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ORIGINAL ARTICLE

Relationship between elevated circulating thrombospondin-1 levels and vascular complications in diabetes mellitus

Na Guo[1†], Linlin Yang[2†], Xiaozheng Wan[1,3], Dongze Qiu[3,4], Wenwen Sun[3,5], Huijuan Ma[2,3]*

[1]Graduate School of Hebei North University, Zhangjiakou, China, [2]Hebei Key Laboratory of Metabolic Diseases, Hebei General Hospital, Shijiazhuang, China, [3]Department of Endocrinology, Hebei General Hospital, Shijiazhuang, China, [4]Graduate School of Hebei Medical University, Shijiazhuang, China, and [5]Graduate School of North China University of Science and Technology, Tangshan, China

Keywords
Diabetes complications, Diabetes mellitus, Thrombospondin-1

***Correspondence**
Huijuan Ma
Tel: +86-180-3283-8686
E-mail address:
huijuanma76@163.com

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ABSTRACT

Aims/Introduction: Thrombospondin-1 (TSP-1) participates in a series of physiological and pathological processes by binding to various receptors regulating cell proliferation, adhesion and apoptosis. Elevated circulating TSP-1 is linked with diabetic vascular complications (DVC). This study aimed to determine the relationship between circulating TSP-1 levels and DVC.

Materials and Methods: A comprehensive search of PubMed, Embase, Web of Science and CNKI databases was carried out. A meta-analysis was carried out to compare circulating TSP-1 levels between diabetes patients without vascular complications (DNVC), diabetes patients with DVC and non-diabetes patients. The correlation between TSP-1 and metabolic parameters was also analyzed. Subgroup analysis was carried out according to complication type, defined as diabetic retinopathy, diabetic nephropathy and diabetic cardiovascular disease (DCVD).

Results: A total of eight studies were included. Compared with non-diabetes patients, diabetic patients, including DNVC and DVC, had significantly higher circulating TSP-1 levels (standardized mean difference [SMD] 2.660, 95% CI 1.17–4.145, $P = 0.000$). DNVC had significantly higher circulating TSP-1 levels than non-diabetes patients (SMD 3.613, 95% CI 1.607–5.619, $P = 0.000$). DVC had significantly higher TSP-1 levels than DNVC (SMD 0.568, 95% CI 0.100–1.036, $P = 0.017$). TSP-1 was significantly positively correlated with fasting plasma glucose (overall Fisher's $z = 0.696$, 95% CI 0.559–0.833) and HbA1c (overall Fisher's $z = 0.849$, 95% CI 0.776–0.923).

Conclusions: Elevated circulating TSP-1 levels are closely related to DVC, especially in diabetic nephropathy and diabetic cardiovascular disease. Circulating TSP-1 detection might be helpful in the timely diagnosis and treatment of DVC.

INTRODUCTION

Diabetes, a metabolic disease characterized by chronically elevated blood glucose levels due to insulin deficiency, is a serious health problem in many developed and developing countries, and has become a major burden to human health[1]. During the chronic progression of high blood glucose, persistent oxidative stress and inflammation challenges the microenvironment of functional cells in the eyes, kidneys and cardiovascular system, thereby inducing dysfunction of multiple organs[2]. For example, approximately one-third of diabetes patients develop renal dysfunction, and ~15–40% of diabetes patients eventually progress to end-stage renal failure, requiring long-term hemodialysis or kidney transplantation[3]. Another major diabetic complication, diabetic retinopathy (DR), has an incidence of >50% in diabetes patients, and has hence been recognized as one of the leading causes of adult visual impairment[4]. Diabetic cardiovascular disease (DCVD) has a mortality rate of ~70–80%[5], and seriously affects the quality of life of diabetes patients[6]. However, due to the lack of biomarkers in the early stage of the disease, diabetes

†These authors contributed equally to this work.
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patients with vascular complications (DVC) can only be diagnosed as such when patients present with symptoms of complications in clinic[7].

Thrombospondin-1 (TSP-1) is a multifunctional, extracellular matrix glycoprotein found in platelets, which is secreted during platelet activation and is closely related to the pathogenesis of many cardiovascular diseases, such as myocardial infarction, atherosclerosis and pulmonary hypertension[8–10]. TSP-1 expression is also increased in many organs in animal models of obesity and diabetes, such as the kidneys[11], adipose tissue[12], heart[13] and blood vessels[14]. Previous research shows a close relationship between TSP-1 and the development of DVC. For example, TSP-1 promotes the pathological events associated with diabetic nephropathy (DN), such as mesangial cell proliferation and increased extracellular matrix production by mesangial cells[15]. By contrast, TSP-1 levels in the aqueous humor and vitreous fluids of the eye are decreased in diabetes patients[16]. These conflicting observations have made the use of TSP-1 in diagnosing and treating DVC difficult. The relationship between TSP-1, especially circulating TSP-1 levels, and DVC is unclear.

Given this context, we carried out a meta-analysis based on circulating TSP-1 levels in DVC, DNVC and NDM to systematically investigate the association between circulating TSP-1 levels and DVC, and determine the utility of TSP-1 as a novel clinical target for the diagnosis and treatment of DVC.

MATERIALS AND METHODS

The present study was registered on the International Prospective Register of Systematic Reviews (PROSPERO); the registration number is CRD42023402960.

Search strategy

We used the following keywords "diabetes," "diabetes mellitus," "DM," "thrombospondin-1," "TSP-1" and "THBS-1" to search four electronic databases (CNKI, PubMed, EMBASE and Web of Science) up to 20 February 2023. There were no language restrictions.

Inclusion and exclusion criteria

Eligible studies were required to meet the following criteria: (1) the study should report circulating TSP-1 levels in patients with diabetic complications, simple diabetes without complications and healthy individuals; (2) the diagnosis of diabetes and diabetic complications should be clearly stated; (3) the study design should be observational; and (4) the study data should be reported as mean ± standard deviation or provide information from which these measures could be derived.

The following criteria were used to exclude studies: (1) duplicate studies from different databases; (2) animal studies, reviews and individual case reports; (3) studies that used previously published research data; and (4) literature with incomplete information or insufficient data to calculate the statistics for the present study.

