Systematic Review

Association of Leptin and Retinol Binding Protein 4 with the Risk of Gestational Diabetes: A Systematic Review and Meta-Analysis of Observational Studies

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Abstract

The positive correlation between serum levels of retinol binding protein 4 (RBP4) and gestational diabetes (GDM) has been proven in the previous meta-analysis on case-control studies. However, its association with serum levels of leptin is not studied in any meta-analysis. Therefore, we performed an updated systematic review of observational studies evaluating the association between serum RBP4 and leptin with the risk of GDM. A systematic search was performed on four databases, including PubMed, Scopus, Web of Science, and Google Scholar, up to March 2021. After screening and deleting duplicates, nine articles met our inclusion criteria. Studies had case-control and cohort design, and included 5074 participants with a mean age range between 18 and 32.65 years (2359 participants for RBP4 and 2715 participants for leptin). Interestingly, this meta-analysis revealed higher levels of RBP4 (OR=2.04; 95% CI: 1.37, 3.04) and leptin (OR=2.32; 95% CI: 1.39, 3.87) are significantly associated with the increased risk of overall GDM. The subgroup analysis approved the results based on the study design, trimester of pregnancy and serum/plasms to investigate the source of heterogeneity. The present meta-analysis determines serum leptin and RBP4 levels as predictors of GDM occurrence. However, studies included in this meta-analysis showed significant heterogeneity.

Keywords: GDM, gestational diabetes mellitus, leptin, meta-analysis, RBP-4, retinol binding protein 4

Introduction

Gestational diabetes mellitus (GDM) is defined as glucose intolerance and hyperglycemia which, recognized for the first time during pregnancy with the increasing rate in recent decades that imposes a lot of financial and health costs for mothers, offspring, and healthcare systems.[1,2] Although, the exact pathophysiology is unclear, physiological insulin resistance is the primary cause that is exacerbated by obesity and more weight gain during pregnancy.[3,4] Moreover, peptides and gestational hormones related to glucose metabolism have dysregulated, that lead to GDM. Routinely, glucose intolerance in GDM diagnosed in the second or third trimester of pregnancy. However, some biomarkers are found in maternal plasma, including adipose tissue-derived factors including leptin, adiponectin, visfatin, omentin-1, FABP-4 (fatty acid-binding protein-4), and retinol binding-protein-4 (RBP4) before the GDM development.[5] A recent systematic review and meta-analysis on case-control studies identified the RBP4 as a modest independent risk factor for GDM in obese and non-obese women. However, further research is suggested to reach concise results.[6] Therefore, identifying related markers of GDM are encouraged for earlier and more specific diagnosis to reduce perinatal morbidity and adverse neonatal outcomes.

RBP4 is an adipokine secreted from white adipose tissue that is traditionally identified as a vitamin A transporter protein to transfer retinol from the liver to peripheral tissues.[7] Leptin

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is another adipokine that regulates energy balance and body weight by acting on the hypothalamus.[8] These adipokines showed a potential role in insulin sensitivity and glucose metabolism.[9] Elevated RBP4 and leptin were associated with an increased risk of glucose intolerance in previous animal and human studies.[6,10,11] It is not clear whether serum leptin level can be a predictor of GDM in pregnancy or not. Moreover, to our knowledge, there is no systematic review and meta-analysis on the observational studies for the importance, weight and role of RBP4 and leptin in GDM occurrence. For this reason, this systematic review and meta-analysis was conducted to determine the importance of these adipokines in GDM occurrence.

Methods

The current study was designed, reported, and conducted based on MOOSE Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies.[12]

Search strategy

We searched online databases including, PubMed, Scopus, Web of Science, and Google Scholar until, March 2021 to identify observational studies that examined the association between circulating RBP4 and leptin levels with GDM risk. The keywords used in the search strategy are as follows: ("Gestational diabetes mellitus" OR "Pregnancy induced diabetes" OR "GDM" OR "gestational diabetes") aND ("Retinol-binding protein" OR "RBP-4" OR "retinol-binding protein-4" OR "retinol binding protein-4" OR "retinol binding protein 4" OR "retinol-binding protein 4" OR RBP4) AND (leptin). No restrictions in terms of publication time or the language of articles were considered. Besides, the reference lists of selected articles were searched to identify studies that might have been missed.

