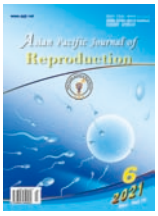

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Neutrophil-lymphocyte ratio in pregnancy-associated maternal complications: A review

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ABSTRACT

Pregnancy associated diseases/disorders are associated with significant maternal and neonatal morbidities and mortalities. Devising/validating cost effective and easily accessible predictive, diagnostic and risk stratification markers are critical to the management and improved outcome in these diseases. Inflammation forms the backbone of most of the routinely encountered maternal complications of pregnancy. Hematological markers can be considered as a direct reflection of the systemic inflammatory milieu. Recently, the neutrophil lymphocyte ratio has been explored for its potential to assess the severity of inflammation and thus the severity of the underlying disorder. The neutrophil-lymphocyte ratio has gained scientific attention as a potential prognostic/predictive marker of acute as well as chronic inflammatory diseases including gynecological and reproductive disorders. This present study reviews the mechanistic role of neutrophils and lymphocytes in fueling or propagating the inflammatory cascades in the three most common maternal complications of pregnancy and the evidence of clinical importance of the neutrophil to lymphocyte ratio in predicting, diagnosing, and prognosticating pregnancy-associated complications.

KEYWORDS: Neutrophil-lymphocyte ratio; Pregnancy; Preeclampsia; Gestational diabetes mellitus; Ectopic pregnancy; Inflammation

1. Introduction

Pregnancy, a physiologically hypermetabolic state, is characterized by increased maternal plasma and altered hematological and biochemical pictures. The induced hypermetabolic and hypervolemic state is beneficial to meet the demands of the developing fetus[1,2]. Normal pregnancy is accompanied by significant hematological changes, including physiological anemia, thrombocytopenia, and neutrophilia[3]. Hematological changes reflect immunomodulation which is central to a successful conception, fetal sustenance, and healthy maternal and neonatal outcomes. Pregnancy involves accepting a semi-allogeneic fetus; hence immune responses play a significant yet unexplained role in the so-called "immunological paradox of pregnancy"[4]. The powerful human defense system is partially tricked during pregnancy which modulates itself to provide maximum protection from environmental hazards yet circumvents immune rejection of a semi allogeneic fetus. These strategies include immune attenuation, suppression, or evasion with the maternal immunological pathways favoring diminished likelihood of activating adaptive immunity, ensuring immune activation is skewed towards immune tolerance, and facilitating the tissue remodeling required to support placental development and function[5]. The proposed mechanisms are the lack of antigen stimulation in maternal lymphocytes, peripheral (relative suppression of cell-mediated immune response accompanied by simultaneous activation of the innate immune response), and decidual (suppression of type Ⅰ cytokine production) suppression of lymphocyte function along with fetal mechanisms to escape maternal immune attack[6,7]. Hematological parameters reflect adaptive changes of pregnancy. Hence, perturbations in a hematological profile can reflect pregnancy outcomes[8].

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Physiological stress in normal pregnancy is characterized by leukocytosis, which manifests as an elevated inflammatory response aimed to effectively balance physiological demands of pregnancy, ranging from immunomodulation, immunosuppression to selective immune tolerance. The significantly elevated neutrophils in pregnancy are attributed to impaired neutrophil apoptosis. The pregnancy-induced alteration in lymphocytes, the defensive cells, remains controversial. A study suggested that lymphocyte counts might increase during pregnancy as a protective measure against infections. However, studies by Eledo *et al* reported gradual lymphocyte suppression with progress in gestational age[9]. In contrast, Chandra *et al* reported a decrease in lymphocyte count during the first and second trimesters and a subsequent increase in the third trimester[10]. The observed lymphopenia is hypothesized to be due to monocytosis ensuring immune tolerance and preventing fetal rejection[3].

Systemic inflammation can be measured by using a variety of biochemical and hematological markers. Recent evidence indicates that measuring the ratio of blood cell subtypes, such as the neutrophil-to-lymphocyte ratio (NLR), might have prognostic significance for diseases related to chronic low-grade inflammation[11].

NLR is an inexpensive inflammatory ratio that can be easily calculated from a simple blood count. Due to the ease of arriving at the ratio and the fundamental nature reflecting an inflammatory load, it is frequently evaluated as a prognostic factor in several medical disciplines[12]. Clinically, a high value has been associated with advanced disease[13]. In the gynecology and obstetrics literature, NLR has been evaluated in gynecological cancers, ovarian hyperstimulation syndrome, premature ovarian insufficiency, endometriosis, hyperemesis gravidarum, gestational diabetes, preeclampsia, pregnancy-associated intrahepatic cholestasis, *etc*[12]. NLR has been used as a predictive tool for maternal and neonatal outcomes[14]. This review focuses on the role of NLR in adverse pregnancy outcomes: preeclampsia, gestational diabetes mellitus (GDM), and ectopic pregnancy.

