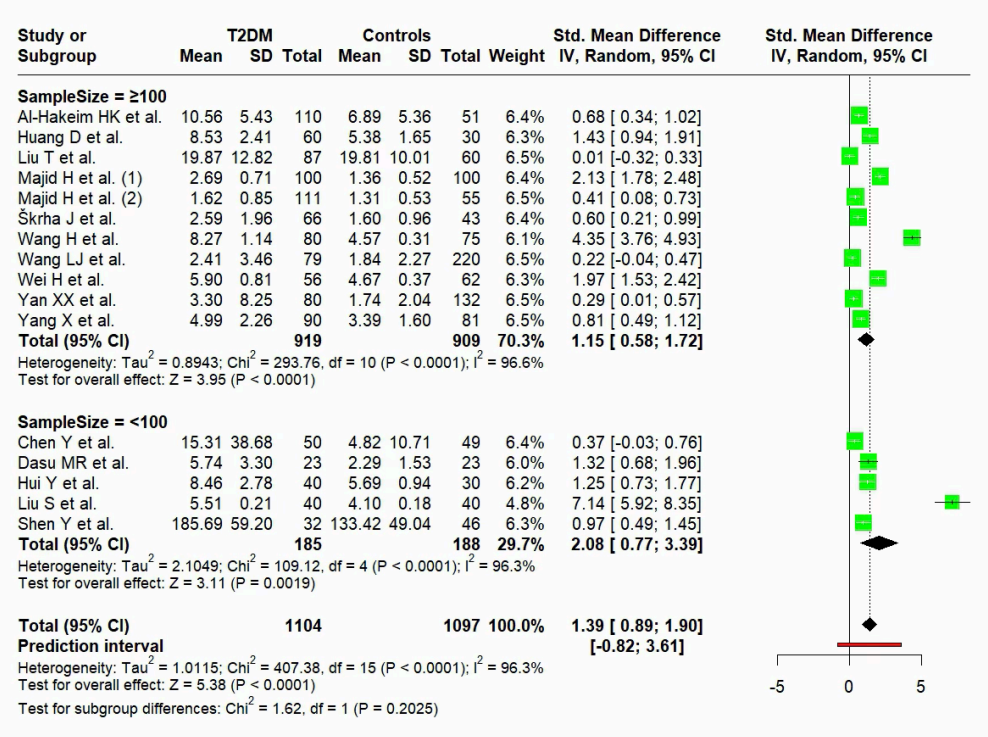


Supplementary Figure 2: Subgroup analysis by geographical region (Country).

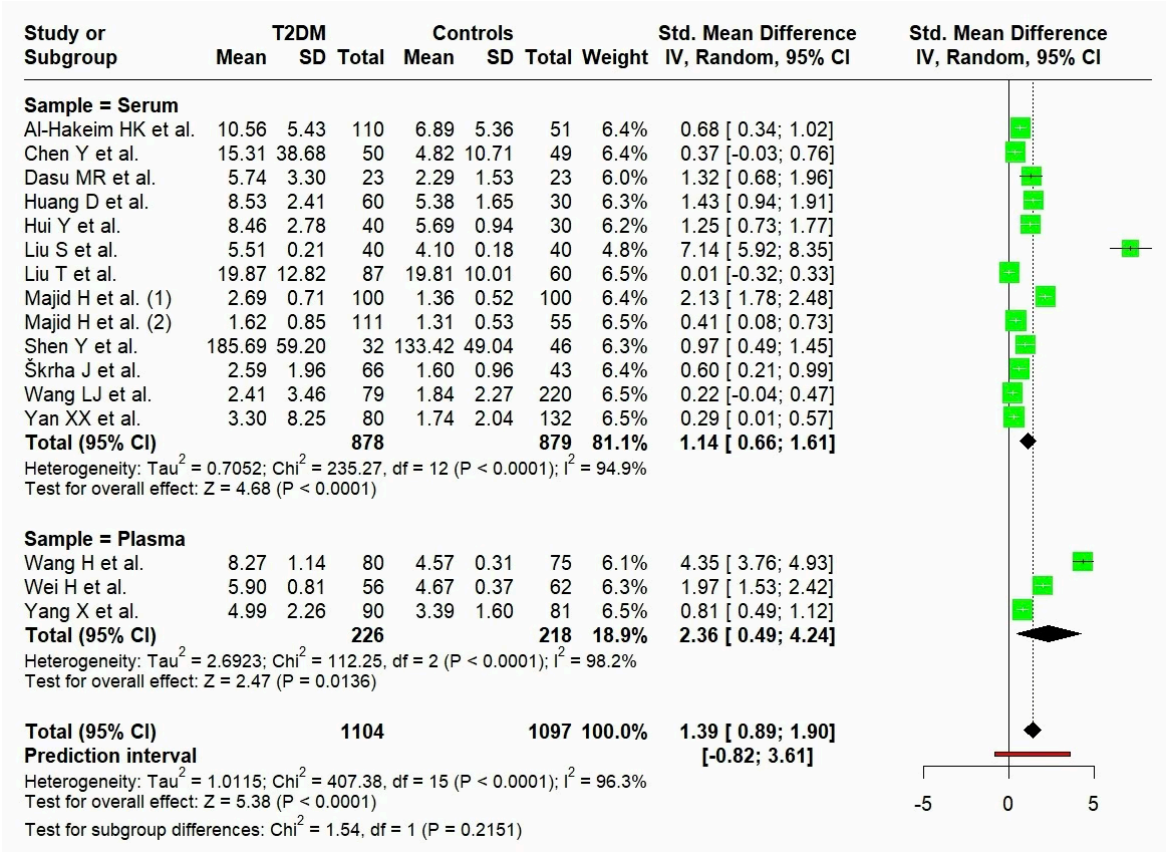
Study or Subgroup	T2DM Mean	T2DM SD	T2DM Total	Controls Mean	Controls SD	Controls Total	Weight	Std. Mean Difference IV, Random, 95% CI
Countries = Other countries								
Al-Hakeim HK et al.	10.56	5.43	110	6.89	5.36	51	6.4%	0.68 [0.34; 1.02]
Dasu MR et al.	5.74	3.30	23	2.29	1.53	23	6.0%	1.32 [0.68; 1.96]
Majid H et al. (1)	2.69	0.71	100	1.36	0.52	100	6.4%	2.13 [1.78; 2.48]
Majid H et al. (2)	1.62	0.85	111	1.31	0.53	55	6.5%	0.41 [0.08; 0.73]
Škrha J et al.	2.59	1.96	66	1.60	0.96	43	6.4%	0.60 [0.21; 0.99]
Total (95% CI)			**410**			**272**	**31.7%**	**1.02 [0.34; 1.69]**
Heterogeneity: Tau^2 = 0.5489; Chi^2 = 61.5, df = 4 (P < 0.0001); I^2 = 93.5%								