Data extraction and quality assessment

We had two researchers independently search and screen the literature, and collect and cross-check relevant data. If the results were inconsistent, these researchers would discuss the paper to reach a conclusion or it would be judged by a third, senior researcher. Standardized data extraction tables were used to extract data from the included literature and were summarized independently. In addition to screening the literature and extracting data, the two researchers used the Newcastle–Ottawa Scale to assess the quality of the included studies. The Newcastle–Ottawa Scale includes three levels of study quality: low = 0–3; medium = 4–6; and high = 7–9. The final score was calculated based on the average score given by the two researchers.

The following data were collected: (1) first author's name; (2) year of publication; (3) country; (4) sample size; (5) age; (6) sex of participants; (7) sample size of case group and control group; (8) circulating TSP-1 levels in the case group and control group (represented by mean ± standard deviation); (9) measurement method and assay range of circulating TSP-1; and (10) Pearson's correlation coefficients between TSP-1 and metabolic parameters.

Statistical analysis

We carefully sorted and checked the data according to the requirements of a meta-analysis. Stata/SE15.0 software (StataCorp, College Station, TX, USA) was used for statistical analysis. Standardized mean differences (SMDs) and 95% confidence intervals (CIs) were used for quantitative analysis of selected studies. Correlation coefficient values were converted by Fisher's *r*-to-*z* transformation to obtain approximately normally distributed

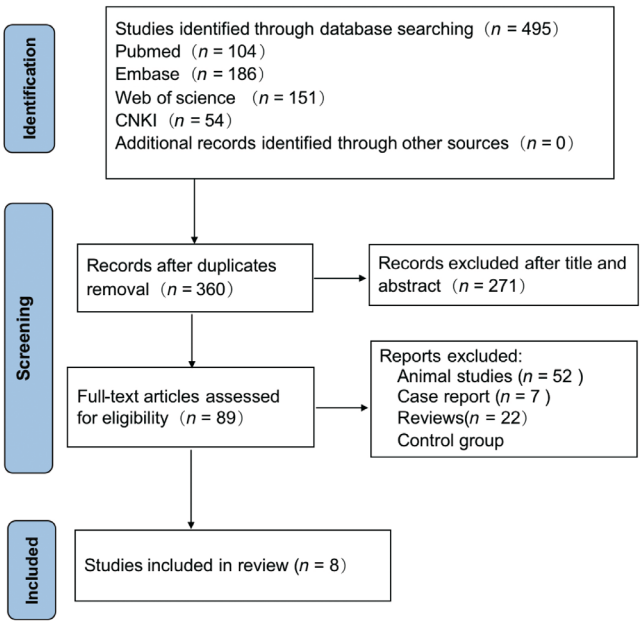

Figure 1 | Literature screening process and results.

Table 1 | Characteristics of the included studies

First author	Year	Country	Sample size (*n*)	Study design	Outcome	Group	*n*	M/F	Age (mean ± SD or range)	TSP-1 (mean ± SD)	Measurement method	Assay range	FPG (*r*)	FINS (*r*)	HbA1c (*r*)	NOS score
Lian[17]	2016	China	178	Case–control	DR	DR	54	31/23	54.07 ± 8.96	329.61 ± 70.01	ELISA	1.563–100 ng/mL	0.601	– 0.514	0.63	7
						DNVC	64	33/31	51.55 ± 7.27	287.10 ± 49.12						
						HP	60	32/28	50.01 ± 7.88	108.56 ± 11.00						
Bian[18]	2017	China	120	Case–control	DR	PDR	29	14/15	49.55 ± 10.18	329.26 ± 37.81	ELISA	0.78–50 ng/mL	0.57	0.288	0.693	7
						NPDR	31	14/17	52.48 ± 11.78	284.66 ± 38.74						
						DNVC	31	16/15	49.97 ± 11.67	261.12 ± 38.11						
						HP	29	15/14	50.45 ± 9.46	121.60 ± 10.78						
Wang[19]	2017	China	87	Case–control	DR	DR	48	25/23	40–72	96.67 ± 22.61	ELISA	NR	NR	NR	NR	6
						DNVC	24	12/12	40–67	164.54 ± 57.68						
						HP	15	8/7	25–62	249.56 ± 83.64						
Ning[20]	2015	China	269	Case–control	DN	DN	90	46/44	53.34 ± 10.12	333.14 ± 69.20	ELISA	1.563–100 ng/mL	0.515	– 0.502	0.692	7
						DNVC	92	47/45	52.21 ± 11.33	281.54 ± 48.92						
						HP	87	44/43	50.33 ± 9.14	106.84 ± 10.11						
Qi[21]	2016	China	286	Case–control	DN	DN	94	50/44	53.2 ± 5.8	345.26 ± 70.12	ELISA	0.78–50 ng/mL	NR	NR	NR	7
						DM	92	49/43	53.6 ± 5.9	275.63 ± 45.29						
						HP	100	55/45	53.0 ± 5.6	102.47 ± 10.25						
Bian[22]	2017	China	158	Case–control	DN	DN	56	26/30	53.88 ± 10.92	310.82 ± 45.62	ELISA	0.78–50 ng/mL	0.71	0.255	0.746	7
						DNVC	52	28/24	50.60 ± 11.79	262.59 ± 36.48						
						HP	50	26/24	50.16 ± 9.86	121.83 ± 11.28						
Choi[23]	2012	Korea	257	Case–control	CVD	DCVD	103	62/41	65.8 ± 9.7	579.00 ± 106.10	ELISA	7.8–500 ng/mL	NR	NR	NR	6
						DNVC	46	16/30	64.1 ± 9.1	532.00 ± 123.80						
						HP	108	72/36	63.0 ± 11.7	518.00 ± 126.80						
Ma[24]	2020	China	277	Case–control	CVD	DCVD	99	NR	NR	329.62 ± 70.02	ELISA	NR	NR	NR	NR	7
						DNVC	98	NR	NR	287.11 ± 49.13						
						HP	80	47/33	NR	108.57 ± 11.01						

DCVD, diabetic cardiovascular disease; DN, diabetic nephropathy; DR, diabetic retinopathy; FINS, fasting insulin; FPG, fasting plasma glucose; NR, not reported; *r*, Pearson's correlation coefficient.

