Meta-Analysis

Association Between Neutrophil-Lymphocyte Ratio and Gestational Diabetes—A Systematic Review and Meta-Analysis

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Abstract

A growing body of evidence shows that the neutrophil-lymphocyte ratio (NLR) is a surrogate index of systemic inflammation in several chronic diseases. Conflicting associations between NLR and gestational diabetes mellitus (GDM) have been reported in individual studies. This meta-analysis sought to investigate the association between NLR and GDM. The PubMed, EMBASE, and Google Scholar databases were searched to identify relevant articles. The pooled standardized mean difference with 95% CI was calculated using a random-effects model. Subgroup and meta-regression analysis were carried out to control for the effects of GDM diagnostic criteria, ethnicity, body mass index (BMI), and age. Eleven eligible articles were included, containing 1271 participants with GDM and 1504 controls. Pooled outcomes indicated a higher NLR in GDM pregnancies than in normoglycemic controls (SMD = 0.584; 95% CI, 0.339–0.830; *P* < .001), although extensive heterogeneity between studies was noted. Subgroup analysis revealed that the higher pooled estimate in GDM was not affected by diagnostic criteria, ethnicity, or BMI, although matching for BMI reduced heterogeneity between studies. This meta-analysis supports the higher NLR in GDM described by some individual studies.

Key Words: gestational diabetes, neutrophil-lymphocyte ratio, systemic inflammation

Gestational diabetes (GDM) describes glucose intolerance that is first diagnosed during pregnancy [1]. Although it is generally transient and asymptomatic in its clinical course, it may lead to the development of a wide range of fetal complications. GDM increases the risk of miscarriage, macrosomia, shoulder dystocia, neonatal hypoglycemia, hyperbilirubinemia, and stillbirth, and is thus associated with higher rates of cesarean birth and

operative vaginal delivery [2, 3]. In addition to adverse fetal outcomes, GDM is linked to long-term maternal effects. These include an increased recurrence rate in subsequent pregnancies, and an increased rate of progression to cardiometabolic disorders including type 2 diabetes mellitus (T2DM), atherosclerotic disease, and metabolic syndrome [4, 5]. Infants born to mothers with GDM also exhibit long-term health effects including impaired glucose tolerance and obesity [6, 7].

The pathophysiology of GDM is complex and overlaps with that of T2DM, with both disorders sharing a common underlying genetic architecture [8]. Human pregnancy is accompanied by extensive changes that accommodate the metabolic demand of the growing fetal-placental unit. In late pregnancy, insulin resistance and compensatory hyperinsulinemia develop because of reduced insulin sensitivity at adipose and muscle tissue. The adaptive metabolic reprogramming in pregnancy is driven by placenta-derived hormones, such as human placental lactogen, prolactin, and human placental growth hormone, as well as a wide array of adipocytokines [9, 10]. In GDM, an inadequate insulin secretory response leads to the development of hyperglycemia [11, 12].

Chronic low-grade inflammation plays a critical role in the development both of GDM and T2DM [13, 14]. A broad array of adipocytokines, including interleukin 6, adiponectin, leptin, resistin, and visfatin regulate insulin resistance, lipid metabolism, and β-cell function. Several studies have investigated the association between these cytokines and GDM [15-19]. However, the clinical utility of serum cytokine assays is limited by challenges in analytic methodology, their low specificity, and the absence of defined quantitative cutoff thresholds. Functionally, inflammatory cytokine networks interact with postinsulin receptor signal transduction cascades to block tyrosine phosphorylation of insulin receptor substrate, thus disrupting signaling through the insulin receptor–insulin receptor substrate–PI3K pathway [20, 21].

Recently, combined indices of systemic inflammation that are obtainable from routine whole blood counts have attracted considerable interest as disease biomarkers. The neutrophil-lymphocyte ratio (NLR) is a low-cost, widely available parameter that has been investigated as a reliable proxy marker of systemic inflammation in a spectrum of chronic diseases. NLR levels positively correlate with interleukin-6 and interleukin-8 levels in liver cirrhosis and ovarian cancer [22, 23]. Meta-analyses have shown that a high NLR correlates with worse outcome in pulmonary embolism, cardiovascular disease, and several malignancies [24-29]. In the context of metabolic disorders, studies have shown that the NLR is a predictive marker both of prediabetes and T2DM and is associated with

microalbuminuria, cerebrovascular disease, and kidney disease in T2DM [30-32]. Several studies have investigated the utility of NLR in GDM, with inconsistent or incongruent results. Some researchers have observed elevated NLR in GDM cases compared to controls [33-35], whereas others have not [36, 37]. The contrasting associations can be attributed to several factors, including statistical power, population heterogeneity, and differences in GDM diagnostic criteria. However, the role of NLR in GDM has not been studied through a systematic review of the available literature. Therefore, we conducted a comprehensive systematic review and meta-analysis of studies to explore the association of NLR with GDM from pooled data.

