Review Article

Serum visfatin in Type 2 Diabetes Mellitus - A systematic review and meta-analysis

Shivani Harikrishnan[1,2], Sangeetha Shyam[3,4], Suan Phaik Khoo[5]

Recent studies have theorised that visfatin plays a significant role in the development and progression of Type 2 Diabetes Mellitus (T2DM). Some studies indicate that levels of serum visfatin are increased in subjects with T2DM whereas other studies dispute this claim. Since the results of these studies remain inconsistent, a systematic review and meta-analysis were performed. A search of PubMed, Ebsco-MEDLINE, Scopus, Science Direct, and Cochrane was conducted up till February 2019. Data analysis was performed using Review Manager 5.3. The standardised mean difference (SMD) with a 95% confidence interval (CI) was used to pool the effect size. The Newcastle Ottawa scale (NOS) was used to evaluate bias in the selected studies and a funnel plot was used to assess publication bias. A total of 17 studies were included in this systematic review and meta-analysis. Overall, levels of serum visfatin in subjects with T2DM were significantly higher when compared to the healthy adults (SMD: 1.68 95% CI [1.22,2.14], p<0.00001, I^2=92%). Sensitivity and subgroup analyses did not decrease heterogeneity. Among subjects with T2DM, those with additional comorbidity showed moderately increased levels of serum visfatin when compared to the subjects without comorbidity (SMD: 0.73 95% CI [0.14,1.32], p< 0.00001, I^2=92%). Sensitivity and subgroup analyses performed did not significantly decrease heterogeneity. Levels of serum visfatin are increased in subjects with T2DM when compared to healthy adults. Levels of serum visfatin are increased in subjects with T2DM with comorbidity when compared to subjects with T2DM without comorbidity. However, these findings must be interpreted with caution as high heterogeneity (I^2=92%) was observed.

Keywords: *Serum visfatin, Type 2 Diabetes Mellitus, Systematic Review, Meta-Analysis*

Introduction

Type 2 Diabetes Mellitus (T2DM), a chronic metabolic disorder, is one of the most common diseases in the world today with over 500 million individuals diagnosed till date[1]. It is characterised primarily by insulin resistance and pancreatic beta cell dysfunction[2]. Individuals with T2DM are more susceptible and often present with different forms of acute and chronic complications and comorbidities. This can substantially lower their quality of life, generate an enormous social and economic burden, and can sometimes even lead to a premature death[2,3]. The increased morbidity and mortality that is commonly seen in individuals with T2DM can be attributed to the insidious onset and late recognition characteristics of this disease[3].

Previously known as a disease of affluence due to its predominance in the western world, T2DM is now quickly spreading all over the globe due to rapid urbanisation. With urbanisation comes environmental and lifestyle changes, some of which have been known to trigger the development of T2DM. A sedentary lifestyle and a poor diet have been shown to increase Body Mass Index (BMI). These factors coupled with age, gender and ethnicity increase the risk of T2DM[4,5]. Therefore, it is important to identify and study the potential diagnostic and prognostic factors, as well as therapeutic targets in order to slow down the incidence of this disease.

[1]School of Medicine, International Medical University, Kuala Lumpur, Malaysia
[2]Warwick Medical School, University of Warwick, Coventry, United Kingdom
[3]Division of Nutrition and Dietetics, School of Health Sciences, International Medical University, Kuala Lumpur, Malaysia
[4]Centre for Translational Research, IMU Institute for Research, Development and Innovation (IRDI), Kuala Lumpur, Malaysia
[5]School of Dentistry, International Medical University, Kuala Lumpur, Malaysia

Address for Correspondence:
Prof Dr. Khoo Suan Phaik, School of Dentistry, International Medical University, Kuala Lumpur, Malaysia.
Email: suanphaik_khoo@imu.edu.my

Adipose tissue has recently been described as a highly active endocrine and metabolic organ with an important role in lipid and glucose metabolism. Adipose tissue produces hormones and cytokines, which are collectively known as 'adipocytokines or adipokines'. These include tumor necrosis factor-alpha, adiponectin, leptin, resistin and visfatin[4,6].

Visfatin, also known as pre-B cell colony enhancing factor (PBEF) and nicotinamide phosphoribosyl transferase (Nampt), is a newly discovered adipocytokine[7]. Fukuhara et al, demonstrated that visfatin has insulin-mimetic effects such as the inhibition of hepatic glucose release, an increase in glucose uptake in myocytes and adipocytes, and an increase in triglyceride synthesis[7]. However, since these results could not be subsequently confirmed, this paper was later retracted[7]. Present-day data proposes that visfatin is important to normal insulin secretion although this mechanism and its association with the development and progression of T2DM remain unclear[8].

The cause – effect relationship between T2DM and visfatin is a controversial one[8]. Some of this controversy stems from whether or not there is a rise in levels of serum visfatin in individuals with T2DM. While some studies show compelling evidence that levels of serum visfatin are increased in subjects with T2DM[9-23] when compared to healthy adults, others have reported no significant association[24,25].

In order to understand whether levels of serum visfatin are different between subjects with T2DM and healthy adults, and to evaluate if the presence of an accompanying comorbidity has an effect on these levels, this comprehensive systematic review and meta-analysis was conducted. Additionally, this study is a step towards evaluating the potential of serum visfatin as a prognostic and diagnostic marker for T2DM to complement existing therapy or diagnostics.

