Review article

Circulating netrin-1 levels in type 2 diabetes mellitus: A systematic review and meta-analysis

Roshan Kumar Mahat[1*], Vedika Rathore[2], Mritunjay Kumar Mishra[3]

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

[2]*Department of Biochemistry, Shyam Shah Medical College, Rewa, India*

[3]*Department of Biochemistry, GMERS Medical College, Rajpipla, India*

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[*]***Corresponding Author:***
Roshan Kumar Mahat
Associate 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

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Abstract

Background: Netrin-1, a laminin-related guidance cue protein with emerging immunomodulatory roles, has shown conflicting associations with type 2 diabetes mellitus (T2DM) in human studies. We conducted a systematic review and meta-analysis to evaluate circulating netrin-1 levels in individuals with T2DM compared to healthy controls.

Methods: A comprehensive search of MEDLINE/PubMed, Scopus, and Europe PMC up to March 17, 2025, was performed for studies reporting netrin-1 levels in T2DM patients. Eligible studies were observational and provided extractable quantitative data. Study quality was assessed using the Newcastle-Ottawa Scale and JBI checklist. Standardized mean differences (SMDs) with 95% confidence intervals (CIs) were calculated using a random-effects model. Heterogeneity, subgroup, sensitivity, and publication bias analyses were performed using R software.

Results: Twenty studies involving 1,798 articles were included. Pooled analysis revealed significantly elevated netrin-1 levels in T2DM (SMD = 0.57; 95% CI: 0.03–1.11; $p = 0.0393$), with substantial heterogeneity ($I^2 = 96.4\%$). Subgroup analyses indicated geographic and diagnostic criteria as key moderators. Egyptian studies and those using ADA guidelines reported increased netrin-1, while Indian and WHO-based studies reported reductions. The 95% prediction interval (–2.03 to 3.18) reflected wide variability. No significant publication bias was detected (LFK index = –0.49).

Conclusion: This meta-analysis supports a potential link between netrin-1 and T2DM, suggesting its role as a biomarker of metabolic inflammation. However, substantial heterogeneity and study-level differences necessitate further standardized, longitudinal research to clarify its clinical relevance.

Introduction

Type 2 diabetes mellitus (T2DM) is a rapidly growing global health concern, characterized by chronic hyperglycemia resulting from insulin resistance and progressive β-cell dysfunction. According to recent estimates, approximately 537 million adults worldwide (aged 20–79 years) are currently living with diabetes, a number projected to rise to 643 million by 2030 and 783 million by 2045 [1]. The pathophysiology of T2DM is multifactorial, involving metabolic dysregulation, lipotoxicity, and chronic low-grade inflammation. Sustained elevations in glucose and free fatty acid levels contribute to β-cell apoptosis and heightened production of pro-inflammatory mediators, which further exacerbate insulin resistance and disease progression [2].

Netrin-1 (NTN-1), originally identified as a member of the neuronal guidance cue family, has garnered increasing attention for its immunomodulatory and tissue-protective properties. This secreted laminin-related protein exerts both chemoattractive and chemorepulsive effects, mediated through interactions with a diverse set of receptors, including UNC5 homologs (UNC5A-D), deleted in colorectal carcinoma (DCC), neogenin-1 (NEO-1), A2B adenosine receptor (A2BAR), CD146, and integrins [3,4]. Although first discovered in the central nervous system, netrin-1 is widely expressed in peripheral tissues such as the vascular endothelium, pancreas, liver, spleen, kidney, and lungs [5]. Emerging evidence suggests its involvement in various inflammation-associated diseases, including cardiovascular and hepatic disorders, cancer, obesity, and ischemia-reperfusion injury, highlighting its systemic role in immune regulation and tissue homeostasis [6-9].

In the context of metabolic disease, netrin-1 has been implicated in attenuating vascular inflammation by reducing endothelial adhesion and monocyte infiltration- processes central to atherogenesis. Experimental models also demonstrate its antiangiogenic and vasodilatory effects via enhanced nitric oxide production, suggesting a protective role in ischemic and inflammatory settings [10]. Given these properties, netrin-1 may serve as a key link between metabolic inflammation and the development or progression of T2DM.

Despite these mechanistic insights, clinical studies evaluating circulating netrin-1 levels in patients with T2DM have yielded inconsistent results. Some investigations report elevated levels in diabetic individuals, potentially as a compensatory anti-inflammatory response [11, 12], while others demonstrate reduced levels, implying a possible deficit in endogenous protection [13, 14]. A prior meta-analysis by Behnoush et al. (2023) synthesized data from 19 studies investigating netrin-1 levels across diverse glycemic states and diabetes-related complications. However, only 8 of these studies specifically focused on individuals with T2DM, thereby limiting the strength of conclusions that could be drawn for this population [15].

To address this gap, the present systematic review and meta-analysis synthesizes evidence from 20 eligible studies investigating circulating netrin-1 levels in individuals with T2DM. By integrating these data, we aim to provide a comprehensive evaluation of the association between netrin-1 and T2DM, with the goal of elucidating its potential utility as a biomarker or therapeutic target in diabetes-associated inflammation and metabolic dysregulation.

Materials and Methods

The study adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [16] and was prospectively registered with the International Prospective Register of Systematic Reviews (PROSPERO) under reference number CRD42023449622.

Search strategy and study selection

MEDLINE/PubMed, Scopus, and Europe PMC databases were systematically searched for studies reporting netrin-1 levels in individuals with type 2 diabetes mellitus, covering the period from the inception of these databases until 17 March 2025. The search was restricted to human studies and publications in the English language. The following search terms were utilized: “Type 2 Diabetes Mellitus”, “Diabetes Mellitus”, “Type 2 Diabetes”, “Diabetes”, “Diabetics”, “Type 2 DM”, “T2DM”, “T2D”, “Netrin-1”, “NTN1” and “NT-1”. A detailed search strategy for each database is presented in Supplementary Table 1, additionally, the bibliographies of relevant articles were manually searched. Titles and abstracts of all retrieved articles were independently screened by all reviewers to identify articles for full-text review and subsequent inclusion. Discrepancies were resolved through consensus-based discussion. Articles that satisfied the inclusion criteria were selected for the meta-analysis.

Eligibility criteria

Inclusion Criteria: The PECOS format was employed to delineate the inclusion criteria: P (Population): individuals diagnosed with type 2 diabetes mellitus and healthy control participants; E (Exposure): patients with type 2 diabetes mellitus; C (Comparator): non-diabetic healthy control subjects; O (Outcome): levels of netrin-1; and S (Study Design): observational studies that provide clear and extractable data regarding netrin-1.

The exclusion criteria were as follows:

1. Publications categorized as case reports or reviews (including systematic reviews or meta-analyses), editorials, commentaries, and conference abstracts;
2. Studies involving animal models or cell lines;
3. Papers that did not provide raw data;
4. Research articles lacking full text; and
5. Unpublished or ongoing trials. The inclusion and exclusion criteria were established following extensive discussions among the authors of this study.

Data extraction

Two independent researchers (RKM and VR) carried out the initial data extraction process. The information obtained encompassed the first author's name, year of publication, country of study, study design, diagnostic criteria for T2DM, the number of T2DM cases and healthy controls, netrin-1 levels in both groups, participant age, and other pertinent details. In instances of missing or incomplete data, the corresponding author of the respective study was contacted for clarification. A third investigator (MKM) subsequently verified the accuracy of the extracted data. Any discrepancies identified were resolved through discussion among all investigators.