Test for overall effect: Z = 2.95 (P = 0.0031)								
Countries = China								
Chen Y et al.	15.31	38.68	50	4.82	10.71	49	6.4%	0.37 [-0.03; 0.76]
Huang D et al.	8.53	2.41	60	5.38	1.65	30	6.3%	1.43 [0.94; 1.91]
Hui Y et al.	8.46	2.78	40	5.69	0.94	30	6.2%	1.25 [0.73; 1.77]
Liu S et al.	5.51	0.21	40	4.10	0.18	40	4.8%	7.14 [5.92; 8.35]
Liu T et al.	19.87	12.82	87	19.81	10.01	60	6.5%	0.01 [-0.32; 0.33]
Shen Y et al.	185.69	59.20	32	133.42	49.04	46	6.3%	0.97 [0.49; 1.45]
Wang H et al.	8.27	1.14	80	4.57	0.31	75	6.1%	4.35 [3.76; 4.93]
Wang LJ et al.	2.41	3.46	79	1.84	2.27	220	6.5%	0.22 [-0.04; 0.47]
Wei H et al.	5.90	0.81	56	4.67	0.37	62	6.3%	1.97 [1.53; 2.42]
Yan XX et al.	3.30	8.25	80	1.74	2.04	132	6.5%	0.29 [0.01; 0.57]