Inclusion criteria

studies with the following criteria were included: 1) observational studies with prospective, case-control, or cross-sectional designs; 2) studies that considered investigated the relationship between circulating RBP4 and leptin levels and GDM risk; 3) those performed on adults (≥18 years); and 4) those studies that reported odds ratios (ORs) or relative risks (RRs) or hazard ratios (HRs) along with 95% confidence intervals (CIs) for the association circulating RBP4 and leptin levels and GDM risk. If findings from one dataset were published in >1 paper, we selected the most recent version; otherwise, the one with the greatest number of cases was included.

Exclusion criteria

We excluded letters, comments, short communications, reviews, meta-analyses, ecological studies, and animal studies. Studies that investigated the relation between Placental or cord-blood RBP4 or leptin levels and GDM risk were also excluded.

Data extraction

Required data from each eligible study were extracted by two independent investigators, and any disagreements were reconciled by discussion. Any reported ORs or HRs or RRs and corresponding 95% CIs for the association circulating RBP4 and leptin levels and GDM risk were extracted from each study. In addition to effect sizes, the following information was extracted: first author's name, year of publication, country of origin, demographic characteristics of participants (age range or mean age), trimester of pregnancy, number of participants and cases, methods used for RBP-4 or leptin assessment and the diagnosis of GDM, and confounding variables adjusted in the statistical analysis. If a study reported its findings based on several trimesters of pregnancy or any other variables, we considered that study as two separate studies.

Quality assessment

The quality of studies included in the current meta-analysis was assessed using the Newcastle Ottawa Scale (NOS) designed for non-randomized studies.[13] According to this scale, a maximum of nine points would be awarded to each study according to the following parameters: four points for the selection of participants, two points for comparability, and three points for the assessment of outcomes. Studies achieving nine points were considered to provide the highest quality.

Statistical analysis

The ORs, RRs, and HRs (and 95% CIs) were considered the effect sizes (ESs) of all studies. We calculated the summary ES to compare the highest versus lowest categories of circulating RBP4 and leptin levels. A random-effects model was used to take between-study heterogeneity into account.[14] By the random-effects model, we also calculated both Q-statistic and I^2 values as the indicators of heterogeneity. I^2 values of >50% were considered significant between-study heterogeneity.[15] In case of significant between-study heterogeneity, we conducted a subgroup analysis based on the participants' trimester of pregnancy. Publication bias was examined using Egger's regression asymmetry test.[16] A trim-and-fill method was used to detect the effect of probable missing studies on the overall effect. We also performed a sensitivity analysis in which each study was excluded to examine the influence of that study on the overall estimate. Statistical analyses were conducted using STATA version 14.0. A P value <0.05 was considered statistically significant for all tests.

Results

Literature search

1785 articles were identified in the initial search. After the exclusion of duplicate papers and those that did not meet the inclusion criteria, we identified 121 full-text articles of potentially relevant studies. After a full-text review, we excluded additional 28 studies because they considered cord-blood RBP4 or leptin levels, not serum or plasma RBP4 and leptin levels, as the exposure variable. Fifteen studies assessed the polymorphisms of RBP4 and leptin about the risk of GDM and therefore were excluded, and we excluded 69 studies because they did not report adequate data on outcomes. After the above-mentioned exclusions, nine papers, including two papers from prospective design,[17,18] six papers from

case-control design[19-24] and one from cross-sectional design[25] were included in the meta-analysis. Five articles with eight arms investigate the association of circulating RBP4 levels and GDM risk[20-23,26] and six articles with eight arms investigate the association of circulating leptin levels with GDM risk.[17,19,20,24-26] A flow diagram of the study selection is shown in Figure 1.

Characteristics of included studies

Characteristics of included studies are provided in Tables 1 and 2. The total number of participants in these studies ranged from 96 to 828 people, with a mean age range between 18 and 32.65 years. Among total of 5074 participants, 2359 participants were studied for the association between circulating RBP4 levels and GDM risk and 2715 participants were related to the association between circulating leptin levels and GDM risk. The total number of GDM cases was 1548. Among nine articles (16 results), three papers were from the United States,[17,20,23] and six papers were from non-US countries. Serum leptin (n = 3),[19,24,26] plasma leptin (n = 3),[17,20,25] serum RBP4 (n = 2),[23,26] and plasma RBP4 (n = 3)[20-22] were assessed. Two papers assessed plasma RBP4 in the first and second trimester of pregnancy[20,21] and one study evaluated both serum RBP4 and serum leptin in the second and third trimester.[26] In all papers, effect sizes were adjusted for BMI. The NOS scores of the included studies ranged between 7 and 9. Looking at the variation of NOS score and considering the score of 7 as the median for a total score of NOS, all papers had a score of ≥7, defined as high-quality studies [Supplementary Tables 1-3].