Quality assessment

The methodological quality of the included case-control studies was assessed using the Newcastle-Ottawa Scale (NOS), which evaluates studies across three domains: selection, comparability, and outcome assessment. A maximum score of 9 points could be awarded. Based on the NOS score, studies were categorized as low quality (≤4 points), moderate quality (5–6 points), or high quality (≥7 points) [17]. For cross-sectional studies, the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Analytical Cross-Sectional Studies was utilized. Studies that received ≤49% "yes" responses were deemed to possess a high risk of bias (low quality), those with 50–69% were classified as having a moderate risk of bias (moderate quality), and those with ≥70% were considered to have a low risk of bias (high quality) [18]. Two reviewers (RKM and VR) conducted an independent assessment of the study quality. A third reviewer (MKM) verified these evaluations, and any discrepancies were resolved through consensus discussions.

Statistical analysis

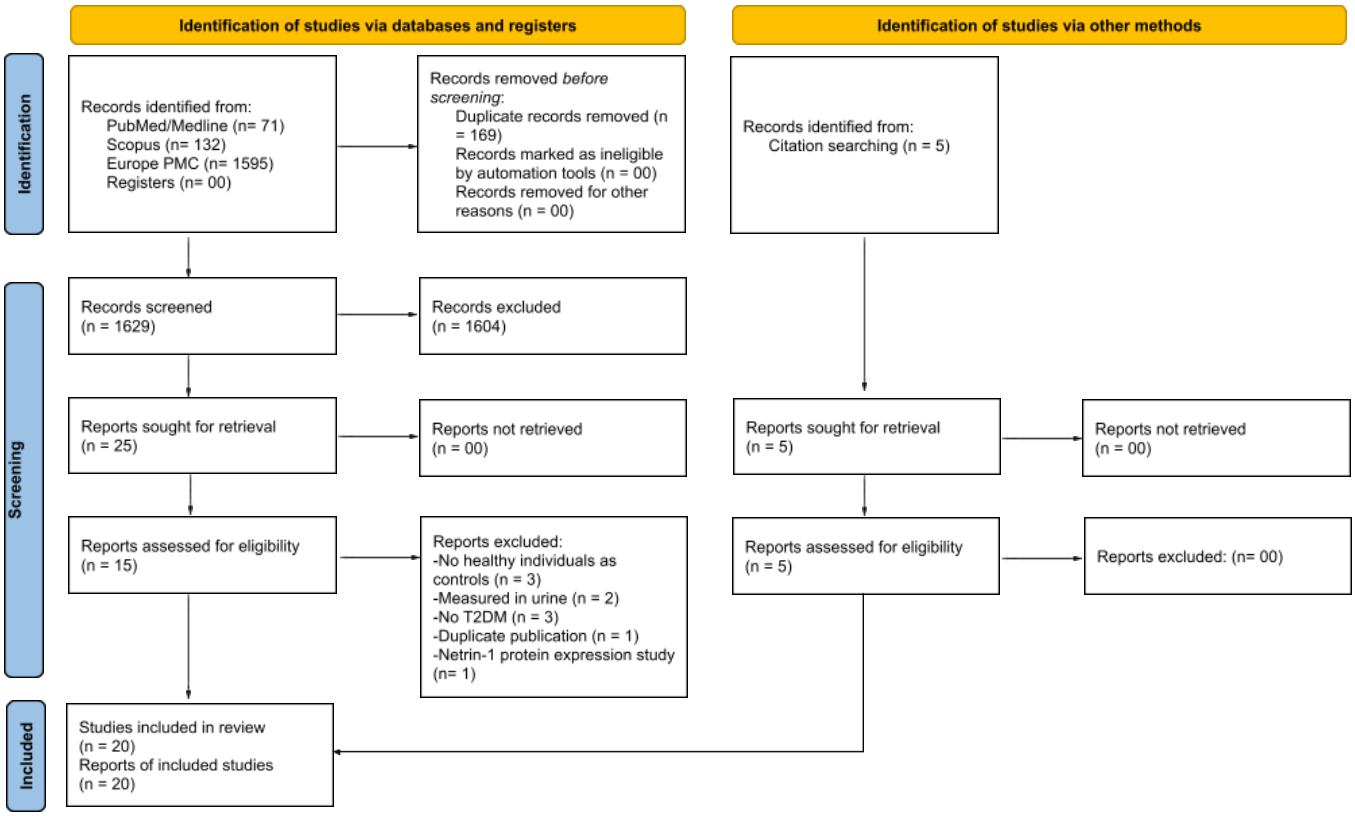
All statistical analyses were conducted using R software (version 4.4.1), specifically employing the "meta" and "metasens" packages. The comparison of circulating netrin-1 concentrations between individuals diagnosed with T2DM and healthy controls was performed by calculating standardized mean differences (SMD) along with 95% confidence intervals (CIs). Furthermore, a 95% prediction interval (PI) was included to provide an estimate of the potential range of netrin-1 values in future studies. A random-effects model was selected for the meta-analysis to accommodate the anticipated clinical heterogeneity among the included studies. For datasets presented as medians with interquartile ranges (IQRs), these values were converted into means and standard deviations (SDs) using established conversion techniques available through the meta-analysis accelerator platform [19]. To evaluate the consistency and reliability of the findings, sensitivity analyses were conducted. Study heterogeneity was assessed utilizing the Cochrane Q test and I^2 statistic, with thresholds of $p < 0.10$ and $I^2 > 50\%$ denoting substantial heterogeneity. To explore sources of heterogeneity, subgroup analyses were performed based on geographical region, diagnostic criteria, sample size, biological sample type, and study design. To assess potential publication bias, Doi plots were visually inspected, and asymmetry was quantitatively measured employing the Luis Furuya-Kanamori (LFK) index [20]. The interpretation of the LFK index adhered to established guidelines: values ranging from −1 to +1 were deemed to reflect symmetry, values between ±1 and ±2 indicated slight asymmetry, and values exceeding ±2 were indicative of substantial asymmetry. A p-value of less than 0.05 was considered statistically significant unless otherwise specified.

Results

Characteristics of included studies

A total of 1,798 articles were initially identified through the implemented search strategy. Following the removal of duplicates, 1,629 records remained for screening. Of these, 25 articles were subjected to full-text review, leading to the exclusion of 10 studies based on the criteria outlined in Figure 1. Furthermore, an additional five studies were identified through citation tracking. Ultimately, 20 studies met all inclusion criteria and were incorporated into the systematic review and meta-analysis [11-14, 21-36]. The comprehensive selection process is illustrated in the PRISMA flow diagram (Figure 1).

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



Of the studies included in the analysis, seven were from Egypt [12, 21, 22, 24, 28, 32, 34], five from Iraq [11, 13, 14, 31, 33], two from India [27, 35], and one each from Mexico [23], China [25], Spain [26], Bulgaria [29], Turkey [30], and Korea [36]. Thirteen studies employed a cross-sectional design [14, 21-23, 25, 27-29, 32-36], while seven followed a case-control format [11-13, 24, 26, 30, 31]. Ten studies diagnosed T2DM using the American Diabetes Association (ADA) criteria [11, 12, 21-24, 26, 34-36], one used World Health Organization (WHO) guidelines [25], and the remaining nine did not specify their diagnostic approach [13, 14, 27-33]. Netrin-1 levels were assessed in serum samples in 18 studies [11-14, 21-24, 27-36] and in plasma samples in 2 studies [25, 26]. The publication years of the included studies ranged from 2016 to 2024. Detailed characteristics of each study are summarized in Table 1. The quality results of included studies are also presented in Table 1.

