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การเพิ่มประสิทธิภาพการทำนายภาวะเบาหวานขณะตั้งครรภ์ด้วยแบบจำลองร่วมของอัตราส่วนนิวโตรฟิลต่อลิมโฟไซต์ ฮีโมโกลบิน และอายุมารดาของหญิงตั้งครรภ์ในภาคใต้

Enhancing the Prediction of Gestational Diabetes Mellitus Using a Combined Model of Inverse Neutrophil-to-Lymphocyte Ratio, Hemoglobin Level, and Maternal Age among Pregnant Women in Southern Thailand

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บทคัดย่อ

การศึกษาย้อนหลังนี้มีวัตถุประสงค์เพื่อประเมินประสิทธิภาพในการวินิจฉัยของค่า NLR ระดับฮีโมโกลบิน และอายุมารดา พร้อมทั้งสร้างแบบจำลองการทำนายร่วมเพื่อใช้คัดกรอง GDM ในหญิงตั้งครรภ์ทางภาคใต้ของประเทศไทย จำนวน 180 ราย (กลุ่ม GDM 62 ราย และกลุ่มควบคุม 118 ราย) วิเคราะห์ข้อมูลทั่วไปและค่าพารามิเตอร์ทางโลหิตวิทยา วิเคราะห์ข้อมูลด้วยสถิติเชิงพรรณนา สถิติถดถอยโลจิสติกพหุตัวแปร และวิเคราะห์ด้วยกราฟ ROC (Receiver Operating Characteristic) ผลการวิจัยพบว่า

1. การเปรียบเทียบพารามิเตอร์ทางโลหิตวิทยาพบว่า ระดับฮีโมโกลบินกลุ่ม GDM สูงกว่ากลุ่มปกติ ในขณะที่อัตราส่วน NLR ต่ำกว่ากลุ่มปกติอย่างมีนัยสำคัญทางสถิติ (*p-value* < .05)

2. การประเมินปัจจัยทำนายด้วยการวิเคราะห์พหุตัวแปร พบว่า อายุมารดา ≥ 30 ปี (aOR = 1.090; 95% CI: 1.028 - 1.156) ระดับฮีโมโกลบิน ≥ 11.8 g/dL (aOR = 1.710; 95% CI: 1.218 - 2.400) และอัตราส่วน NLR < 3.15 (aOR = 0.632; 95% CI: 0.454 - 0.880) เป็นปัจจัยเสี่ยงอิสระต่อการเกิดภาวะ GDM (*p-value* < .05)

3. การสร้างแบบจำลองการทำนาย GDM พบว่า การใช้ปัจจัยเสี่ยงอิสระร่วมกัน (AUC = 0.734) สามารถจำแนกกลุ่ม GDM ออกจากกลุ่มปกติได้มากกว่าการใช้อัตราส่วน NLR เพียงอย่างเดียว (AUC = 0.623) เมื่อนำมาสร้างแบบประเมินความเสี่ยงอย่างง่ายด้วยช่วงคะแนน 0–5 พบว่า หญิงตั้งครรภ์ที่มีคะแนนสูงสุดเท่ากับ 5 คะแนน มีโอกาสเกิด GDM ร้อยละ 61.1 เมื่อเทียบกับกลุ่มที่มีความเสี่ยงต่ำ (0 คะแนน) ซึ่งสามารถทำนายได้ร้อยละ 20.80

การสร้างแบบจำลองร่วมจากอัตราส่วน NLR ร่วมกับระดับฮีโมโกลบินและอายุมารดา ช่วยเพิ่มความแม่นยำในการทำนาย GDM ได้อย่างมีนัยสำคัญ แบบประเมินความเสี่ยง GDM อย่างง่ายที่พัฒนาขึ้นสามารถใช้เป็นเครื่องมือที่ใช้ได้จริง มีความคุ้มค่า และไม่รุกรานร่างกาย สำหรับการคัดกรองความเสี่ยงเบื้องต้น ซึ่งมีประโยชน์อย่างยิ่งในบริบทที่มีทรัพยากรจำกัด เพื่อคัดกรองหญิงตั้งครรภ์กลุ่มเสี่ยงสูงเข้าสู่กระบวนการวินิจฉัยยืนยันได้อย่างรวดเร็ว

คำสำคัญ: เบาหวานขณะตั้งครรภ์ อัตราส่วนนิวโตรฟิลต่อลิมโฟไซต์ ระดับฮีโมโกลบิน อายุมารดา แบบจำลองการทำนายร่วม

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Abstract

This retrospective study aimed to evaluate factors such as the neutrophil-to-lymphocyte ratio (NLR), hemoglobin level, and maternal age, in order to develop a new combined predictive model for screening gestational diabetes mellitus (GDM) among pregnant women in Southern Thailand. A total of 180 participants were included (62 with GDM and 118 controls). General characteristics and hematological parameters were analyzed using descriptive statistics, multivariate logistic regression, and receiver operating characteristic (ROC) curve analysis. The results were as follows.

1. Compared to controls, hemoglobin levels were significantly higher in the GDM group, while NLR values were significantly lower (*p-value* < .05).

2. Multivariate analysis identified maternal age ≥ 30 years (aOR = 1.090; 95% CI: 1.028–1.156), hemoglobin ≥ 11.8 g/dL (aOR = 1.710; 95% CI: 1.218–2.400), and NLR < 3.15 (aOR = 0.632; 95% CI: 0.454–0.880) as independent risk factors for GDM (*p-value* < .05).

3. The combined predictive model (AUC = 0.734) demonstrated greater discriminatory ability than NLR alone (AUC = 0.623). A simple risk score ranging from 0–5 was developed; pregnant women with the maximum score of 5 had a 61.1% probability of GDM compared to 20.8% in the lowest-risk group (score = 0).

The combined model incorporating NLR, hemoglobin level, and maternal age significantly improved the accuracy of GDM prediction. The developed simple risk assessment tool is practical, cost-effective, and non-invasive, making it particularly valuable in resource-limited settings for early screening of high-risk pregnant women, therefore facilitating timely confirmatory diagnosis.

Keywords: Gestational Diabetes Mellitus, Neutrophil-to-Lymphocyte Ratio, Hemoglobin Level, Maternal Age, Combined Predictive Model

Introduction

Gestational diabetes mellitus (GDM) is a common metabolic disorder defined as glucose intolerance first recognized during pregnancy, often leading to significant postpartum complications. The prevalence of GDM varies widely between 1% and 28% worldwide, depending on diagnostic criteria, screening methods, and demographic factors (Ogurtsova, Guariguata, Barengo, Ruiz, Sacre, Karuranga et al., 2022; Sirirat, Boriboonhirunsarn, Ruangvutilert, Yapan, 2022). Physiologically, GDM occurs when the body's capacity to adapt to its altered state diminishes, leading to inadequate insulin production by the endocrine system to meet the increased demand (Plows, Stanley, Baker, Reynolds, Vickers, 2018). As the primary endocrine disease during pregnancy, GDM is linked to long-term adverse effects, including type 2 diabetes mellitus (T2DM), obesity, and cardiovascular issues for both the mother and the fetus (Moon, Jang, Kim, 2022). Furthermore, fetal complications such as macrosomia, shoulder dystocia, neonatal hypoglycemia, hyperbilirubinemia, and surgical delivery are frequently associated with GDM pregnancies. (Kanthiya, Luangdansakul, Wacharasint, Prommas, Smanchat, 2013).

Currently, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria are widely used for diagnosing GDM (Metzger, