Review Article

Association of Circulating and Urinary Calprotectin (S100A8/A9, MRP8/14) Levels with Type 2 Diabetes Mellitus and its Complications: A Systematic Review and Meta-analysis

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Keywords

Calprotectin, Type 2 diabetes mellitus, Diabetic complications, Meta-analysis

Abstract

Background: Calprotectin is a myeloid-derived damage-associated molecular pattern implicated in metabolic inflammation and vascular injury. Evidence regarding circulating and urinary calprotectin in type 2 diabetes mellitus (T2DM) and its complications remains inconsistent.

Methods: PubMed, Scopus, and Web of Science were searched up to 25th October 2025. Human observational studies reporting quantitative calprotectin levels were included. Standardized mean differences (SMDs) with 95% confidence intervals (CIs) were calculated using a random-effects model. Heterogeneity was assessed using I^2 and Cochran's Q. Sensitivity analyses, prediction intervals, and publication bias evaluation using the Doi plot and LFK index were performed.

Results: Seventeen studies were included in the meta-analysis. Circulating calprotectin levels were significantly higher in patients with T2DM compared with healthy controls (SMD = 1.08, 95% CI: 0.77–1.39; $p < 0.0001$; $I^2 = 78.2\%$). Sensitivity analyses confirmed the robustness of this association, although small-study effects were suggested. Higher circulating calprotectin levels were observed in T2DM patients with peripheral neuropathy (SMD = 1.02; 95% CI: 0.06–1.98) and macrovascular complications (SMD = 0.43; 95% CI: 0.08–0.77) compared with those without complications, but these associations showed considerable heterogeneity and limited stability. No significant difference in circulating calprotectin levels was observed between T2DM patients with microalbuminuria and normoalbuminuria. Urinary calprotectin levels did not differ significantly between T2DM patients and controls.

Conclusions: Circulating calprotectin is elevated in T2DM, supporting its role as a biomarker of metabolic inflammation. Associations with neuropathy and macrovascular disease are suggestive but heterogeneous, while evidence for urinary calprotectin remains inconclusive.

Introduction

Diabetes mellitus (DM) represents a significant global public health challenge and ranks among the most prevalent non-communicable diseases worldwide. Current estimates indicate that approximately 537 million adults, accounting for 10.5% of the global population, are affected by diabetes, with projections suggesting an increase to 784 million by the year 2045 [1]. Type 2 diabetes mellitus (T2DM), the predominant form of the disease, is characterized by substantial heterogeneity in clinical presentation and disease progression. This variability often results in delayed diagnosis, multiple pathophysiological abnormalities, and differing susceptibilities to complications. These complications are broadly classified into microvascular complications, which include retinopathy, neuropathy, and nephropathy, and macrovascular complications, such as cardiovascular, cerebrovascular, and peripheral vascular diseases [2].

Calprotectin, also known as myeloid-related protein 8/14 (MRP8/14), is a stable heterodimer constituted of calcium-binding proteins MRP8 (Calgranulin A, S100A8) and MRP14 (Calgranulin B, S100A9) [3]. MRP8 and MRP14 belonging to the S100 protein family, are predominantly expressed in cells of myeloid lineage, particularly in monocytes/macrophages and neutrophils [4] with additional expression observed in platelets [5]. MRP8/14 exhibits both intracellular and extracellular functions. Intracellularly, it regulates calcium homeostasis, interacts with cytoskeletal and microtubule components, and contributes to intracellular trafficking within phagocytes [6]. It's essential role in leukocyte transmigration has recently been demonstrated in murine models [7]. When released, calprotectin functions as a damage-associated pattern molecules (DAMP), promoting the inflammatory response [6]. Calprotectin is actively secreted during phagocyte stress responses [8] and has long been acknowledged as a robust biomarker of inflammation [9]. Recent evidence identifies calprotectin as an endogenous activator of Toll-like receptor 4 (TLR4) and a ligand for the receptor for advanced glycation end products (RAGE) [10, 11], both of which play pivotal roles in the pathophysiological pathways that drive microvascular and macrovascular complications in T2DM.

Although numerous individual studies have explored circulating and urinary calprotectin levels in patients with T2DM and its associated complications, the existing evidence remains fragmented, and no comprehensive pooled analysis has been conducted to date. Consequently, this systematic review and meta-analysis aimed to synthesize the available data to elucidate the association between calprotectin and T2DM along with its complications. Specifically, we aimed to compare circulating and urinary calprotectin levels between individuals with T2DM and healthy controls, as well as to examine whether calprotectin concentrations differ between T2DM patients with complications and those without complications.

Materials and Methods

This meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [12] and was prospectively registered with PROSPERO under the registration number CRD420251176512.

Data sources and search strategy

A comprehensive literature search was conducted in MEDLINE/PubMed, Scopus, and Web of Science, encompassing all studies published up to 25 October 2025. The search strategy utilized a combination of keywords and medical subject headings, including type 2 diabetes mellitus, diabetes mellitus, type 2 diabetes, diabetes, diabetics, type 2 DM, T2DM, T2D, calprotectin, myeloid-related protein 8/14, MRP 8/14, S100A8/A9 and leukocyte protein L1. The complete search strategy is detailed in Supplementary Table 1. Furthermore, the reference lists of eligible articles and pertinent review papers were manually screened to identify additional studies. Subsequent to the database searches, two independent reviewers (RKM and MKM) initially screened the titles and abstracts to exclude studies that did not meet the inclusion criteria. The full texts of potentially eligible articles were then assessed in detail to confirm their suitability for inclusion in the systematic review. Any disagreements between the reviewers were resolved through discussion among the authors until a consensus was achieved.

Inclusion and Exclusion criteria

Study selection was guided by the Population, Exposure, Comparator, Outcomes, and Study Design (PECOS) framework. Population (P): Adults diagnosed with type 2 diabetes mellitus (T2DM), with or without diabetes-related complications. Exposure (E): Quantitative measurements of circulating (serum or plasma) or urinary calprotectin levels (S100A8/A9 or MRP8/14). Comparator (C): Healthy, non-diabetic individuals or subjects with normal glucose tolerance (NGT); and/or T2DM patients without complications for analyses involving complication subgroups. Outcomes (O): Differences in circulating calprotectin levels between T2DM patients and healthy controls, and between T2DM patients with and without complications; differences in urinary calprotectin levels among these groups; and comparisons across specific diabetic complications when reported. Study Design (S): Observational human studies, including cross-sectional, case–control, and cohort studies, as well as interventional studies that provided baseline quantitative calprotectin data.

Exclusion criteria encompassed studies involving type 1 diabetes, gestational diabetes, animal or in vitro models, studies lacking an appropriate control or comparator group, and those that did not include quantitative calprotectin measurements (e.g., qualitative assessments, reviews, case reports, editorials). Furthermore, duplicate publications or overlapping datasets, conference abstracts lacking accessible full texts, and studies

with inadequate extractable data were also omitted from this review.

Data extraction

Data from each included study were extracted according to predefined criteria, which included the following elements: the first author's surname, year of publication, country of study, study design, study population, diagnostic criteria for diabetes, sample characteristics, study quality, and key findings. In instances where critical information was missing or ambiguous, the corresponding authors were contacted for clarification. Numerical data presented solely in graphical format were extracted utilizing WebPlotDigitizer (Version 5). The data extraction process was carried out independently by two reviewers, with all extracted information subsequently verified by a third reviewer to ensure accuracy. Any discrepancies identified during the extraction process were resolved through discussion and consensus among the reviewers.

Quality assessment

The methodological quality of case–control and prospective cohort studies was assessed using the Newcastle–Ottawa Scale (NOS) [13], while cross-sectional studies were evaluated using the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Analytical Cross-Sectional Studies [14]. For studies assessed using the NOS, a maximum of 9 points could be awarded, and study quality was categorized as low quality (≤4 points), moderate quality (5–6 points), or high quality (≥7 points). For studies assessed using the JBI checklist, quality was determined based on the proportion of affirmative ("yes") responses. Studies achieving ≤49% "yes" responses were classified as having a high risk of bias (low quality), those with 50–69% were considered to have a moderate risk of bias (moderate quality), and those with ≥70% were deemed to have a low risk of bias (high quality). Two reviewers (RKM and MKM) independently assessed the methodological quality of the included studies. A third reviewer (NKPS) subsequently verified the assessments, and any discrepancies were resolved through consensus following discussion among all reviewers.