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

Author	Year	Country	Study design	Diagnostic criteria of diabetes	Type 2 DM		Controls		Sample	Quality	Main findings
					Age (Years) Mean±SD or Range	N	Age (Years) Mean±SD or Range	N			
Al-Shakour et al. [11]	2024	Iraq	C-C	ADA	53.60 ± 9.10	81	52.60 ± 9.67	79	Serum	High	The average serum Netrin-1 level was significantly elevated in patients with type 2 diabetes mellitus compared to the control group.
Assi et al. [13]	2021	Iraq	C-C	NR	54.44±11.190	45	49.21±14.91	45	Serum	Moderate	Netrin-1 levels were significantly decreased in newly diagnosed patients with type 2 diabetes compared to the control group.

Badran et al. [21]	2024	Egypt	C-S	ADA	54.90±10.18	60	54.29 ±10.64	30	Serum	High	Patients with type 2 diabetes and diabetic nephropathy (DN) had significantly lower levels of Netrin-1 compared to those with type 2 diabetes without nephropathy; however, there was no statistically significant difference between the DN group and the control group.
Elkholy et al. [22]	2021	Egypt	C-S	ADA	52.10±9.54	135	50.3±8.1	45	Serum	High	The mean serum netrin-1 levels were significantly elevated in diabetic nephropathy (DN) patients with microalbuminuria and macroalbuminuria compared to the control group. Among these, the highest levels were found in patients with macroalbuminuria, and the differences between these groups were statistically significant. However, no statistically significant difference in netrin-1 levels was found between normoalbuminuric type 2 diabetes mellitus (T2DM) patients and the control group.
Fadel et al. [12]	2021	Egypt	C-C	ADA	50.36 ±7.57	100	52.22 ± 8.72	80	Serum	High	Patients with uncomplicated type 2 diabetes mellitus (T2DM) and those with complications both showed significantly increased netrin-1 levels compared to the control group. Additionally, patients with complicated T2DM had significantly higher netrin-1 levels compared to those without complications.
Garcia Galindo et al. [23]	2023	Mexico	C-S	ADA	51.3±5.5	30	22.8 ± 4.3	30	Serum	High	Ntn1 levels were significantly elevated in newly diagnosed patients with type 2 diabetes.

Ibrahem et al. [24]	2024	Egypt	C-C	ADA	53.24 ± 11.76	34	45.60 ± 10.60	20	Serum	High	Serum netrin-1 levels were significantly higher in patients with type 2 diabetes mellitus compared to the control group.
Khazeil et al. [14]	2020	Iraq	C-S	NR	54.11±2.44	58	42.7 ± 2.1	30	Serum	Moderate	Netrin-1 levels were found to be lower on average in individuals with type 2 diabetes mellitus (T2DM) compared to the control group.
Liu et al. [25]	2016	China	C-S	WHO	52.7 (11.08)	30	52.96 (11.65)	26	Plasma	High	Netrin-1 levels were significantly decreased in patients with type 2 diabetes mellitus compared to the control group.
Mentxaka et al. [26]	2022	Spain	C-C	ADA	47±2	41	42±5	18	Plasma	High	Circulating levels of NTN-1 were significantly elevated in individuals with obese type 2 diabetes compared to lean control individuals.
Mondal et al. [27]	2024	India	C-S	NR	50.96±9.57	72	43.07±10.53	45	Serum	Moderate	Serum netrin-1 concentrations were markedly lower in patients with type 2 diabetes without small nerve fiber damage (DN-) and with small nerve fiber damage (DN+) compared to the control group.
Naiem et al. [28]	2024	Egypt	C-S	NR	53.53±8.60	90	53.07±8.4	30	Serum	High	Plasma netrin-1 levels are significantly increased in patients with diabetic nephropathy, including those with microalbuminuria or macroalbuminuria, highlighting its potential as an early biomarker for the diagnosis of diabetic nephropathy.
Nedeva et al. [29]	2020	Bulgaria	C-S	NR	54.18±10.86	39	49.38±12.12	42	Serum	High	Lower levels of Netrin-1 were reported in patients with newly diagnosed type 2 diabetes compared to the healthy control group.

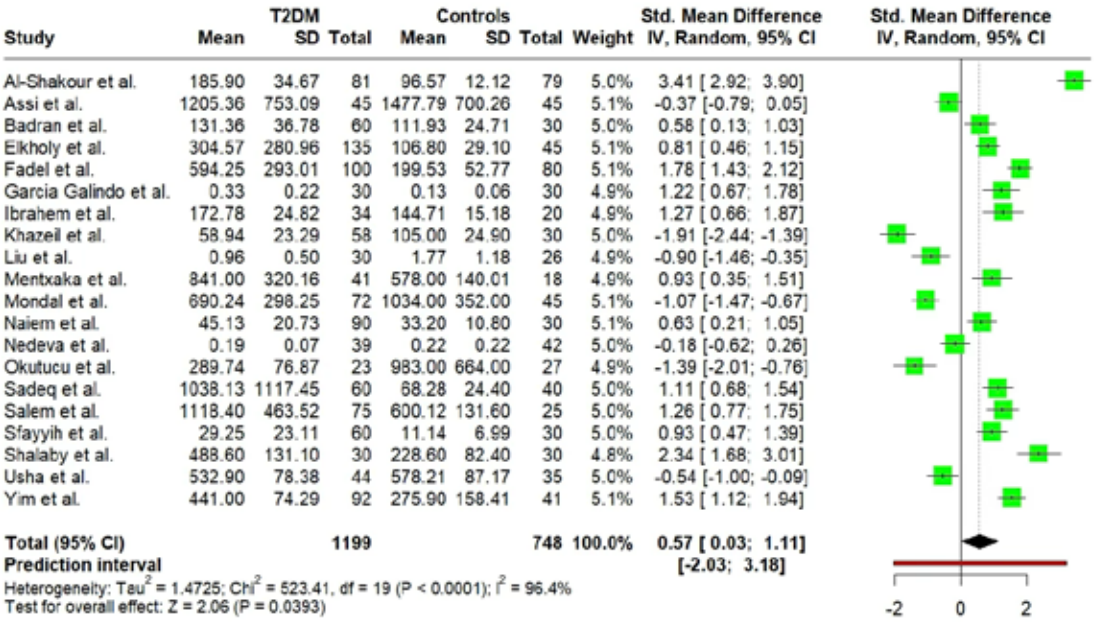
Okutucu et al. [30]	2021	Turkey	C-C	NR	67.35±10.32	23	65.74 ± 7.17	27	Serum	High	Patients with proliferative and non-proliferative diabetic retinopathy exhibited significantly lower serum netrin-1 levels compared to the control group.
Sadeq et al. [31]	2021	Iraq	C-C	NR	54.18±8.11	60	53.42±9.93	40	Serum	High	Netrin-1 levels were significantly elevated in patients with type 2 diabetes mellitus (with and without diabetic retinopathy) compared to healthy controls.
Salem et al. [32]	2022	Egypt	C-S	NR	49.96±8.37	75	48.32 ± 8.21	25	Serum	Moderate	Netrin-1 levels were significantly higher in diabetic patients with macroalbuminuria and microalbuminuria compared to normoalbuminuric patients and the control group.
Sfayyih et al. [33]	2024	Iraq	C-S	NR	R= 34-56	60	R= 34-56	30	Serum	Moderate	Netrin-1 levels were significantly elevated in patients with type 2 diabetes compared to the healthy control group.
Shalaby et al. [34]	2021	Egypt	C-S	ADA	44.7±6.2	30	44.3 ±7.3	30	Serum	High	Serum netrin-1 concentrations were elevated in individuals with newly diagnosed type 2 diabetes.
Usha et al. [35]	2023	India	C-S	ADA	49.38±2.29	44	47.78±2.69	35	Serum	High	There was no significant difference in serum netrin-1 levels between patients with type 2 diabetes mellitus and healthy controls.
Yim et al. [36]	2018	Korea	C-S	ADA	52.6±13.4	92	40.9± 14.5	41	Serum	High	Serum netrin-1 levels were significantly elevated in subjects with type 2 diabetes compared to healthy controls.