Methodology

Literature Search

The PRISMA 2009 protocol for reporting systematic reviews and meta-analysis was prospectively followed. Ethical approval was not sought since there was no need to handle individual patient data. PubMed, Ebsco MEDLINE, Science Direct, Scopus, and Cochrane were systematically searched using a Boolean code - 'serum' AND 'visfatin' AND 'type 2 diabetes mellitus'. The following filters were added to narrow down the results of our search. The English filter was applied in PubMed and the search was confined to research articles in ScienceDirect. For Ebsco MEDLINE, the search was limited to academic journals, all adults above the age of 19, English, and with the major heading including the keywords diabetes mellitus, type 2. In Scopus, the English language and only research articles filter were used. No filters were applied in the Cochrane database.

Study Selection

Two reviewers (SH and SGS) independently performed a thorough eligibility assessment based on the title and abstract of a paper following the inclusion and exclusion criteria which are as follows. For a study to be included in our meta-analysis it must (1) be in English (2) involve both healthy participants and those diagnosed with Type 2 Diabetes Mellitus and report serum levels of visfatin as such separately (3) involve adults over the age of 18 (4) be an observational study. Any studies that included a Type 2 Diabetic group and a group with additional comorbidities and did not report serum

visfatin levels separately were excluded. Studies that did not use a healthy control group and review articles, conference abstracts, letters, and book chapters were excluded as well. Any disagreements on the admissibility of a paper were resolved by a third reviewer (SPK).

Data Extraction

A predesigned data extraction form was used to collect comprehensive information from each included study. Details collected are as follows - (1) author, (2) location (3) gender (4) age (5) study design (6) comorbidities (if any) (7) participant numbers (case/control) (8) method of measurement of visfatin (9) T2DM definition (10) BMI (11) HbA1c levels 1(12) serum visfatin in healthy controls (13) visfatin levels in T2DM (14) visfatin levels in T2DM plus comorbidities (if there are any comorbidities).

Assessment of quality

The quality of included eligible studies regarding the role of serum visfatin levels in T2DM was evaluated based on Newcastle–Ottawa Scale (NOS), which assesses a study based on selection, the comparability of the groups, and the ascertainment of the exposure or outcome of interest with the use of a rating system. One star is allocated when a feature of quality is present, and total scores are ranged between 0 and 9. Scores between 0 to 3 were regarded as low-quality studies, 4 to 6 were regarded as moderate quality studies and 7 to 9 were regarded as high-quality studies.

Conversion of Data

The unit of measurement for the levels of serum visfatin in this study was ng/ml with mean ±SD for statistical expression. Since not all studies were reported in this format, some units had to be converted. Three studies[12,16,25] that presented their data as median (interquartile range) were converted to mean ±standard deviation (SD) using a method devised by Hozo et al[26]. One study[21] that expressed levels of serum visfatin in µg/l was converted to ng/ml using standard measurements of conversion. Another study[19] which expressed the levels of serum visfatin as mean ± standard error of the mean (SEM) was converted to the preferred format of mean ± SD using the Review Manager Calculator[27]. Another study[21] that presented its data in log ng/ml format was converted to ng/ml using the inverse function in a calculator. Data expressed in a box plot was extracted by two independent reviewers before being used in this study. The data from the boxplot[25] is expressed as median (interquartile range) which was then converted to mean ±SD using an equation devised by Hozo et al[26].

Statistical Analysis

Data analysis was performed using Review Manager 5.3. All data were calculated as Standard Mean Deviation (SMD) with 95% CI. SMD was used as not all our data had been originally expressed using the same unit of measurement. The effect size was calculated using the random-effects model as we anticipated heterogeneity between the papers. Heterogeneity was assessed by the I^2 test. The results of the I^2 test were interpreted according to Cochrane Handbook of Systematic reviews and meta-analysis which suggest that 0% to 40% indicates heterogeneity might not be important, 30% to 60% may represent moderate heterogeneity, 50% to 90% may represent substantial heterogeneity and 75% to 100% represents considerable heterogeneity.

To investigate any specific source of heterogeneity, a sensitivity analysis was carried by sequentially removing each study. This was done in order to evaluate the influence of a study on the pooled results. If heterogeneity remained high, a subgroup analysis was undertaken to

find the root cause. Secondary assessments were made by performing comparative sub-group analysis to identify potential source of heterogeneity within T2DM subjects. This included sub-group analysis of T2DM subjects differing by age groups (≥ 50 years and < 50years), gender, study location (Europe, Asia, Africa), duration of T2DM, glycaemic control, treatment modality presence of obesity (BMI ≥ 25 and ≥ 30), cardiovascular disease and metabolic syndrome, when such information was explicitly available in literature.

Publication Bias

We used the funnel-plot method to test for publication bias. In this method, the difference in standard mean changes is plotted against their standard errors.

Results

Search results

A search of the aforementioned databases resulted in 336 citations. After adjusting for 73 duplicates, we ended up with 263 citations. Out of this, 243 papers were excluded after reviewing abstracts as they did not meet inclusion criteria. The remaining 20 studies were examined more closely, and 3 articles were further excluded. The 17 studies that fit our criteria were included in our meta-analysis. Out of these 17 studies, 8 papers had additional information to carry out a meta-analysis to determine serum visfatin levels in those with T2DM and comorbidities. Figure I shows the PRISMA flowchart for this analysis.