Data synthesis and analysis

Data analyses were performed using R software version 4.4.1 (R Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria). Statistical procedures were conducted with the "meta" package (version 8.0-2) and "metasens" package (version 1.5-2). For studies presenting calprotectin data as medians with interquartile ranges (IQRs), corresponding means and standard deviations (SDs) were estimated using the Meta-analysis Accelerator [15]. In the study by Pedersen et al. [16], calprotectin values in healthy controls were reported as medians with 2.5th–97.5th percentiles due to non-normal distribution; because no additional dispersion metrics were available, the arithmetic mean was approximated by using the median as the mean, and an SD was estimated under the assumption of approximate normality. These converted values were used solely for quantitative pooling. Pooled estimates were expressed as standardized mean differences (SMDs) with 95% confidence intervals (CIs). A random-effects model was employed to calculate overall effect sizes. Forest plots and drapery plots were generated to visually summarize pooled analyses. Additionally, prediction intervals (PIs) were computed to quantify the expected dispersion of true effect sizes across future studies. Heterogeneity was assessed using Cochran's Q test and the inconsistency statistic (I^2), where $I^2 > 50\%$ indicated substantial heterogeneity. A p value < 0.10 for Cochran's Q test was considered evidence of significant heterogeneity. Given the presence of notable heterogeneity, leave-one-out sensitivity analyses were conducted to evaluate the influence of individual studies on the pooled estimate. Potential publication bias was assessed using doi plots, and asymmetry was quantified using the Luis Furuya-Kanamori (LFK) index [17], where values within ±1 indicated no asymmetry, between ±1 and ±2 suggested minor asymmetry, and > ±2 denoted major asymmetry. Subgroup analyses were performed to explore sources of heterogeneity based on diagnostic criteria (ADA, WHO, not reported), country, sample size (≥100 vs <100), and type of biological sample (serum vs plasma). For Pedersen et al. [16], plasma values from T2DM patients were classified under the plasma subgroup. Although controls were measured in serum, the study demonstrated no significant difference between serum and plasma calprotectin concentrations (p = 0.191), therefore it was appropriate to classify the comparison under the plasma subgroup. With the exception of Cochran's Q test, p-values of 0.05 or less were considered statistically significant.

Results

Characteristics of included studies

The literature search across major databases identified 171 records from PubMed/MEDLINE, 219 from Scopus, and 205 from Web of Science, yielding a total of 595 records. After removal of 250 duplicate records, 345 unique records remained for screening. During the title and abstract screening phase, 288 records were excluded, leaving 57 articles for full-text retrieval and assessment. All 57 full-text articles were successfully retrieved and assessed for eligibility. Of these, 43 studies were excluded for the following reasons: publication in a non-English language (n = 4); measurement of calprotectin in biological samples other than blood or urine, or absence of circulating calprotectin data (n = 8); studies conducted exclusively in pregnant women (n = 1); conference abstracts or oral presentations without accessible full text (n = 6); editorials (n = 1); absence of calprotectin data (n = 8); studies not specific to type 2 diabetes mellitus (n = 5); separate measurement of S100A8 and S100A9 rather than the MRP8/14 complex (n = 3); lack of group-wise calprotectin data (n = 5); and data not extractable for quantitative synthesis (n = 2). An additional 3

records were identified through citation searching. A total of 17 studies met the eligibility criteria and were included in the final systematic review and meta-analysis [16, 18-33]. The details of the search process are shown in Figure 1. The study characteristics as well as qualities of the included studies are shown in Table 1.

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

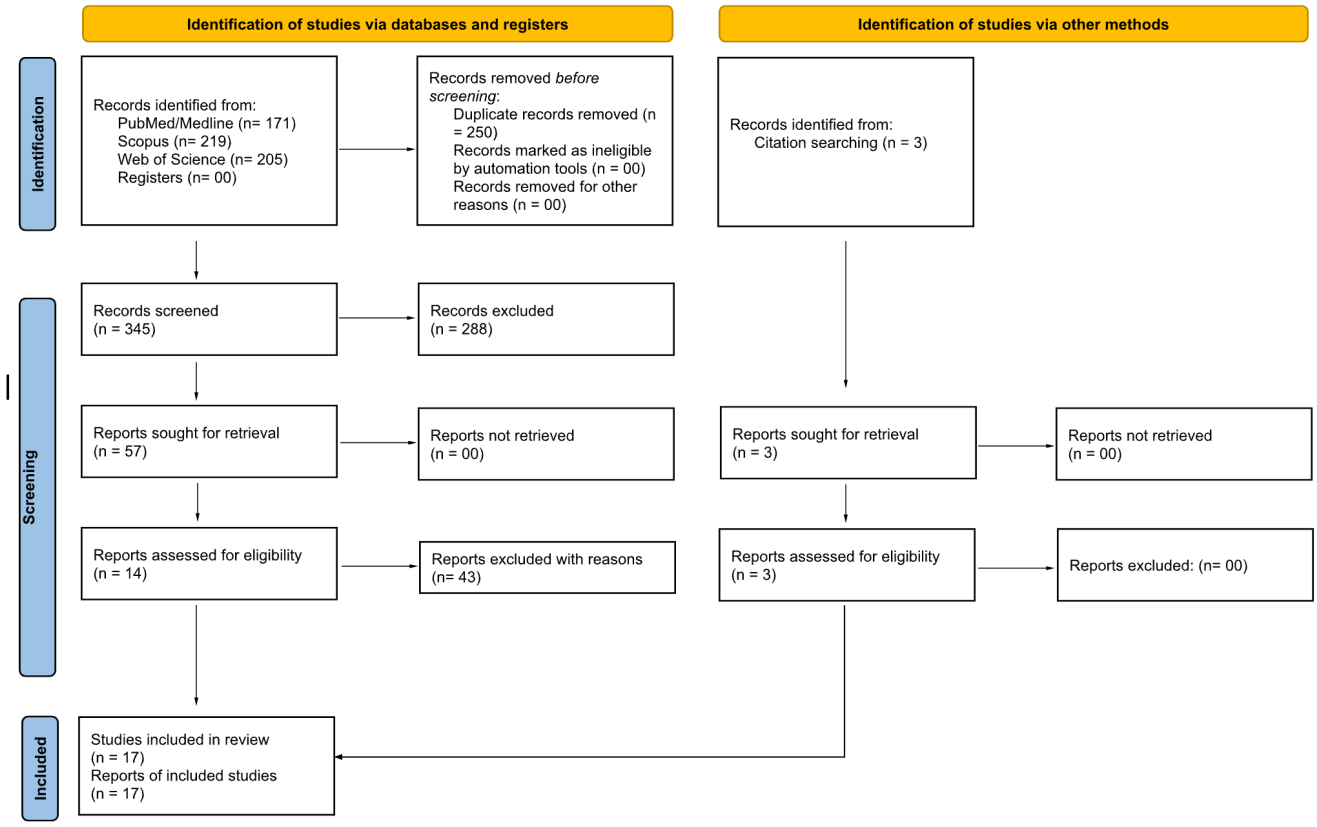


Table 1: Baseline characteristics of studies included in meta-analysis.

Author	Year	Country	Study design	Study subjects	Diagnostic criteria of type 2 DM	Sample	Quality	Main findings
Afify et al. [18]	2022	Egypt	C-C	20 healthy controls, 20 T2DM without diabetic peripheral neuropathy (No-DPN), 20 T2DM with neuropathy (DPN)	NR	Serum	High	Serum calprotectin levels were significantly elevated in patients with T2DM compared to control subjects. Moreover, these levels were found to be highest in T2DM patients with diabetic peripheral neuropathy (DPN) in comparison to those without neuropathy.
El-adawy et al. [19]	2017	Egypt	C-C	20 healthy controls and 20 T2DM	ADA	Serum	High	Calprotectin levels were significantly elevated in individuals with T2DM compared to control subjects.
El-hafez et al. [20]	2021	Egypt	C-C	15 controls, 15 T2DM without neuropathy, 60 T2DM with neuropathy	NR	Serum	Moderate	Calprotectin levels were significantly elevated in individuals with diabetic neuropathy compared to those without neuropathy and control subjects.