C-C: Case-Control; C-S: Cross-Sectional; ADA: American Diabetes Association; WHO: World Health Organization; NR: Not Reported; N: Number of T2DM/Control Subjects; SD: Standard Deviation.

Meta-analysis

Using a random-effects model, the pooled SMD in netrin-1 levels between T2DM and control groups was 0.57 (95% CI: 0.03 to 1.11, p = 0.0393), indicating a statistically significant elevation of netrin-1 in patients with T2DM. Substantial heterogeneity was observed among the included studies (I^2 = 96.4%, τ^2 = 1.4725, p < 0.0001), suggesting considerable variability in study results. The 95% prediction interval ranged from –2.03 to 3.18, indicating that the effect size in future studies may vary and, in some cases, may not demonstrate a significant difference or may even favor controls. Figure 2 displays a forest plot comparing netrin-1 values between patients with T2DM and those without.

Figure 2: Forest plot showing comparison of circulating netrin-1 levels between T2DM and controls.

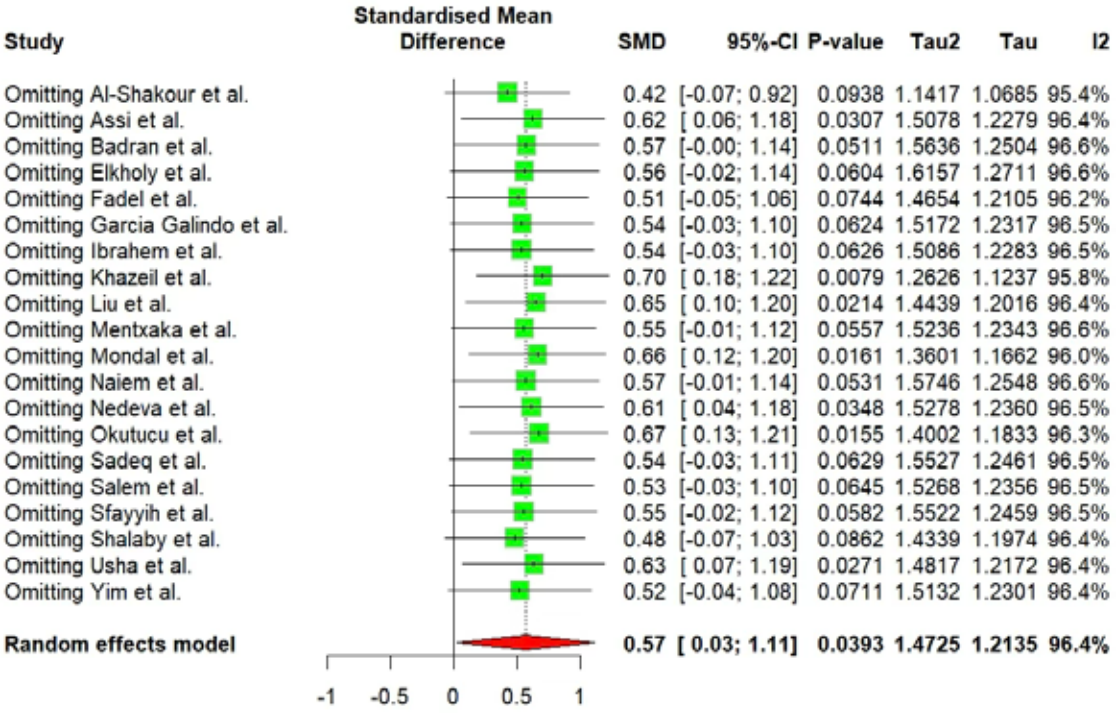


A drapery plot was also constructed to visually represent the meta-analysis findings by displaying each study's p-value in relation to its corresponding effect size (Supplementary Figure 1).

Sensitivity analysis

Across all iterations, the pooled SMD remained positive and relatively stable, ranging from 0.42 (95% CI: –0.07 to 0.92) upon exclusion of Al-Shakour et al. [11] to 0.70 (95% CI: 0.18 to 1.22) when Khazeil et al. [14] was excluded. Although none of the exclusions meaningfully changed the direction of the overall effect, the statistical significance of the association approached non-significance when studies such as Al-Shakour et al. [11], Badran et al. [21], Elkholy et al. [22], Fadel et al. [12], Garcia Galindo et al. [23], Ibrahem et al. [24], Mentxaka et al. [26], Naiem et al. [28], Sadeq et al. [31], Salem et al. [32], Sfayyih et al. [33], Shalaby et al. [34], and Yim et al. [36] were excluded individually. Heterogeneity remained consistently high across all iterations (I^2 = 95.4% to 96.6%, Tau^2 = 1.3601 to 1.6157), indicating persistent between-study variability regardless of individual study omission (Figure 3).

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



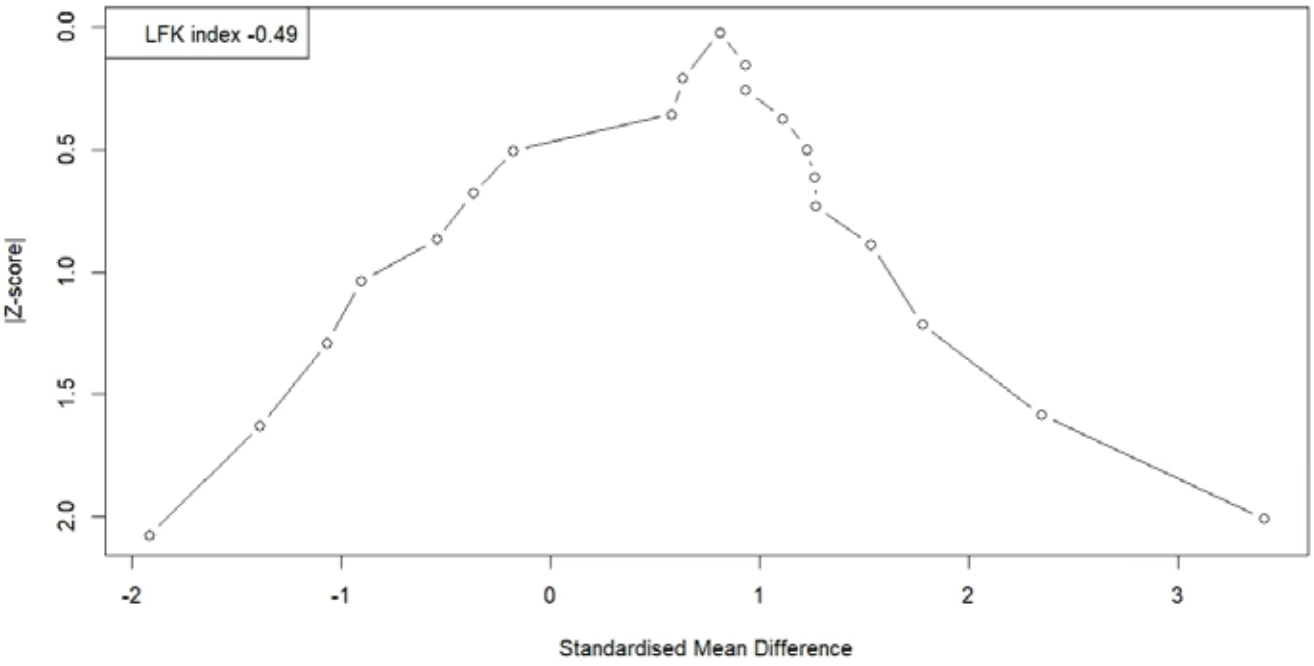
Subgroup analysis

When stratified by country, studies from Egypt showed a significantly elevated pooled SMD in netrin-1 levels among individuals with T2DM compared to controls (SMD: 1.21; 95% CI: 0.77 to 1.66), whereas studies from India demonstrated a significantly decreased pooled effect (SMD: –0.82; 95% CI: –1.33 to –0.31). Studies from Iraq and other countries yielded non-significant pooled effects. The difference among country subgroups was statistically significant ($\chi^2 = 34.60$, $p < 0.0001$), suggesting that geographic location may be a key moderator of effect size (Supplementary Figure 2). Subgroup analysis by diagnostic criteria revealed that studies adhering to the ADA criteria reported a significant increase in netrin-1 levels (SMD: 1.33; 95% CI: 0.68 to 1.97), while studies with unreported criteria showed no significant difference (SMD: –0.10; 95% CI: –0.82 to 0.61), and studies using WHO criteria reported significantly lower netrin-1 levels in T2DM (SMD: –0.90; 95% CI: –1.46 to –0.35). The difference among diagnostic subgroups was also statistically significant ($\chi^2 = 26.72$, $p < 0.0001$) (Supplementary Figure 3). When categorized by sample size, studies with ≥100 participants showed a significant association (SMD: 1.18; 95% CI: 0.35 to 2.00), whereas studies with <100 participants reported a non-significant effect (SMD: 0.16; 95% CI: –0.48 to 0.79). However, the difference between these subgroups was marginally non-significant ($\chi^2 = 3.69$, $p = 0.0546$) (Supplementary Figure 4). Subgroup analysis by biological sample type revealed a significant association in serum-based studies (SMD: 0.63; 95% CI: 0.06 to 1.21), whereas plasma-based studies did not show a significant association (SMD: 0.01; 95% CI: –1.79 to 1.81); nonetheless, this difference was not statistically significant ($\chi^2 = 0.42$, $p = 0.5188$) (Supplementary Figure 5). Finally, analysis by study design showed non-significant associations in both case-control studies (SMD: 0.97; 95% CI: –0.10 to 2.03) and cross-sectional studies (SMD: 0.36; 95% CI: –0.24 to 0.95), with no significant subgroup difference observed ($\chi^2 = 0.96$, $p = 0.3265$) (Supplementary Figure 6). These findings suggest that geographic origin and diagnostic criteria may be major contributors to the observed heterogeneity, while sample size, sample type, and study design appear to have a more modest impact.

Publication bias

Publication bias was assessed utilizing the Doi plot in conjunction with the LFK index. The Doi plot illustrates a symmetrical distribution of studies around the central axis, with a calculated LFK index of –0.49. This value falls within the acceptable range of ±1, suggesting the absence of significant asymmetry in the Doi plot (Figure 4). Consequently, there is no evidence of publication bias influencing the pooled results of netrin-1 levels in individuals diagnosed with T2DM.

Figure 4: Doi plot showing no evidence of publication bias.



Discussion

This systematic review and meta-analysis synthesize contemporary evidence concerning circulating netrin-1 levels in individuals diagnosed with T2DM. The pooled analysis demonstrated a statistically significant elevation of netrin-1 levels in T2DM patients when compared to healthy controls, indicating a potential role for netrin-1 as a biomarker for diabetes-related inflammation and metabolic dysfunction. Nonetheless, considerable heterogeneity across studies and inconsistent findings in subgroup analyses underscore the complexity of netrin-1's biological role and the impact of various study characteristics.

Our findings diverge from the meta-analysis conducted by Behnoush et al. [15], which found no overall difference in netrin-1 levels between patients with diabetes and healthy controls. However, they did report significantly elevated netrin-1 levels in diabetic individuals with microalbuminuria and macroalbuminuria compared to those with normoalbuminuric. Furthermore, they observed reduced netrin-1 levels in individuals with prediabetes compared to healthy controls. In contrast, our results align with several individual studies that document increased serum netrin-1 concentrations in patients with T2DM and its associated complications. For instance, Elkholy et al. [22] demonstrated significantly elevated netrin-1 levels in patients suffering from diabetic nephropathy, with both serum and urinary measurements suggesting its potential utility for early detection. Mentxaka et al. [26] similarly found increased circulating netrin-1 levels in patients with obesity and T2DM, which exhibited a positive correlation with insulin and HOMA-IR, and a negative correlation with QUICKI. Fadel et al. [12] reported higher netrin-1 levels in T2DM patients experiencing vascular complications, thereby underscoring its diagnostic utility in conjunction with inflammatory markers such as VCAM-1. Additionally, Garcia Galindo et al. [23] established a positive correlation between netrin-1 levels and hs-CRP, further emphasizing its role in T2DM-related low-grade inflammation. Despite these findings, discrepancies persist in the literature. Usha et al. [35] reported no significant difference in netrin-1 levels between T2DM patients and controls; however, they identified a significant negative correlation between netrin-1 and QUICKI. Yim et al. [36] observed elevated serum netrin-1 levels in individuals with impaired fasting glucose or T2DM, interpreting this as a potential compensatory response. Their analysis also revealed significant positive correlations with fasting glucose, HbA1c, and HOMA-IR. Conversely, studies conducted by Liu et al. [25] indicated lower plasma netrin-1 levels in newly diagnosed T2DM patients, suggesting that netrin-1 expression may be diminished in the early stages of the disease or as a result of impaired compensatory mechanisms. In their investigation, netrin-1 was negatively associated with fasting and postprandial glucose, HbA1c, triglycerides, and HOMA-IR. Similarly, Nedeva et al. [29] reported significantly lower serum netrin-1 levels in individuals with obesity, prediabetes, and T2DM compared to healthy controls. These inconsistencies may arise from variations in population characteristics, duration of diabetes, sample sizes, disease phenotypes, and methodological differences, particularly regarding the use of various ELISA kits for netrin-1 measurement. Furthermore, the diagnostic criteria employed appeared to influence the results; studies utilizing ADA guidelines generally reported elevated netrin-1 levels, whereas

those employing WHO criteria or unspecified diagnostic standards exhibited inconsistent or inverse trends.

Biologically, netrin-1 exhibits dual roles, functioning as an anti-inflammatory mediator by inhibiting leukocyte adhesion and migration, while also being implicated in insulin resistance and metabolic dysfunction [15]. Garcia Galindo et al. [23] proposed a mechanistic model in which a loss of receptor affinity (e.g., Unc5b) leads to unchecked secretion of netrin-1, thereby amplifying inflammatory cascades via high-sensitivity C-reactive protein (hs-CRP) and nuclear factor kappa B (NF-κB), which contribute to insulin resistance and the progression of T2DM. Furthermore, preclinical studies highlight tissue-specific effects; for instance, Ramkhelawon et al. [37] demonstrated that in obese mice, the expression of netrin-1 and UNC5B was elevated in visceral adipose tissue, promoting macrophage retention and inflammation. Deletion of hematopoietic netrin-1 improved insulin sensitivity by reducing adipose inflammation. Wu et al. [38] further suggested that the impact of netrin-1 varies depending on receptor subtype and concentration, adding an additional layer of complexity. Notably, netrin-1 levels also appear to vary with body composition. Nedeva et al. [8] observed that netrin-1 is elevated in lean individuals with diabetes but decreased in their obese counterparts, which may partly account for the inconsistent findings across clinical studies. Despite these mechanistic insights, a critical gap remains in the literature: no longitudinal studies have yet evaluated changes in netrin-1 levels throughout the course of diabetes development or insulin resistance. Consequently, the precise role of netrin-1 in the pathophysiology of T2DM remains incompletely elucidated.