A comprehensive search (including research articles published till May 2021) was performed in the following scientific databases: PubMed/MEDLINE (USNLM), Scopus (ELSEVIER) and Web of Science to find all relevant articles on NLR in reproduction. The search strategy included the keywords such as: “preeclampsia”, “NLR”, “gestational diabetes mellitus”, “hemolysis, elevated liver enzymes, low platelets (HELLP) count syndrome”, “ectopic pregnancy”, “inflammation” which were combined using Boolean operators. Also, a random search in Google, Google scholar was conducted to obtain published articles on mechanism of neutrophil and lymphocyte perturbation or significance of NLR in disorders of pregnancy and reproduction.

2. NLR in preeclampsia

2.1. Preeclampsia and HELLP syndrome

Preeclampsia, a common gestational disorder, is clinically diagnosed by new-onset hypertension developing after 20 weeks of gestation (systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg) with or without proteinuria. In the absence of proteinuria, a diagnosis of preeclampsia is made in the presence of one or more of the cardinal signs, *i.e*., thrombocytopenia, renal insufficiency, elevated liver enzymes, pulmonary edema, cerebral, or visual disturbances. Preeclampsia and its associated complications (HELLP syndrome & eclampsia) remain one of the leading causes of maternal and perinatal morbidity and mortality[15,16].

Traditionally, preeclampsia is considered to progress through a “two-stage” theory; an abnormal spiral artery remodeling in early pregnancy causes placental hypoxia (stage 1) and the ischemic placenta releasing large amounts of soluble factors, such as reactive oxygen species (ROS), proinflammatory cytokines, and anti-angiogenic factors, into the maternal circulation, leading to the clinical manifestations and complications of the disease (stage 2)[15].

Evidence reiterates the presence of an exaggerated inflammatory response (abnormal cytokine production and neutrophil activation) and attributes the immune imbalance to the clinical manifestations of preeclampsia[15,17]. NLR, a simple marker of inflammation, is being widely assessed for its diagnostic, staging, prognostic and predictive potential in preeclampsia and associated hypertensive disorders of pregnancy.

2.2. Neutrophils in preeclampsia

2.2.1. Increase in the number of neutrophils

Numerous studies indicate leukocyte and neutrophil counts (leukocytosis, evidence of increased inflammatory response) were slightly elevated in mild preeclampsia, while severe preeclampsia is characterized by marked elevation of neutrophils. Inflammatory mediators [interleukin (IL)-6, tumor necrosis factor (TNF)-alpha] are attributed to the occurrence of neutrophilia and it is reported that neutrophilia is also associated with the disease severity, progression to HELLP syndrome, reversible posterior encephalopathy syndrome, and adverse maternal and fetal outcomes[18].

2.2.2. Delayed apoptosis

Delayed neutrophil apoptosis triggered by a heightened inflammatory response is believed to contribute to neutrophilia during pregnancy and preeclampsia. It is yet to be determined if delayed apoptosis is a compensatory reaction or exaggerated response to the severity of the disease. Studies are needed to determine the degree of delayed neutrophil apoptosis and its contribution to endothelial activation or dysfunction observed in preeclampsia.

This observed resistance to neutrophil apoptosis is also postulated to partly explain why the clinical syndrome of preeclampsia persists even after expulsion of the placenta (initiating stimulus) post cesarean section and draws similarities between preeclampsia and the systemic inflammatory immune response[18–20].

2.2.3. Neutrophil activation

Evidence supporting marked neutrophil activation mimicking systemic inflammatory response syndrome in preeclampsia has been directly implicated in hepatic necrosis, adult respiratory distress syndrome, and associated organ damages[20].

Neutrophil activation requires binding and transmigration of neutrophils through the endothelium. Neutrophil recruitment in the endothelium involves P-selectin and the release of platelet-activating factors from the endothelium. Potential mechanisms of neutrophil activation include upregulation of cellular adhesion molecules, increased generation of TNF-alpha, and endothelial activation. IL-6 and endothelin-1 activate neutrophils for superoxide production and contribute to arteriopathy and endothelial damage. Once activated, neutrophil undergoes degranulation, which in turn mediates vascular damage. Further, leukotrienes are synthesized, and superoxide is generated in a respiratory burst. Superoxide anions initiate lipid peroxidation and consequently endothelial cell lysis. However, it is still undetermined if activation of neutrophils is the cause or the consequence of endothelial damage[21].

2.2.4. NETosis

Neutrophil extracellular traps (NETs) were first implicated in preeclampsia with the detection of increased NETs in the intervillous space of placentae derived from patients (the first evidence indicating that NETs may be associated even with non-infectious human pathologies). Experimental studies have reported that placental debris activates polymorphic neutrophils *in vitro* and triggers the generation of NETs in a time and dose-dependent manner[22].

NETs have been detected in the intervillous space of normal placentae, too. However, preeclamptic placentae showed drastically elevated NETs (in few cases appearing to fill the entire intervillous space). NETs also contribute to the widespread systemic damage to the maternal endothelium observed in preeclampsia. However, it is currently unclear whether NETs represent an initiating lesion in preeclampsia are the result of another underlying placental deficiency[22].

2.3. Lymphocytes in preeclampsia

The immuno-tolerant state of the mother in normal pregnancy is changed to cellular hyperreactivity in preeclampsia. Various arms (innate as well as adaptive) of the immune systems undergo alterations (partial inactivation of attack arms and over activation of tolerance arms) to induce an immune tolerogenic shift to protect fetus from rejection and culmination of a successful pregnancy. This delicate toloregenic shift/dip is disrupted/perturbed in preeclampsia, resulting in an inappropriately overactive immune system. This disbalance leads to inappropriate placentation, growth and leads to the development of feto-restrictive and materno-toxic hypertensive environment in preeclampsia[23].