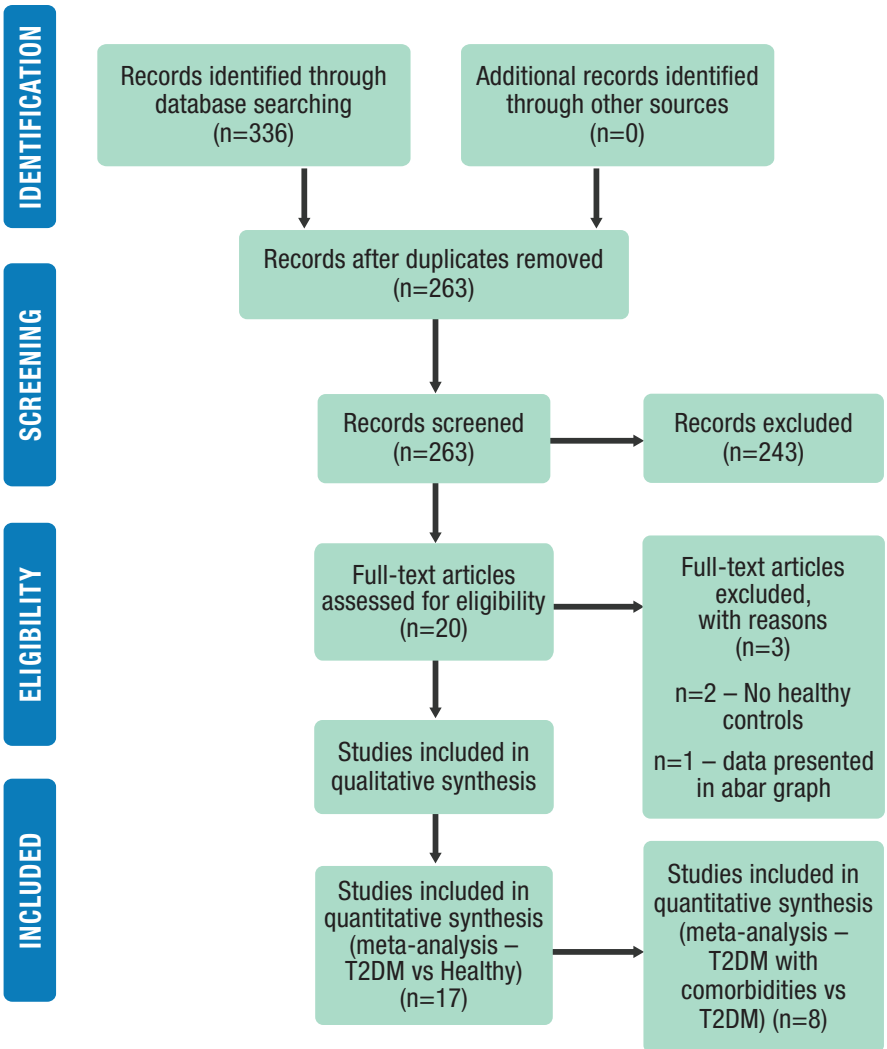

Figure I: PRISMA flowchart

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Characteristics of included studies

Out of the 17 studies that were selected for this meta-analysis, 15 were cross-sectional studies and two were open-labelled drug therapy trials. These studies involved a total of 1,445 participants out of which 763 had T2DM. The remaining 682 participants were healthy controls. Two studies explored the effects of drugs: thiazolidinedione's[17] and rosiglitazone[21] on levels of serum visfatin in subjects with T2DM and healthy adults. Fifteen studies explored the relationship of levels of serum visfatin in T2DM when compared to healthy adults. The summary of key findings of papers only comparing levels of serum visfatin in subjects with T2DM and healthy adults are shown in Table I.

Table I: Characteristics of studies that compare levels of serum visfatin in subjects with T2DM and healthy adults

S. No/ Ref No	Author/Year	Location	Study Design	Participants (Case/Control)	Method of visfatin measurement	T2DM Definition	Serum visfatin level in patients without T2DM (ng/ml) mean+SD	Serum visfatin level in patients with T2DM (ng/ml) mean+SD	Newcastle-Ottawa scale
1 [9]	Hetta et al, 2018	Saudi Arabia	Hospital Based Cross Sectional	80/40	EIA	WHO criteria for diagnosis of diabetes (WHO 2007)	19.0±8.2	40.33±9.98	5
2 [10]	Legakis et al,2016	Greece	Cross sectional	37/43	EIA	75g OGTT	2.9±0.7	5.0±2.1	5
3 [11]	Esteghamati et al,2010	Iran	Cross sectional	76/76	ELISA	Self-Reported	3.6±2.2	5.5±2.4	5
4 [12]	Retnakaran et al,2008	Thailand	Cross sectional	50/79	ELISA	Self-Reported	2.2±0.5	2.8±0.7	6
5 [13]	Lopez-Bermejo et al,2006	Spain	Prospective Study	35/118	EIA	American Diabetes Association	15.0±0.8	17.0±2.0	5
6 [15]	Celebi et al,2017	Turkey	Cross sectional study	20/20	ELISA	American Diabetes Association (2003)	0.6±0.6	3.2±2.2	5
7 [21]	McGee et al,2011	Coventry	Open labeled drug therapy trial	30/34	EIA	Self-Reported	1.1±0.1	1.4±0.1	4
8 [20]	El Shaer et al,2012	Egypt	Cross sectional	60/20	ELISA	Self-Reported	14.4±4.2	22.9±17.5	5
9 [17]	Hammarstedt et al,2006	Sweden, Finland	Open labeled drug therapy trial	7/6	EIA	Self-Reported	21.5±8.3	42.0±19.9	4

Abbreviations:

ELISA-enzyme-linked immunosorbent assay; EIA: enzyme immunoassay; OGTT - oral glucose tolerance test.