Yang X et al.	4.99	2.26	90	3.39	1.60	81	6.5%	0.81 [0.49; 1.12]
Total (95% CI)			**694**			**825**	**68.3%**	**1.59 [0.89; 2.29]**
Heterogeneity: Tau^2 = 1.3288; Chi^2 = 343.27, df = 10 (P < 0.0001); I^2 = 97.1%								
Test for overall effect: Z = 4.47 (P < 0.0001)								
Total (95% CI)			**1104**			**1097**	**100.0%**	**1.39 [0.89; 1.90]**
Prediction interval								**[-0.82; 3.61]**
Heterogeneity: Tau^2 = 1.0115; Chi^2 = 407.38, df = 15 (P < 0.0001); I^2 = 96.3%								
Test for overall effect: Z = 5.38 (P < 0.0001)								
Test for subgroup differences: Chi^2 = 1.34, df = 1 (P = 0.2464)								



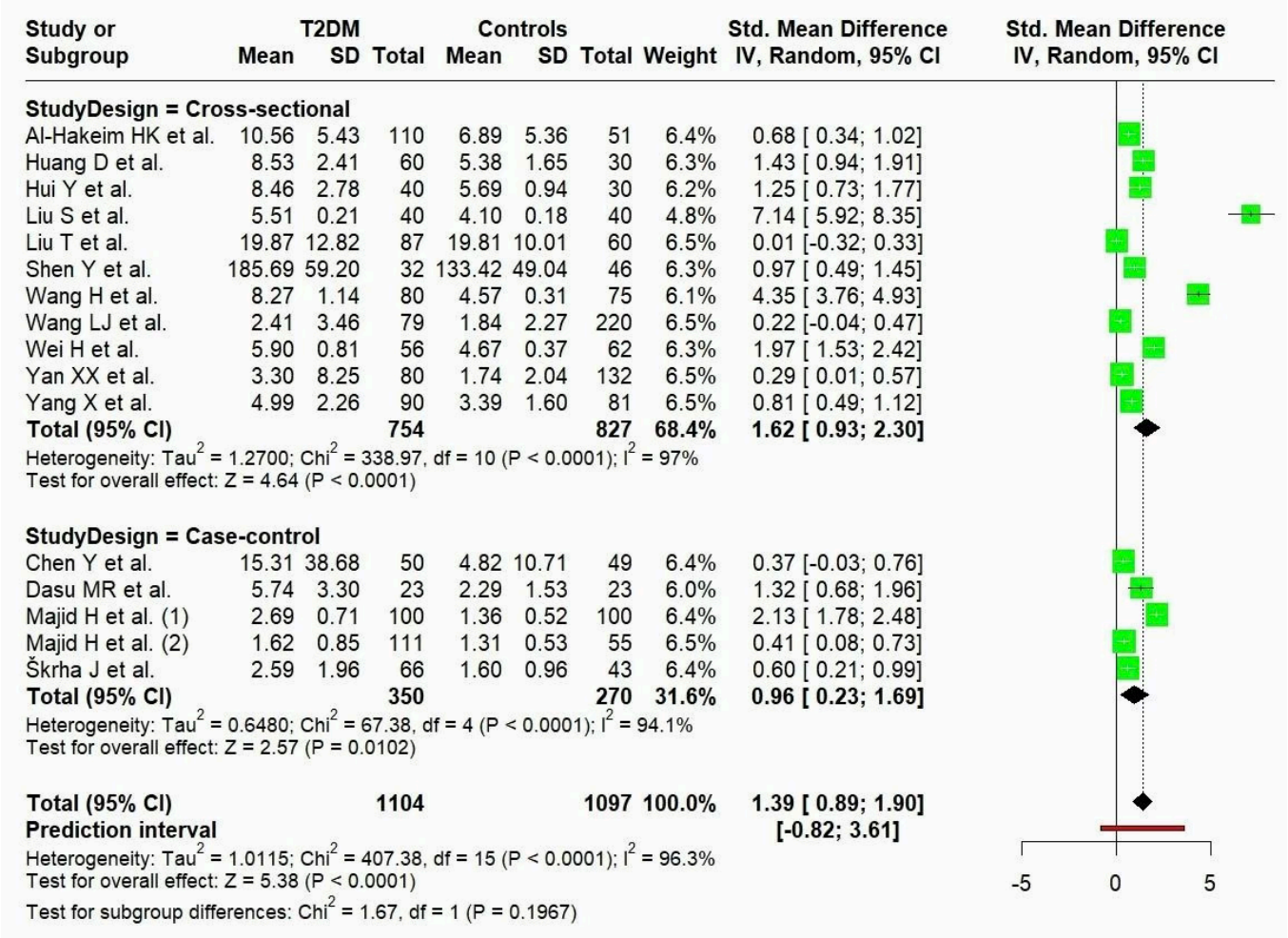
Supplementary Figure 3: Subgroup analysis by sample size.



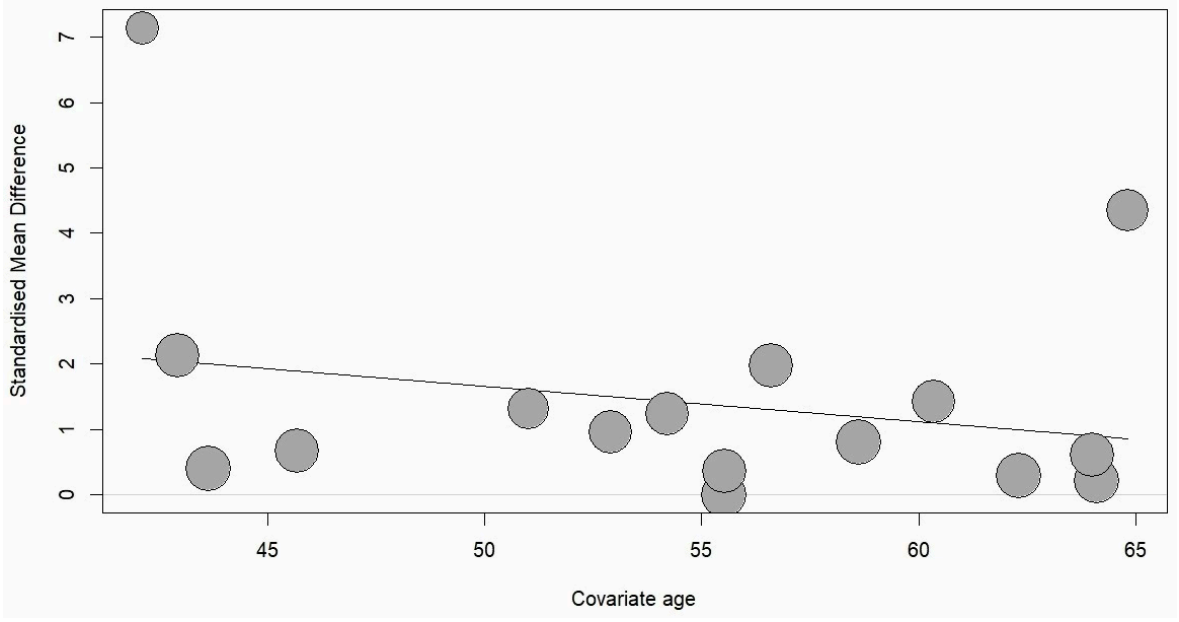
Supplementary Figure 4: Subgroup analysis by biological sample type.



