SYSTEMATIC REVIEW

Angiogenesis outcomes of metformin utilization in diabetes mellitus: a systematic review and meta-analysis

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Abstract

Objective: : To review relevant literature regarding the role of metformin in angiogenesis among diabetic patients.

Method: The systematic review and meta-analysis conducted from May to September 2022, and comprised search on Medline, ScienceDirect, ProQuest, Web of Science, EBSCOhost and Cochrane Library databases. The studies included were published in the English language and were human studies having angiogenesis endothelial markers as the outcomes of interest among patients of type 2 diabetes mellitus undergoing metformin therapy. Endothelial markers, including vascular endothelial growth factor, von-Willebrand-factor, plasminogen activator inhibitor-1, soluble vascular adhesion molecule-1, intercellular adhesion molecule-1, soluble endothelial-selectin, tissue plasminogen activator, urinary albumin excretion, platelet endothelial cell adhesion molecule-1 and thrombin-activatable fibrinolysis inhibitor, were assessed as angiogenesis outcomes. Data was statistically analysed using Review Manager 5.4.

Results: Of the 413 studies identified, 8(1.9%) were included; 5(62.5%) randomised control trials, 2(25.0%) cross-sectional, and 1(12.5%) cohort studies, with overall 1199 patients. Among the outcomes, von-Willebrand-factor (p=0.01), soluble vascular adhesion molecule-1 (p<0.00001), intercellular adhesion molecule-1 (p=0.0003), soluble endothelial-selectin (p=0.007), and tissue plasminogen activator (p<0.00001) showed significantly lower levels after metformin treatment using the random effect methods.

Conclusion: Metformin was found to have an additional effect of endothelial function improvement.

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Introduction

An imbalance in the production of vasodilators and vasoconstrictors, which affects smooth muscle cell proliferation, coagulation and platelet action factors, and the expression of pro-inflammatory cytokines is known as endothelial dysfunction. Nitric oxide (NO), prostacyclin (PGI2), von Willebrand factor (vWF), tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) are among the molecules that endothelial cells produce to carry out the task.[1]

Any blood vessel can experience endothelial dysfunction, including the cardiovascular system. Endothelial damage in the cardiovascular system can be made worse by risk factors, like smoking, diabetes mellitus (DM), hypertension (HTN), etc. Atherosclerosis, which impairs arterial function, frequently coexists with endothelial impairment related to the cardiovascular system.[2] The body must engage in angiogenesis to create new blood vessels when blood vessels are injured.

The key mechanisms through which metformin, a biguanide class drug, works include inhibition of gluconeogenesis and increase of insulin sensitivity. It is anticipated that several of these medications will promote angiogenesis by increasing NO signalling.[3] NO signalling is triggered by the angiogenic factors adenosine monophosphate (AMP) and endothelial NO synthase (eNOS), which are activated. Metformin is also claimed to have anti-oxidative and anti-inflammatory properties that affect blood vessels.[4]

The current systematic review was planned to review relevant literature regarding the role of metformin in angiogenesis among diabetic patients.

Materials and Methods

The systematic review and meta-analysis was conducted

from May to September 2022, and comprised search on Medline, ScienceDirect, ProQuest, Web of Science, EBSCOhost and Cochrane Library databases. The review was done in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[5] This review was registered with the International Prospective Register of Systematic Reviews (PROSPERO) database (https://www.crd.york.ac.uk/prospero/) (CRD42022307837).[6]

The studies included were published in the English language and were human studies having angiogenesis endothelial markers as the outcomes of interest among patients of type 2 DM (T2DM) undergoing metformin therapy. Review articles, case series, unpublished articles, abstract-only, and irrelevant articles were excluded. The key words and Boolean operators used in the search were Metformin OR Biguanide AND diabetes mellitus AND VEGF OR vascular endothelial growth factor OR PECAM1 OR PECAM-1 OR platelet endothelial cell adhesion molecule-1 OR CD31 OR cluster of differentiation 31 OR vWF OR von Willebrand factor OR NOS3 OR nitric oxide synthase 3 OR intercellular adhesion molecule OR ICAM. The identified studies were entered into Rayyan systematic review software (https://www.rayyan.ai) for initial screening.[7]

The studies were systematically searched for eligibility by 4 authors. Data was collected regarding first authors' names, publication year, study design, country of origin, patients' age, sample size, metformin intervention, and angiogenesis outcomes. The other authors examined the data with the aim of clearing up any confusion. Any disagreement was resolved through team discussion.