Out of these 15 studies, eight studies also explored the levels of serum visfatin in T2DM patients who had a comorbidity. Out of the eight studies, the comorbidity in two studies was metabolic syndrome[14,18], four studies with cardiovascular disease[16,22-24] and two studies with obesity[19,25]. The summary of key findings from papers comparing levels of serum visfatin in subjects with T2DM with a comorbidity and subjects with T2DM without a comorbidity are shown in Table II. The NOS score of all the studies assessed was between four and six indicating moderate quality.

Table II: Characteristics of studies that compare levels of serum visfatin in subjects with T2DM with comorbidity and subjects with T2DM without comorbidity

S. No/ Ref No	Author/ Year	Location	Study Design	Comorbidity	Participants [Case (T2DM)/ Case with Comorbidity/ Control]	Method of visfatin measurement	T2DM Definition	Serum visfatin level in healthy control (ng/ml)	Serum visfatin level in patients with T2DM (ng/ml)	Serum visfatin in patients with T2DM and comorbidity	Newcastle-Ottawa scale
1 [14]	Ahmed et al,2015	Saudi Arabia	Cross sectional	Metabolic syndrome	39/48/29/	ELISA	Self-Reported	18.4±6.0	28.2±15.1	58.9±29.9	5
2 [16]	Bilovol et al,2020	Ukraine	Cross sectional	Arterial Hypertension	61/125/20	ELISA	American Diabetes Association	17.0±2.0	39.0±3.5	42.0±2.7	5
3 [24]	Ahmed et al,2018	Egypt	Cross sectional	Cardio-Vascular disease	40/40/20	ELISA	Self-Reported	1.2±0.20	1.4±0.7	3.9±3.3	5
4 [18]	Haddad et al,2018	Iraq	Cross sectional	Metabolic Syndrome	44/22/22	EIA	WHO criteria (Alberti Zimmet, 1998)	52.5±14.1	63.7±8.3	56.0±10.6	6
5 [22]	Motawi et al,2014	Egypt	Cross sectional	Cardiovascular Disease	44/46/60	EIA	Self-Reported	17±4.1	29.8±6.3	41.0±7.9	5
6 [23]	Alghasham et al,2008	Saudi Arabia	Cross sectional	Microangiopathy	29/33/22	ELISA	American Diabetes Association criteria 1998	8.9±4.2	14.3±5.8	10.8±6.3	6
7 [25]	Kara et al,	Turkey	Cross sectional	Obesity	20/25/20	ELISA	American Diabetes Society criteria	6.0±0.5	8.0±1.0	11.3±2.8	6
8 [19]	El Mesallamy et al 2010	Egypt	Cross sectional	Obesity	37/19/19	ELISA	Self-Reported	9.4 + 8.6	25.9±3.4	45.4±20.1	6

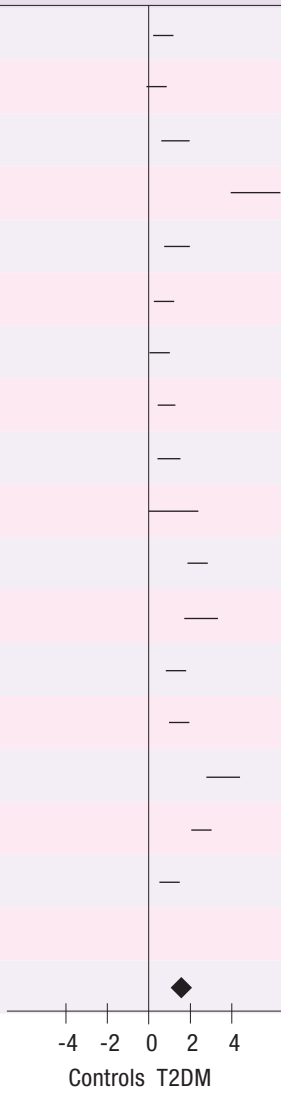
Meta-analysis results

The results of the meta-analysis are discussed under two sections (i) Levels of serum visfatin in subjects with T2DM vs healthy adults and (ii) Levels of serum visfatin in subjects with T2DM with and without a comorbidity

(i) Levels of serum visfatin in subjects with T2DM vs healthy adults

There is a significant increase in levels of serum visfatin in subjects with T2DM in comparison to healthy adults (SMD: 1.68, 95% CI [1.22,2.14], P<0.00001). However, pooled studies showed significant heterogeneity ($I^{\wedge 2}$= 92%). (Figure II)