Findings from the systematic review

Out of 8 results on the association between circulating RBP4 levels and the risk of GDM, five indicated a positive association,[21,22,26] and others illustrated no significant association. In terms of leptin levels and GDM risk, two studies reported no significant association[24,25] while others indicated a significant positive association.

Findings from the meta-analysis on RBP4 levels and risk of GDM

Five studies (8 results) with 2359 participants and 707 GDM cases were included in this association.[20-23,26] The summary effect size for the risk of overall GDM, comparing the highest versus the lowest levels of RBP4, showed a significant positive association between RBP4 levels and GDM risk. (OR = 2.04;95%CI: 1.37-3.04, $P < 0.001$), [Figure 2]. However, there was evidence of significant heterogeneity

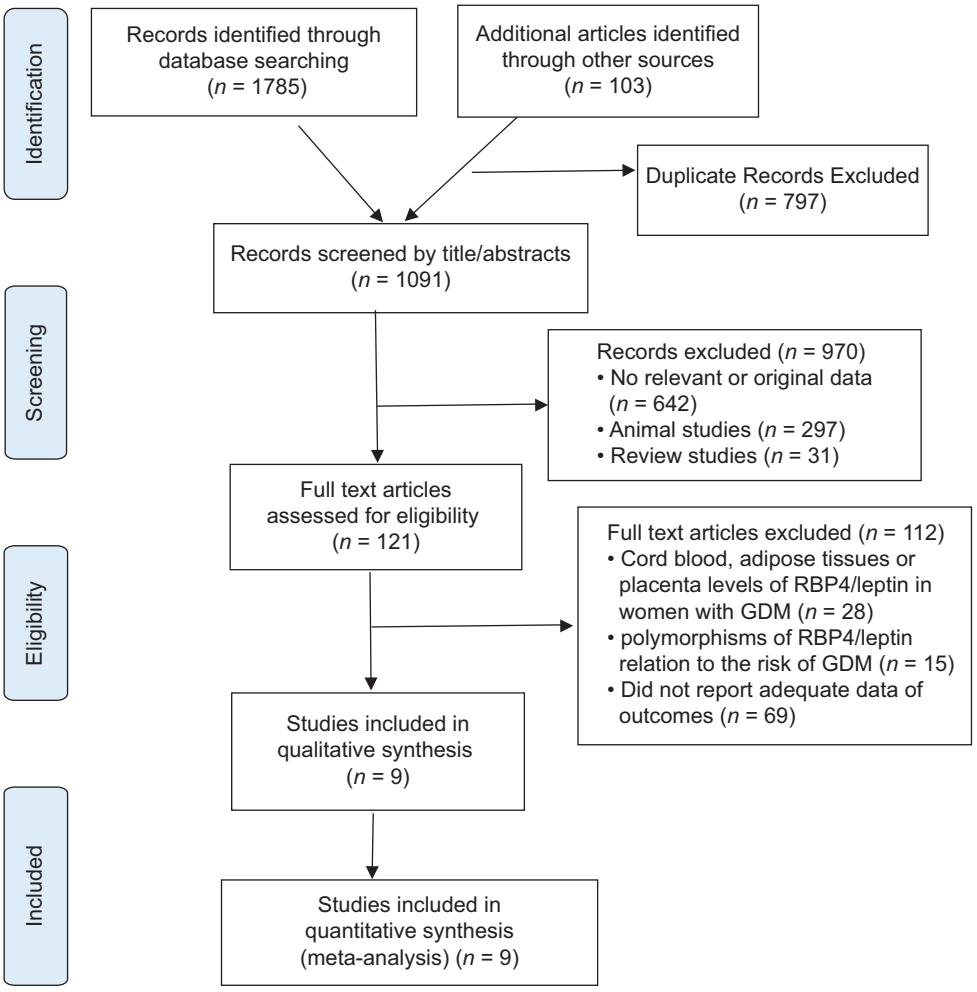

Figure 1: Flowchart of study selection included in this meta-analysis

Table 1: Characteristics of included studies for association of circulating RBP4 levels and GDM risk