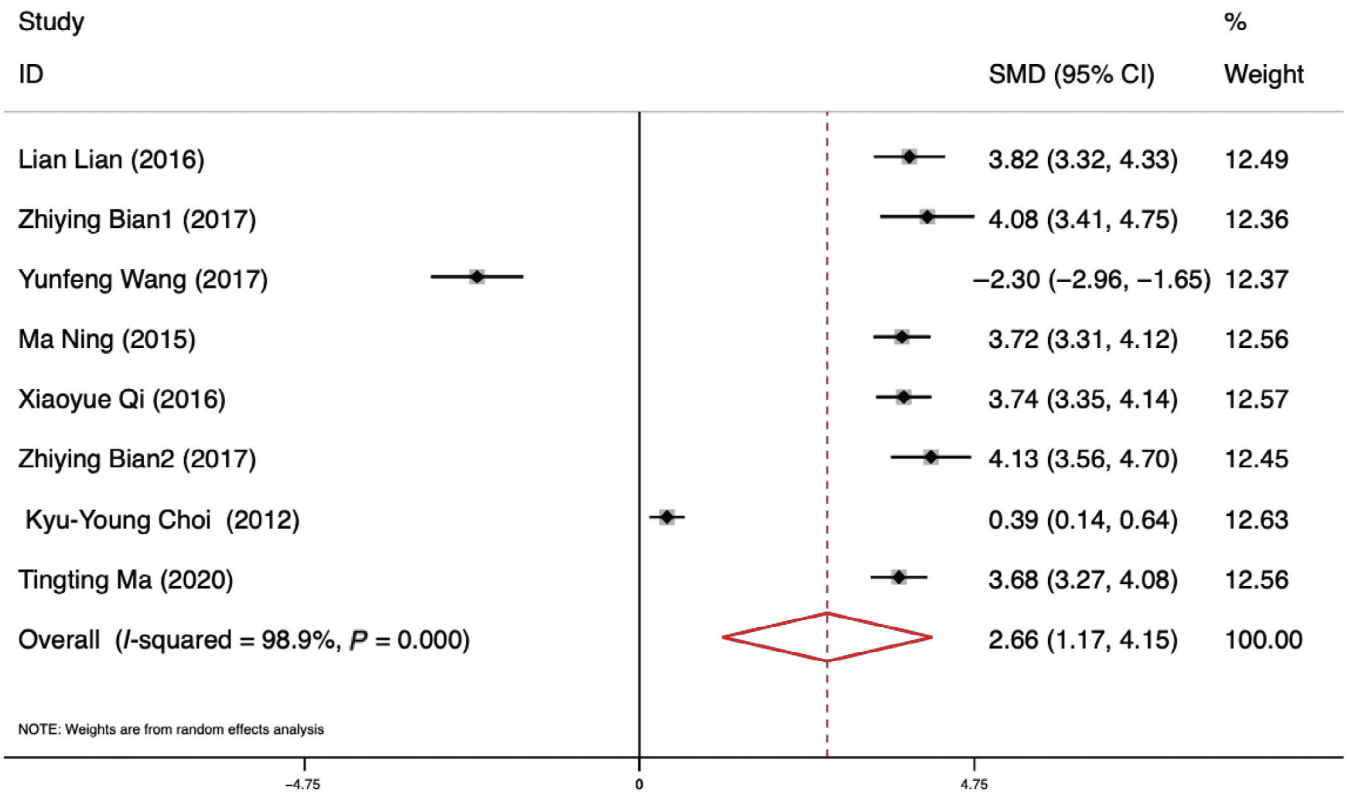

Figure 2 | Forest plot of circulating thrombospondin-1 levels in patients containing diabetic vascular complications and diabetes patients without vascular complications compared with non-diabetes patients. CI, confidence interval; SMD, standardized mean difference.

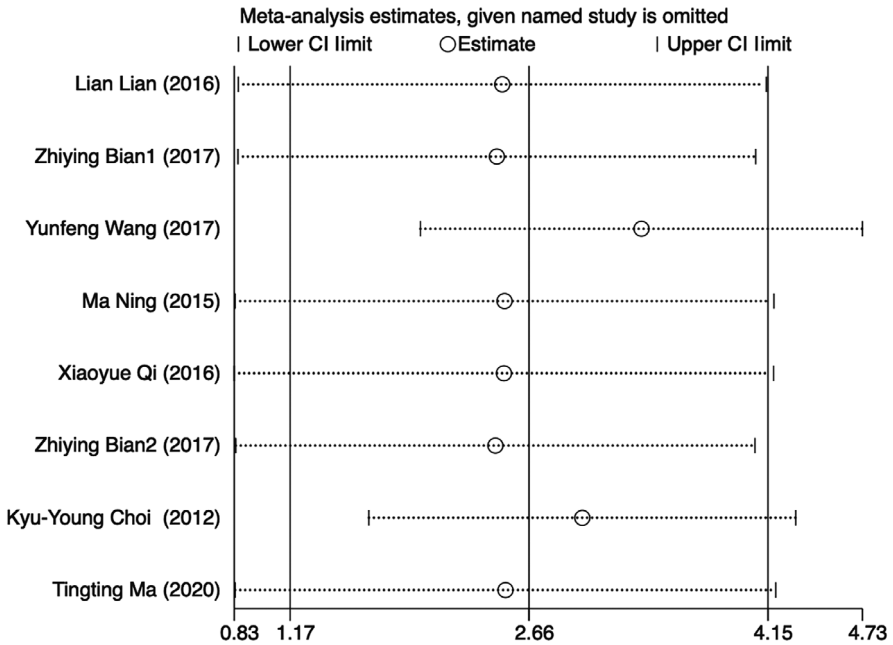

Figure 3 | Results of the sensitivity analysis of the eight studies. CI, confidence interval.

z-values to further calculate 95% CIs. The I^2-test was used to assess heterogeneity between studies. When $I^2 \leq 50\%$, heterogeneity was considered statistically insignificant, and a fixed effects model was used for analysis. Otherwise, a random effects model was used for analysis. Egger's test was used to evaluate publication bias, with $P < 0.05$ defining significant publication bias. Sensitivity analysis was used to evaluate robustness of the results.