	Type 2 Diabetes Mellitus			Healthy Control				Std Mean Difference	Std Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Ahmed 2015	28.2	15.1	39	18.4	6	28	6.2%	0.80 [0.29, 1.30]	
Ahmed 2018	1.44	0.71	40	1.17	0.2	20	6.1%	0.45 [-0.09, 0.99]	
Alghasham 2008	14.3	5.77	29	8.92	4.16	22	6.0%	1.03 [0.44, 1.62]	
Bilovol 2020	39	3.5	61	17	2	20	4.6%	6.80 [5.61, 7.99]	
Celebi 2017	3.2	2.2	20	0.6	0.6	20	5.7%	1.58 [0.86, 2.30]	
El Mesallamy 2010	25.9	20.9247	37	9.37	8.63062	19	6.0%	0.92 [0.34, 1.50]	
El Shaer 2012	22.9	17.5	60	14.4	4.2	20	6.1%	0.55 [0.03, 1.06]	
Esteghamati 2010	5.49	2.4	76	3.58	2.2	76	6.4%	0.83 [0.49, 1.16]	
Haddad 2018	63.71	8.3	44	52.46	14.05	22	6.1%	1.05 [0.51, 1.60]	
Hammarstedt 2006	42	19.9	7	21.5	8.3	6	4.5%	1.21 [-0.01, 2.44]	
Hetta 2018	40.33	9.98	80	19.03	8.22	40	6.2%	2.24 [1.77, 2.72]	
Kara 2014	8	1	20	6	0.5	20	5.4%	2.48 [1.64, 3.32]	
Legakis 2016	4.968	2.138	37	2.891	0.6168	43	6.2%	1.35 [0.86, 1.84]	
Lopez-Bermejo 2006	17	2	35	15	0.833	118	6.3%	1.66 [1.24, 2.08]	
McGee 2010	1.36	0.095	30	1.056	0.0725	34	5.5%	3.58 [2.78, 4.39]	
Motawi 2014	29.82	6.26	44	17	4.11	60	6.1%	2.48 [1.96, 3.00]	
Retnakaran 2008	2.75	0.695	50	2.22	0.4533	79	6.4%	0.94 [0.57, 1.32]	
Total (95% CI)			709			647	100.0%	1.68 [1.22, 2.14]	



Heterogeneity. Tau* = 0.83, Chi* = 194.80, df = 16 (P < 0.00001), I* = 92%
Test for overall effect Z = 7.12 (P < 0.00001)

Figure II: Data analysis - Levels of serum visfatin in subjects with T2DM vs healthy adults

Sensitivity Analysis

Sensitivity analysis was conducted by excluding each study one by one. This was done in order to evaluate the influence of each study on the pooled results. Only one study (Bilovol 2020[16]) slightly reduced the heterogeneity (from 92% to 87%). Sensitivity analysis of included studies did not significantly decrease pooled heterogeneity.

Subgroup Analysis

BMI - Subjects with T2DM were separated into subgroups to reflect overweight (BMI ≥ 25) and obese (BMI ≥ 30) categories as per the World Health Organization (WHO) definition[28]. Subjects with T2DM with BMI greater ≥ to 25 showed a significant increase in levels of serum visfatin compared to healthy adults (SMD: 1.42, 95% CI [1.04,1.80]) as did T2DM cases with BMI < 25 (SMD: 4.62[0.38,8.85]). Levels of serum visfatin were significantly higher in subjects with T2DM compared to healthy adults when the BMI of the subjects with T2DM was ≥ 30 (SMD: 1.53, 95% CI [1.02,2.04]) as well as when it was ≤ 30 (SMD: 2.11, 95% CI [1.15,3.06]).

Age - Subgroup analysis based on age was separated into different age groups reflecting the advancement of age among subjects with T2DM. Subjects with T2DM who were ≥ 50 years of age (SMD: 1.88, 95% CI [1.26,2.50]) and < 50 years of age (SMD: 1.53, 95% CI [0.74,2.32]) showed a significant increase in levels of serum visfatin when compared to healthy adults. Subjects with T2DM who were ≥ 55 years of age (SMD: 1.36, 95% CI [0,84,1.87]) and < 55 years of age (SMD: 1.89, 95% CI [1.26,2.53]) showed a significant increase in levels of serum visfatin when compared to healthy adults. Subjects with T2DM who were ≥ 60 years of age (SMD: 1.86, 95% CI [0.76,2.96]) and < 60 years of age (SMD: 1.75, 95% CI [1.22,2.28]) showed a significant increase in levels of serum visfatin when compared to healthy adults.

Gender - Groups with more women subjects with T2DM (SMD: 1.36, 95% CI [0.52, 2.20]) and groups with more men subjects with T2DM (SMD: 1.94, 95% CI [1.29,2.58] showed significantly higher levels of serum visfatin when compared to healthy adults.

Location of Study - Subjects with T2DM in studies conducted in Europe (SMD: 2.87, 95% CI [1.41,4.32], Asia (SMD: 1.32, 95% CI [0.90,1.74]) and Africa (SMD: 1.10, 95% CI [0.15,2.05]) have significantly higher levels of serum visfatin compared to healthy adults.

Duration of T2DM - Subjects who were described as newly diagnosed with T2DM (SMD: 1.11, 95% CI [0.59,1.63]) and subjects as having had a previous/ long-standing diagnosis of T2DM (SMD: 2.06, 95% CI [1,40,2.72]) had significantly increased levels of serum visfatin when compared to healthy adults.