The findings from the subgroup analyses further highlight the variability in circulating netrin-1 levels across diverse studies. Notably, research conducted in Egypt consistently reported elevated levels of netrin-1 among individuals with T2DM, whereas studies from India-including those by Mondal et al. [27] -tended to indicate reduced levels. This geographical variation may reflect underlying differences in population genetics, environmental exposures, or disease phenotypes. Furthermore, the choice of diagnostic criteria appeared to influence the observed associations: studies utilizing the ADA guidelines more frequently demonstrated elevated netrin-1 levels, whereas those employing WHO criteria or lacking specific diagnostic standards often exhibited null or inverse associations.

Despite the overall positive association observed in our findings, the results were characterized by considerable heterogeneity. The sensitivity analysis indicated that no single study exerted a disproportionate influence on the overall results; however, the statistical significance of the findings diminished upon the exclusion of studies such as Al-Shakour et al. [11], Badran et al. [21], Elkholy et al. [22], Fadel et al. [12], Garcia Galindo et al. [23], Ibrahem et al. [24], Mentxaka et al. [26], Naiem et al. [28], Sadeq et al. [31], Salem et al. [32], Sfayyih et al. [33], Shalaby et al. [34], and Yim et al. [36]. The extensive prediction interval (–2.03 to 3.18) and the accompanying drapery plot visualization further elucidate the variability and dispersion of effect estimates, thereby reinforcing the existence of genuine differences between studies.

Interestingly, our analysis did not reveal any evidence of publication bias, as supported by the symmetrical Doi plot and an LFK index of –0.49, which falls within the threshold for no asymmetry. This enhances confidence in the overall findings despite heterogeneity.

This meta-analysis is the most comprehensive to date focusing exclusively on circulating netrin-1 in T2DM, incorporating diverse geographic populations, sample types, and study designs. The use of rigorous subgroup and sensitivity analyses enhances the reliability and transparency of findings. Additionally, visualization tools such as forest plots, drapery plots, and Doi plots provide multiple perspectives on data distribution and bias. However, several limitations must be acknowledged. First, the majority of the studies included in this review were cross-sectional in nature, which restricts the ability to draw causal inferences. Second, substantial heterogeneity persists despite subgroup analyses. Third, discrepancies among the studies were noted, which may be attributable to a variety of factors, including differences in sample size, patient characteristics, and measurement techniques. Additionally, there may be variations in the duration of diabetes and its progression among the patient groups. Lastly, the results may have been influenced by the conversion of non-normally distributed data to a normal distribution, potentially leading to variations in the findings.

Conclusion

In conclusion, while elevated netrin-1 levels are associated with T2DM in pooled analyses, variability across studies warrants cautious interpretation. Future research should aim for standardized methodologies, larger multicentre cohorts, and longitudinal designs to clarify netrin-1's role in the pathophysiology and progression of type 2 diabetes.

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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

Roshan Kumar Mahat: Conceptualization, Methodology, Software, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration.
Vedika Rathore: Methodology, Investigation, Data Curation, Validation, Writing - Review & Editing.
Mritunjay Kumar Mishra: Methodology, Investigation, Data Curation, Writing - Review & Editing.
All authors have read and approved the final manuscript.

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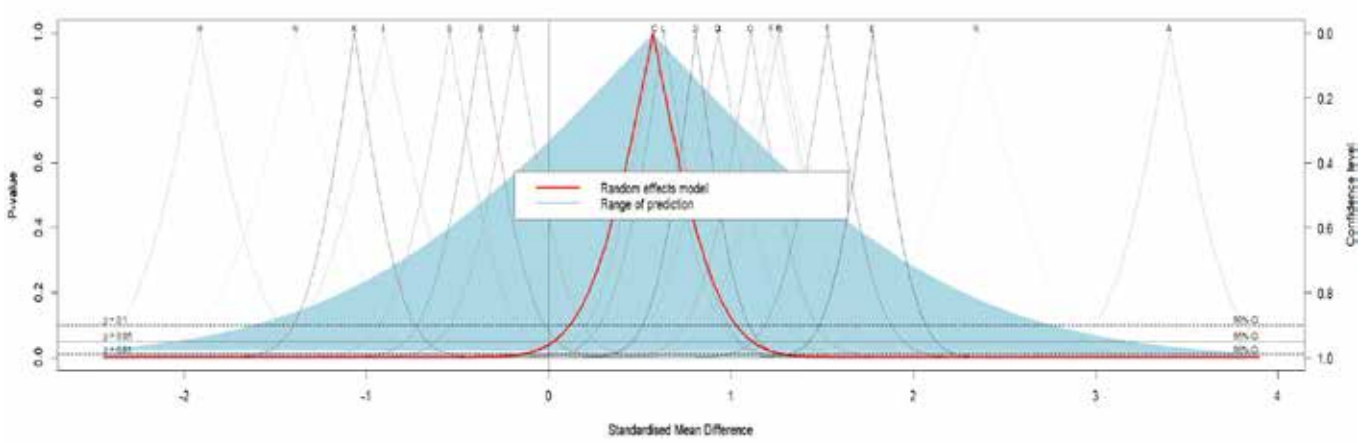
Supplementary files

Supplementary Table 1: Search strategies in databases.