RESULTS

Characteristics of included studies

Our search identified 495 records. These included 135 duplicate articles that were removed, leaving 360 articles to be screened by title and abstract. Out of the 360 articles screened, 271 articles were excluded, because they were not related to the content of our meta-analysis. Of the remaining 89 articles, 52 were carried out in animal or cell culture models involving the mechanism of TSP-1 involvement in diabetes, seven were case reports and 22 were reviews or conference abstracts where the full text could not be found. All of these articles were excluded, and eight studies[17–24] finally met the inclusion criteria of meta-analysis. All eight studies were case–control studies. The flowchart of the article selection process is shown in Figure 1. These studies were published between 2012 and 2020, and included a total of 604 DVC, 499 DNVC and 529 NDM. The basic characteristics of the studies are shown in Table 1. One study assigned DR into proliferative and non-proliferative stages; this data was merged. The included articles were rated as moderate or high quality according to the Newcastle–Ottawa Scale.

Circulating TSP-1 concentrations in DVC and DNVC compared with NDM

We pooled patients with DVC and DNVC from eight studies, and compared circulating TSP-1 concentrations with NDM. As there was significant heterogeneity between the studies ($I^2 = 98.9\%$, $P = 0.000$), a random effects model was used. As shown in Figure 2, compared with NDM, DVC and DNVC had significantly higher circulating TSP-1 levels (SMD 2.660, 95% CI 1.174–4.145, $P = 0.000$). Egger's test showed no publication bias ($P = 0.348$). Sensitivity analysis showed that excluding any individual study did not significantly affect the overall results (Figure 3).

Circulating TSP-1 concentrations in DNVC compared with NDM

A random effects model was used to merge study data ($I^2 = 98.8\%$, $P = 0.000$). Circulating TSP-1 was significantly higher in DNVC than NDM (SMD 3.613, 95% CI 1.607–5.619,

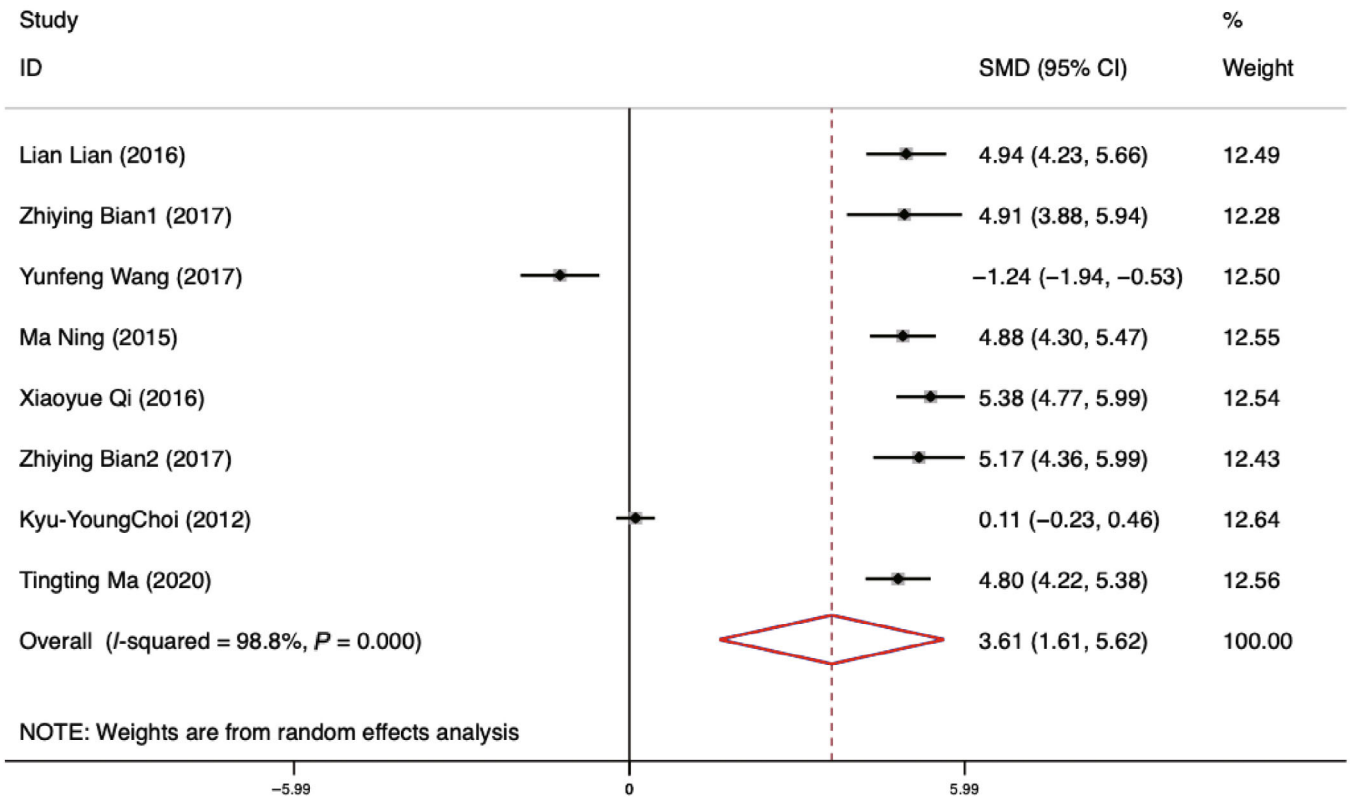

Figure 4 | Forest plot of circulating thrombospondin-1 levels in patients with diabetes patients without vascular complications compared with non-diabetes patients. CI, confidence interval; SMD, standardized mean difference.

P = 0.000; Figure 4). Egger's test showed no publication bias (P = 0.109).