Materials and Methods

Literature Search

A systematic literature search of eligible studies evaluating the association between NLR and GDM was conducted using PubMed/MEDLINE, Google Scholar, and EMBASE databases from their inception until December 2020. A comprehensive search strategy was based on the following combinations of free text key words and medical subject heading terms: "gestational diabetes" or "GDM" or "pregnancy-induced diabetes" or "gestational diabetes mellitus" and "neutrophil lymphocyte ratio" or "NLR" or "neutrophil-to-lymphocyte ratio."

Original published observational studies evaluating NLR in GDM pregnancies to a comparison group of women with normoglycemic pregnancies were eligible for inclusion in this meta-analysis. We excluded studies with 1) insufficient or ambiguous data for meta-analysis; 2) articles not written in English; 3) overlapping or duplicate data; and 4) conference proceedings, reviews, case reports, editorial letters, or comments.

Data Extraction

Articles identified from the literature search were screened for duplicates. All studies deemed to be potentially eligible for inclusion were reviewed and had data extracted by 2 independent investigators, and any discrepancies were resolved by consensus.

The following information was extracted from each eligible article: 1) primary author and year of publication; 2) the total number of participants including the number of controls and GDM cases; 3) country of data source; 4) GDM diagnostic criteria; 5) the gestational age at the time of sampling; 6) mean and SD of age, prepregnancy body mass index (BMI), and NLR in GDM and control groups; 7) where available, data on parity, gravidity, and

macrosomia; and 8) high-sensitivity C-reactive protein (hsCRP). This meta-analysis is reported in accordance with the Preferred Reporting Items for Systemic Reviews and Meta-Analysis guidelines [38]—supplementary Fig. 1 [39].

Quality Assessment

The methodological quality of studies was determined using a modified version of the Newcastle-Ottawa Scale for case-control studies [40].This tool evaluates studies on the basis of 3 components—the selection of study groups, their comparability, and the exposure of interest. The Newcastle-Ottawa Scale components were customized to fit this meta-analysis with regards to defining the control group and adjusting for confounding factors. One item of the exposure category was excluded (nonresponse rate) because it was not applicable to this analysis. Therefore, the exposure category was awarded a maximum of 2 points. We considered age and BMI differences between GDM and normoglycemic cohorts to be essential confounding factors for the comparability category, given their established association with the GDM phenotype. Studies with a score of 6 or greater indicated high quality.

Statistical Analysis

Results are presented as standardized mean differences (SMDs) and corresponding 95% CI. Pooled estimates were calculated using the using the DerSimonian and Laird random-effects model [41]. Statistical heterogeneity in SMD values among studies was evaluated using the Cochran Q and Higgins I^2 statistics. Heterogeneity was quantified using the equation $I^2 = 100\% \times (Q - df)/Q$, where df indicates degree of freedom and I^2 indicates the degree of inconsistency between studies and whether the percentage of total variation across studies is due to heterogeneity or chance. Statistically significant heterogeneity was considered when the P value was less than .1 and the I^2 value more than 50% [42].

We conducted subgroup analysis and meta-regression analysis for categorical and continuous variables respectively to explore potential sources of heterogeneity apart from random error. Subgroups were defined by GDM diagnosis criteria and study geographic location. The effect of quantitative variables, including age, study size, and BMI, was assessed by meta-regression. To evaluate the contribution of each study on the overall effect, sensitivity analysis was performed by sequentially omitting each individual study [43].

Publication bias was assessed by visual inspection of Begg funnel plot and the Doi plot. The Doi plot uses a rank-based measure (*Z* score) of precision plotted against the effect size. Asymmetrical Doi plots suggest the presence of publication bias. The Luis Furuya–Kanamori (LFK) index quantifies Doi plot asymmetry, with LFK indices exceeding ±2 indicating major asymmetry [44, 45]. The LFK index has a higher sensitivity and diagnostic accuracy for detection of publication bias than the Egger regression test, especially when the number of studies is small.

All statistical tests were 2-sided, and a *P* value of less than .05 was considered statistically significant. The meta-analysis was conducted using MetaXL version 3.0 (EpiGear; http://www.epigear.com).