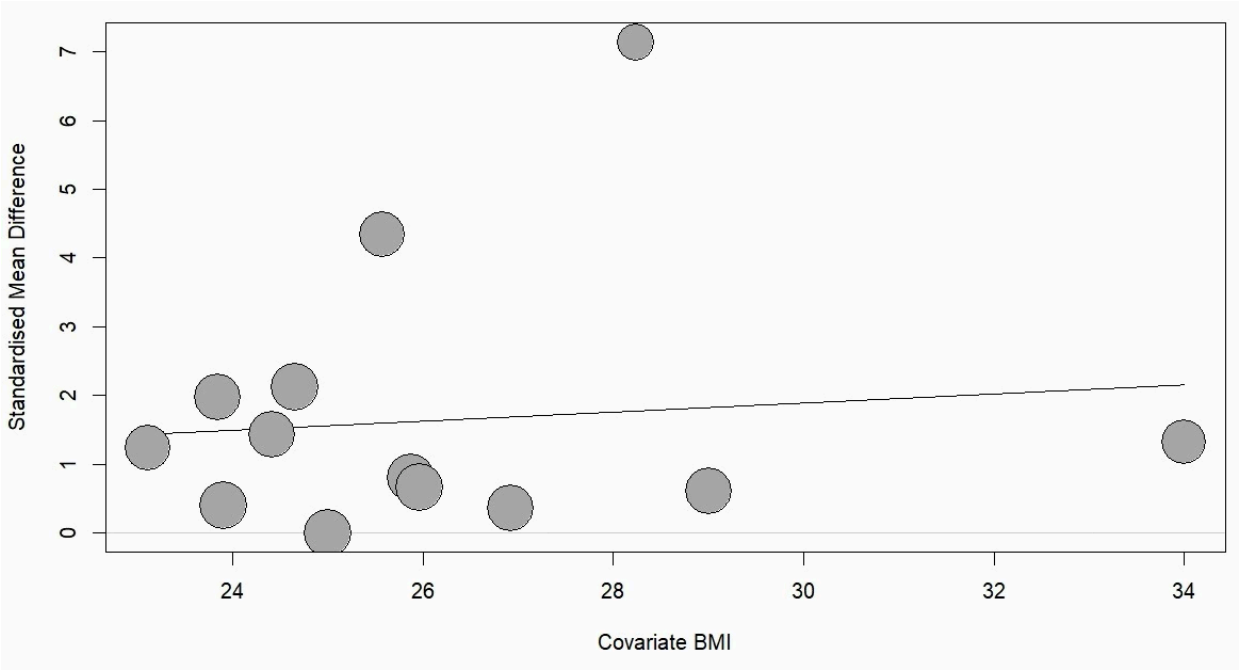
Supplementary Figure 5: Subgroup analysis by study design.