Circulating TSP-1 concentrations in DR, DN and DCVD compared with DNVC

Circulating TSP-1 was significantly higher in DVC than DNVC (SMD 0.568, 95% CI 0.100–1.036, P = 0.017; Figure 5). There was no publication bias (P = 0.131), but significant heterogeneity (I^2 = 92.4%, P = 0.000). Subgroup analysis according to the type of diabetes complications was carried out; although there was no difference between patients with DR and DNVC (SMD 0.009, 95% CI −1.531 to 1.550, P = 0.991), circulating TSP-1 in patients with DN (SMD 1.051, 95% CI 0.840–1.263, P = 0.000) and DCVD (SMD 0.580, 95% CI 0.306–0.854, P = 0.000) were significantly higher than in DNVC (Figure 6).

The correlation between TSP-1 and FPG

Four studies reported associations between TSP-1 and glycemic parameters. Pooled results from these studies showed a positive correlation between TSP-1 and FPG (overall Fisher's z = 0.696, 95% CI 0.559–0.833). Heterogeneity was significant between included studies (I^2 = 70.2%, P = 0.018; Figure 7).

The correlation between TSP-1 and fasting insulin

Four studies showed that no correlation was observed between circulating TSP-1 and fasting insulin (overall Fisher's z = −0.143, 95% CI −0.610 to 0.323). There was significant heterogeneity between studies (I^2 = 97.4%, P = 0.000; Figure 8).

The correlation between TSP-1 and glycated hemoglobin

Data from four studies showed a significant positive correlation between TSP-1 and glycated hemoglobin (overall Fisher's z = 0.849, 95% CI 0.776–0.923; Figure 9). Between-study heterogeneity was not significant (I^2 = 26.4%, P = 0.253).

DISCUSSION

In the present study, we assessed the association between TSP-1 and DVC. A meta-analysis was carried out to compare circulating TSP-1 levels between DNVC, DVC and NDM. Circulating TSP-1 levels in distinct DVCs, and the correlation between TSP-1 and metabolic parameters was also investigated.

We observed that circulating TSP-1 was significantly positively correlated with FPG and glycated hemoglobin, which is in line with the effect of glucose on stimulating TSP-1 production[25]. *In vitro* research using blood vessel cells (fibroblasts[26], vascular smooth muscle cells[27] and mesangial

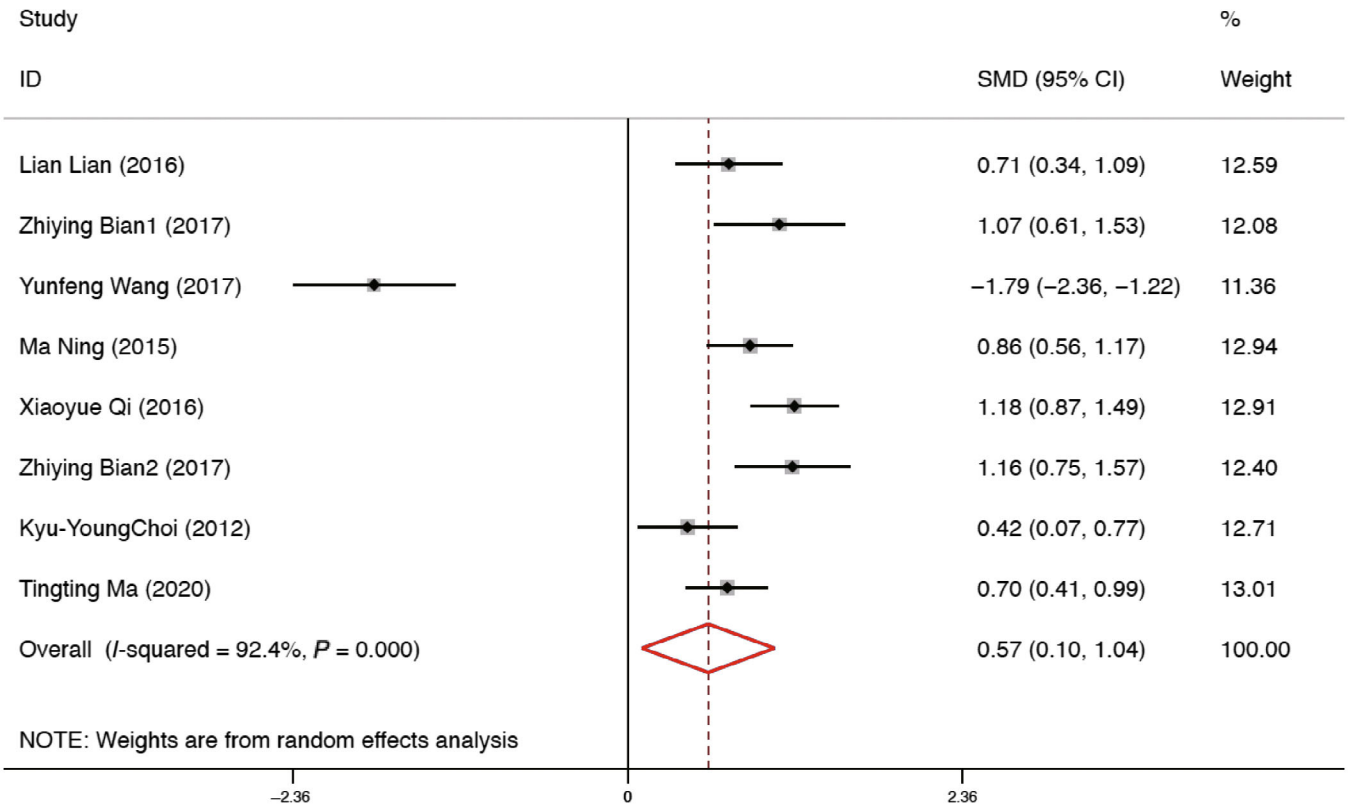

Figure 5 | Forest plot of circulating thrombospondin-1 levels in patients with diabetic vascular complications compared with diabetes patients without vascular complications.