The risk of bias was accessed by 2 reviewers using Cochrane risk of bias tool for randomised trial (RoB) version 2. Randomisation, intended interventions, measurement of outcomes, missing outcomes and selective reporting were assessed in ROB and rated as low, some concerns, and high-risk categories.[8] For cross-sectional and cohort studies, the Newcastle-Ottawa Scale (NOS) assessment tool was used.[9]

Data was subjected to statistical analysis using Review Manager version 5.4. Heterogeneous studies were tested using the I2 test. The random effect model was used since the I2 test result presented >50% moderate-to-high heterogeneity. For dichotomous outcome, the risk ratio (RR) was pooled using the Mantel-Haenszel method.[10] Only three randomised controlled trials (RCTs) were analysed with the geometric mean, which was converted into standard deviation mean differences manually using the formula SD = N x (upper limit - lower limit) / 3.92. The standard deviation was divided by 3.92 because it used 95% confidence interval (CI).[11] Statistical analyses were considered significant with $p<0.05$. Odds ratio (OR) with 95% confidence interval (CI) were calculated, as appropriate. Begg and funnel plots were used statistically and visually for publication bias.

Results

Of the 413 studies identified, 8(1.9%) were included (Figure 1); 5(62.5%) RCTs, 2(25.0%%) cross-sectional studies, and 1(12.5%) cohort study (Supplementary 1). Cohort and cross-sectional studies showed high-quality results, with scores ranging 7-9. Among the RCTs, 3(37.5%) showed low risk of bias, while 2(25.0%) showed some concerns (Supplementary 2).

Of the 1,119 patients in the studies reviewed, 578(51.7%) were females. The number of participants in metformin and non-metformin groups was 650(58%) and 469(42%), respectively. Some studies reported that patients in the metformin group were more likely to be older.[11-13]

Diagnosis of T2DM was established using the American

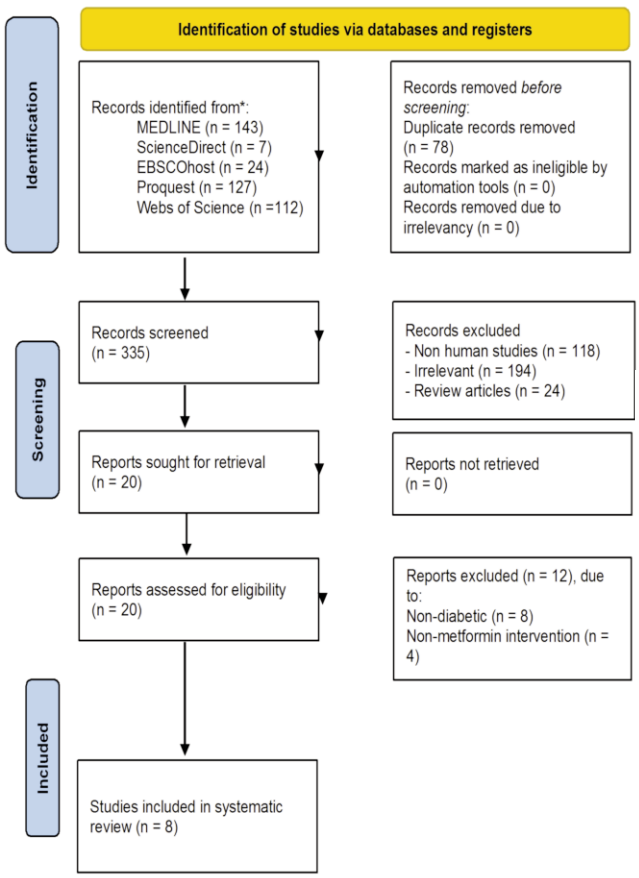
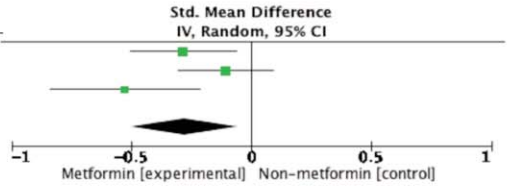


Figure-1:Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.