2.3.1. Role of T lymphocytes in preeclampsia

T lymphocyte perturbations commonly implicated in preeclampsia are as follows: a) low regulatory T cell (Treg) activity; b) shift towards T helper cell (Th-1) responses; c) presence of Th-17 lymphocytes[24]. It is hypothesized that the physiological shift from a Th1 to a Th2 phenotype at the fetal-maternal interface appears to be absent/disturbed in this syndrome[25].

Studies have reported reduced numbers and functional capacity of Tregs in preeclampsia compared with normal pregnancies[25,26]. Increased levels of serum endoglin could block transforming growth factor signals essential for Treg functions[27]. The predominance of Th17 cells in preeclampsia, the concomitant decrease in Treg function, and the resultant altered balance in the Th17: Treg ratio may be triggered by perturbations in circulating cytokines (IL-6 and IL-1)[25]. The reticular activating system functional elements of T lymphocytes produce angiotensin-2 at sites of inflammation, promoting chemotaxis of natural killer cells and T cells (Figure 1).

2.3.2. Role of B lymphocytes in preeclampsia

B lymphocytes play a vital role in the tailored immune mechanisms (the balance between protection and tolerance towards a semiallogenic fetus) essential for a successful pregnancy[28]. Protective antibodies such as antipaternal antibodies (antibodies directed to paternal HLA antigens) are essential to ensure a successful pregnancy, the absence of which leads to recurrent spontaneous abortions[29].

A successful pregnancy is also marked by increased production of asymmetric antibodies and immunoglobulin G (IgG) characterized by post-transcriptional addition of a high mannose oligosaccharide group in one Fab fragment, hindering effective bivalent antigen-antibody binding. These asymmetric antigens with relatively high affinity but altered binding properties fail to form insoluble antigen-antibody complexes and hence cannot activate immune effector functions (complement fixation, phagocytosis, and cytotoxicity) rendering increased immune tolerance[29].

Further, pregnancy favors increased differentiation of Breg cells (IL-10 producing immunoregulatory cells) and marginal zone B cells (compensatory strategy to ensure adequate defense)[30,31].

However, perturbation in B cell functions and resultant alloantibodies has been associated with obstetric complications[28]. Hence, controlled suppression of B cell lymphopoiesis and the subsequent B cell lymphopenia are proposed to be the fundamental mechanisms to ensure immune tolerance during pregnancy[30,31].