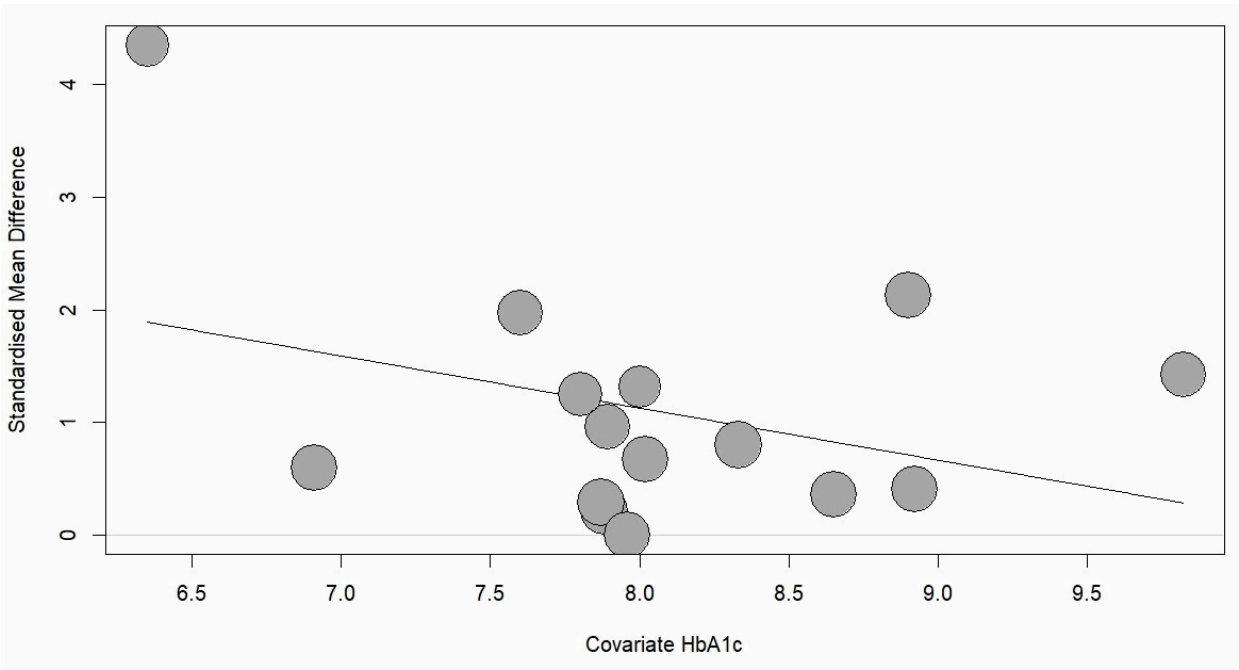
Supplementary Figure 6: Bubble plot of the meta-regression analysis evaluating the association between mean age and the standardized mean difference (SMD) of circulating HMGB1 levels in patients with T2DM.



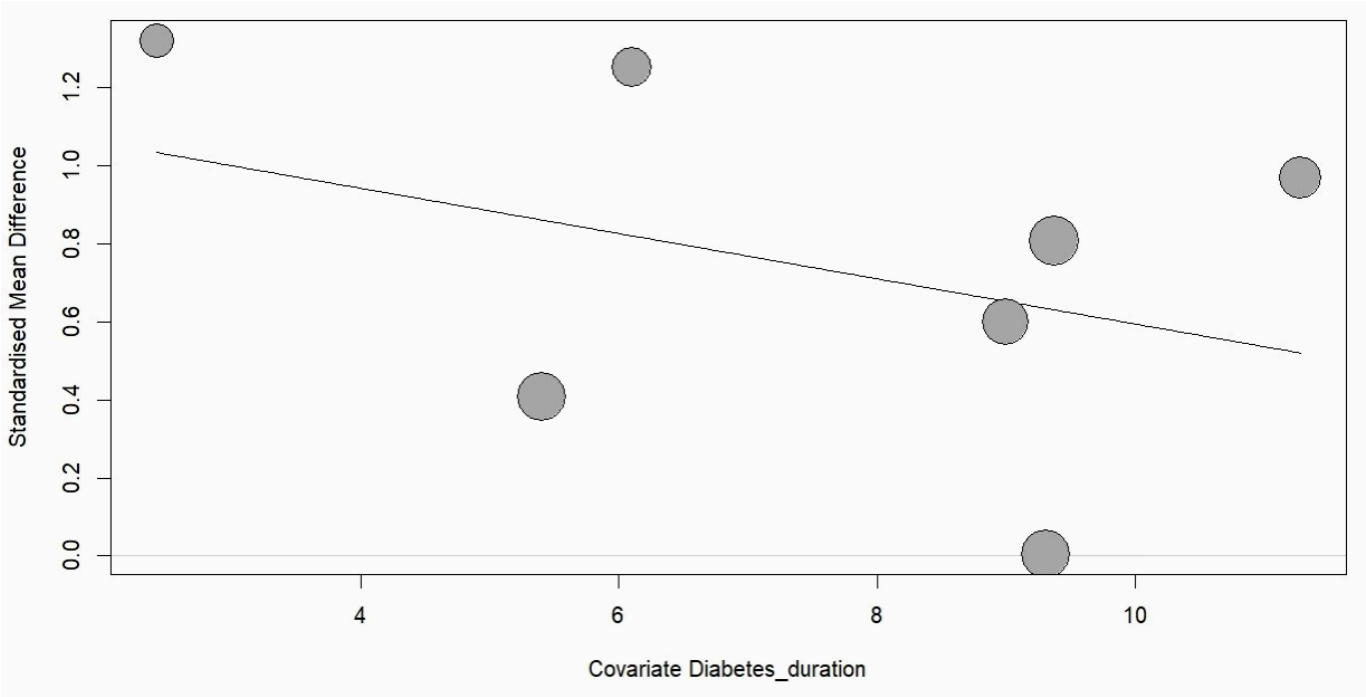
Supplementary Figure 7: Bubble plot of the meta-regression analysis evaluating the association between BMI and the standardized mean difference (SMD) of circulating HMGB1 levels in patients with T2DM.