Author	Year	Country	Study design	Study subjects	Diagnostic criteria of type 2 DM	Sample	Quality	Main findings
Gao et al. [21]	2020	China	C-S	35 T2DM and 43 controls	WHO	Serum	High	Serum calprotectin levels in T2DM patients were significantly higher than those in healthy controls.
Gobejishvili et al. [22]	2024	USA	C-C	17 T2DM adults and 11 healthy controls	NR	Serum	Moderate	Calprotectin levels are elevated in individuals with T2DM when compared to non-diabetic controls; however, this difference is statistically insignificant.
Gu et al. [23]	2020	China	C-S	80 T2DM (20 normoalbuminuria, 20 microalbuminuria, 20 macroalbuminuria with normal serum creatinine and 20 macroalbuminuria with increased serum creatinine)	NR	Serum	High	As diabetic kidney disease progressed, there was a gradual increase in the serum levels of MRP8/14.
Halawa et al. [24]	2020	Egypt	C-C	60 T2DM (30 with PAD & 30 without PAD) and 30 controls	NR	Plasma and urine	High	Both plasma and urinary calprotectin levels were significantly elevated in individuals with T2DM compared to control subjects. Additionally, plasma calprotectin levels were notably higher in T2DM patients with PAD. However, the urinary calprotectin levels in T2DM patients with PAD were comparable to those in T2DM patients without PAD.
Hamzah et al. [25]	2020	Iraq	C-C	60 T2DM and 60 controls	WHO	Serum	Moderate	Patients with T2DM exhibited significantly elevated levels of calprotectin in comparison to healthy control subjects.
Hassan et al. [26]	2025	Iraq	C-C	40 T2DM and 20 controls	NR	Serum	Moderate	There was a significant increase in calprotectin levels in individuals with T2DM compared to those in the healthy control group.
Lylloff et al. [27]	2017	Denmark	Prospective cohort study	18 subjects with NGT and 29 with T2DM	NR	Plasma	High	Patients with T2DM exhibit elevated plasma levels of calprotectin in comparison to individuals with normal glucose tolerance.
Murad et al. [28]	2019	Saudi Arabia	C-S	200 T2DM and 50 controls	ADA	Urine	High	Urinary calprotectin is unable to differentiate between individuals with treated-controlled T2DM and those with treated-uncontrolled T2DM, nor can it distinguish between diabetic patients and non-diabetic control subjects.
Pedersen et al. [16]	2014	Denmark	C-S	305 T2DM (183 with CVD & 122 without CVD) and 120 healthy controls	WHO	Serum (controls), Plasma (T2DM)	High	The median plasma calprotectin concentration was significantly elevated in individuals with T2DM compared to the healthy reference population; however, there was no significant difference in median plasma calprotectin concentrations between patients with and without cardiovascular disease (CVD).
Peng et al. [29]	2011	China	C-S	375 T2DM (205 with CAD and 170 without CAD)	WHO	Serum	High	Diabetic patients with coronary artery disease exhibited significantly elevated plasma levels of MRP8/14 in comparison to control subjects.

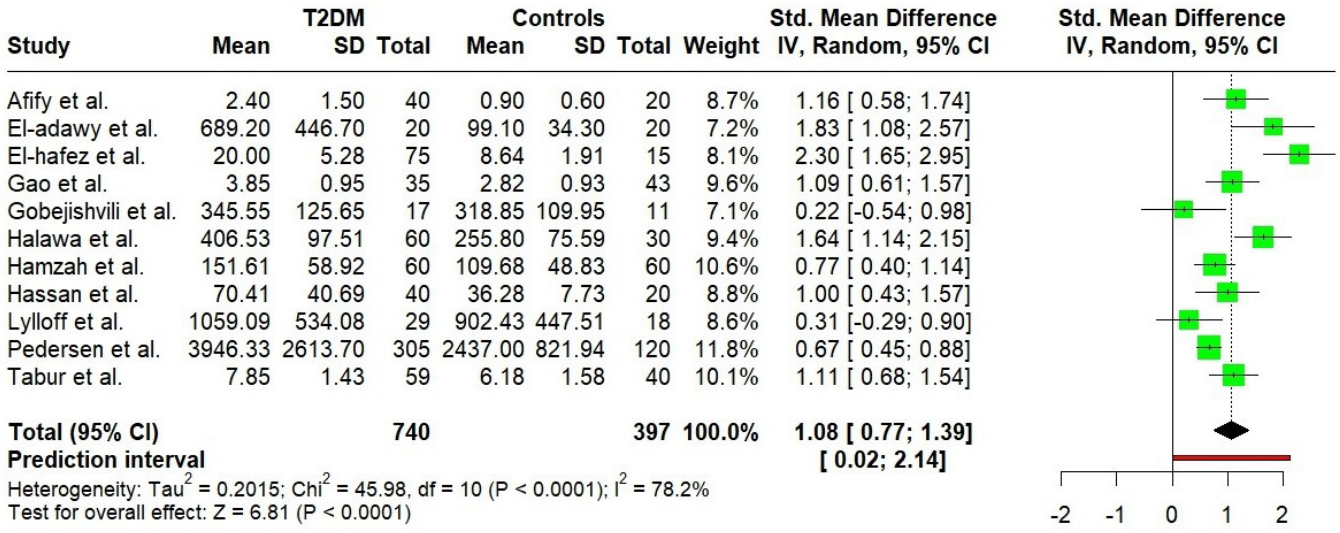
Salem et al. [30]	2024	Iraq	C-S	126 T2DM (63 with DPN and 63 without DPN)	NR	Serum	High	There were no statistically significant differences in the mean serum calprotectin levels between diabetic patients with and without DPN.
Schmaderer et al. [31]	2014	Germany	C-S	86 T2DM (46 with normoalbuminuria & 40 with microalbuminuria)	ADA	Serum	High	MRP8/14 levels were significantly elevated in the microalbuminuric cohort of patients with diabetes when compared to the normoalbuminuric cohort.
Tabur et al. [32]	2015	Turkey	C-C	59 T2DM (29 with DPN and 30 without DPN) and 40 healthy controls	NR	Serum	High	Serum calprotectin levels were significantly elevated in both patients with neuropathy and those without, compared to healthy controls. Furthermore, serum calprotectin levels were higher in diabetic patients with neuropathy than in those without neuropathy.
Velayutham et al. [33]	2021	India	C-S	126 T2DM (63 with DPN and 63 without DPN)	NR	Serum	High	The serum calprotectin levels were significantly elevated in patients with diabetic peripheral neuropathy compared to those without neuropathy.

C-C: Case-Control; C-S: Cross-Sectional; ADA: American Diabetes Association; WHO: World Health Organization; NR: Not Reported; T2DM: Type 2 Diabetes Mellitus; DPN: Diabetic Peripheral Neuropathy; PAD: Peripheral Artery Disease; CVD: Cardiovascular Disease; NGT: Normal Glucose Tolerance; CAD: Coronary Artery Disease; MRP8/14: Myeloid-related Protein 8/14.

Calprotectin in T2DM and controls

The meta-analysis of 11 studies [16, 18-22, 24-27, 32] comparing circulating calprotectin levels between individuals with T2DM (n = 740) and healthy controls (n = 397) demonstrated a significantly higher pooled standardized mean difference (SMD) among patients with T2DM (SMD = 1.08; 95% CI: 0.77–1.39; $p < 0.0001$), as shown in the overall forest plot (Figure 2).

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



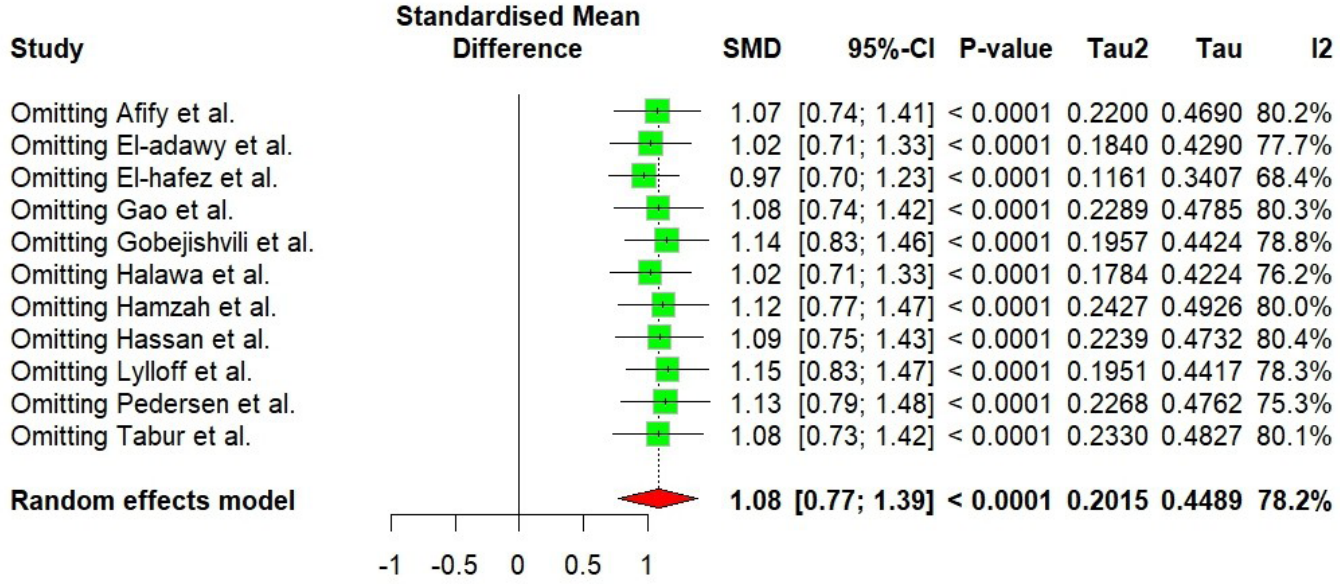
Considerable heterogeneity was present ($I^2 = 78.2\%$), and the 95% prediction interval (0.02–2.14) indicated that future studies are expected to show effects consistently favoring elevated calprotectin in T2DM, though with varying magnitude. A drapery plot illustrating the meta-analysis results using the p-value functions of individual studies is presented in Supplementary Figure 1.