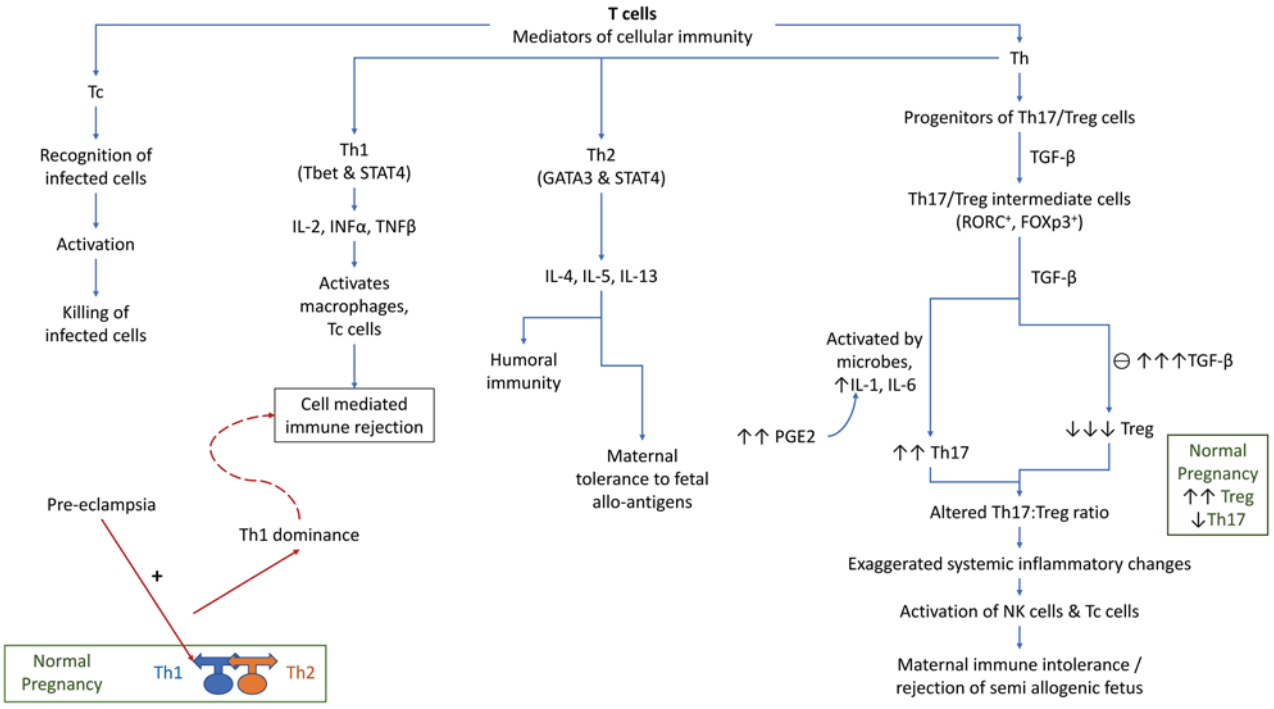

Figure 1. Role of T cells in preeclampsia. Tc: cytotoxic T cells; Th: T helper cells; Treg: regulatory T cell; IL: interleukin, INF: interferon; TNF: tumour necrosis factor; STAT: signal transducers and activators of transcription; TGF: transforming growth factor; PGE2: prostaglandin E2; ROR: retineic acid receptor related orphan nuclear receptor; FOXp3: forkhead box p3.

2.4. Presence of self–reactive B lymphocytes

2.4.1. B lymphocytes secreting antiphospholipid antibodies

Antiphospholipid antibodies (lupus anticoagulant, anticardiolipin antibodies, anti-beta2-glycoprotein-Ⅰ) have been associated with several adverse obstetrics outcomes (intrauterine fetal death, spontaneous abortions, deep vein thrombosis, and preeclampsia). Antiphospholipid syndrome is a hypercoagulable state that triggers blood clots/thrombosis, leading to significantly high adverse maternal and fetal outcomes[29].

2.4.2. Angiotensin II type I receptor autoantibodies producing B lymphocytes

B cells are implicated in preeclampsia due to the production of autoantibodies against adreno-receptors and AT1-R[22]. AT1 autoantibodies (IgG isotype) are seen in 70%–95% of women with preeclampsia. These antibodies bind to trophoblast and vascular cells receptors, inducing the production of soluble Fms-like tyrosine kinase-1 and serum endoglin. Stimulation of the IL-6 production induces endothelin-1. AT1-AA binds with a relatively high affinity to the AT1 receptor and mimics the natural ligand of the angiotensin type Ⅰ receptor[29].

AT1 autoantibodies may also promote vasoconstriction and hypertension. AT1 antibodies cross the placental barrier and contribute to intrauterine growth restriction by inducing apoptosis in the placenta. Adreno-receptor autoantibodies have been demonstrated in severe preeclampsia[25]. Further studies should aim to identify triggers and mechanisms by which these antibodies act in severe preeclampsia.

2.4.3. Upregulation of B cell activation factor (BAFF) and perturbations in Breg cell concentration/functions

BAFF, a cytokine essential for B cell survival and differentiation (Breg cells to naïve B cells), critically modulates B cell function during pregnancy[28]. Although low in circulation, alterations in numbers and function of Breg cells (aids in the prevention of auto/alloantibodies, induction/maintenance of Tregs, modification of T helper cell responses, inhibition of effector cell responses) have been extensively explored and implicated in systemic and neuro-immunological autoimmune diseases, graft rejection and cancers; hence alterations in Breg differentiation signaling ought to be critical to the complex immune balance of pregnancy[32,33].

Further, BAFF is shown to promote $CD19^{+}CD5^{+}B1$ cell survival, source of polyreactive antibodies (murine model), which are known to produce AT1 AA implicated in preeclampsia[28,29]. Maternal serum BAFF is reported to decrease with the initiation of pregnancy and continues to further decrease with advancing gestation (ensuring quiescence of autoreactive/alloreactive B cells)[28,32]. It is reported that BAFF circulating levels are elevated since the first trimester of pregnancy in patients developing hypertensive disorders of pregnancy[28].

2.4.4. Effect of hormonal dysregulation on B cells

High circulating levels of human chorionic gonadotropin (hCG)