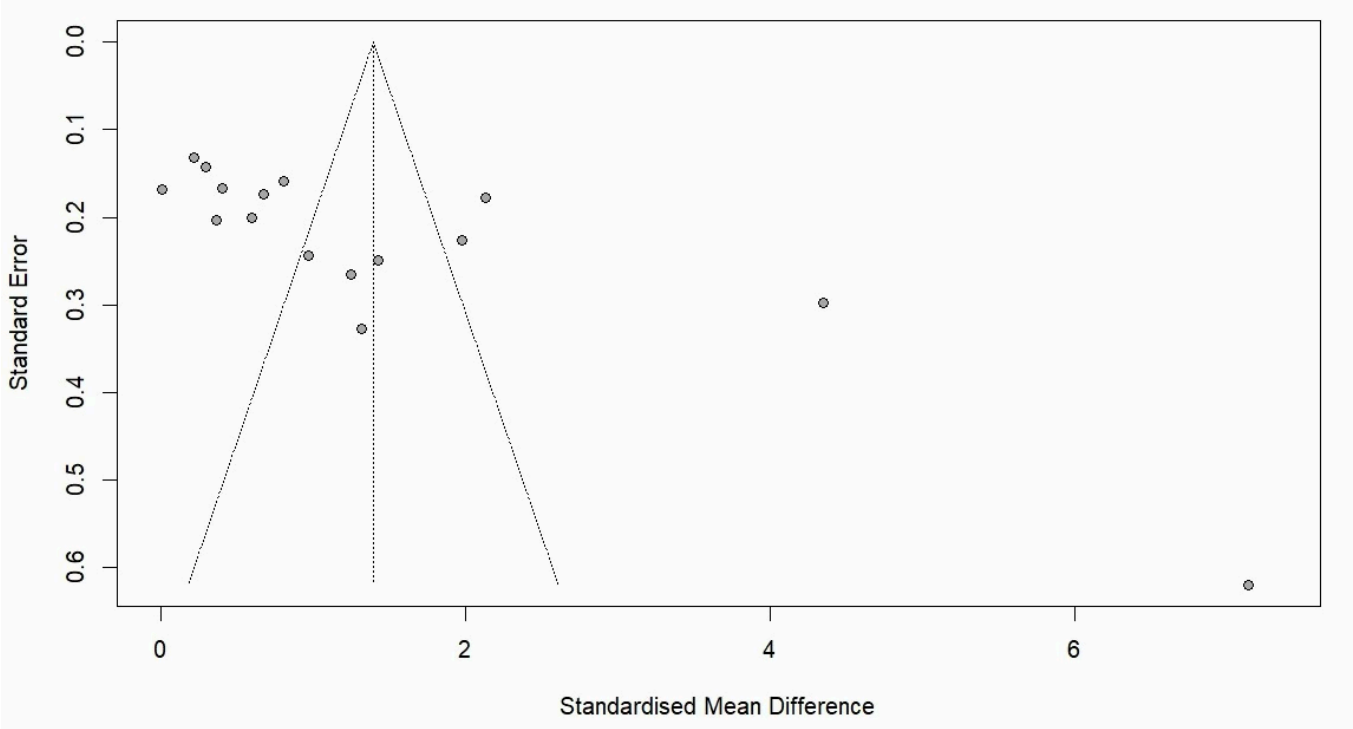
Supplementary Figure 8: Bubble plot of the meta-regression analysis evaluating the association between HbA1c and the standardized mean difference (SMD) of circulating HMGB1 levels in patients with T2DM.