Subgroup analyses provided insights into sources of heterogeneity. Stratification by country revealed the highest effect size among studies from Egypt (SMD = 1.71; 95% CI: 1.25–2.17), followed by moderate effects in other countries including China, USA, and Turkey (SMD = 0.90; 95%

CI: 0.44–1.36), Iraq (SMD = 0.84; 95% CI: 0.53–1.15), and Denmark (SMD = 0.60; 95% CI: 0.31–0.88), with a statistically significant subgroup difference (p = 0.0009) (Supplementary Figure 2). Studies with smaller sample sizes (<100) reported larger effect sizes (SMD = 1.18; 95% CI: 0.80–1.57) compared with those enrolling ≥100 participants (SMD = 0.69; 95% CI: 0.51–0.88; p = 0.0246), suggesting a small-study effect (Supplementary Figure 3). Subgrouping by biospecimen type showed that serum-based studies yielded higher pooled estimates (SMD = 1.17; 95% CI: 0.80–1.53) than plasma-based studies (SMD = 0.87; 95% CI: 0.20–1.55), although the difference was not statistically significant (p = 0.4526) (Supplementary Figure 4). Finally, subgrouping by diagnostic criteria indicated meaningful variation: studies not reporting diagnostic criteria produced higher pooled effects (SMD = 1.12; 95% CI: 0.64–1.60), whereas those adopting WHO criteria showed lower effect sizes (SMD = 0.76; 95% CI: 0.56–0.97), with a significant subgroup difference (p = 0.0156) (Supplementary Figure 5). Collectively, these figures demonstrate a consistent and significant elevation of circulating calprotectin levels in T2DM across multiple analytical layers, while highlighting potential contributors to between-study heterogeneity.

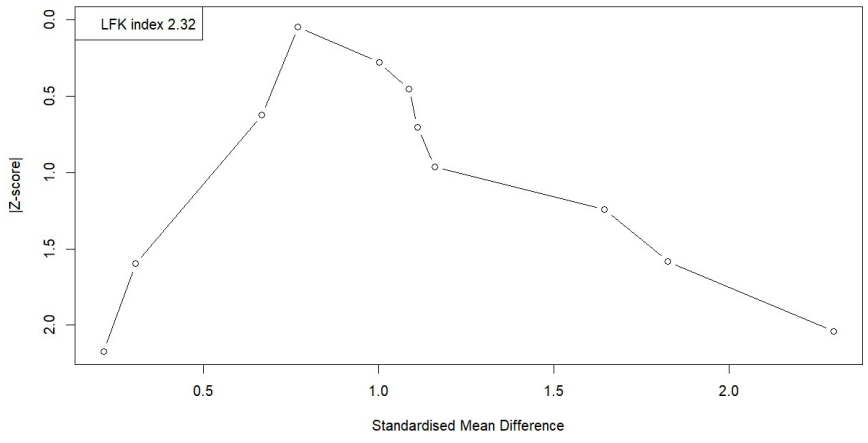
Sensitivity analysis using a leave-one-out approach demonstrated that the overall association between circulating calprotectin levels and T2DM was highly stable. Sequential omission of individual studies yielded pooled SMDs ranging from 0.97 to 1.15, with all estimates remaining statistically significant and heterogeneity values changing only minimally, indicating that no single study disproportionately influenced the overall effect (Figure 3).

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



Assessment of potential publication bias using the DOI plot and LFK index revealed marked asymmetry (LFK = 2.32), suggesting the presence of small-study effects (Figure 4).

Figure 4: Doi plot showing publication bias.



Despite this asymmetry, the robustness of the sensitivity analysis supports the reliability of the pooled findings, reinforcing the conclusion that circulating calprotectin levels are consistently elevated in T2DM across studies.

Calprotectin levels in T2DM complications

T2DM with peripheral neuropathy

Across five studies [18, 20, 30, 32, 33] comparing individuals with and without diabetic peripheral neuropathy (DPN), circulating calprotectin levels were higher in those with DPN, with a pooled SMD of 1.02 (95% CI: 0.06–1.98; p = 0.0382). However, substantial heterogeneity was present (I^2 = 94.7%), indicating marked variability across studies (Supplementary Figure 6). The leave-one-out sensitivity analysis showed that the pooled effect was not stable, with effect sizes ranging widely from 0.69 to 1.31 and the loss of statistical significance when several studies were omitted (Supplementary Figure 7). This suggests that the overall association is sensitive to individual datasets and should be interpreted cautiously. The DOI plot demonstrated minor asymmetry (LFK = 1.64), consistent with small-study effects but not indicative of major publication bias (Supplementary Figure 8). Together, these findings support a possible association between elevated calprotectin and DPN, but highlight that the evidence is heterogeneous and not robust across analytical scenarios.

T2DM with microalbuminuria

Across two studies [23, 31] involving 60 individuals with microalbuminuria (MAU) and 66 with normoalbuminuria (NAU), calprotectin levels did not differ significantly between groups (SMD = 0.43; 95% CI: −0.06 to 0.91; p = 0.0850). Heterogeneity was low to moderate (I^2 = 41.7%) (Supplementary Figure 9). These findings indicate that circulating calprotectin does not appear to distinguish microalbuminuria from normoalbuminuria in T2DM.

T2DM with macrovascular complications

Across three studies [16, 24, 29] involving 418 individuals with macrovascular complications and 322 without, calprotectin levels were significantly higher in the macrovascular group (SMD = 0.43; 95% CI: 0.08–0.77; p = 0.0148). Moderate heterogeneity was present (I^2 = 76.6%) (Supplementary Figure 10). The leave-one-out sensitivity analysis revealed that the observed association between calprotectin and macrovascular complications in T2DM is only partially robust. Although the direction of effect remained positive in all iterations, statistical significance was lost in two of the three leave-one-out scenarios, indicating that the pooled estimate is sensitive to the inclusion of specific studies. Moderate–high heterogeneity persisted across all iterations (Supplementary Figure 11). These results suggest that while calprotectin is elevated in individuals with macrovascular complications, the strength of this association should be interpreted cautiously.

Urinary calprotectin in T2DM

Across two studies [24, 28] evaluating urinary calprotectin in T2DM (n = 260) versus controls (n = 80), the pooled effect showed no significant difference between groups (SMD = 1.19; 95% CI: −1.28 to 3.65; p = 0.3450). The studies reported highly inconsistent results, reflected in extreme heterogeneity (I^2 = 98.3%) (Supplementary Figure 12). Overall, current evidence does not demonstrate a reliable elevation of urinary calprotectin in T2DM.

Discussion

In this systematic review and meta-analysis, we conducted a comprehensive evaluation of circulating and urinary calprotectin (S100A8/A9, MRP8/14) levels in individuals diagnosed with T2DM and associated complications. The principal finding of this analysis indicates that circulating calprotectin levels are significantly elevated in patients with T2DM in comparison to healthy controls, demonstrating a large pooled effect size and consistent directionality across multiple sensitivity and subgroup analyses. These results provide strong support for the notion that calprotectin serves as an indicator of the heightened inflammatory environment characteristic of T2DM, thereby underscoring its potential utility as a biomarker for metabolic inflammation.

Calprotectin is highly expressed in activated neutrophils and monocytes, and it is released during cellular stress, inflammation, and endothelial activation. As a damage-associated molecular pattern (DAMP) molecule, it plays a significant role in amplifying innate immune responses through its interaction with Toll-like receptor 4 (TLR4) and the receptor for advanced glycation end products (RAGE) [3, 4, 6, 8-11]. Both receptors are critically involved in the pathogenesis of insulin resistance, endothelial dysfunction, and atherogenesis in T2DM [34, 35]. The markedly elevated levels of circulating calprotectin observed in patients with T2DM in our pooled analysis are, therefore, biologically plausible and align with studies that identify chronic low-grade inflammation as a defining characteristic of T2DM [36, 37]. Furthermore, emerging evidence indicates that calprotectin may play an active role in metabolic dysregulation rather than merely serving as a marker of inflammation. Increased expression of S100A8/A9 has been documented in the visceral adipose tissue of individuals with T2DM, showing a correlation with macrophage infiltration and indices of insulin resistance [38]. Consequently, elevated circulating calprotectin may function both as a biomarker and a mediator of metabolic inflammation in T2DM.

Despite the strong overall association, considerable heterogeneity was observed across the studies. Subgroup analyses identified several factors contributing to this variability, including geographic region, sample size, and the diagnostic criteria employed for T2DM. Larger effect sizes were noted in studies conducted in Egypt and within smaller cohorts, indicating potential small-study effects and population-

specific inflammatory profiles. Notably, sensitivity analyses demonstrated that the overall association remained stable and statistically significant regardless of the exclusion of individual studies, thereby reinforcing the robustness of the primary finding.