Results

Eleven studies comprising 1271 participants with GDM and 1504 controls were included [33-37, 46-51]. Their characteristics are summarized in Table 1. The studies differed in GDM diagnostic criteria, cohort size, and matching for age and prepregnancy BMI between the case and control groups. Wang et al separately stratified a hyperglycemia first diagnosed in pregnancy cohort into GDM and a diabetes in pregnancy cohort. In keeping with the specific objectives of this meta-analysis, findings from the GDM group are presented [34]. Sargin et al distinguished between GDM, impaired glucose tolerance, and only screen-positive cases, and reported only findings from the GDM group [36]. Sun et al controlled for age, BMI, and parity as confounders by adopting a 1:1 case-control matching procedure, and presented findings from the matched subanalysis [48]. Similarly, findings from the subcohort matched for age, diabetes family history, acanthosis nigricans, education level, and socioeconomic status reported by Basu et al are used in this meta-analysis [47]. The quality assessment of all the published studies that evaluated the association between NLR in GDM vs normoglycemic pregnancies is shown in Supplementary Table 1 [39].

Two studies did not identify a statistically significant difference in NLR between GDM cases and matched controls [36, 37]. Only 2 studies reported hsCRP, while Wang and colleagues reported CRP levels semiquantitatively [34, 35, 50]. In these studies, CRP levels were significantly elevated in patients with GDM. The Homeostatic Model Assessment of Insulin Resistance was reported in only one study [49]. Clinical data on parity and gravidity, and key pregnancy outcomes relevant to GDM—such as macrosomia—were inconsistently reported in the included studies. Summary statistics identified a significantly higher mean NLR in GDM cases (3.74 ± 0.67) compared to controls (3.11 ± 0.61), *P* = .036—Fig. 1.

Table 1. Characteristics of included studies

Study	Country	GDM criteria	Cohort size		GDM			Controls		
			GDM	Controls	Age, y	BMI	NLR	Age, y	BMI	NLR
Aktulay 2015 [46]	Turkey	Carpenter Coustan	29	29	29.3 ± 5.2	29.1 ± 5.2	5.1 ± 1.9	27.0 ± 6.1	27.1 ± 3.3	3.3 ± 0.9
Basu 2018 [47]	India	DIPSI	114	114	25.7 ± 4.8	22.7 ± 3.9	2.8 ± 0.9	24.3 ± 4.3	25.6 ± 4.1	1.79 ± 0.5
Dincgez Cakmak 2019 [50]	Turkey	Carpenter Coustan	60	75	29.4 ± 5.4	27.6 ± 2.1	3.4 ± 1.3	28.6 ± 5.4	25.6 ± 1.9	2.94 ± 0.85
Fashami 2020 [37]	Iran	IADPSG	110	110	29.3 ± 5.9	25.4 ± 2.8	3.2 ± 1.9	27.9 ± 6.2	25.6 ± 2.7	3.06 ± 1.04
Liu 2020 [35]	China	IADPSG	58	62	29.8 ± 5.1	24.5 ± 4.3	3.4 ± 0.9	30.2 ± 4.8	25.1 ± 4.3	3.00 ± 0.8
Sahbaz 2016 [51]	Turkey	Carpenter Coustan	59	60	31.4 ± 5.4	26.3 ± 2.2	4.3 ± 1.5	26.8 ± 4.1	24.4 ± 1.4	3.76 ± 1.84
Sargin 2016 [36]	Turkey	Carpenter Coustan	144	304	30.9 ± 4.8	30.1 ± 1.9	3.5 ± 1.2	27.51 ± 5.22	26.21 ± 2.0	3.44 ± 1.19
Sun 2020 [48]	China	IADPSG	200	200	30.0 ± 5.0	23.4 ± 3.5	3.9 ± 1.3	29.00 ± 5.00	23.4 ± 3.5	3.45 ± 1.21
Wang 2020 [34]	China	IADPSG	153	172	29.1 ± 4.3	28.4 ± 3.6	4.4 ± 1.3	27.39 ± 3.31	26.6 ± 3.8	3.62 ± 0.91
Yang 2015 [49]	China	IADPSG	302	310	30.6 ± 4.0	21.2 ± 1.8	3.7 ± 0.3	27.0 ± 3.3	20.5 ± 2.2	3.67 ± 0.28
Yilmaz 2014 [33]	Turkey	Carpenter Coustan	42	68	30.4 ± 3.4	29.2 ± 2.5	3.0 ± 0.8	26.75 ± 3.19	24.9 ± 3.0	2.26 ± 0.43

Abbreviations: BMI, body mass index; DIPSI, Diabetes in Pregnancy Study Group India; GDM, gestational diabetes mellitus; IADPSG, International Association of Diabetes and Pregnancy Study Group; NLR, neutrophil-lymphocyte ratio.