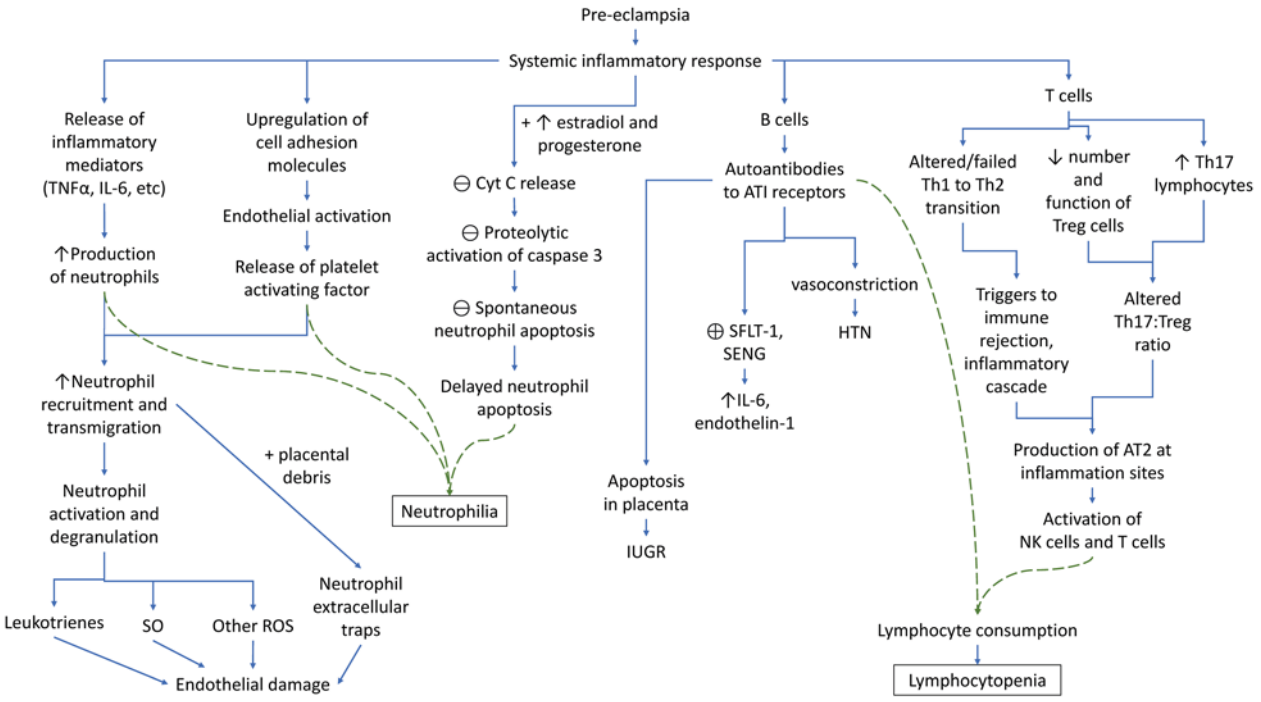

Figure 2. Neutrophil to lymphocyte ratio in preeclampsia. TNF alpha: tumour necrosis factor alpha; IL-6: interleukin 6; Cyt C: cytochrome C; AT1 receptors: angiotensin Ⅱ type Ⅰ receptors; sFlt: soluble fms-like tyrosine kinase 1; sEng: serum endoglin; HTN: hypertension; Th: T helper cells; IUGR: intrauterine growth retardation.

have been strongly associated with perturbed $CD19^{+}CD5^{+}$ in *in-vitro* models. $CD19^{+}CD5^{+}$ cells express many hCG receptors, and higher placental hCG levels characterize preeclampsia. $CD19^{+}CD5^{+}$ cells have also been implicated in the production of AT1 AA[34,35].

2.5. Significance of NLR in preeclampsia

Hematological perturbations have been recognized as systemic inflammatory response markers and may have predictive and prognostic value in inflammatory diseases and preeclampsia[18]. The above-explained mechanisms make neutrophils and lymphocytes essential determinants of the preeclampsia pathophysiology. Neutrophilia (due to increased production, activation & decreased apoptosis) and imbalances in the lymphocyte population together seem to account for an increased NLR ratio in preeclampsia. A study by Sachan *et al* showed a significantly higher NLR ratio in preeclampsia when compared to healthy controls, even in early pregnancy[36]. In contrast, a study by Yucel *et al* reported a significantly lower platelet lymphocyte ratio (PLR) ratio in severe preeclampsia compared to the controls but no significant differences in NLR ratios[37]. While NLR is reported to be significantly high in patients with preeclampsia, establishing robust cut-offs for classification of the severity needs future extensive studies[38] (Figure 2).

Kang *et al* suggest that NLR might be a useful laboratory marker for clinical prediction and evaluation of disease severity in preeclampsia. However, the authors also highlight the fact that available pieces of evidence are drawn from case-control studies. Therefore, prospective cohort studies are essential to accurately determine the utility of NLR, the optimal timing of analysis, and predictive cut-off values in clinical settings[39]. Sanchan *et al* reported that NLR was higher among women with preeclampsia when compared to healthy pregnant women even at early gestation and NLR at a cut-off value of >3.35 showed a significant diagnostic accuracy between the controls and mild preeclamptic women with an area under curve (AUC) of 0.75, 52.9% sensitivity and 74.5% specificity. A cut-off value of NLR 3.42 could effectively differentiate between mild and severe preeclampsia with a sensitivity of 81.3%, specificity of 64.7%, and AUC of 8.94[36]. According to Kirbas *et al*, NLR at 4.01 cut-off accurately predicted preeclampsia with sensitivity, specificity rates of 79.1% and 38.7%, positive and negative predictive values of 73.6% and 72.3%, and AUC of 0.568[40]. Kurtoglu *et al* showed NLR at 4.48 cut off predicted preeclampsia with 57.7% sensitivity, 63% specificity, 73.5% positive predictive value, 455% negative predictive value, 59.6% accuracy, and an area under curve (AUC) of 0.596[41]. Oylumlu *et al* reported that increased NLR and echocardiographic epicardial fat thickness were independent predictors of preeclampsia with 83.3% sensitivity, 81.5% specificity at NLR ≥4.1. with 0.925 AUC[42].

NLR >5.6 at early second trimester has been reported to predict the development of preeclampsia with a sensitivity of 73.4% and a specificity of 88.6%, and severe preeclampsia with sensitivity 93.3%

and specificity 86.6%, respectively[43].