Our analysis indicates that circulating calprotectin levels are elevated in patients with T2DM who exhibit peripheral neuropathy and macrovascular complications, in comparison to those without such complications; however, these associations were not as robust as the primary comparison. The relationship with diabetic peripheral neuropathy (DPN) was marked by significant heterogeneity and sensitivity to individual studies, suggesting that the current evidence remains preliminary. Nonetheless, this finding is consistent with experimental data that implicate S100A8/A9-mediated neuroinflammation and microvascular dysfunction in the pathogenesis of diabetic neuropathy [18, 20, 32].

In our meta-analysis, we observed that patients with macrovascular complications exhibited elevated levels of calprotectin. Calprotectin is predominantly synthesized by immune cells of myeloid lineage, particularly macrophages and neutrophilic granulocytes, which may facilitate atherosclerotic plaque development through multiple mechanisms. These mechanisms include the activation of endothelial cells that line blood vessels, leading to a disruption of their structural integrity, promotion of apoptosis, and the production of reactive oxygen species. Consequently, this cascade results in oxidative stress and the oxidation of low-density lipoproteins. Furthermore, heightened levels of calprotectin in the bloodstream are believed to augment the activation of the receptor for advanced glycation end products (RAGE), which subsequently initiates various inflammatory and coagulation responses, thereby exacerbating atherosclerosis. The pathogenesis of atherosclerosis is also characterized by inflammation of the endothelial and perivascular tissues, encompassing processes such as vascular fibrosis and calcification [39]. Elevated concentrations of S100A8/A9 have been documented in patients with T2DM who have coronary artery disease, correlating with the severity of coronary artery disease [29]. Thus, increased levels of calprotectin in T2DM patients with macrovascular disease may indicate heightened vascular inflammation and enhanced plaque vulnerability. However, given the limited number of studies and the partial instability observed in sensitivity analyses, these findings warrant cautious interpretation.

In the present meta-analysis, circulating calprotectin levels did not exhibit a statistically significant difference between patients with T2DM presenting with microalbuminuria and those with normoalbuminuria. However, individual studies have reported heterogeneous findings. Schmaderer et al. [31] demonstrated significantly elevated serum MRP8/14 (calprotectin) levels in T2DM patients with microalbuminuria compared to normoalbuminuric individuals, positing that enhanced macrophage transmigration—evidenced by increased circulating MRP8/14—may signify an early event in the pathogenesis of diabetic microvascular injury. The systemic elevation of MRP8/14, a macrophage-derived inflammatory mediator, supports the hypothesis of chronic low-grade inflammation during the initial stages of diabetic nephropathy. Conversely, Gu et al. [23] observed no significant difference in calprotectin concentrations between T2DM patients with normoalbuminuria and those with microalbuminuria, although markedly higher levels were noted in individuals with macroalbuminuria. This stepwise increase across stages of albuminuria suggests that circulating calprotectin may serve as a marker of disease severity rather than an indicator of early renal involvement, indicating a potentially stronger correlation with advanced microcirculatory damage in diabetic kidney disease (DKD). Collectively, these discrepant findings underscore the dynamic role of MRP8/14 in the progression of DKD and may partially elucidate the lack of significant association observed in pooled analyses at the microalbuminuria stage. Evidence regarding urinary calprotectin in T2DM remains limited and inconsistent. The two available studies exhibited considerable heterogeneity and did not reveal a statistically significant pooled difference between T2DM patients and controls.

The strengths of this meta-analysis include adherence to the PRISMA guidelines, prospective registration with PROSPERO, a comprehensive literature search, a rigorous quality assessment, and extensive sensitivity and subgroup analyses. However, several limitations must be acknowledged. A majority of the included studies employed cross-sectional designs, which precludes the establishment of causal inferences. Notably, significant heterogeneity and evidence of small-study effects were observed, particularly within complication-specific analyses. Furthermore, incomplete adjustment for confounding variables, such as obesity, glycemic control, and medication usage, may have influenced the results. Lastly, the limited number of studies investigating urinary calprotectin and specific complications constrains the generalizability of these findings.

Conclusions

In conclusion, this systematic review and meta-analysis elucidates that circulating calprotectin levels are significantly elevated in individuals with Type 2 Diabetes Mellitus (T2DM), thereby reinforcing its potential role as a biomarker of chronic low-grade inflammation in diabetes. Preliminary evidence indicates that elevated calprotectin levels may be associated with macrovascular complications and peripheral neuropathy; however, these associations necessitate validation through larger, rigorously designed prospective studies. Future research should prioritize longitudinal assessments of its prognostic value and investigate the potential of calprotectin as a therapeutic target in the context of metabolic inflammation.

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Declaration of Conflict of interests
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Ethical approval
This study is a systematic review and meta-analysis. No ethical approval is required.

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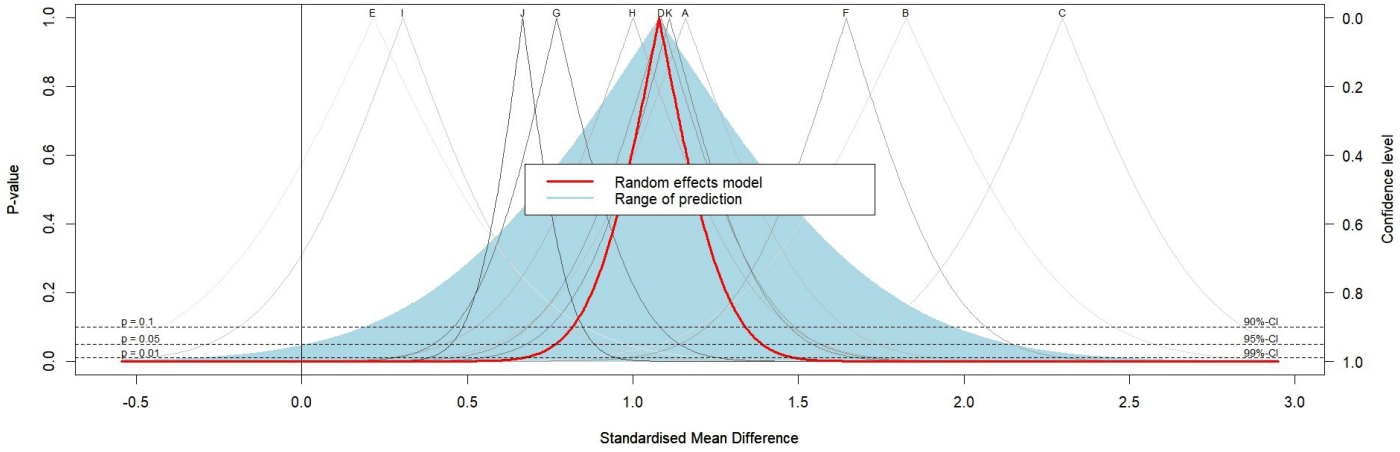
Supplementary files

Supplementary Table 1: Search strategies in databases.