(A) vWF level's

Study	Metformin Mean	Metformin SD	Metformin Total	Non-metformin Mean	Non-metformin SD	Non-metformin Total	Weight	Std. Mean Difference IV, Random, 95% CI
De Jager et al., 2005	-6	18.74	150	0	22.8	163	35.4%	-0.29 [-0.51, -0.06]
De Jager et al., 2014	-1	98	196	12	139.2	194	38.2%	-0.11 [-0.31, 0.09]
Lund et al., 2008	-11	13.94	83	-3	16.17	82	26.5%	-0.53 [-0.84, -0.22]
Total (95% CI)			429			439	100.0%	-0.28 [-0.50, -0.06]

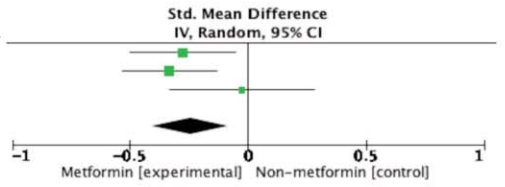
Heterogeneity: Tau² = 0.02; Chi² = 5.14, df = 2 (P = 0.08); I² = 61%
Test for overall effect: Z = 2.49 (P = 0.01)



(B) PAI-1

Study	Metformin Mean	Metformin SD	Metformin Total	Non-metformin Mean	Non-metformin SD	Non-metformin Total	Weight	Std. Mean Difference IV, Random, 95% CI
De Jager et al., 2005	79.4	63.42	150	98.8	75.23	163	36.0%	-0.28 [-0.50, -0.05]
De Jager et al., 2014	33.6	38.21	196	48.7	51.17	194	41.5%	-0.33 [-0.53, 0.13]
Lund et al., 2008	17.4	180.12	83	22.7	226.62	82	22.5%	-0.03 [-0.33, -0.28]
Total (95% CI)			429			439	100.0%	-0.24 [-0.41, -0.08]

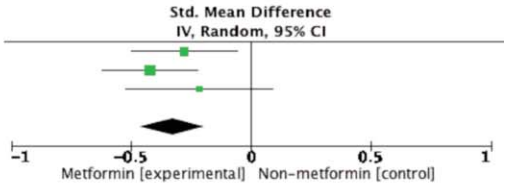
Heterogeneity: Tau² = 0.01; Chi² = 2.80, df = 2 (P = 0.25); I² = 29%
Test for overall effect: Z = 2.97 (P = 0.003)



(C) sVCAM-1

Study	Metformin Mean	Metformin SD	Metformin Total	Non-metformin Mean	Non-metformin SD	Non-metformin Total	Weight	Std. Mean Difference IV, Random, 95% CI
De Jager et al., 2005	-4	15.62	150	0	13.03	163	36.2%	-0.28 [-0.50, -0.06]
De Jager et al., 2014	-1	14.29	196	5	14.21	194	44.6%	-0.42 [-0.62, 0.22]
Lund et al., 2008	-6	9.3	83	-4	9.24	82	19.2%	-0.21 [-0.52, 0.09]
Total (95% CI)			429			439	100.0%	-0.33 [-0.46, -0.20]

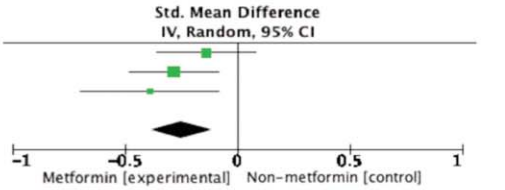
Heterogeneity: Tau² = 0.01; Chi² = 1.53, df = 2 (P = 0.47); I² = 0%
Test for overall effect: Z = 4.82 (P < 0.00001)