Authors	Country	Age range (y)	Sample size	Cases	Design of study	trimester of pregnancy	Exposure	Exposure assessment	Outcome	Outcome assessment	Comparison	OR, RR or HR (95%CI)	Adjustment
Jin *et al.* 2020	China	29	270	135	Nested case-control	first	RBP4	Plasma RBP4	GDM	IADPSG	Q4 vs. Q1	OR: 3.71* (95% CI: 1.27-10.84)	total cholesterol, triglyceride, HDL, LDL, GFR, ALT, AST, daily intake of calories and weekly physical activity time,
	China	29	270	135	Nested case-control	second	RBP4	Plasma RBP4	GDM	IADPSG	Q4 vs. Q1	OR: 3.3* (95% CI: 1.03-10.52)	
Francis *et al.* 2020	USA	30.5	214	107	Nested case-control	first	RBP4	Plasma RBP4	GDM	Carpenter. Coustan	Q4 vs. Q1	OR: 1.69 (95%CI: 0.83-3.45)	maternal age and gestational week of blood collection, family history of diabetes and parity
			214	107	Nested case-control	second	RBP4	Plasma RBP4	GDM	Carpenter. Coustan	Q4 vs. Q1		
Du *et al.* 2019	china	26	827	104	Case-control	first	RBP4	Plasma RBP4	GDM	IADPSG	Q4 vs. Q1	OR: 6.36* (95%CI: 3.21-10.04)	maternal age, pre-gestational BMI, gestational age at sampling, smoking, ethnicity, pre-existing hypertension and CVD, gestational weeks at admission, conception status, family history of diabetes, physical activity, SBP, DBP, total cholesterol, triglyceride, HDL, FPG, CRP, insulin, IL-6, adiponectin,
Zhang *et al.* 2016	china	28.78	280	40	prospective cohort	second	RBP4	serum RBP4	GDM	IADPSG	NR	OR: 1.35* (95%CI: 1.07-1.72)	Pre-pregnancy body mass index, age and weight gain
			280	40	prospective cohort	third	RBP4	serum RBP4	GDM	IADPSG	NR	OR: 1.70* (95% CI: 1.23-2.35)	
Abetew *et al.* 2013	USA	>18	360	173	Nested case-control	second	RBP4	serum RBP4	GDM	ADA	Q4 vs. Q1	OR: 1.53 (95%CI: 0.81-2.89)	maternal age, race/ethnicity, family history of diabetes and pre-pregnancy overweight

Table 2: Characteristics of included studies for association of circulating leptin levels and GDM risk.

Authors	Country	Age range (y)	Sample size	Cases	Design of study	trimester of pregnancy	Exposure	Exposure assessment	Outcome	Outcome assessment	Comparison	OR, RR or HR (95%CI)	Adjustment
Francis *et al*. 2020	USA	30.5	214	107	Nested case-control	first	leptin	Plasma leptin	GDM	Carpenter. Coustan	Q4 vs. Q1	OR: 3.39* (95% CI: 1.52-7.55)	matching factors matched within a range (maternal age and gestational week of blood collection) family history of diabetes, and parity
			214	107	Nested case-control	second	leptin	Plasma leptin	GDM	Carpenter. Coustan	Q4 vs. Q1	OR: 3.45* (95% CI: 1.43-8.32)	
O`Malley *et al*. 2020	Ireland	>18	196	105	prospective cross-sectional	second	leptin	Plasma leptin	GDM	IADPSG	NR	OR: 0.5 (95% CI: 0.3-1.1)	Maternal obesity
Mosavat *et al*. 2018	Malaysia	32.65	96	53	prospective case-control	second	leptin	serum leptin	GDM	UMMC	T3 vs. T1	OR: 2.6 (95% CI: 0.3-1.1)	maternal age, gestational age, and BMI
Zhang *et al*. 2016	china	28.78	280	40	prospective cohort	second	leptin	serum leptin	GDM	IADPSG	NR	OR: 3.23* (95% CI: 1.19-5.60)	Pre-pregnancy body mass index, age and weight gain
			280	40	prospective cohort	third	leptin	serum leptin	GDM	IADPSG	NR	OR: 2.27* (95% CI: 1.29-2.27)	
Fatima *et al*. 2017	Pakistan	26.4	508	208	Case-Control	second	leptin	serum leptin	GDM	IADPSG	NR	OR: 2.57* (95% CI: 1.50-4.42)	NR
Qiu *et al*. 2004	USA	31.9	823	47	Cohort	second	leptin	Plasma leptin	GDM	ADA	T3 vs. T1	OR: 4.7* (95% CI: 1.2-18)	parity, first-degree family history of non–insulin-dependent diabetes, and body mass index at blood collection.