HbA1c Levels - Subjects with T2DM with HbA1c levels ≥ to 7% (SMD: 1.48, 95% CI [0.85,2.12]) and < 7% (SMD: 2.45, 95% CI [1.15,3.74]) had significantly increased levels of serum visfatin in comparison to healthy adults.

Treatment Modality - Subjects with T2DM in studies using participants using insulin therapy (SMD: 1.29 [0.60,1.98]) and subjects in studies who used participants using only oral hypoglycemics (SMD: 1.77, 95% CI [1.03,2.51]) had significantly increased levels of serum visfatin when compared to healthy adults.

The results of the subgroup analysis are summarized in Table III.

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Table III: Subgroup analysis – Levels of serum visfatin in subjects with T2DM vs healthy participants

SUBGROUP	CATEGORY	SMD	95% CI	I^2
BMI	≥25	1.42	[1.04,1.80]	86%
	<25	4.62	[0.38,8.85]	97%
	≥30	1.53	[1.02,2.04]	86%
	<30	2.11	[1.15,3.06]	94%
Age	≥50	1.88	[1.26,2.50]	93%
	<50	1.53	[0.74,2.32]	91%
	≥55	1.36	[0.84, 1.87]	73%
	<55	1.89	[1.26,2.53]	93%
	≥60	1.86	[0.76,2.96]	81%
	<65	1.75	[1.22,2.28]	93%
Gender	Men	1.94	[1.29,2.58]	94%
	Women	1.36	[0.52,2.20]	92%
Location of Study	Europe	2.87	[1.41,4.32]	95%
	Asia	1.32	[0.90,1.74]	82%
	Africa	1.10	[0.15,2.05]	92%
Duration of T2DM	Newly Diagnosed	1.11	[0.59,1.63]	45%
	Established	2.06	[1,40,2.72]	94%
HbA1c Levels	≥7%	1.48	[0.85,2.12]	89%
	<7%	2.45	[1.15,3.74]	95%
Treatment Type	Insulin	1.29	[0.60,1.98]	89%
	Oral Hypo-glycemic	1.77	[1.03,2.51]	92%

(ii) Levels of serum visfatin in subjects with T2DM with and without a comorbidity

There is a moderate increase in the levels of serum visfatin in subjects with T2DM with a comorbidity, compared to subjects with T2DM without a comorbidity. (SMD: 0.73, 95% CI [0.14,1.32], P<0.00001). However, pooled studies showed high heterogeneity (I^2=92%) (Figure III.)

	T2DM with comorbidities			T2DM				Std Mean Difference	Std Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Ahmed 2015	58.9	29.9	48	28.2	15.1	39	12.7%	1.25 [0.78, 1.71]	
Ahmed 2018	3.92	3.32	40	1.44	0.71	40	12.7%	1.02 [0.56, 1.49]	
Alghasham 2008	10.81	6.29	33	14.3	5.77	29	12.5%	-0.57 [-1.08, -0.06]	
Bilovol 2020	42	2.6667	125	39	3.5	61	13.2%	1.01 [0.68, 1.33]	
El Mesallamy 2010	45.4	20.05094	19	25.9	20.9247	37	12.2%	0.93 [0.35, 1.51]	
Haddad 2018	56.03	10.58	22	63.71	8.3	44	12.4%	-0.83 [-1.37, -0.30]	
Kara 2014	11.25	2.75	25	8	1	20	11.7%	1.48 [0.81, 2.15]	
Motawi 2014	41.03	7.86	46	29.82	6.26	44	12.7%	1.56 [1.09, 2.03]	
Total (95% CI)			358			314	100.0%	0.73 [0.14, 1.32]	

Heterogeneity. Tau* = 0.66, Chi* = 83.89, df = 7 (P < 0.00001), I* = 92%
Test for overall effect Z = 2.43 (P = 0.02)

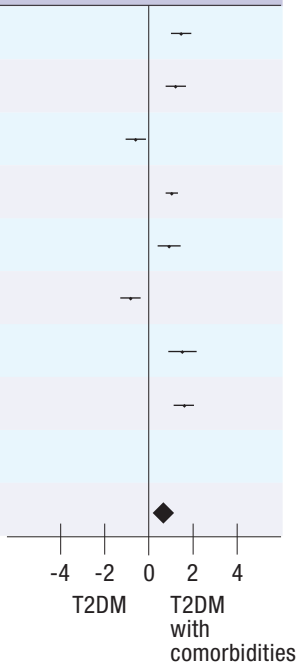

Figure III: Data Analysis - Levels of serum visfatin in subjects with T2DM with a comorbidity vs subjects with T2DM without a comorbidity

Sensitivity Analysis

Only two studies (Algasham 2008 and Haddad 2018) slightly reduced the heterogeneity of the pooled studies (from 92% to 79% and from 92% to 89% respectively). Sensitivity analysis of included studies did not significantly decrease pooled heterogeneity.

Subgroup Analysis

Obesity - Subjects with T2DM with a comorbidity showed significantly higher levels of visfatin when compared to subjects with T2DM without obesity. (SMD: 1.18, 95% CI [0.65,1.71]).