Associations Between Neutrophil-Lymphocyte Ratio and Gestational Diabetes Mellitus

A meta-analysis of 11 studies investigating the association between GDM and NLR was carried out. A random-effects model was applied because of significant heterogeneity between studies (I^2 = 89.12%). In the pooled analysis, a significant increase in NLR was observed between the GDM and control groups (SMD = 0.584; 95% CI, 0.339-0.830, SE = 0.125; P < .001)—Fig. 2. There was extreme heterogeneity between studies in the overall analysis (I^2 = 89.21; P < .001).

Subgroup Analysis

To identify potential sources of heterogeneity, subgroup analysis was performed according to 1) GDM diagnosis criteria, 2) study geographic location, and 3) studies controlling for BMI differences between controls and cases. NLR was significantly higher in GDM cases compared to normoglycemic controls irrespective of whether GDM was diagnosed with the International Association of Diabetes and Pregnancy Study Group (IADPSG; SMD = 0.388; 95% CI, 0.227-0.550; SE = 0.083; P < .001) or the Carpenter and Coustan (SMD = 0.629; 95% CI, 0.176-1.082; SE = 0.231; P = .007) criteria—Fig. 3. Similarly, subgroup analysis by geographic location revealed significantly higher NLR in GDM in studies both from China (SMD = 0.442; 95% CI, 0.292-0.593; SE = 0.077; P < .001) and Turkey (SMD = 0.629; 95% CI, 0.176-1.082; SE = 0.231; P = .007). A significantly higher NLR was detected in GDM cases irrespective of whether the investigators matched for BMI, although heterogeneity was higher in the BMI-unmatched studies (I^2 = 93.11%) vs the BMI-matched studies (I^2 = 67.14%)—Supplementary Fig. 2 [39]. We also explored the pooled SMD across studies stratified by BMI category in the GDM cohort. A significantly higher NLR was observed both in the normal weight (SMD = 0.675; 95% CI, 0.300-1.050; SE = 0.191; P < .001) and in the overweight cohort (SMD = 0.362; 95% CI, 0.244-0.479; SE = 0.006; P < .001)—Supplementary Fig. 3 [39]. Of note, no heterogeneity was observed in the 3 studies incorporating lean GDM, whereas significant heterogeneity was observed in the studies using overweight GDM women.