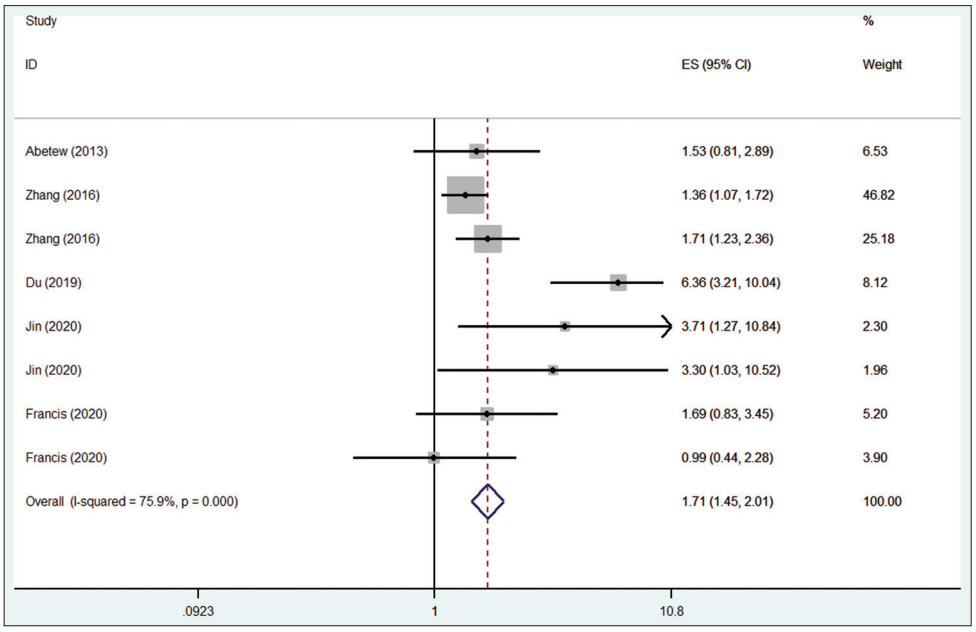

Figure 2: Random-effects model estimates for the association of RBP-4 and GDM risk