(D) ICAM-1

Study	Metformin Mean	Metformin SD	Metformin Total	Non-metformin Mean	Non-metformin SD	Non-metformin Total	Weight	Std. Mean Difference IV, Random, 95% CI
De Jager et al., 2005	-2	12.5	150	0	16.29	163	36.3%	-0.14 [-0.36, 0.09]
De Jager et al., 2014	-1	17.86	196	4	17.76	194	44.9%	-0.28 [-0.48, -0.08]
Lund et al., 2008	-7	13.94	83	-2	11.54	82	18,8%	-0.39 [-0.70, -0.08]
Total (95% CI)			429			439	100.0%	-0.25 [-0.38, -0.11]

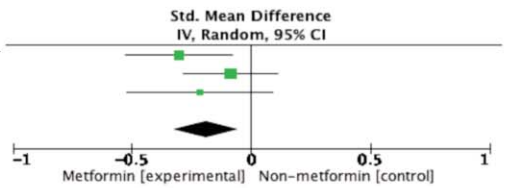
Heterogeneity: Tau² = 0.01; Chi² = 1.87, df = 2 (P = 0.39); I² = 0%
Test for overall effect: Z = 3.64 (P = 0.0003)



E) sE-selectin

Study	Metformin Mean	Metformin SD	Metformin Total	Non-metformin Mean	Non-metformin SD	Non-metformin Total	Weight	Std. Mean Difference IV, Random, 95% CI
De Jager et al., 2005	-5	15.625	150	1	22.8	163	35.8%	-0.30 [-0.53, -0.08]
De Jager et al., 2014	-5	21.43	196	-3	24.85	194	44.7%	-0.09 [-0.28, 0.11]
Lund et al., 2008	-11	18.6	83	-7	18.47	82	19.4%	-0.21 [-0.52, 0.09]
Total (95% CI)			429			439	100.0%	-0.19 [-0.33, -0.05]

Heterogeneity: Tau² = 0.00; Chi² = 2.08, df = 2 (P = 0.35); I² = 4%
Test for overall effect: Z = 2.72 (P = 0.007)



(F) tPA.

Study	Metformin Mean	Metformin SD	Metformin Total	Non-metformin Mean	Non-metformin SD	Non-metformin Total	Weight	Std. Mean Difference IV, Random, 95% CI
De Jager et al., 2005	-15	18.75	150	1	22.8	163	36.1%	-0.76 [-0.99, -0.53,]
De Jager et al., 2014	-10	28.57	196	6	35.51	194	44.5%	-0.50 [-0.70, -0.29]
Lund et al., 2008	-1.76	4.25	83	-0.33	4.3	82	19.4%	-0.33 [-0.64, -0.03]
Total (95% CI)			429			439	100.0%	-0.62 [-0.88, -0.36]

Heterogeneity: Tau² = 0.02; Chi² = 2.91, df = 1 (P = 0.09); I² = 66%
Test for overall effect: Z = 4.69 (P < 0.00001)

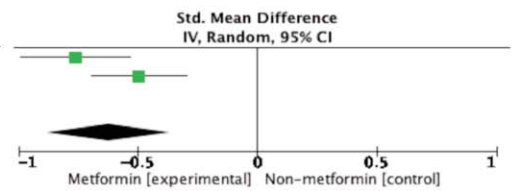


Figure-2: Forest plot related to angiogenesis outcomes after treatment with metformin.
vWF: Von Willebrand factor, PAI-1: Plasminogen activator inhibitor-1, sVCAM-1: Soluble vascular cell adhesion molecule-1, ICAM-1: Intercellular adhesion molecule-1, sE-selectin: Soluble endothelial-selectin, tPA: Tissue plasminogen activator.

Table 1: Meta-analysis based on endothelial markers post-treatment.

Variable	Studies	Study Design	Total patients	Statistical Method	Effect Estimated	p-value	I2
vWF	3	Randomised Controlled Trial	868	Std. Mean Difference (IV, Random, 95% CI)	-0.10 [-0.23, 0.04]	0.15	0%
PAI-1	3	Randomised Controlled Trial	868	Std. Mean Difference (IV, Random, 95% CI)	-0.24 [-0.41, -0.08]	0.003	29%
sVCAM-1	3	Randomised Controlled Trial	868	Std. Mean Difference (IV, Random, 95% CI)	-0.04 [-0.18, 0.09]	0.53	0%
ICAM-1	3	Randomised Controlled Trial	868	Std. Mean Difference (IV, Random, 95% CI)	-0.06 [-0.20, 0.07]	0.34	0%
sE-selectin	3	Randomised Controlled Trial	868	Std. Mean Difference (IV, Random, 95% CI)	0.02 [-0.11, 0.15]	0.79	0%
tPA	2	Randomised Controlled Trial	703	Std. Mean Difference (IV, Random, 95% CI)	-0.16 [-0.40, -0.09]	0.21	63%
Urinary albumin excretion	3	Randomised Controlled Trial	868	Std. Mean Difference (IV, Random, 95% CI)	0.00 [-0.13, 0.14]	0.96	0%

ICAM-1: Intercellular adhesion molecule-1, PAI-1: Plasminogen activator inhibitor-1, sE-selectin: Soluble endothelial-selectin, sVCAM-1: Soluble vascular cell adhesion molecule-1, tPA: Tissue plasminogen activator, vWF: Von Willebrand factor, CI: Confidence interval.

Table-2: Meta-analysis based on change in endothelial markers post-treatment compared to pre-treatment.

Variable	Studies (n)	Study Design	Total patients	Statistical Method	Effect Estimated	p-value	I2
vWF	3	Randomised Controlled Trial	868	Std. Mean Difference (IV, Random, 95% CI)	-0.28 [-0.50, -0.06]	0.01	61%
PAI-1	3	Randomised Controlled Trial	868	Std. Mean Difference (IV, Random, 95% CI)	-0.16 [-0.65, 0.34]	0.53	92%
sVCAM-1	3	Randomised Controlled Trial	868	Std. Mean Difference (IV, Random, 95% CI)	-0.33 [-0.46, -0.20]	<0.00001	0%
ICAM-1	3	Randomised Controlled Trial	868	Std. Mean Difference (IV, Random, 95% CI)	-0.25 [-0.38, -0.11]	0.0003	0%
sE-selectin	3	Randomised Controlled Trial	868	Std. Mean Difference (IV, Random, 95% CI)	-0.19 [-0.33, -0.05]	0.007	4%
tPA	2	Randomised Controlled Trial	703	Std. Mean Difference (IV, Random, 95% CI)	-0.62 [-0.88, -0.36]	<0.00001	66%
Urinary albumin excretion	3	Randomised Controlled Trial	868	Std. Mean Difference (IV, Random, 95% CI)	-0.05[-0.08, 0.19]	0.43	0%

ICAM-1: Intercellular adhesion molecule-1, PAI-1: Plasminogen activator inhibitor-1, sE-selectin: Soluble endothelial-selectin, sVCAM-1: Soluble vascular cell adhesion molecule-1, tPA: Tissue plasminogen activator, vWF: Von Willebrand factor, CI: Confidence interval.

Diabetes Association 2019 criteria of fasting plasma glucose (FPG) >126 mg/dL (7.0mmol/L). Fasting was defined as no caloric intake of plasma glucose >200mg/dL (11.1mmol/L) for at least 8 hours, or 2 hours for oral glucose tolerance test (OGTT).[14] The test should be performed as described by the World Health Organisation (WHO) using a glucose load equivalent to 75g anhydrous glucose dissolved in water.[15] Glycated haemoglobin (HbA1c) >6.5% (48mmol/mol) was performed in laboratory settings using the method approved by the National Glycohaemoglobin Standardisation Programme (NGSP) for Diabetes Control

and Complications Trial (DCCT) testing.[16] Patients with typical symptoms of hyperglycaemia or hyperglycaemic crisis could have incidental plasma glucose levels >200mg/dL (11.1mmol. L).[14]

With respect to the dose of metformin, 2(22.2%) studies used 1-3x 850mg a day, 2(22.2%) studies used the initial dose of 500mg a day, then titrated to 1000mg twice a day, 1(11.1%) study used 1000mg twice a day, 1(11.1%) study used 1.5-2 g per day, while 2(22.2%) studies did not report the dosage. No study explained the reasons for choosing a specific dose. Furthermore, 2(22.2%) studies only performed pre-treatment and post-treatment analysis and did not include a non-metformin group.[17,18]

There were [5](55.5%) studies that used other antidiabetic drugs in the control group, including pioglitazone.[12] gliclazide and pioglitazone,19 and repaglinide,[20] or a placebo[10,13]. The duration of intervention ranged 16-52 weeks.[11-13,19,20]

Angiogenesis outcomes were assessed in the current review through vascular endothelial growth factor (VEGF). Moreover, von-Wille-factor (vWf), plasminogen activator inhibitor-1 (PAI-1), soluble vascular adhesion molecule-1 (sVCAM-1), intercellular adhesion molecule-1 (ICAM-1), soluble endothelial-selection (sE-selectin), tissue plasminogen activator (tPA), urinary albumin excretion (UAE), platelet endothelial cell adhesion molecule-1 (PECAM-1) and thrombin-activatable fibrinolysis inhibitor (TAFI) were the endothelial dysfunction markers that correlated to angiogenesis (Supplementary 3).

The cross-sectional studies about VEGF showed no significant positive effect of metformin usage on angiogenesis.[18,21] A prospective cohort study demonstrated that metformin treatment for 12 weeks provided a significant positive angiogenesis effect. It was added to a 4-week medical nutrition treatment and regular exercise programme.[17]

The meta-analysis demonstrated that metformin utilisation was not associated with low levels of vWF marker post-treatment (OR -0.10; 95%CI: -0.23, 0.04; p=0.15) (Table 1). On the other hand, vWF levels reduced after metformin treatment significantly (OR -0.28, 95%CI: -0.50, -0.06]; p=0.01) (Table 2, Figure 2A).

Significantly low PAI-1 levels were measured post-treatment (OR -0.24, 95%CI: -0.41, -0.08]; p = 0.003) (Table 1). PAI-1 levels changed post-treatment non-significant (OR -0.24; 95%CI: -0.41, -0.08; p=0.53) (Table 2, Figure 2B).

The sVCAM-1 level was not significantly low post-treatment (OR -0.04; 95%CI: -0.18, 0.09; p=0.53) (Table 1). The change, however, was significant compared to baseline (OR -0.33, 95%CI: -0.46, -0.20; p<0.00001) (Table 2, Figure 2C.)

The ICAM-1 level was not significantly low post-treatment (OR -0.06; 95%CI: -0.20, 0.07; p=0.34) (Table 1), but the change was significant compared to baseline (OR -0.25; 95%CI: -0.38, -0.11; p=0.0003) (Table 2, Fig 2D.)

Compared to placebo, metformin treatment had no significant effect on sE-selectin post-treatment (OR 0.02; 95%CI: -0.11, 0.15; p=0.79) (Table 1). However, metformin had favourable effects post-treatment compared to baseline (OR -0.19; 95%CI: -0.33, -0.05; p=0.007) (Table 2, Figure 2E).

Metformin treatment showed no significant effect on tPA level (OR -0.16; 95%CI: -0.40, 0.09; p=0.21) (Table 1). However, significantly lower levels of tPA were observed post-treatment compared to baseline (OR -0.62; 95%CI: -0.88, -0.36; p<0.00001) (Table 2, Figure 2F).

There was no significant increase in UAE post-treatment (OR 0.00; 95%CI: -0.13, 0.14]; p=0.96) (Table 1). There was also no significant change in UAE level post-treatment compared to baseline (OR -0.05; 95%CI: -0.08, 0.19; p=0.43).