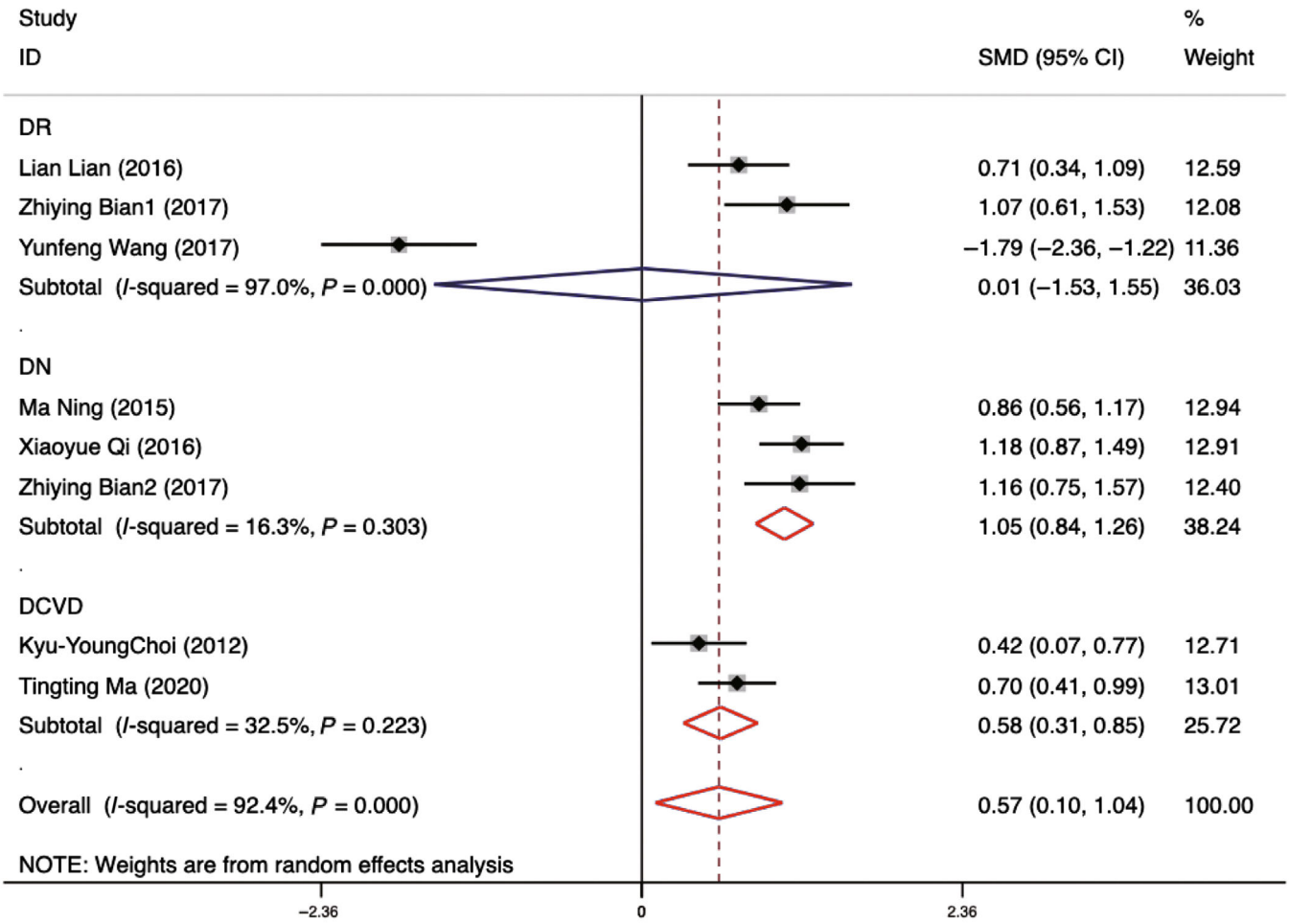

Figure 6 | Subgroup analysis forest plots of circulating thrombospondin-1 levels in patients with diabetic retinopathy, diabetic nephropathy and diabetic cardiovascular disease compared with diabetes patients without vascular complications. CI, confidence interval; SMD, standardized mean difference.

cells[28]) consistently shows upregulated TSP-1 expression in the presence of glucose. Together, results suggest that TSP-1 might link hyperglycemia and accelerated vascular complications in diabetes.

Long-term diabetes results in structural and functional abnormalities of blood vessels, characterized by microvascular and macrovascular diabetic complications[29]. Previous studies describe abnormal extracellular matrix accumulation as a marker for morphological changes of target organs in diabetic complications[30–33]. As a widely expressed glycoprotein, TSP-1 mediates cell communication, as well as complex interactions between cells and the surrounding extracellular matrix[34]. Due to the lack of indicators, it is difficult to diagnose and treat DVC in time. Some previous *in vitro* studies based on cells and animals have found that TSP-1 is closely related to the pathogenesis of vascular complications. The present study results suggested that the level of circulating TSP-1 were elevated in patients with diabetes, and were further elevated in diabetes patients with vascular complications. This change trend of TSP-1 indicates that the level of circulating TSP-1 increases with the progression of diabetes. We conclude that it might be possible to use circulating TSP-1 levels to help diagnose and treat in the occurrence of vascular complications of diabetes in clinical practice.

To further determine the association between TSP-1 and distinct DCV, we analyzed circulating TSP-1 levels in different subgroups. Significantly higher circulating TSP-1 levels were observed in the DN and DCVD groups compared with the DNCV group. DN is a common diabetic complication characterized by increased extracellular matrix accumulation, which is stimulated by the production and activation of transforming growth factor (TGF)-beta[35–37]. *In vitro* studies showed that TSP-1 is an activator of TGF-beta in DN[25,38]. Immunohistological evaluation shows that, in the glomeruli of TSP-1-deficient