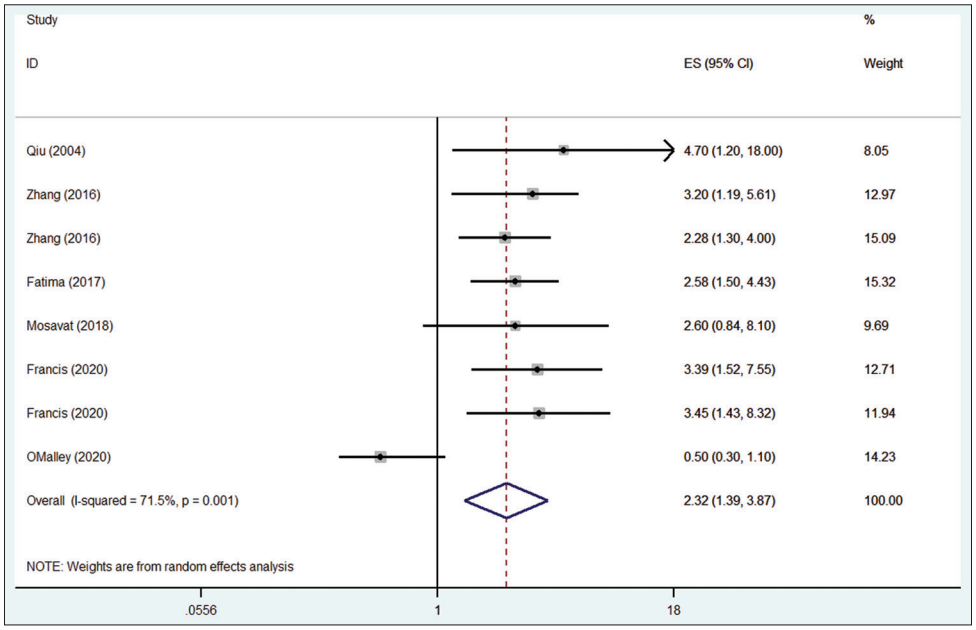

Figure 3: Random-effects model estimates for the association of leptin and GDM risk

between studies (I2 = 75.9%; $P < 0.01$). Findings from subgroup analyses revealed that study design, trimester of pregnancy and type of samples (serum/plasma) explained the between-study heterogeneity. These analyses showed a significant positive association between RBP4 levels and the risk of overall GDM in all subgroups of studies [Table 3].

Findings from the meta-analysis on leptin levels and risk of GDM

Six studies (8 results) with a total of 2715 participants and 841 GDM cases were included in this association.[17,19,20,24,26] The summary effect size for the risk of overall GDM, comparing the highest versus the lowest levels of leptin, indicates a significant positive association. (OR = 2.32; 95%CI: 1.39-3.87, $P < 0.001$), [Figure 3]. However, there was evidence of significant heterogeneity between studies (I2 = 71.5%; $P < 0.01$). Findings from subgroup analyses revealed that study design, trimester of pregnancy, and type of samples (serum/plasma) explained the between-study heterogeneity. These analyses showed a significant positive association between leptin levels and the risk of overall GDM in all subgroups of studies [Table 3].

Sensitivity analyses and publication bias

Sensitivity analysis based on a fixed-effects model showed that excluding any single study from the analysis did not

Table 3: Summary risk of random-effects model estimates for the association circulating RBP4 and leptin with GDM risk

	n[2]	Pooled ES (95% CI)[1]	I^2 (%)[3]	$P_{Q\ test}$[4]
The highest vs. lowest comparison of circulating RBP4 levels				
Overall	8	2.04 (1.37, 3.04)*	75.9	<0.001*
Study design				
Cohort	6	2.39 (1.27, 4.49)*	74.2	0.002
Case-control	2	1.48 (1.19, 1.84)*	19.0	0.267
Trimester of pregnancy				
Trimester 1	3	3.46 (1.43, 8.37)*	75.3	0.017
Trimester 2	4	1.39 (1.12,1.71)*	0	0.406
Trimester 3	1	1.70 (1.23,2.36)*	-	-
circulating RBP4				
Serum	3	1.48 (1.23, 1.77)	0.0	0.536
Plasma	5	2.65 (1.26, 5.59)	75.5	0.003
The highest vs. lowest comparison of circulating leptin levels				
Overall	8	2.32 (1.39, 3.87)	71.5	0.001
Study design				
Cohort	3	2.73 (1.39, 4.20)	0.0	0.55
Case-control	4	2.89 (1.98, 4.21)	0.0	0.916
Cross sectional	1	0.50 (0.26, 0.96)	-	-
Trimester of pregnancy				
Trimester 1	1	3.39 (1.52,7.56)*	-	-
Trimester 2	6	2.23 (1.09, 4.56)*	78.2	<0.001
Trimester 3	1	2.28 (1.09,4.56)*	-	-
circulating leptin				
Serum	4	2.57 (1.84, 3.59)*	0	0.922
Plasma	4	2.17 (0.68,6.88)	85.7	<0.001

[1]CI, confidence interval; ES, effect size. [2]Number of effect sizes. [3]Inconsistency- the percentage of variation across studies due to heterogeneity. [4]Obtained from the Q-test. *P <0.05