A study reported that NLR [(odds ratio (*OR*) 1.43] and PLR (*OR* 1.38) emerged as most powerful predictors of preeclampsia on multivariate analysis with an AUC of 0.716 and 0.705 for NLR and PLR, respectively. The cut-off value of NLR ≥3.08 predicted preeclampsia with the sensitivity of 74.6% and specificity of 70.1% respectively[44]. Another meta-analysis by Wen *et al* reported a pooled sensitivity and specificity of 0.74 and 0.64 and diagnostic *OR* of 8.44 with 0.82 AUC. The authors added that the sample size of the studies was the primary source of heterogeneity, which appropriately concluded that as per evidence, NLR has unsatisfactory specificity but acceptable sensitivity for the diagnosis of preeclampsia. Extensive prospective cohort studies are required to validate the utility of NLR in preeclampsia[45].

3. NLR in gestational diabetes mellitus (GDM)

3.1. GDM

GDM is a unique and transient form of glucose intolerance exclusive to pregnancy. GDM can lead to adverse pregnancy outcomes. Recent evidence demonstrates that GDM is an inflammatory condition involving unbalanced cytokine production. Low-grade chronic inflammation-inducing insulin dysfunction (*e.g.*, defects in insulin sensitivity or inadequate B-cell compensation for insulin resistance) is considered as the leading cause of GDM pathogenesis[46]. The insulin post-receptor and inflammatory cytokine signaling pathways interact to block the normal tyrosine phosphorylation of the insulin receptor substrate by inducing serine/threonine phosphorylation of insulin receptor substrate. Phosphorylation of insulin receptor substrate reduces its ability to bind the insulin receptor, altering adequate glucose homeostasis. Accumulated metabolites derived from deranged glucose and lipid metabolism increases ketogenesis, enhances the release of inflammatory factors, and aggravates insulin resistance[47].

3.2. Neutrophils in GDM

Neutrophils, the first line of defense, are perturbed and increase vulnerability to opportunistic infections in patients with diabetes mellitus. Studies in patients and experimental organisms demonstrate defects of neutrophil chemotactic, phagocytic and microbicidal activities. Other alterations include increased protein leakage, reduced mast cell degranulation, impairment of neutrophil adhesion and migration, production of free radicals, decreased release of cytokines and prostaglandins by neutrophils, and increased leukocyte apoptosis. Metabolic alterations implicated in neutrophil dysfunction include discrepancies in the advanced protein glycosylation, polyol pathway, ROS, and the glycolytic and glutaminolytic pathways[48].

3.2.1. Enhanced release of neutrophil proteases and formation of NETs

GDM is associated with an altered neutrophil response, characterized by excessive pro-NETotic activity. Reports suggest that hyperglycemic states promote a pro-NETotic state. Data report an elevated circulatory TNF alpha level in GDM patients compared to the normoglycemic healthy pregnant women. Evidence indicates that circulatory TNF alpha plays a crucial role in mediating neutrophil activation in cases with GDM. TNF alpha can promote neutrophil migration, facilitating increased placental infiltration, where the primed neutrophils would readily undergo NETosis or degranulation. Exogenously liberated neutrophil elastase causes disturbances in physiological proliferation and increases insulin resistance, explaining pathological alterations like increased fetal size and villous immaturity. In addition, fibrin thrombi have been detected in the syncytiotrophoblast of placentae affected by GDM. Thus, GDM is associated with overt neutrophil activity, resulting in placental infiltration, enhanced NET formation, and neutrophil elastase release, which can profoundly alter placental cell biology *via* the enzymatic degradation of key regulatory signal transduction components[46,49].

3.2.2. Persistent neutrophil activation

A study reports significantly increased neutrophil and monocyte counts in the GDM patients when compared to normal pregnancies. This has been attributed to the exaggerated activation of circulating immune cells that promote inflammation and immune dysfunction. Increased cyclic adenosine monophosphate concentration in β islet cells induced by high glucose promotes neutrophil differentiation and aggregation through the mitochondrial respiratory pathway, further promoting IL-6, ROS, and leukotriene B4 production to contribute to the development of insulin resistance[47].

3.3. Lymphocytes in GDM

Some studies have concluded that insulin resistance may be related to altered signal transduction due to decreased number of T cells[50]. In pregnant women, high plasma glucose may lead to dysregulation of the adipo-insulin axis. Monocyte chemoattractant protein 1 (MCP-1) is upregulated by free fatty acids in high glucose conditions, thus inducing MCP-1-mediated pathologic inflammation and monocyte recruitment. Increased serum very low-density lipoprotein inhibits lymphocyte proliferation by disrupting DNA synthesis, possibly explaining the more pronounced reduction of lymphocyte cell counts in the GDM group with more severe glucose elevations[47].