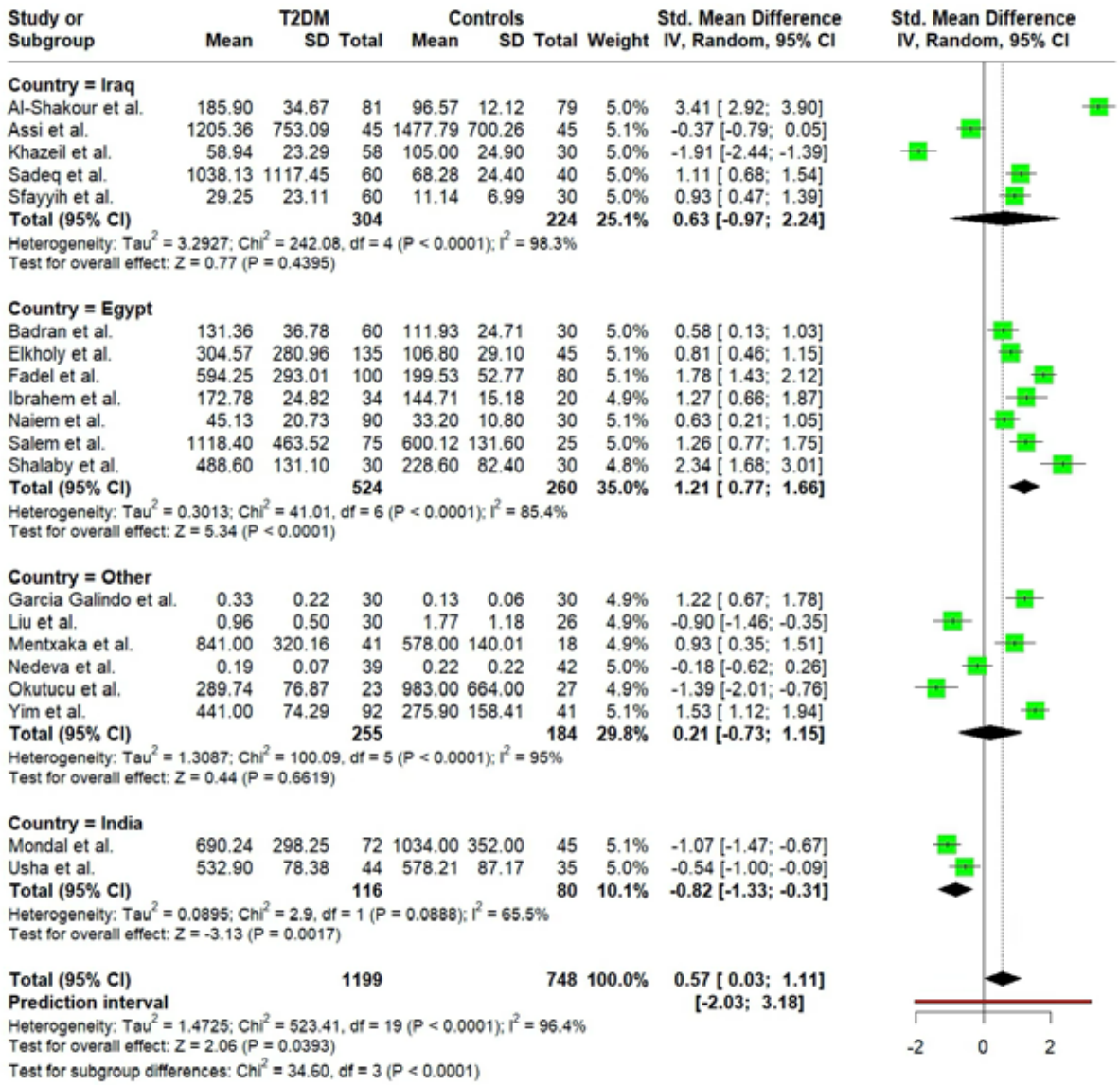
Databases	Search terms	No of articles identified
PubMed/Medline	("Type 2 Diabetes Mellitus"[All Fields] OR "Diabetes Mellitus"[All Fields] OR "Type 2 Diabetes"[All Fields] OR "Diabetes"[All Fields] OR "Diabetics"[All Fields] OR "Type 2 DM"[All Fields] OR "T2DM"[All Fields] OR "T2D"[All Fields]) AND ("Netrin-1"[All Fields] OR "NTN1"[All Fields] OR "NT-1"[All Fields])	71
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 ("Netrin-1" OR "NTN1" OR "NT-1"))	132
Europe PMC	(“Type 2 Diabetes Mellitus” OR “Diabetes Mellitus” OR “Type 2 Diabetes” OR “Diabetes” OR “Diabetics” OR “Type 2 DM” OR “T2DM” OR T2D) AND (“Netrin-1” OR “NTN1” OR “NT-1”)	1595

Supplementary Figure 1: Drapery plot of meta-analysis.

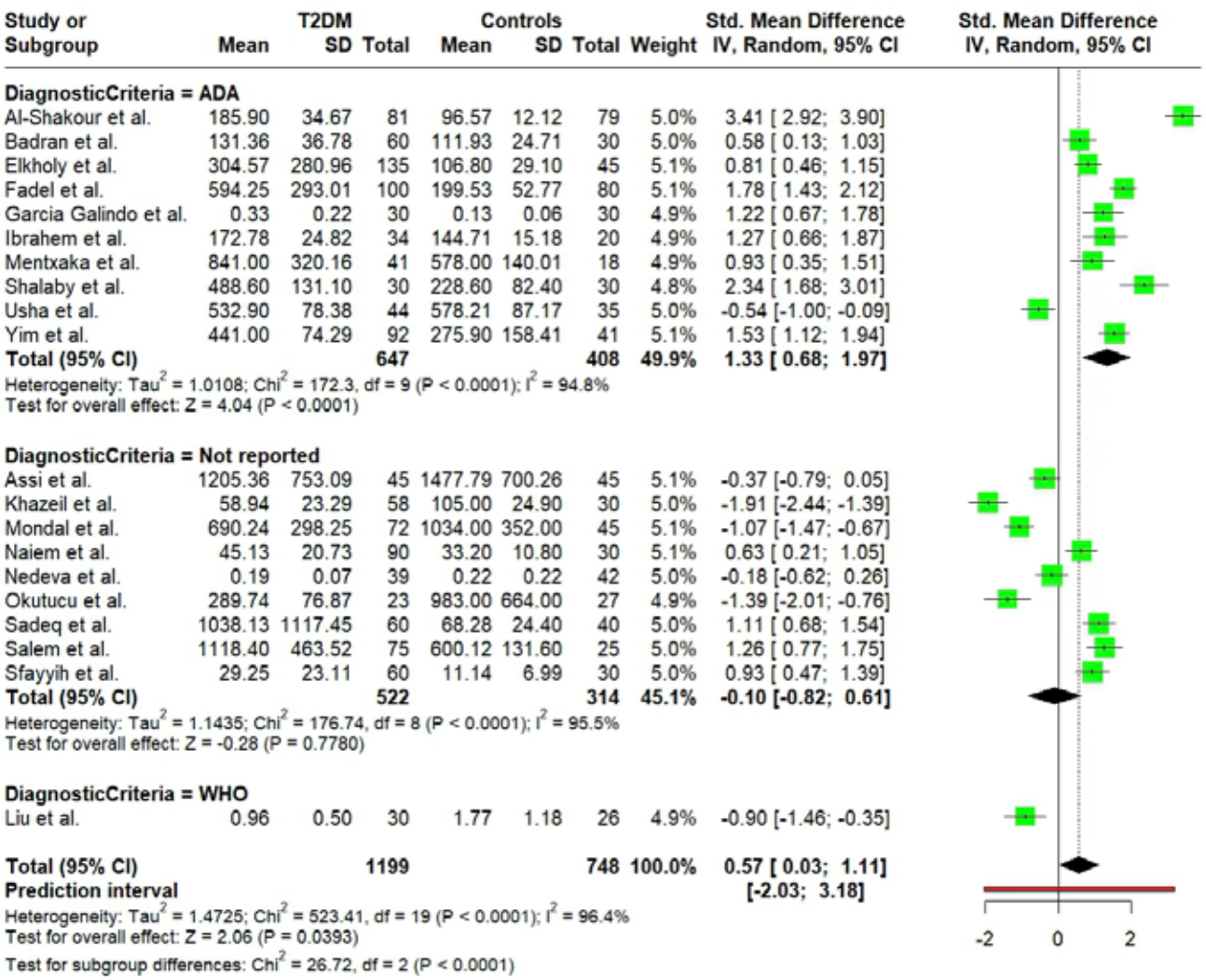


A: Al-Shakour et al. [11]; B: Assi et al. [13]; C: Badran et al. [21]; D: Elkholy et al. [22]; E: Fadel et al. [12]; F: Garcia Galindo et al. [23]; G: Ibrahem et al. [24]; H: Khazeil et al. [14]; I: Liu et al. [25]; J: Mentxaka et al. [25]; K: Mondal et al. [27]; L: Naiem et al. [28]; M: Nedeva et al. [29]; N: Okutucu et al. [30]; O: Sadeq et al. [31]; P: Salem et al. [32]; Q: Sfayyih et al. [33]; R: Shalaby et al. [34]; S: Usha et al. [35]; Yim et al. [36].