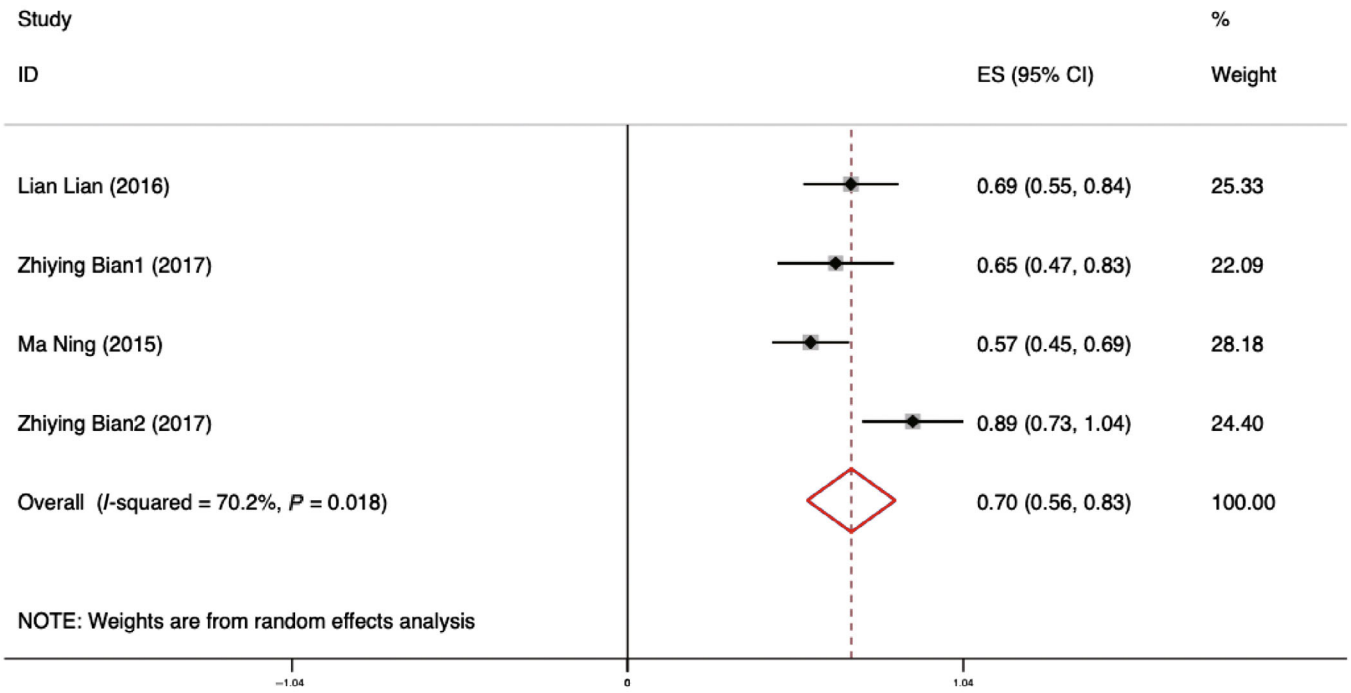

Figure 7 | Forest plot of the correlation between thrombospondin-1 and fasting plasma glucose. CI, confidence interval; ES, Fisher's *z*.

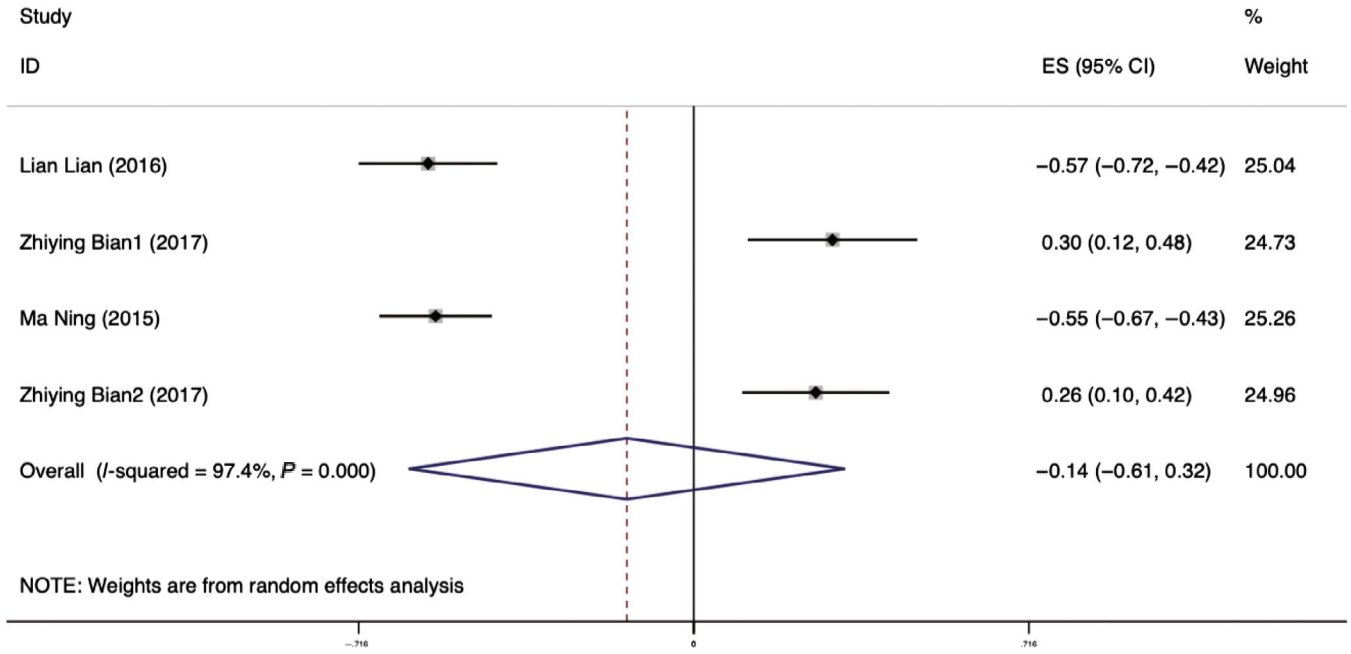

Figure 8 | Forest plots of the correlation between thrombospondin-1 and fasting insulin. CI, confidence interval; ES, Fisher's *z*.

mice, the active form of TGF-beta is significantly reduced[39]. In addition, TSP-1 activates latent TGF-beta in mesangial cells cultured with high glucose concentrations[40]. The present study found significantly higher circulating TSP-1 levels in patients with DN, which further confirms the association between TSP-1 and DN. We also observed significantly higher circulating