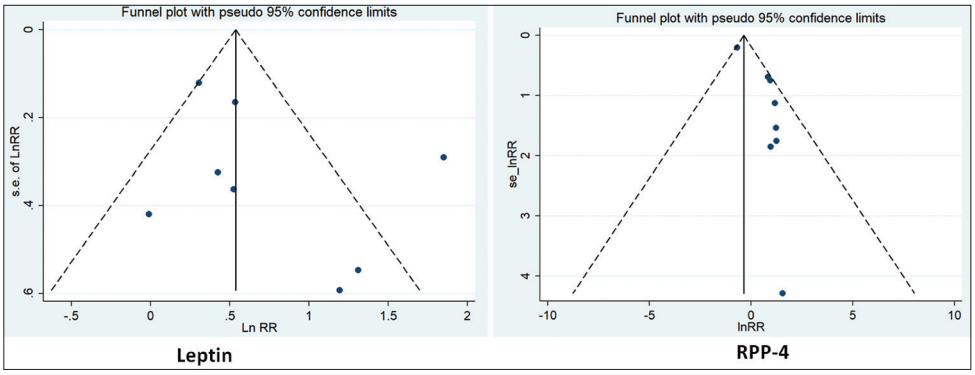

Figure 4: Funnel plots

significantly alter the pooled effect sizes (OR of 1.58 with 95% CI of 0.64-3.86). Based on Egger's test, we found no substantial publication bias for the associations examined in the current meta-analysis, except for the association between plasma leptin and risk of overall GDM (P < 0.001). Funnel plots are depicted to test of publication bias visually [Figure 4].

Discussion

We have determined the association between serum leptin and RBP4 with GDM occurrence according to observational studies. Results of these studies with a total number of 2359 participants for RBP4 and 2715 participants for serum leptin levels showed a significant positive association with risk of overall GDM. Pregnant women with the highest serum levels of RBP4 were 2.04-fold more prone to GDM than those with the lowest levels. Moreover, higher serum leptin levels are associated with 2.32-fold increase in GDM risk. These results determine serum leptin and RBP4 levels as predictors of GDM occurrence. However, studies included in this meta-analysis showed significant heterogeneity. Results were approved after subgroup analysis based on the study design, trimester of pregnancy and type of samples to investigate the source of heterogeneity.

RBP4 and leptin are metabolic risk factors in obesity that are positively associated with body mass index (BMI), fat storage and waist to hip ratio (WHR).[27-30] Moreover, these adipokines are bioactive signaling molecules with autocrine, paracrine, or endocrine actions associated with oxidative stress, inflammation, and insulin resistance.[31,32] RBP4 is a ~21 kDa plasma membrane transporter with a single polypeptide chain, encoded by the RBP4 gene located on chromosome 10 (10q23–q24) that is almost entirely bound to thyroxine-binding transthyretin (TTR) for transporting retinol into other tissues.[33] Circulating and tissue RBP4 has been associated with systemic insulin resistance and may link adipose tissue dysfunction to glucose intolerance.[34,35] Alongside, the RBP4-membrane receptor STRA6 also contributes to the etiology of insulin resistance by inducing the suppressor of cytokine signaling 3 (SOCS3), an inhibitor of insulin signaling.[36] RBP4 affect various organs that result in insulin resistance. In adipose tissue, dephosphorylate the insulin receptor substrate 1 (IRS1) which decreases insulin sensitivity and GLUT4 translocation by deactivation of PI3K and Akt signaling pathways. Moreover, it activates JAK2/STAT5 signaling pathway that impairs insulin signaling in adipose tissues. In the liver, phosphoenolpyruvate carboxykinase (PECK) is expressed by RAR/RXR signaling pathways that lead to an increase in hepatic glucose production (gluconeogenesis) and a decrease in glucose tolerance.[37] Interestingly, according to previous genetic studies, regions near the RBP4 locus on human chromosome 10q have been linked to higher diabetes risk in independent populations, suggesting that elevated RBP4 can be an independent risk factor for glucose intolerance.[38,39] Leptin is a 16-kDa protein hormone secreted by adipocytes. However, it is secreted from other organs, including the mammary gland, ovary, skeletal muscle, stomach, pituitary gland, and lymphoid tissue in low amounts.[40] Leptin production is induced by insulin; however, cortisol is a suppressor of this adipokine.[41,42] Two signaling pathways are regulated by leptin is associated with insulin sensitivity, including the phosphatidylinositol 3-kinase/ak strain transforming/protein kinase (PI3K/AKT) and mitogen-activated-protein-kinase/extracellular signal-regulated kinases (MAPK/ERK) signaling pathways. Leptin phosphorylates the JAK2 that activates the AKT through IRS1 and PI3K phosphorylation. This signaling pathway leads to the forkhead box protein O1 (FOXO1) activation.[43] The FOXO1 expression increases the abundance of the insulin receptors, glucose transporter-4, ribosomal protein S6, mammalian target of rapamycin (mTOR) and raptor.[44] Moreover, β cell function and mass, as well as insulin secretion are regulated through the PI3K/AKT pathway in the pancreas.[45] Therefore, PI3K/AKT activation is a therapeutic target for insulin resistance regulated by leptin. The JAK2 phosphorylation upregulates the Ras (family of small GTPase) and Raf (raf proto-oncogene serine/threonine-protein kinase) that activate the MAPK/ERK signaling pathway.[43] The MAPK/ERK regulates cellular insulin sensitivity controlling the expression of the insulin-like receptor (inr) gene through the Ets-1 pointed orthologue.[46] Therefore, we expect that obese people that have more fat produce higher levels of leptin. It must be noted that leptin acts by binding to its membrane receptor (LepR). There are six isoforms of LepRs that with different actions due to various intracellular domains. LepRb is responsible for the main biologic actions of leptin that is predominantly expressed in the central nervous system. When leptin binds to LepRb, JAK2 is recruited, activated, and promoted auto-phosphorylation and phosphorylation of three tyrosine residues of LepRb (Y985, Y1077 and Y1138).[47] The *db/db* mice, the genetic model of obesity, lack exclusively the LepRb named leptin resistant.[48] Therefore, not only leptin production is critical for its effect on glucose metabolism, but also binding capacity to the receptors is essential for its biological actions.