To further investigate the impact of confounding factors on differences in the NLR between GDM and controls, a univariate meta-regression analysis was conducted. The outcome variable was the SMD of the NLR, and the covariates included age in years, prepregnancy BMI, year of publication, and study size. Age in GDM cases and controls was found to be significantly and negatively associated with the
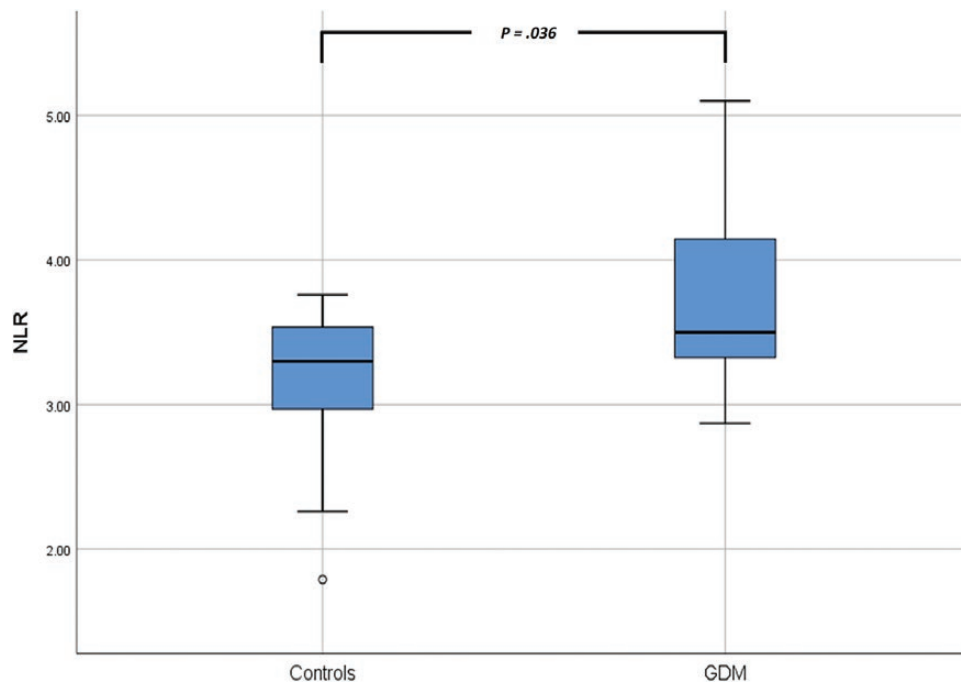
Figure 1. Box and whisker plot comparing mean neutrophil-lymphocyte ratio (NLR) in gestational diabetes mellitus (GDM) cases to normoglycemic controls. Independent samples *t* test identified a significantly higher mean NLR in GDM cases (3.74 ± 0.67) compared to controls (3.11 ± 0.61). *P* = .036.
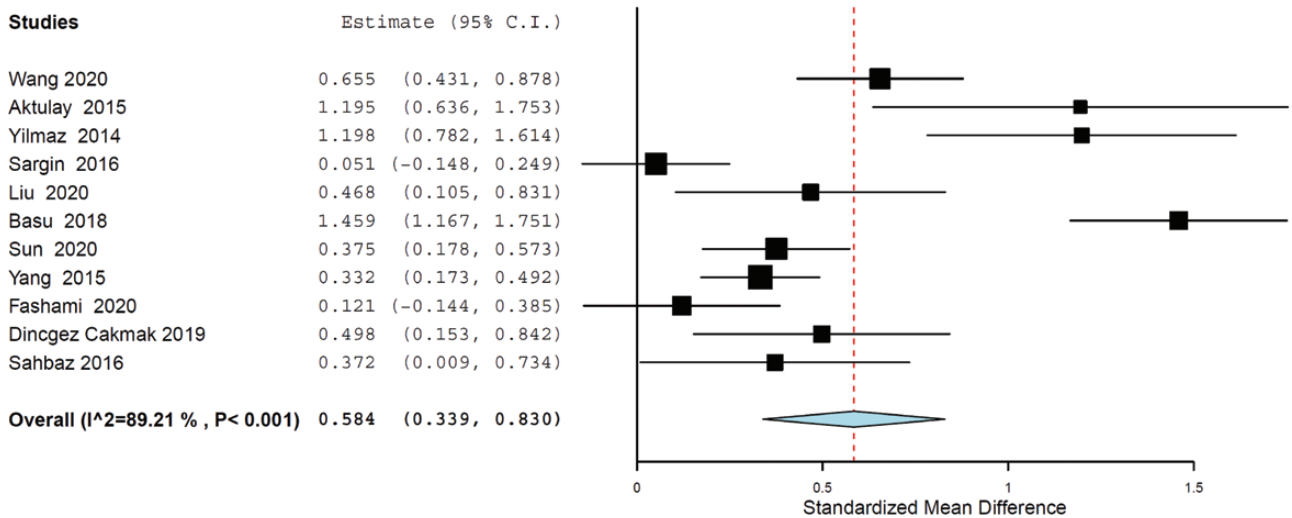

Figure 2. Forest plot showing a comparison of the neutrophil-lymphocyte ratio between gestational diabetes mellitus (GDM) cases and controls. Results are presented as standardized mean differences (SMDs) and corresponding 95% CI. A significant increase in NLR was observed between GDM when compared to normoglycemic controls (SMD = 0.606; 95% CI, 0.341 to 0.871; SE = 0.135; *P* < .001).

pooled SMD (*P* < .05), whereas the other quantitative variables showed no effect on the overall SMD—Table 2. Meta-regression plots are provided in Supplementary Fig. 4 [39].