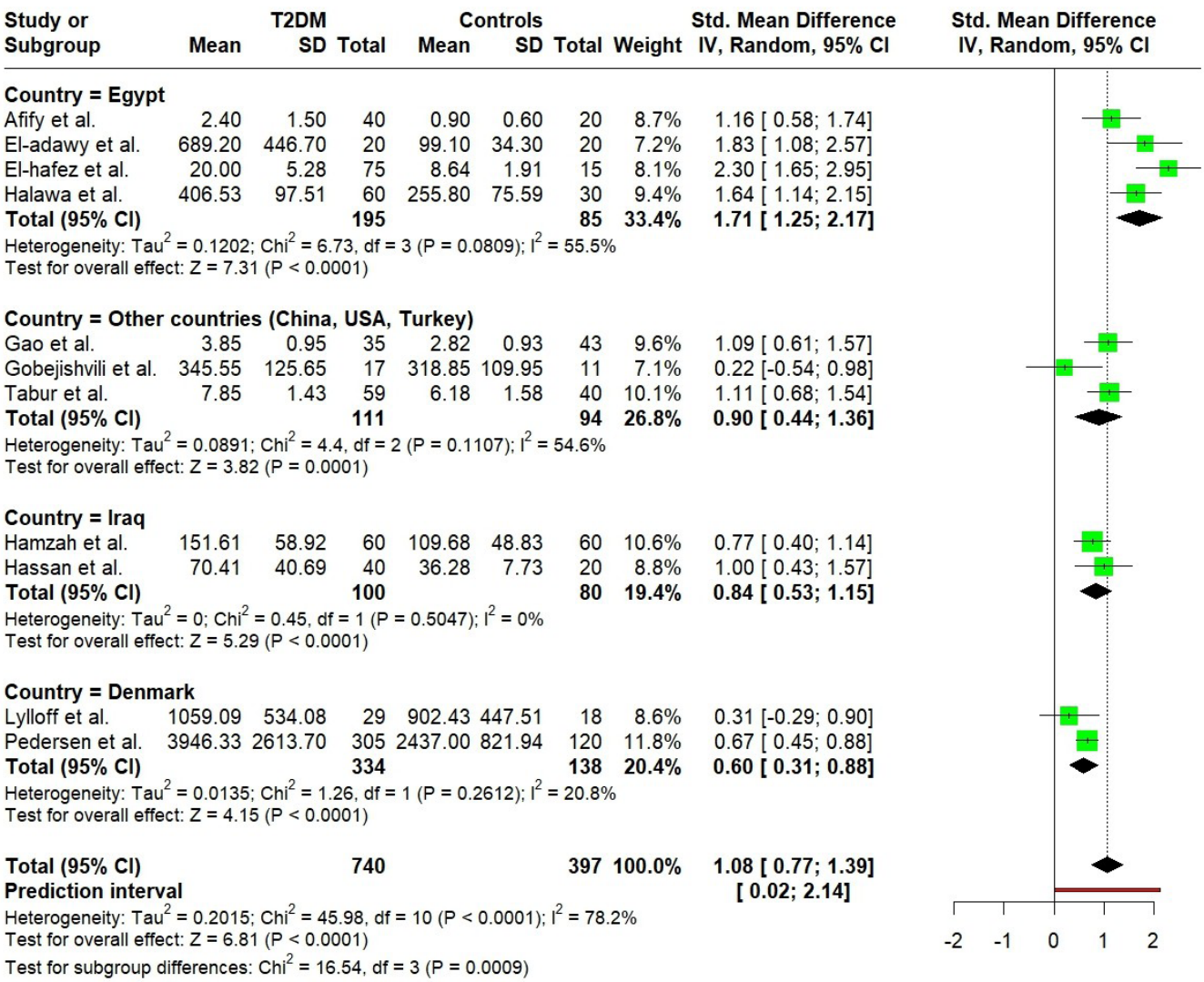
Databases	Search terms	No of articles identified
PubMed/Medline	("type 2 diabetes mellitus"[All Fields] OR "diabetes mellitus"[All Fields] OR "type 2 diabetes"[All Fields] OR "diabetes"[All Fields] OR "diabetics"[All Fields] OR "type 2 DM"[All Fields] OR "T2DM"[All Fields] OR "T2D"[All Fields]) AND ("calprotectin"[All Fields] OR "myeloid-related protein 8/14"[All Fields] OR "MRP 8/14"[All Fields] OR "S100A8/A9"[All Fields] OR "leukocyte protein L1"[All Fields])	171
Scopus	TITLE-ABS-KEY (("type 2 diabetes mellitus" OR "diabetes mellitus" OR "type 2 diabetes" OR "diabetes" OR "diabetics" OR "type 2 DM" OR "T2DM" OR T2D) AND ("calprotectin" OR "myeloid-related protein 8/14" OR "MRP 8/14" OR "S100A8/A9" OR "leukocyte protein L1"))	219
Web of Science	("type 2 diabetes mellitus" OR "diabetes mellitus" OR "type 2 diabetes" OR "diabetes" OR "diabetics" OR "type 2 DM" OR "T2DM" OR T2D) AND ("calprotectin" OR "myeloid-related protein 8/14" OR "MRP 8/14" OR "S100A8/A9" OR "leukocyte protein L1")	205

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

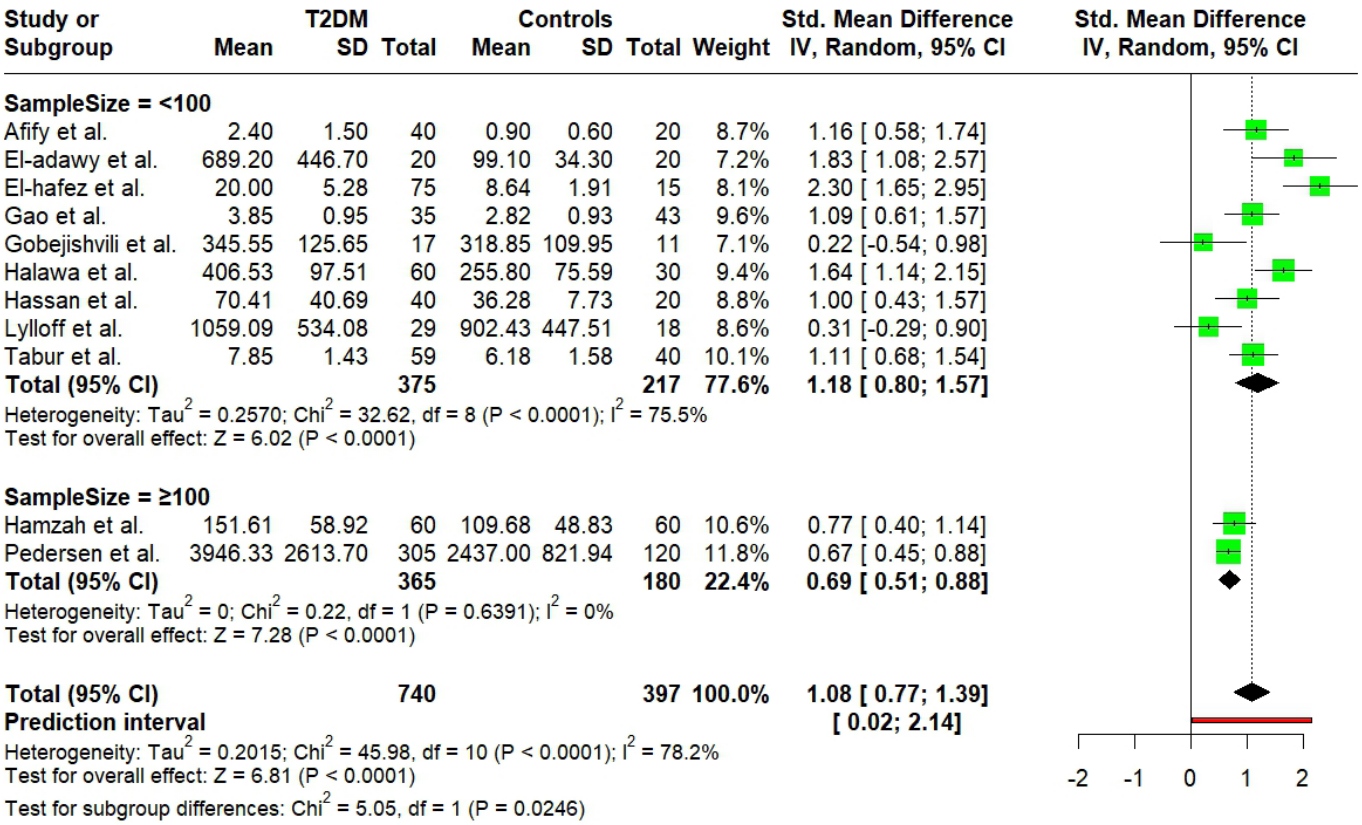


A: Afify et al. [18]; B: El-adawy et al. [19]; C: El-hafez et al. [20]; D: Gao et al. [21]; E: Gobejishvili et al. [22]; F: Halawa et al. [24]; G: Hamzah et al. [25] H: Hassan et al. [26]; I: Lylloff et al. [27]; J: Pedersen et al. [16]; K: Tabur et al. [32]

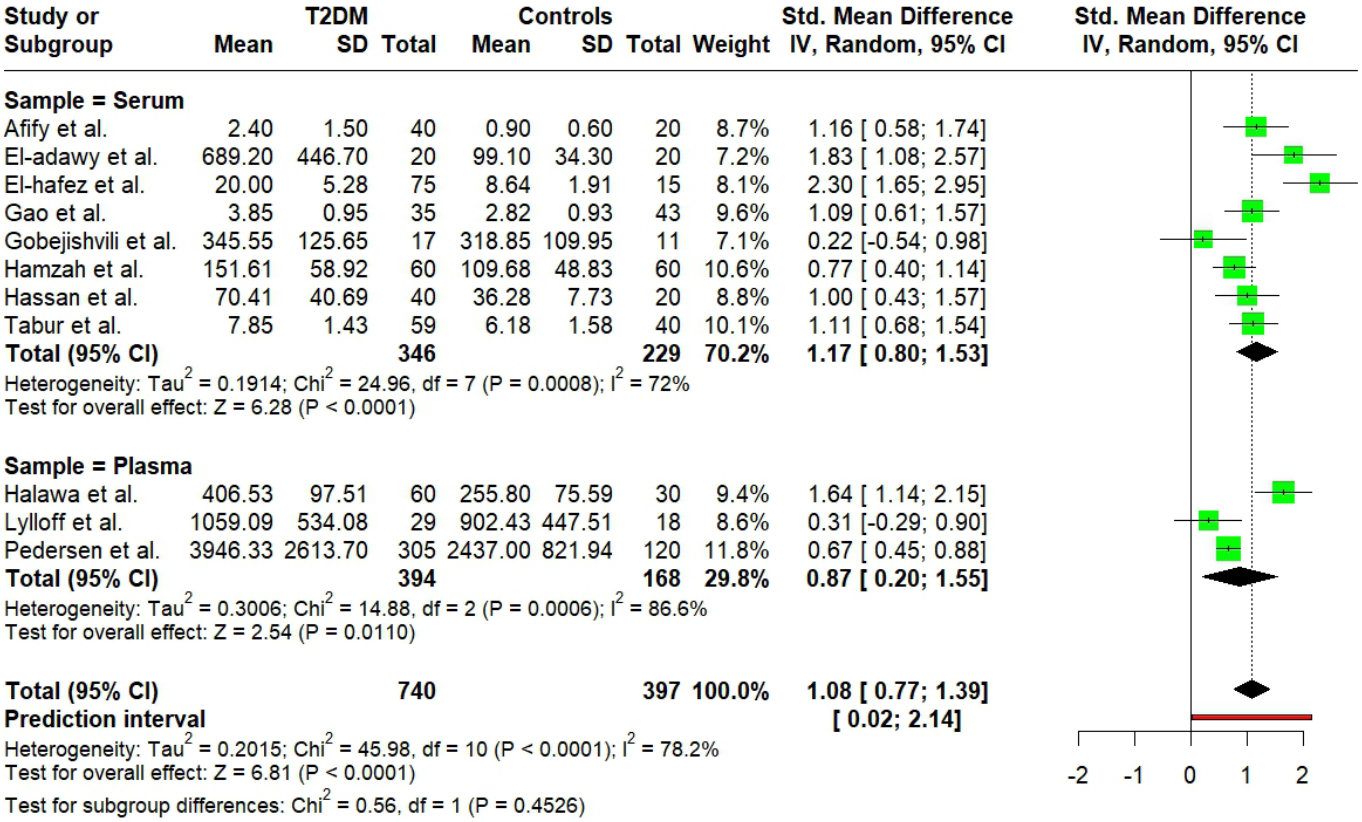
Supplementary Figure 2: Subgroup analysis by country.