In conclusion, the results of the present systematic review and meta-analysis showed that serum levels of RBP4 and leptin could be used as the predictive markers of GDM in pregnancy. However, further high-quality studies are needed to establish these results.

Author contributions

SS and SNM designed and searched systematically for the study. MH and TB reviewed and selected the articles and extracted data from articles under the supervision of EY. EY and TB: performed quality assessment of the trials. SS and MH performed data analysis and interpretation. SNM and SS drafted the manuscript. EY revised the article for important intellectual content.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: Quality assessment of case-control studies included in the current systematic review and meta-analysis on the association between circulating RBP4 and leptin levels and GDM risk[1]

Study	Selection				Comparability		Exposure			Final score
	Case definition adequate	Representativeness of the cases	Selection of Controls	Definition of Controls	Main factor	Controls for any additional factor	Ascertainment of exposure	Ascertainment for cases and controls	Non-Response rate	
Abetew *et al*. 2013	*	*	*	*	*	*	*	*	0	8
Fatima *et al*. 2017	*	*	*	0	*	*	*	*	0	7
Mosavat *et al*. 2018	*	*	*	0	*	*	*	*	0	7
Du *et al*. 2019	*	*	*	0	*	*	*	*	0	7
Francis *et al*. 2020	*	*	*	*	*	*	*	*	*	9
Jin *et al*. 2020	*	*	*	*	*	*	*	*	0	8

[1]Quality assessment was done based on the Newcastle-Ottawa scale (NOS)

Supplementary Table 2: Quality assessment of prospective cohort studies included in the current systematic review and meta-analysis on the association circulating RBP4 and leptin levels and GDM risk[1]

Study	Selection				Comparability		Outcome			Final score
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome of interest was not present at the start of the study	Main factor	Controls for any additional factor	Assessment of outcome	Follow-up long enough	Adequacy of follow-up of cohorts	
Qiu *et al*. 2004	*	*	*	*	*	*	*	*	*	9
Zhang *et al*. 2016	*	*	*	*	*	*	*	*	*	9

[1]Quality assessment was done based on the Newcastle-Ottawa scale (NOS)

Supplementary Table 3: Quality assessment of prospective cross-sectional study included in the current systematic review and meta-analysis on the association between circulating RBP4 and leptin levels and GDM risk[1]

Study	Selection				Comparability		Exposure		Final score
	Representativeness of the sample	Sample size	Non_ respondents	Ascertainment of exposure (risk factors)	Main factor	Controls for any additional factor	Assessment of outcome	Statistical test	
O`Malley *et al*. 2020	*	*	*	*	*	*	**	*	9

[1]Quality assessment was done based on the Newcastle-Ottawa scale (NOS)