Sensitivity Analysis and Publication Bias

The stability of the meta-analysis was evaluated by sensitivity analysis. When single studies were sequentially removed, no significant effect on the pooled SMD was observed, with an effect size ranging from 0.47 to 0.64, suggesting that the results of the meta-analysis were stable—Table 3. Visual inspection of the funnel plot shows divergence from the expected shape and reveals asymmetry. The Doi plot and LFK index similarly demonstrated major asymmetry, suggestive of publication bias or small-study effects (LFK index = 2.28). No asymmetry was detected when the 5 studies defining GDM by IADPSG criteria were considered (LFK index = 0.41)—Supplementary Fig. 5
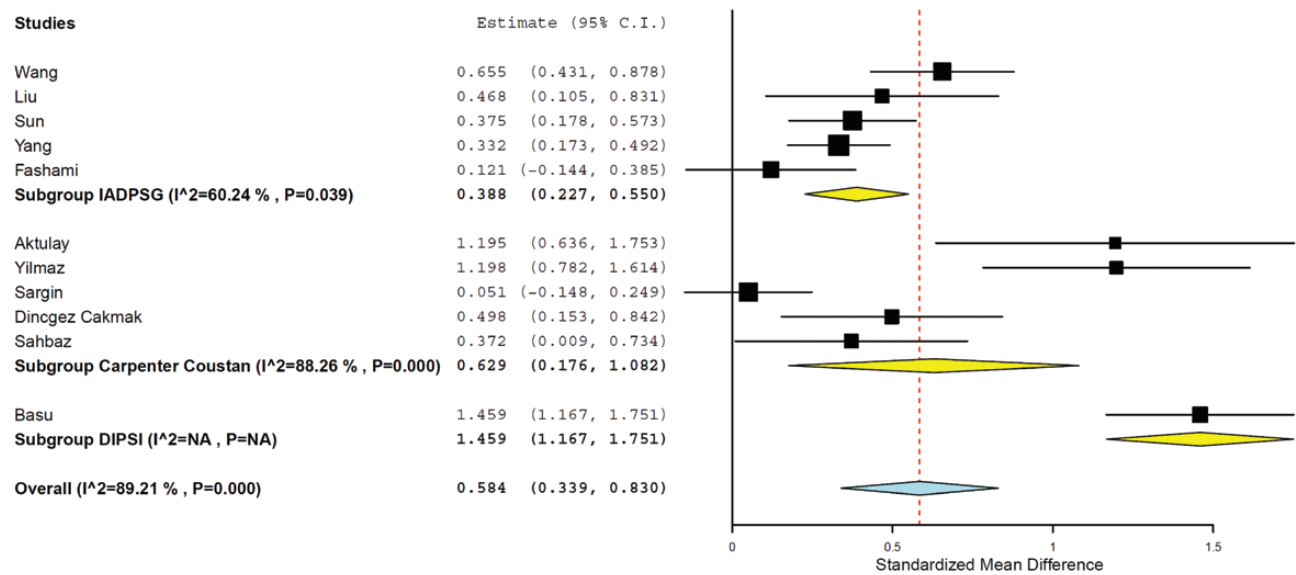
Figure 3. Subgroup analysis stratified by gestational diabetes mellitus (GDM) diagnostic criteria. Neutrophil-lymphocyte ratio was significantly higher in GDM cases compared to normoglycemic controls irrespective of whether GDM was diagnosed with the International Association of Diabetes and Pregnancy Study Group (IADPSG) or Carpenter-Coustan criteria. DIPSI, Diabetes in Pregnancy Study Group India.

Table 2. Coefficient and associated P values for body mass index, age, sample size, and publication year in meta–regression analysis comparing neutrophil-lymphocyte ratio between gestational diabetes mellitus cases and controls

Covariate	Coefficient (95% CI)	P
BMI GDM	0.004 (–0.086 to 0.094)	.934
BMI controls	0.069 (–0.071 to 0.208)	.334
Age GDM	–0.205 (–0.329 to –0.081)	.001
Age controls	–0.182 (–0.324 to –0.041)	.01
Study size	–0.001 (–0.003 to 0.00)	.09
Publication y	–0.050 (–0.162 to 0.062)	.384

Age in GDM cases and controls was found to significantly affect the NLR ($P < .05$), whereas other quantitative variables showed no effect.
Abbreviations: BMI, body mass index; GDM, gestational diabetes mellitus; NLR, neutrophil-lymphocyte ratio.

[39]. Major asymmetry persisted when the studies defining GDM by the Carpenter Coustan criteria were considered.

Discussion

This review combines evidence from studies investigating NLR in pregnant women with GDM compared to normoglycemic controls. Through comprehensive analysis of 11 studies and their meta-analysis, we provide evidence supporting a higher NLR in GDM than in euglycemic pregnancies… This finding is congruent with the established role of systemic inflammation in the development of GDM. However, it warrants cautious clinical interpretation in view of the nonspecific nature of the NLR and the extensive degree of interstudy heterogeneity identified in the pooled analysis.

The evidence presented here is significant for several reasons. It represents the first attempt, to our knowledge, to pool NLR estimates in GDM in a meta-analysis. It should therefore provide a more robust estimate of the association than that provided by individual studies, which often comprise relatively small numbers of participants. This meta-analysis is also strengthened by using a comprehensive search strategy with strict inclusion and exclusion criteria. Additionally, various demographic and clinical parameters were evaluated as possible confounders of the observed between-study variance to increase the robustness of the meta-analysis.