Discussion

The condition of hyperglycaemia in T2DM can cause a reduction of number and function of endothelial progenitor cells (EPCs).[22] A long-standing level of excessive blood sugar impairs the growth of endothelial cells, leading eventually to the damage of blood vessels. In response to this, the body should physiologically form new blood vessels in addition to the existing ones, and this process is called angiogenesis,[23] which needs both pro- and anti-angiogenic factors to work in equilibrium. Multiple factors, such as hyperglycaemia-induced oxidative stress, cytokines, and inflammatory factors, are also responsible for angiogenesis. VEGF is one of the pro-angiogenic factors and must be increased to promote angiogenesis by activating endothelial cells.[24] The expression of endothelial markers, such as VCAM-1 and ICAM-1, can be used to describe the condition of endothelial dysfunction, thus representing angiogenesis disturbance.[25]

Abnormal angiogenesis in DM could be a subsequent event after endothelial dysfunction that increases the risk of cardiovascular disease (CVD) in DM patients.[26] The abnormality of vascular smooth muscle cell function, immune regulation, platelet aggregation, inflammation and thrombosis are the mechanisms that convert endothelial dysfunction into angiogenesis

incompetence.[3] Angiogenesis is an adaptive response required for endothelial repair, whose incompetence is common in DM patients, increasing the risk of cardiovascular complications.[27]

Endothelial dysfunction and angiogenesis incompetence in T2DM patients usually occurs progressively.[22] The workup of endothelial function can be further evaluated by using physiological blood markers that were thought to reflect the function of endothelial cells. Increased circulating adhesion molecules, such as sE-selectin, ICAM-1 and VCAM-1, are indicators of early endothelial damage. These adhesion molecule blood levels have also been shown to predict future cardiovascular events.[23]

In addition to its hypoglycaemic effects, metformin has been shown to have other therapeutic effects, including improved vasodilation and reduced inflammation. Exposure of endothelial cells to metformin inhibits expression of endothelial adhesion molecules from inflammatory processes.[11]

A study in 2018 found that metformin could treat endothelial dysfunction in hyperglycaemia patients by enhancing the NO system, endothelin-derived hyperpolarising factor, and sirtuin 1. In addition, metformin's anti-angiogenic effect is still far from the clinical dose of 250mg per day, which requires further investigation as high doses of metformin lead to toxicity and side-effects.[28]

The United Kingdom Prospective Diabetes Study (UKPDS) stated that the cardiovascular benefits of metformin in T2DM extend beyond its blood sugar-lowering effect.[29] A review of non-randomised trials demonstrated that metformin improved markers of vascular damage by increasing the circulating EPCs and bringing pro-angiogenic cells (PACs) to the level of healthy controls.[27] These current findings were consistent, showing additional benefits in improving endothelial function, as shown by reduced anti-angiogenic levels after metformin treatment. Therefore, it is recommended that these markers may be considered for laboratory work up in T2DM patients receiving metformin treatment to examine their endothelial function.

The current meta-analysis had several limitations. First, it could merely analyse the association between metformin and angiogenesis that was represented by a few endothelial markers. Furthermore, each endothelial marker could only be analysed from 2-3 studies, thus not all the studies were included. Second, metformin intervention was not identical across the studies; neither in terms of the dosage, nor with respect to therapy dynamics. Most of the studies had metformin as monotherapy, while 2 studies had polytherapy, and a study did not clearly state the parameter. Nevertheless, the meta-analysis comprised several RCTs, which means the validity of the study was considerable. Third, three included studies in this systematic review and meta-analysis were outdated, but the data were still relevance to our research.

Conclusion

Metformin was found to have additional benefits in the improvement of endothelial function. It was significantly demonstrated by reduced anti-angiogenic levels after metformin treatment in T2DM patients. Hence, further laboratory work-up and clinical trials in T2DM patients receiving metformin treatment are required for their endothelial function examination.

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Conflict of Interest: None.

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