Cardiovascular Disease - Subjects with T2DM with cardiovascular disease showed moderately higher values of visfatin compared to subjects with T2DM without a comorbidity. (SMD: 0.76, 95% CI [-0.03,1.56]).

Metabolic Syndrome - Subjects with metabolic syndrome and T2DM showed slightly higher values of visfatin compared to subjects with T2DM without a comorbidity (SMD: 0.21, 95% CI [-1.83,2.25]).

These results are summarized in Table IV.

Table IV: Sub-group analysis: Level of serum visfatin in subjects with T2DM with a comorbidity vs subjects with T2DM without a comorbidity

SUBGROUP	CATEGORY	SMD	95% CI	I^2
Type of Comorbidity	Obesity	1.18	[0.65, 1.71]	32%
	Cardiovascular Disease	0.76	[-0.03, 1.56]	92%
	Metabolic Syndrome	0.21	[-1.83, 2.25]	97%

Publication Bias

Visual inspection of the funnel plot revealed asymmetry. Hence, publication bias cannot be excluded, and it cannot be established that a non-publication of negative or inconclusive data did not impact this meta-analysis (Figure IV).

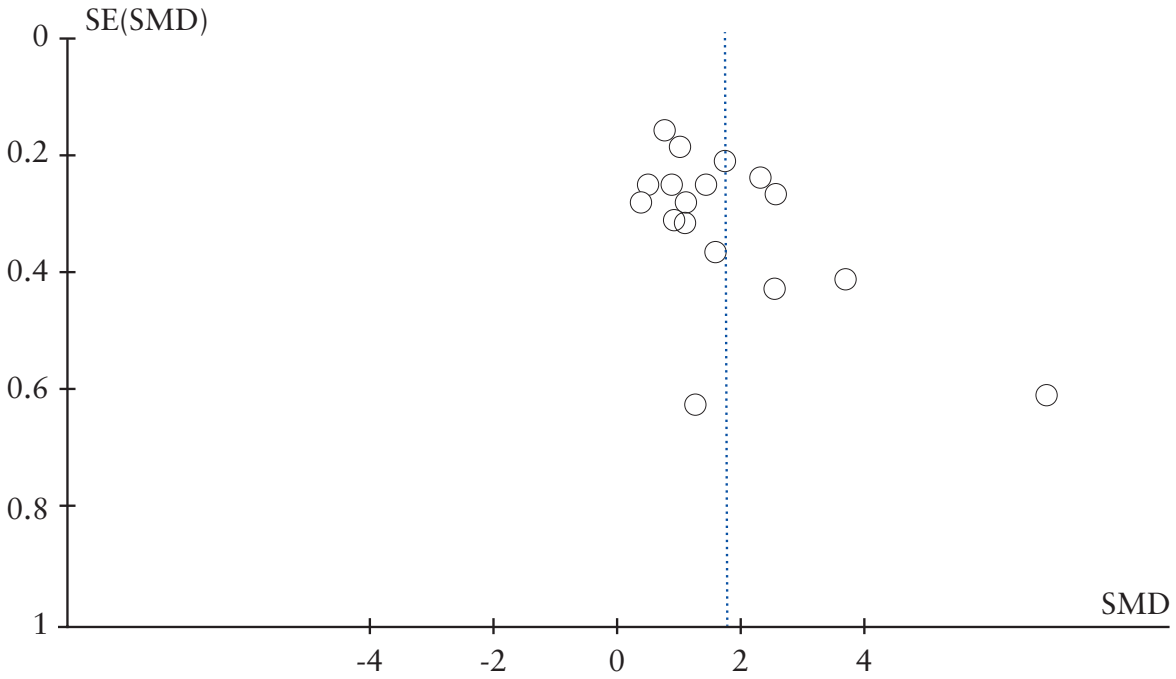

Figure IV: Funnel plot indicative of publication bias

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Discussion

The results of this study showed an increased level of serum visfatin in T2DM groups when compared to healthy controls (SMD: 1.68, 95% CI: [1.22, 2.14]). These increased levels of serum visfatin could be due to the effects of hyperglycaemia. One study found that an infusion of glucose caused an increase of circulating visfatin levels[29]. Likewise, Lopez et al. showed that in individuals with poor insulin secretion there was an increase in visfatin when an intravenous glucose tolerance test was performed[13]. Moreover, a meta-analysis showed a positive correlation between visfatin and insulin resistance which is a core factor in the development of T2DM[30]. This information indicates that in a hyperglycaemic environment, visfatin production can be stimulated. Since individuals with T2DM have hyperglycaemia because of insulin resistance or beta cell dysfunction, this could lead to the rise in serum levels of visfatin in T2DM.

To understand the high heterogeneity in this study (I^2=92%), a sensitivity analysis was performed by sequentially excluding each study one at a time. The sensitivity analysis performed showed that the exclusion of any included study did not significantly decrease or negate heterogeneity. One study had an effect size that was far apart from the effect sizes of the rest of the studies[16]. A sensitivity analysis undertaken to exclude this study[16] showed a small decrease in heterogeneity (from 92% to 87%). This indicates that while this study contributes to heterogeneity, it is not its root cause. Therefore, subgroup analyses were performed to investigate the origin of heterogeneity.