In subgroup analysis, we show that the pooled difference in NLR between cases and controls was significantly larger when GDM was diagnosed using the Carpenter and Coustan criteria rather than the IADPSG criteria, although both subgroups still demonstrated high between-study heterogeneity. This finding can be possibly attributed to the lower diagnostic cutoff applied at the one-step 75-g oral glucose tolerance test recommended by the IADPSG guidelines [52]. The IADPSG thresholds are derived from the seminal Hyperglycemia and Adverse Pregnancy Outcome study and capture mild hyperglycemia that is associated with adverse neonatal outcomes [53]. It is likely that the detection of milder hyperglycemia in pregnancy using the IADPSG parameters dilutes the phenotypic severity of GDM when compared to the 2-step Carpenter and Coustan criteria.

This meta-analysis identified significant variation among individual studies included in the pooled estimates. Notably, the extent of heterogeneity was minimally affected

Table 3. Sensitivity analysis

Excluded study	Pooled SMD [95% CI]	Cochran Q	P
Aktulay 2015 [46]	0.54 (0.29-0.79)	85.65	< .05
Basu 2018 [47]	0.47 (0.28-0.66)	43.96	< .05
Dincgez Cakmak 2019 [50]	0.59 (0.33-0.86)	92.35	< .05
Fashami 2020 [37]	0.63 (0.37-0.90)	85.65	< .05
Liu 2020 [35]	0.60 (0.33-0.86)	92.41	< .05
Sahbaz 2016 [51]	0.61 (0.34-0.87)	92.19	< .05
Sargin 2016 [36]	0.64 (0.39-0.89)	73.48	< .05
Sun 2020 [48]	0.61 (0.33-0.89)	91.65	< .05
Wang 2020 [34]	0.58 (0.31-0.85)	88.98	< .05
Yang 2015 [49]	0.62 (0.33-0.90)	89.39	< .05
Yilmaz 2014 [33]	0.53 (0.28-0.77)	79.84	< .05

No individual study significantly affected the pooled estimate.

Abbreviation: SMD, standardized mean difference.

by subgroup analysis controlling for GDM diagnostic criteria and BMI. The residual heterogeneity can be attributed to unmeasured or incomplete adjustment for confounding factors. Uncontrolled confounding factors can significantly bias any causal inference in observational studies and should be recognized as potential sources of systemic errors in epidemiologic studies [54, 55]. In the context of this meta-analysis, it is essential to emphasize that empirical quantitative conclusions on the NLR in GDM are currently limited by interstudy heterogeneity. It is plausible to postulate that this heterogeneity arises because of the combined effects of several factors—including publication bias, selective outcome reporting, variable differences in study design and patient ascertainment criteria, the limited number of available studies, and spuriously inflated effects in small studies. Notably, several meta-analyses investigating the association of NLR with different clinical end points exhibited identical levels of interstudy heterogeneity [56-58].

Subgroup analysis also revealed a lower difference in NLR between GDM and controls and a lower between-study heterogeneity in studies originating from China. Possibly, such differences can be partially accounted for by population-specific risk factors that affect the risk and progression of dysglycemia. These include variations in population genetics, demographics, and the background prevalence of T2DM between populations [59, 60]. Racial or ethnic-specific variation in adiposity and body composition can in part account for the observed differences. Asians have higher body and truncal fat proportions than White individuals matched for age and BMI [61]. BMI captures considerable heterogeneity as a phenotypic marker of adiposity and body shape across ethnicities that should be considered when critically evaluating population studies [62].

The 11 studies included in this review hail from 4 countries and capture wide disparity in GDM characteristics and risk factors. GDM prevalence stands at 16.3% in Europe, and ranges from 1.2% in India to greater than 24% in China [63-65]. Studies report significant associations between GDM and both age and adiposity. In addition, considerable heterogeneity in sociodemographic GDM predictors is described in studies from different geographic regions. Risk factors such as parity, education level, ethnicity, economic status, and physical activity are variably implicated in GDM risk in different population studies [64-67]. These variables can represent potential hidden confounders driving interstudy heterogeneity in this meta-analysis.

The findings from this analysis also suggest that BMI is a potential confounder in NLR interpretation in GDM. The univariate meta-regression analysis showed no significant association between BMI and the pooled SMD in the overall analysis. However, we show that studies matching for BMI exhibited lower heterogeneity, and that no heterogeneity was observed in the studies using lean GDM women. A positive correlation between NLR and BMI has been described in the literature, potentially reflecting a systemic chronic proinflammatory state induced by excess adiposity [68, 69].