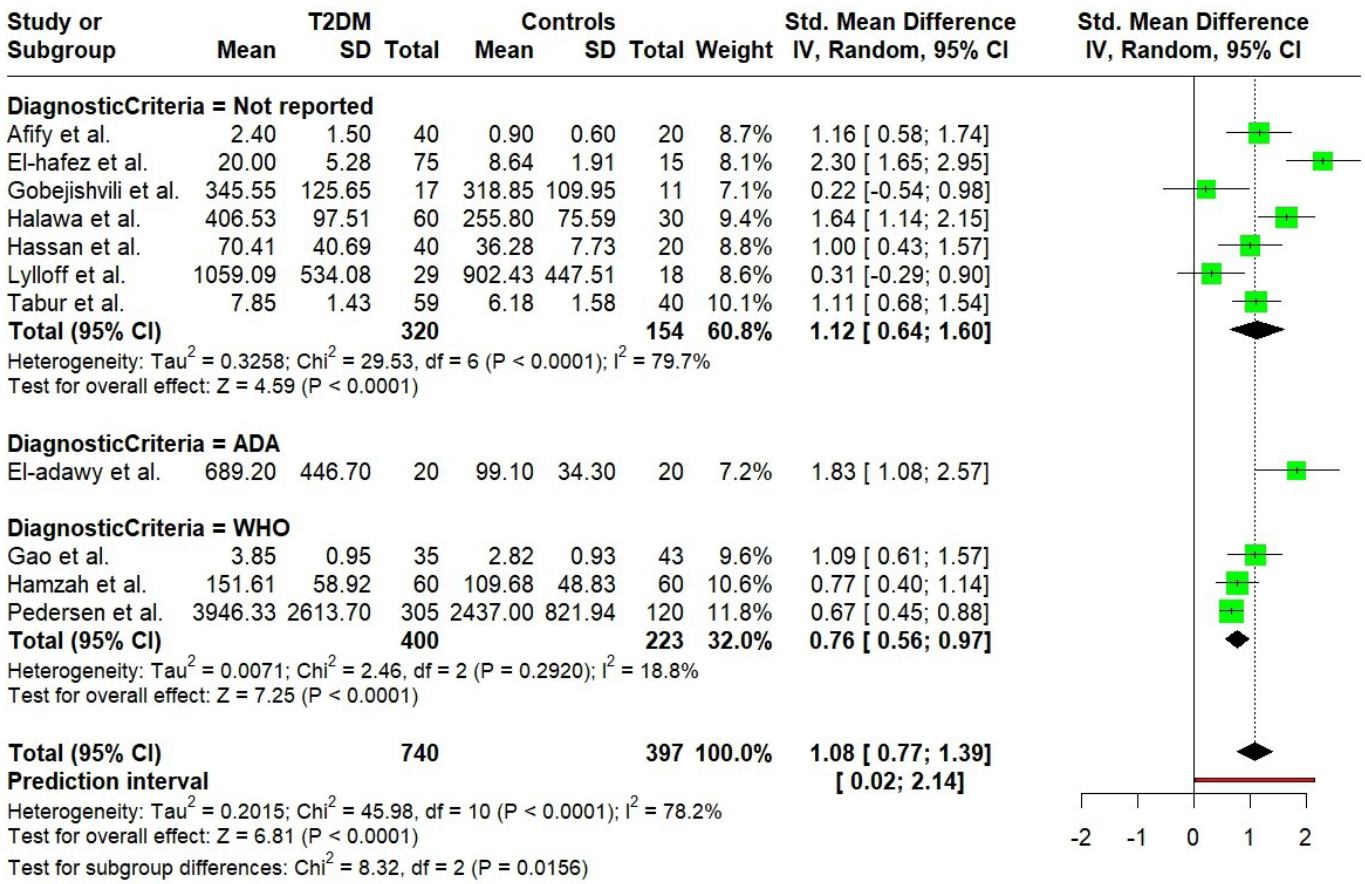
Supplementary Figure 3: Subgroup analysis by sample size.



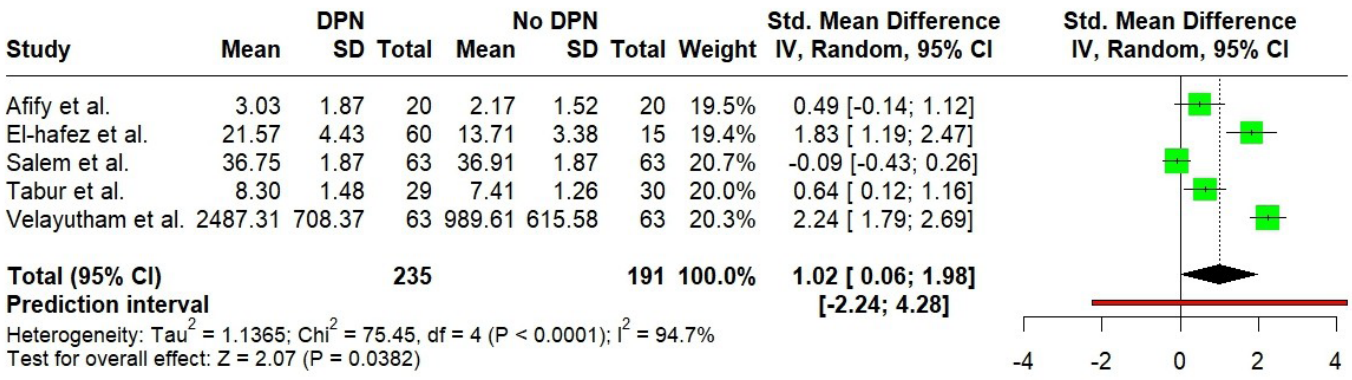
Supplementary Figure 4: Subgroup analysis by sample type.



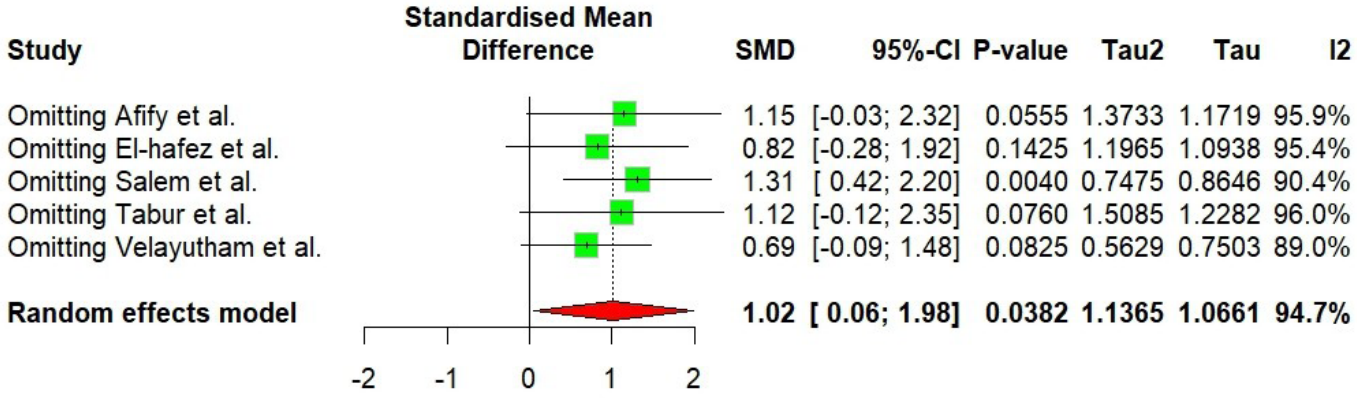
Supplementary Figure 5: Subgroup analysis by diagnostic criteria.