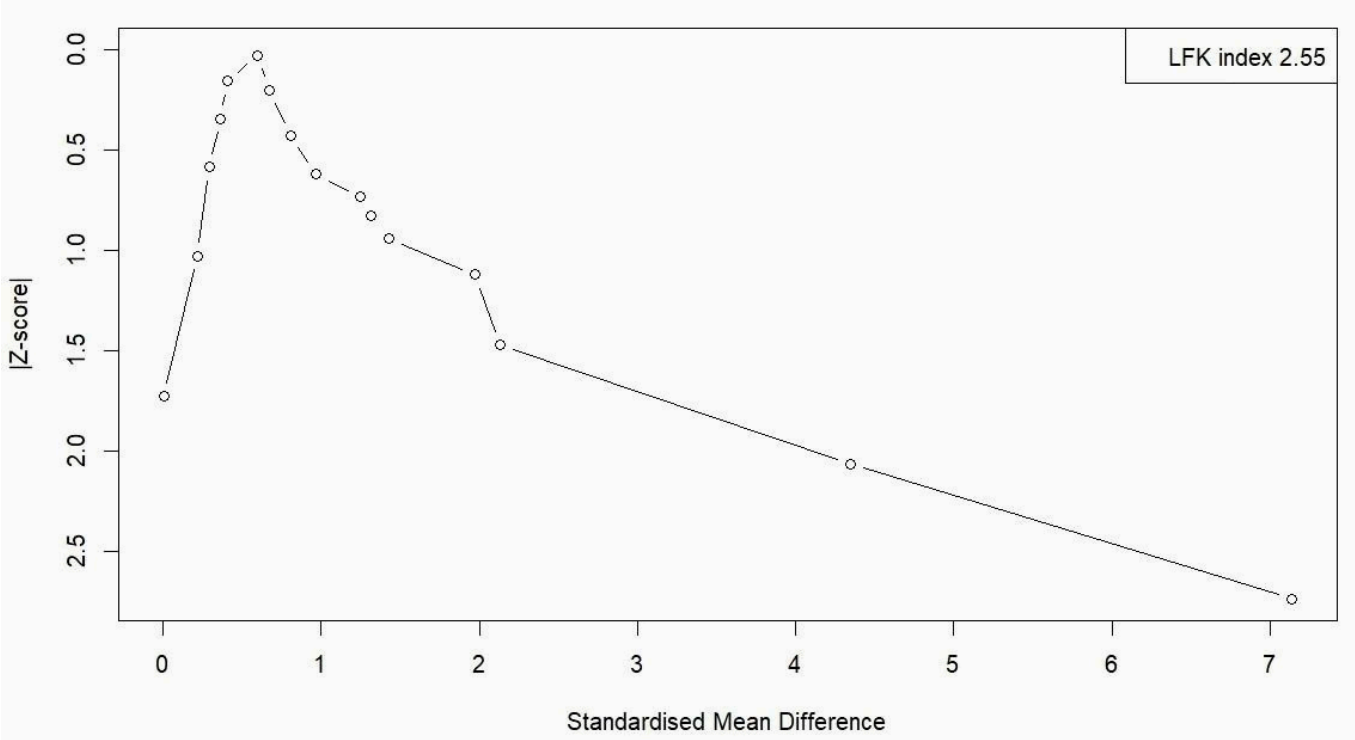
Supplementary Figure 9: Bubble plot of the meta-regression analysis evaluating the association between diabetes duration and the standardized mean difference (SMD) of circulating HMGB1 levels in patients with T2DM.



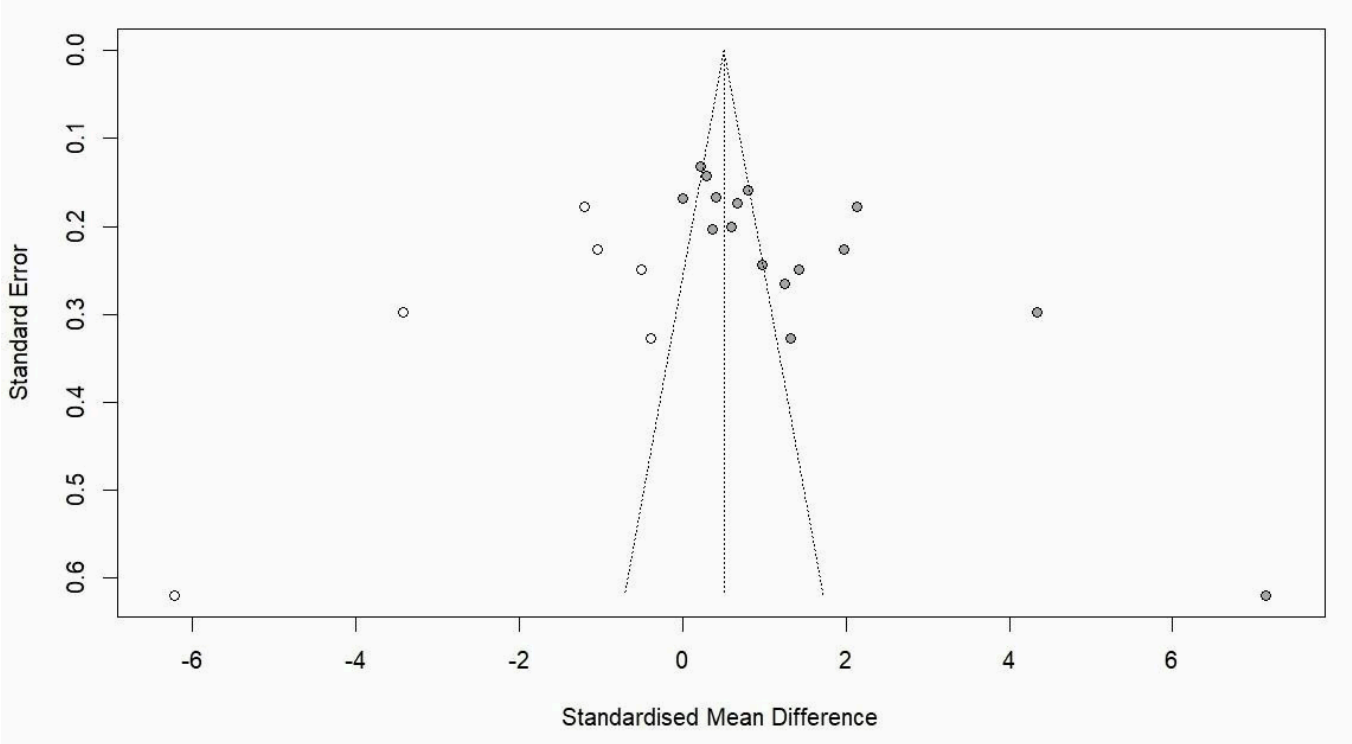
Supplementary Figure 10: Funnel plot showing publication bias in T2DM.