The negative correlation between NLR and increasing female age has been reported in several studies [70, 71]. Sex-specific differences in leukocyte composition with age exist, attributed to the effects of estrogen and progesterone on promoting neutrophil recruitment and delaying apoptosis [72, 73]. Additionally, during pregnancy, the NLR varies between trimesters, reaching a maximum value in the second trimester [74]. It is thus likely that the NLR values reported in cross-sectional studies on GDM are strongly confounded both by maternal and gestational age.

In addition to its variable association with GDM, the NLR has been investigated in relation to other obstetric complications, including hyperemesis gravidarum, preeclampsia, pregnancy-associated intrahepatic cholestasis, and pregnancy outcome [75-78]. A large retrospective population study showed no difference in NLR between high-risk and

normal-risk pregnancy groups, but demonstrated a weak positive correlation between NLR and patient age [74]. Critically, the interpretation of NLR in pregnancies complicated by GDM requires consideration of several factors, including diagnostic criteria, ethnicity, BMI, and patient age as potential confounders.

The observed differences in NLR and their putative association with GDM need to be interpreted in the context of the hematologic changes accompanying pregnancy and diabetes. Normal pregnancy is accompanied by increased leukocyte counts in the second and third trimesters [79]. Prospective studies have shown that leukocyte counts in early pregnancy are independently associated with GDM risk, and levels of proinflammatory cytokines and chemokines are elevated in GDM [80-83]. Within the normal range, elevated leukocyte counts have been associated with T2DM risk, microvascular complications, and reduced insulin sensitivity [84, 85]. The PROMISE cohort study showed that leukocyte subtypes were independently associated with insulin resistance but the NLR was not associated with β-cell dysfunction [86]. Physiologically, neutrophils are directly related to the development of insulin resistance, through imbalance of the neutrophil elastase and its inhibitor α1-antitrypsin that regulate adenosine monophosphate–activated protein kinase C signaling [87, 88]. Increased neutrophil activity and elevated neutrophil elastase in GDM placentae has been described [89]. Lou et al reported elevated plasma levels of neutrophil gelatinase-associated lipocalin both in lean and obese women with GDM [90].

In addition to the constraints imposed by broad interstudy heterogeneity, the findings from this review should be considered in the context of several other limitations. Studies reported a single NLR value at the time of the oral glucose tolerance test in the second trimester of pregnancy but did not assess its progression through gestation and beyond. Although GDM is typically considered a transient state, its metabolic derangement is potentially long-lasting, with investigations documenting increased carotid intima media thickness and persistent subclinical inflammation in women with previous GDM [91, 92]. Crucial pregnancy outcomes (miscarriage, birth weight) were not described by some studies, thus limiting interpretation of the cause-effect relation between NLR and GDM. Secondly, key biochemical indices of systemic inflammation and insulin resistance, such as hsCRP and Homeostatic Model Assessment of Insulin Resistance, were missing in several of the studies included in this meta-analysis. A single measurement of NLR does not fully capture the systemic proinflammatory state in GDM, particularly during the dynamic metabolic and hematologic changes of pregnancy. Thirdly, we included studies published only in the English language, and some studies may not have been included in this meta-analysis (articles missing key data, conference proceedings, case reports, etc). Furthermore, although the NLR is a simple biomarker of systemic inflammation, no universal cutoff reference value derived from healthy populations is in use. Forget et al report a mean NLR of 1.65 (range, 0.78-3.53) in an active, healthy, adult nongeriatric population [93]. Racial and ethnic variations in NLR in large North American and Central American cohorts have been recently documented [71, 94]. This limits the direct comparison of studies reporting NLR in different populations. The pooled analysis of different studies restricts interpretation of the cause-effect relationship between NLR and GDM. Recently, Rias et al showed that environmental exposures such as secondhand smoke and physical activity correlate with the NLR, and these confounding factors were not accounted for by the studies incorporated into this review [95].

Conclusion

This meta-analysis provides evidence supporting the significant increase in NLR in pregnancies complicated by GDM. While it seeks to overcome the limitations of individual studies by providing pooled estimates, several factors limit the direct clinical translation of NLR as a simple inexpensive biomarker of GDM. Further studies are needed to define its clinical utility and validity, and to provide robust physiological evidence causally linking the NLR to GDM and its clinical outcomes.

Additional Information

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