3.4. Significance of NLR in GDM

A significant relationship between NLR and blood glucose regulation has been reported in type Ⅱ diabetes mellitus; elevated

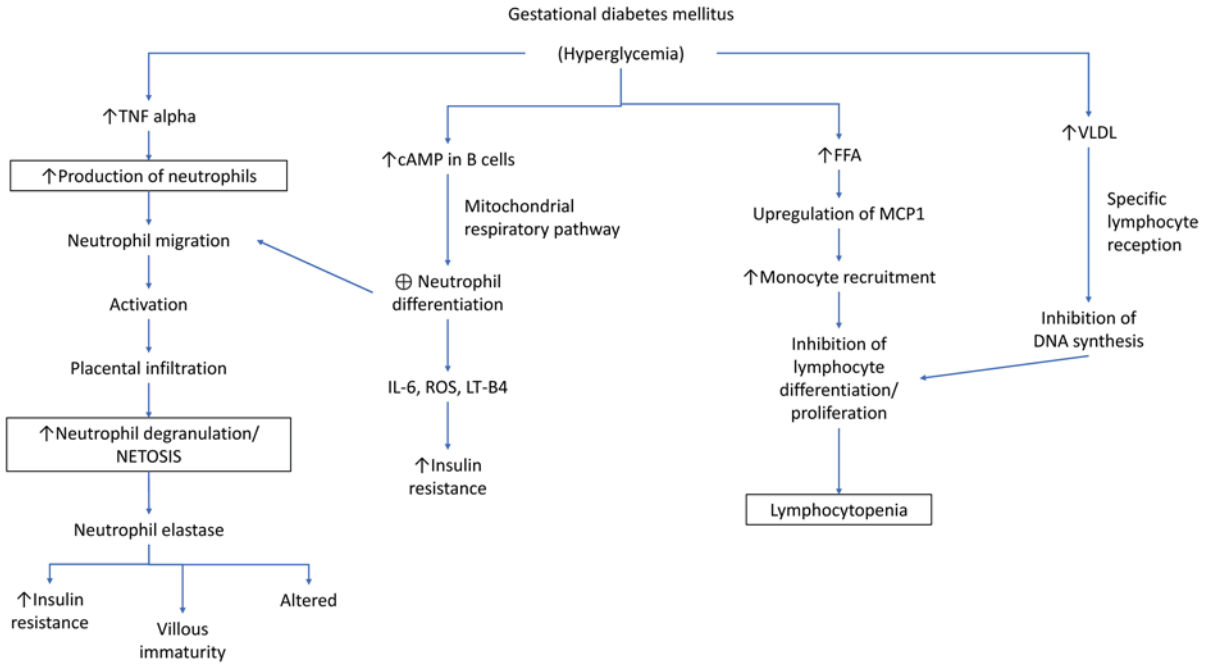

Figure 3. Neutrophil to lymphocyte ratio in gestational diabetes mellitus. cAMP: cyclic adenosine monophosphate; FFA: free fatty acids; VLDL: very low density lipoprotein; IL-6: interleukin 6; ROS: reactive oxygen species; LT-B4: leukotriene B4.

NLR may indicate underlying impaired glucose metabolism, which displays similar pathogenesis to GDM[47] (Figure 3).

Subclinical inflammation and cytokine-induced insulin resistance are the core inflammatory response mechanisms related to GDM[3,4]. Pattanathaiyanon *et al* found a significant impact of increased white blood cell counts on GDM development[14]. Increased NLR in GDM patients has been demonstrated in this study, with elevated NLR levels being proportional to serum glucose. Receiver operating characteristic curve analysis showed better diagnostic performance of NLR than other risk factors in distinguishing GDM from control pregnancies. However, NLR showed low sensitivity, possibly because the threshold of inflammatory/immune responses is elevated even in normal pregnancies, which calls for the development of gestational age-adjusted reference intervals for NLR. A study shows that NLR >4.394 is significantly associated with maternal complications, adverse neonatal outcomes, and preterm delivery, indicating that NLR may be a promising prognostic indicator for GDM. However, in contrast to this, a recent study reported NLR was not a beneficial prognostic marker[47].

Liu *et al* reported higher NLR values among GDM patients when compared to healthy pregnant women with NLR showing an *OR* of 5.5[51]. A study by Ayla Aktulay *et al* reported significantly higher NLR and PLR in GDM compared with the control group[52]. Yilmaz *et al* showed increased NLR to be a powerful and independent predictor (*OR* 5.5) of GDM, with NLR>2.93 showing 76.2% sensitivity and 94.1% specificity in predicting GDM[53]. In 2014, a cross-sectional study indicated that NLR was independently associated with GDM. However, a few subsequent studies with a large sample size failed to find increased NLR in GDM[54]. Sargın *et al* found no statistical differences in NLR and PLR values between GDM patients and healthy pregnant women[55]. According to Wang *et al*, NLR at 4.89 cut-offs accurately predicted preeclampsia with sensitivity, specificity rates of 56.69% and 87.83%, positive and negative predictive values of 87.79% and 57.23% AUC of 0.78[47].

A meta-analysis reported elevated NLR levels in GDM compared to euglycemic pregnancies with a standardized mean difference of 0.584; however, they reported an extensive interstudy heterogeneity[56]. Another meta-analysis by Kamran *et al* reports that higher NLR (weighted mean difference of 0.48) was observed in GDM when compared with healthy pregnancies[57]. Further, a few studies report that the leukocyte, neutrophil, and lymphocyte counts were significantly higher in GDM patients when compared to healthy controls.