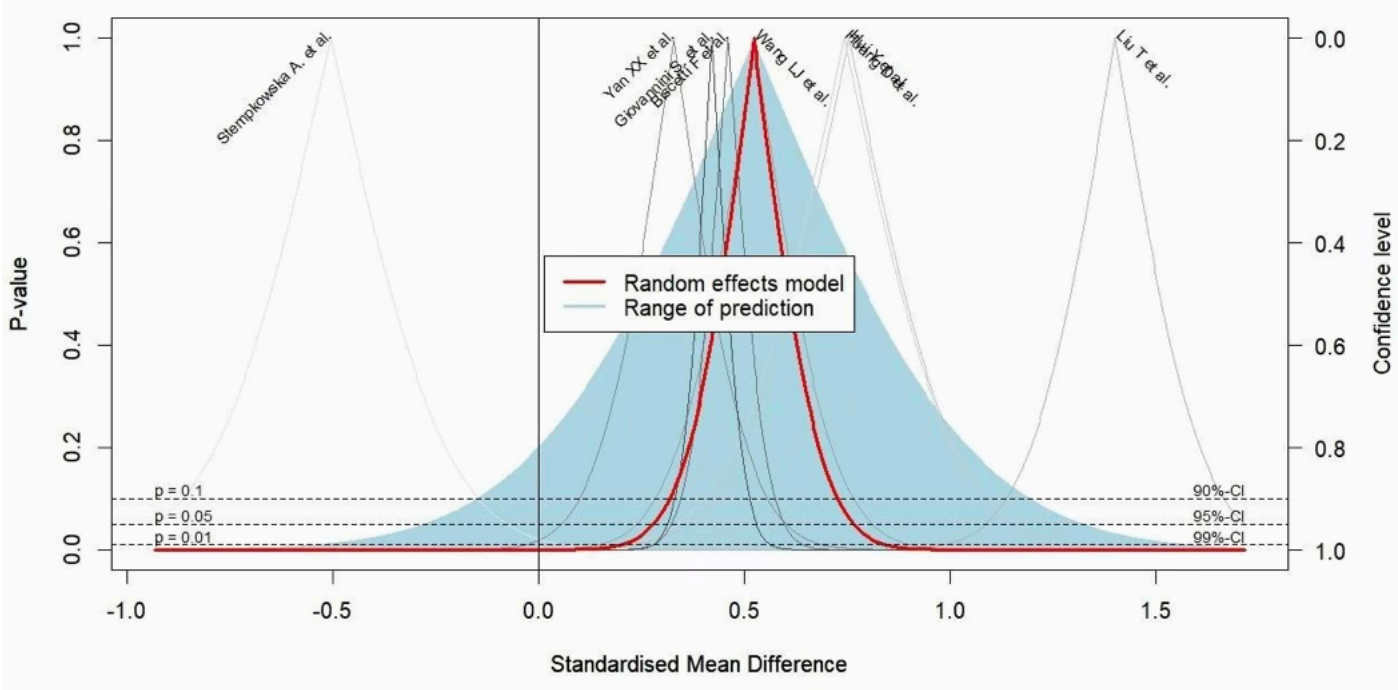
Supplementary Figure 11: Doi plot showing publication bias in T2DM.



Supplementary Figure 12: Trim-and-fill funnel plot. Six missing studies were imputed on the left side. The imputed studies are represented by circles that have no fill colour.



Supplementary Figure 13: Drapery plot depicting the meta-analysis results based on study-specific p-value functions in T2DM with complications (T2DM+C).



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