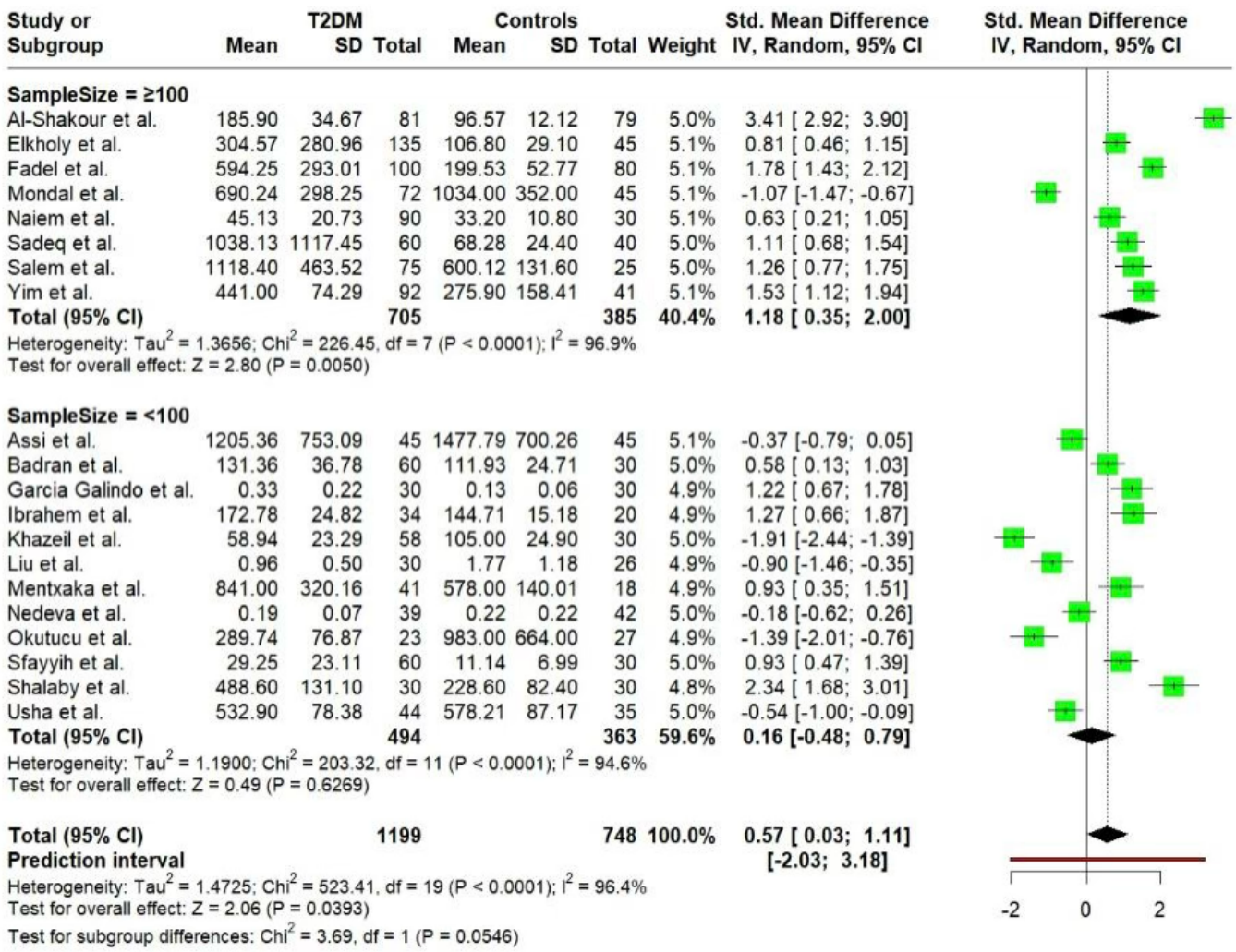
Supplementary Figure 2: Subgroup analysis by geographical region (country).



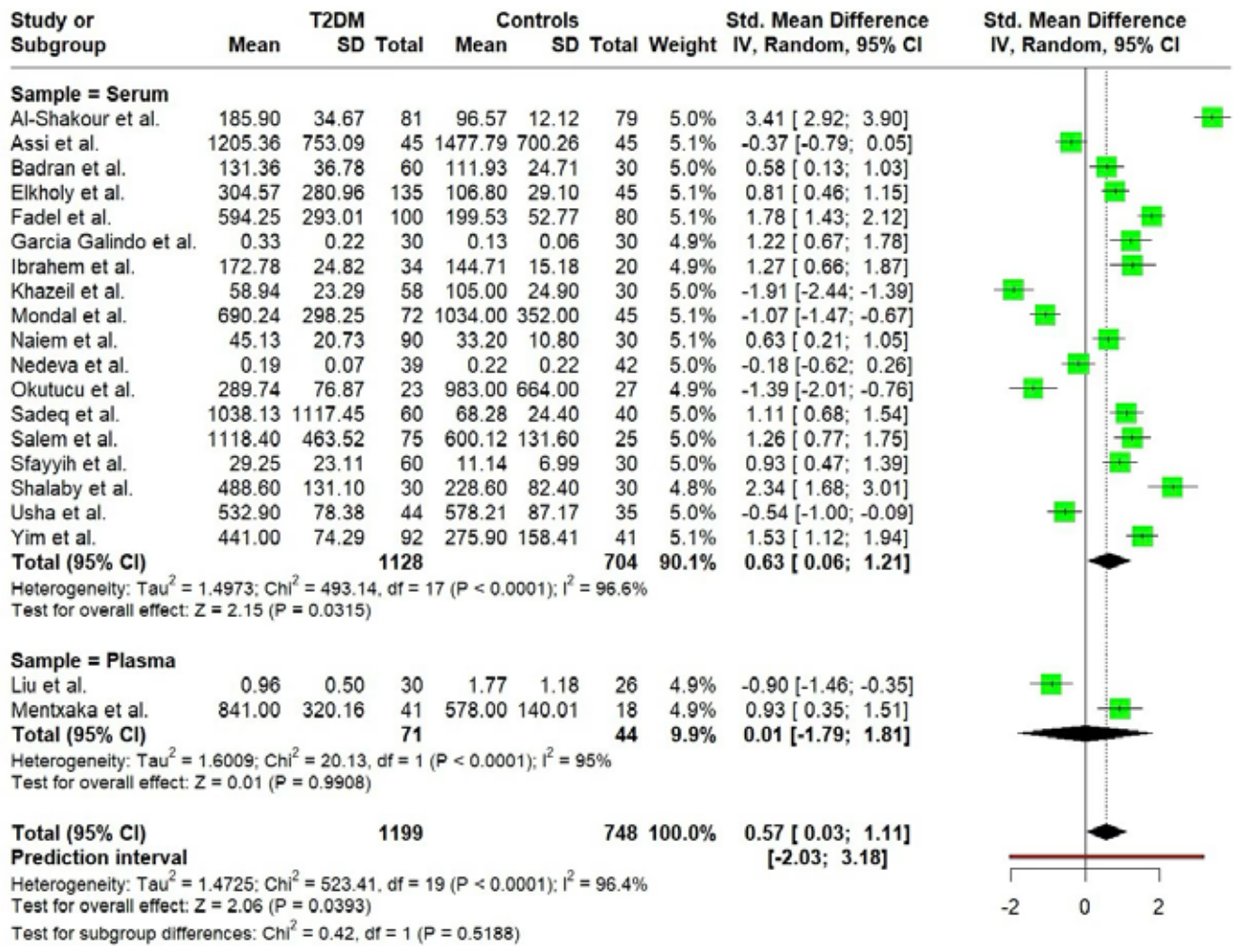
Supplementary Figure 3: Subgroup analysis by diagnostic criteria.



Supplementary Figure 4: Subgroup analysis by sample size.



Supplementary Figure 5: Subgroup analysis by biological sample type.



Supplementary Figure 6: Subgroup analysis by study design.