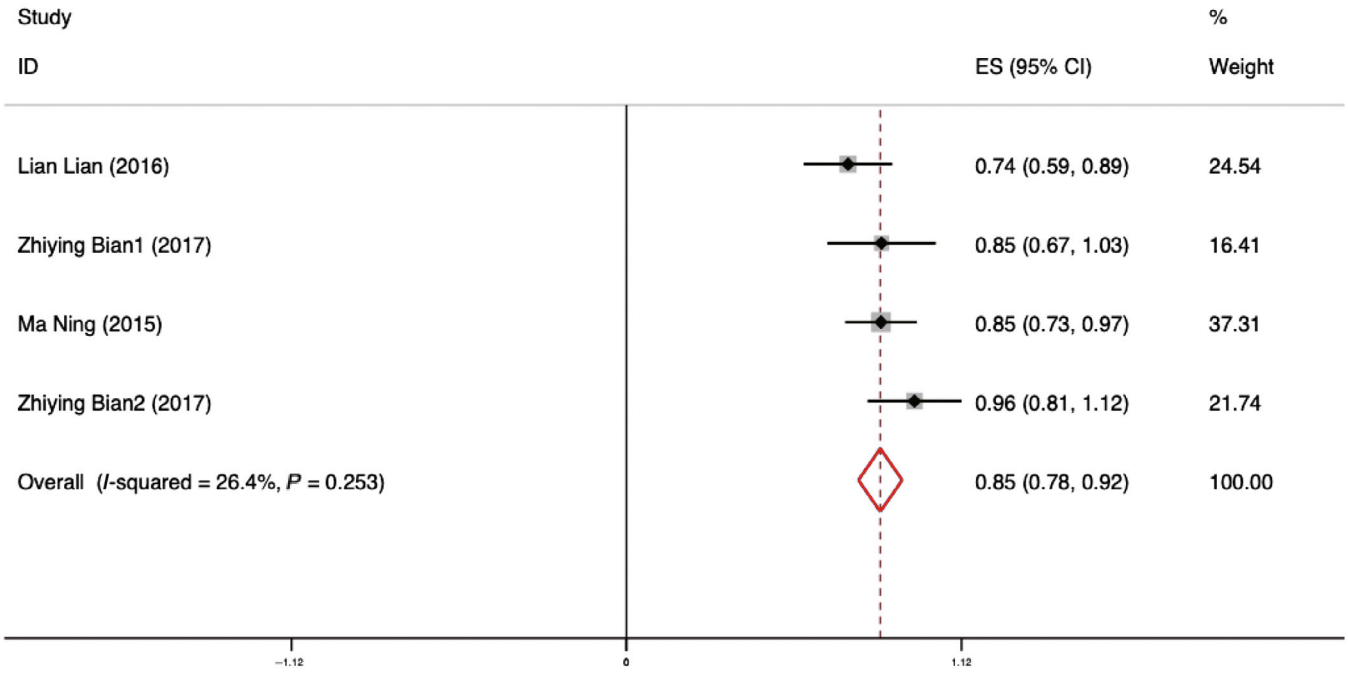

Figure 9 | Forest plots of the correlation between thrombospondin-1 and glycated hemoglobin (HbA1c). CI, confidence interval; ES, Fisher's *z*.

TSP-1 levels in DCVD compared with DNVC. Previous *in vitro* studies suggest that TSP-1 is a potent proatherogenic and antiangiogenic protein. As a multifunctional protein, TSP-1 stimulates vascular smooth muscle cells proliferation[14,41] and triggers the development of atherosclerotic lesions[14].

Unlike the elevated TSP-1 levels in patients with DN and DCVD, there were conflicting observations in patients with DR. Among the three datasets that included patients with DR[15–17], two showed significantly higher circulating TSP-1 levels in patients with DR compared with DNVC, whereas the other dataset reported significantly lower circulating TSP-1 levels in patients with DR than DNVC. Interestingly, there are similar observations in animal models of DR. Sheibani *et al.*[16] reported decreased TSP-1 in the vitreous fluid of diabetic mice, and found that a high-glucose environment suppresses TSP-1 secretion from vascular endothelial cells in a time-dependent manner. By contrast, Xu *et al.*[42] observed elevated TSP-1 expression in the retina of diabetic rats, whereas retinal endothelial cells showed significantly increased TSP-1 after culturing in high glucose for 48 h. No exact mechanism has been found to explain these inconsistent results; however, they might be related to the complex and delicate structure of eyes. Further efforts are needed to comprehensively interpret the cell- and tissue-specific effects of TSP-1 in the pathogenesis of DR.

The present meta-analysis investigated the association between circulating TSP-1 levels and DVC. Although the association between TSP-1 and DR was undetermined due to the cell type and tissue-specific, TSP-1 appears to have diagnostic and therapeutic significance in DVC, especially in patients with DN and DCVD. Further efforts are required to clarify the role of TSP-1 in the pathogenesis of DR.

The present study was not without limitations. First, factors, such as treatment status and blood lipid levels, might have an impact on TSP-1; second, although we did not observe a significant difference across different laboratories, the effect of different TSP-1 measurement methods is still unknown. Third, the severity of complications was not graded in the original data we included in the study; therefore, we were unable to determine the relationship between TSP-1 and different degrees of diabetic complications. Fourth, most of participants included in this study were from China, and our results might be more applicable to the Chinese population. Finally, as all the studies analyzed in this article were cross-sectional studies, whether perturbed TSP-1 occurred before DVC cannot be concluded. Further follow-up design in a larger cohort is warranted to determine the causal relationship between TSP-1 and DVC.

Overall, we found that elevated circulating TSP-1 levels were associated with an increased risk of DN and DCVD. In clinical practice, detection of circulating TSP-1 in diabetes patients might be helpful in the timely diagnosis and treatment of DVC.

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DISCLOSURE

The authors declare no conflict of interest.
Approval of the research protocol: The research protocol was carried out based on the PRISMA guideline.
Informed consent: N/A.
Registry and the registration no. of the study/trial: This meta-analysis was registered with PROSPERO as CRD42023402960 on 8 March 2023.
Animal studies: N/A.

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