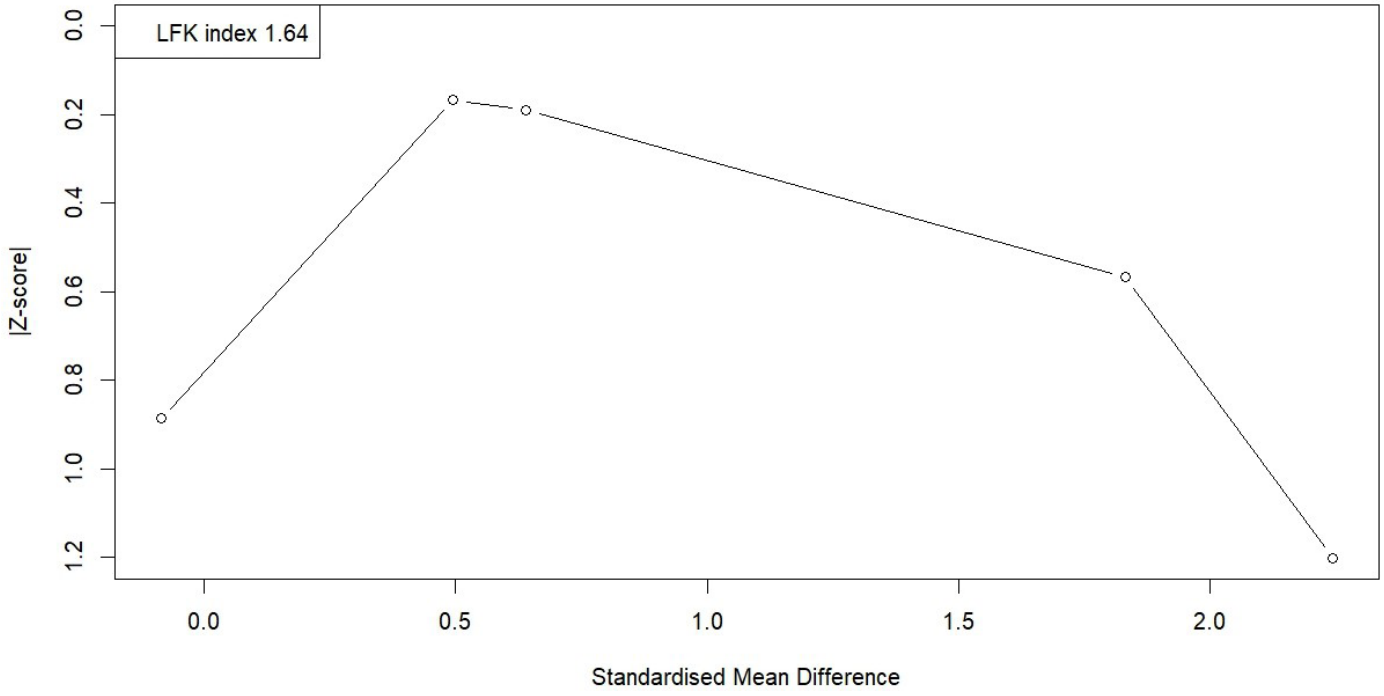
Supplementary Figure 6: Forest plot showing comparison of circulating calprotectin between individuals with and without diabetic peripheral neuropathy. DPN: diabetic peripheral neuropathy.



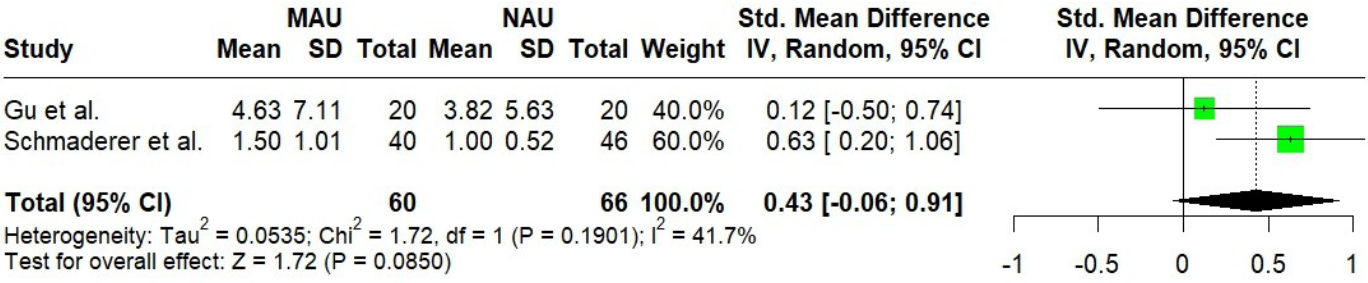
Supplementary Figure 7: Leave-one-out sensitivity analysis illustrating the influence of individual studies on the pooled estimate for diabetic peripheral neuropathy.



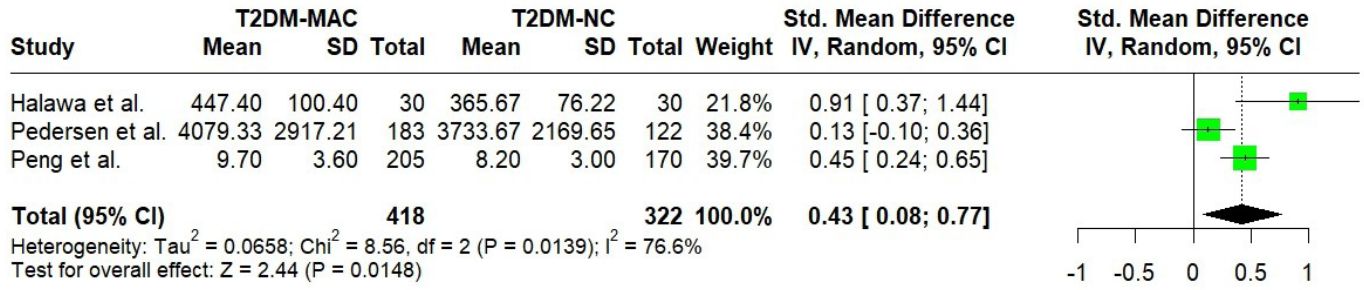
Supplementary Figure 8: Doi plot illustrating potential publication bias in diabetic peripheral neuropathy.



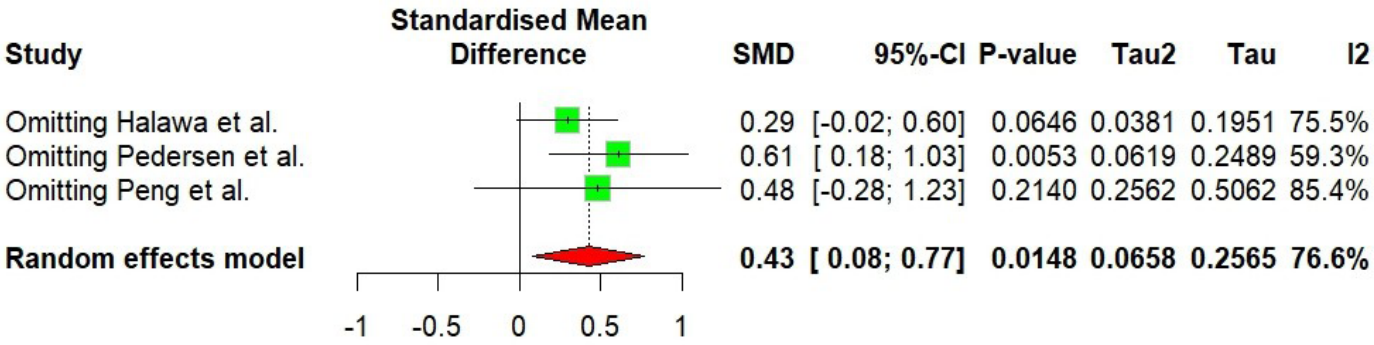
Supplementary Figure 9: Forest plot illustrating differences in circulating calprotectin levels between T2DM patients with normoalbuminuria (NAU) and microalbuminuria (MAU).



Supplementary Figure 10: Forest plot comparing circulating calprotectin levels between T2DM patients with macrovascular complications and those without macrovascular complications. T2DM-MAC: Type 2 diabetes mellitus with macrovascular complications; T2DM-NC: Type 2 diabetes mellitus without complications.



Supplementary Figure 11: Leave-one-out sensitivity analysis illustrating the influence of individual studies on the pooled estimate for T2DM with macrovascular complications.



Supplementary Figure 12: Forest plot showing comparison of urinary calprotectin between T2DM and controls.