This study found an increase in levels of serum visfatin in T2DM patients regardless of their range of BMI. A study by Esteghmati et al inferred that visfatin levels are increased in T2DM independent of BMI. This was also reported by a meta-analysis[30] which suggested that the interaction between visfatin and glucose levels is not influenced by being overweight or obese.

The current meta-analysis found that serum visfatin levels are increased significantly in both subgroups containing subjects who use insulin and subjects who use oral hypoglycaemics. This correlates with studies done by Chen et al and Kadoglou et al which showed that insulin does not impact visfatin synthesis in adipocytes and as a result, there is no difference in serum levels of visfatin between type 2 diabetic subjects treated with insulin infusion or oral hypoglycaemic agents[31,32].

In T2DM, beta cells of the pancreas start to deteriorate due to over exertion because of increased insulin secretion to compensate for insulin resistance[4]. This study shows that serum visfatin levels are increased regardless of whether the subject with T2DM was newly diagnosed or had a previous T2DM diagnosis. It is theorised that serum visfatin could be a product of beta cell deterioration, which is also commonly seen even in newly diagnosed type 2 diabetics[13]. HbA1c level of more than 7% is considered to be linked with beta cell deterioration[33] and the production of visfatin is thought to correlate with beta cell dysfunction. However, this study showed that serum visfatin levels are increased regardless of whether HbA1c levels are <7% or >7%. However, this could simply be because of no correlation between visfatin and HbA1c levels[34].

In subjects with T2DM with a comorbidity, there is an increase in the level of serum visfatin when compared to subjects with T2DM without a comorbidity (SMD: 0.73, 95% CI: [0.14, 1.32]). However, there was considerable pooled heterogeneity (92%).

To understand the persistent high heterogeneity (I^2=92%), a sensitivity analysis was performed. The sensitivity analysis performed showed that the removal of any included study did not significantly decrease or negate heterogeneity. Two studies had effect sizes far apart from the effect sizes of the rest of the studies[18,23]. The sensitivity of these studies showed a small decrease in heterogeneity (from 92% to 87%[18] and from 92% to 89%[23]). This indicates that while these studies may contribute to heterogeneity, they are not its root cause. Therefore, subgroup analyses were done to investigate the origin of heterogeneity.

In subjects with T2DM and obesity, the levels of serum visfatin were shown to be significantly increased when compared to T2DM subjects without a comorbidity (SMD: 1.18, 95% CI [0.65,1.71]). Increased abdominal visceral fat has been thought to produce visfatin[7]. In addition to this, the increased adipose tissue mass seen in obesity is known to produce adipocytokines like visfatin[4]. This could be the cause of the increased levels of serum visfatin as seen in T2DM complicated with obesity.

In subjects with T2DM and cardiovascular disease, levels of serum visfatin are moderately increased in comparison to subjects with T2DM without a comorbidity. Visfatin in addition to being expressed by adipocytes, is also expressed by inflammatory cells such as macrophages. In cardiovascular disease, more specifically coronary artery disease, the levels of serum visfatin have been described to correlate with the circulating inflammatory markers present[35]. Additionally, one study found an increased expression of visfatin in macrophages around areas with carotid plaques[36]. This increased expression of visfatin could contribute to the increased levels of serum visfatin in subjects with T2DM and cardiovascular disease that is seen in this study.

This study showed that in subjects with T2DM with metabolic syndrome, the levels of serum visfatin were slightly higher when compared to subjects with T2DM without a comorbidity. The metabolic syndrome is made up of the collective presentation of multiple cardiovascular risk factors including abdominal obesity, hypertension, insulin resistance, hyperinsulinemia, and dyslipidaemia[37]. Increased macrophage expression caused by inflammation due to cardiovascular disease[36], along with the increase in visceral abdominal fat seen in obesity[7] could be causing the increase in levels of serum visfatin that is seen in subjects with T2DM with metabolic syndrome.

This meta-analysis updates the existing ones in the area by including studies published until 2020. Another strength of this study is that it accounts for heterogeneity through sub analysis of different factors such as age, gender, BMI, treatment modality, location of study, duration of T2DM, level of glycemic control and presence or absence of complications. This meta-analysis shows that the levels of serum visfatin are higher in subjects with T2DM when compared to their healthy counterparts. In addition, this study also shows that levels of serum visfatin are higher when a subject has a comorbidity that complicates T2DM.

The main limitation that was faced in this study was the presence of high heterogeneity which may be attributed to the observational studies used in this systematic review and meta-analysis whereby there may be differences in case-definitions, lack of confounder evaluation as well as differences in population settings, family history, ethnic backgrounds, lifestyle factors

and habits. Additionally, all studies included were of moderate quality and publication bias could not be excluded. Therefore, the findings need to be interpreted with caution.

This meta-analysis was performed as a step towards evaluating the potential of serum visfatin as a prognostic and diagnostic marker for T2DM. We found that levels of serum visfatin were increased in subjects with T2DM when compared to healthy adults, especially among those with comorbidities. However, since the findings have a high level of heterogeneity, they should be interpreted with caution. At this juncture, it may be premature to suggest that visfatin will complement existing means of DM control/diagnosis. We therefore recommend that future studies (1) employ a case-control design with matched confounders like underlying diseases, medication, treatment regimen and (2) use a prospective design to establish a cause–effect relationship between visfatin and T2DM.

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Conflict of Interest

The authors declare no conflicting interest.

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