NLR has been used to predict long-term complications of diabetes and is a predictive factor for hearing loss and the development of diabetic retinopathy, early diabetic nephropathy, and coronary artery disease in patients with diabetes mellitus. However, discrepancies may be attributed to differences in age, demography, duration, and severity of hyperglycemia exposure, and any underlying inflammatory comorbidities in the patient population[55]. NLR warrants cautious clinical interpretation due to the nonspecific nature of the NLR and its implication in varied inflammatory pathophysiologies[56]. Current evidence regarding the role of NLR in GDM is controversial, and prospective cohort studies are needed to reassess the clinical utility of NLR in GDM[54] (Figure 3).

4. NLR in ectopic pregnancy

4.1. Ectopic pregnancy

Ectopic pregnancy is a condition where the fetus is implanted outside the uterine cavity (98% in the fallopian tube). Ectopic pregnancy emerges as one of the leading causes of first-trimester maternal deaths accounting for 6%-10% of pregnancy-related mortality[58].

The level of inflammatory cytokines is increased both regionally and in the systemic circulation in ectopic pregnancy patients. The diagnostic gold standard for ectopic pregnancy is a combination of sonographic adnexal evaluation and serial measurements of β-hCG. A reliable maker that could effectively determine the risk of tubal rupture and stratify patients suitable for a conservative approach has been a pressing need in the obstetrics community. Literature evidence suggests that evaluating systemic inflammatory markers in combination with serum β-hCG levels and ultrasound might help decide the appropriate treatment option, and NLR might serve as an additional marker for the same[59].

4.2. Neutrophils in ectopic pregnancy

Inflammatory signaling mechanisms (leukocytes guided by cytokines, chemokines, and integrins) are involved in directing and engaging the embryo to the implantation site, including ectopic pregnancies. This causes an increase in NLR. Stress-induced neutrophilia appears to be attributed to the redistribution of neutrophils into the systemic circulation[60].

4.3. Lymphocytes in ectopic pregnancy

The observed lymphopenia is believed to be secondary to stress-induced glucocorticoid excess. Excess glucocorticoids alter the distribution of lymphocytes with increased trafficking of lymphocytes from blood to lymphoid tissue and decreased shift from lymphoid tissue to blood[61].

4.4. Significance of NLR in ectopic pregnancy

NLR has emerged as a commonly used marker of systemic inflammatory response. Dogru *et al* reported elevated NLR in ruptured ectopic pregnancies when compared with healthy controls[62]. Donmez *et al* reported that NLR was significantly higher in patients with ruptured ectopic pregnancies, with NLR greater than 4 being associated with 6.9 times higher risk of tubal rupture[62]. Another study involving 142 participants (72 patients and 70 controls) observed that NLR and PLR were significantly higher in patients with tubal rupture[59]. Further, a study concluded that NLR was significantly increased in patients requiring surgery, indicating that NLR could be a reliable risk stratification marker in ectopic pregnancies. Another study reported that ectopic pregnancy patients with tubal rupture had higher NLR than those without tubal rupture; hence, NLR can effectively assist in treatment decisions. Further, a study reported higher NLR in patients non-responsive to methotrexate treatment when compared to methotrexate responsive patients, thus indicating that NLR might be a predictor of methotrexate treatment outcome in ectopic pregnancies[54].

5. NLR and reproduction

NLR is a basic inflammatory ratio that could be determined from a blood count. The ratio is commonly reported and investigated as a predictive markers in a range of diagnostic fields due to its core significance in reflecting an inflammatory load. Inflammatory reproductive diseases/conditions, such as hyperemesis gravidarum, gestational diabetes, pre-eclampsia, pregnancy-associated intrahepatic cholestasis, and other illnesses, have all been linked to an elevation in NLR. NLR might prove to be an accessible predictor of common maternal associated morbidities/complications of pregnancy.

6. Conclusions

Immunological and inflammatory mechanisms lie at the heart of a successful pregnancy, and dysfunction plays a critical role in adverse pregnancy outcomes. The major players of systemic inflammation include the primary cells of the immune system, *i.e.*, the neutrophils and lymphocytes, and hence mechanistically neutrophil-lymphocyte ratio ought to forecast an impending/ongoing inflammatory pathology. Increasing scientific evidence emphasizes that the NLR ratio has a significant role in predicting and prognosticating pregnancy-associated diseases. This simple, easily derivable, inexpensive, and valuable predictor ratio associated with the clinical picture can guide clinicians, caution about the development of pregnancy-associated inflammatory pathologies, and timely referral of these patients to the tertiary care center to avoid maternal and fetal complications. The prospective study should further explore the utility of this ratio in pregnancy-associated diseases in well-designed gestational age-adjusted reference ranges and clinical cut-offs.

7. Future prospects

Trimester wise establishment of reference ranges for normal pregnancy, developing cut-off values for predicting and prognostication of high-risk pregnancy with maternal complications and development of high risk delta NLR values in serial NLR measurements.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Authors' contributions

Ms. Monalisa Biswas carried out article extraction and critical appraisal, synthesis of results from various articles, manuscript writing and editing. Dr. Vijetha Shenoy Belle conducted article extraction and critical appraisal, manuscript editing and review. Dr. Nihaal Maripini created figures for mechanistic role of NLR in various maternal complications and manuscript editing. Dr. Krishnananda Prabhu was involved in conception, intellectual inputs, review supervision, manuscript editing and review.

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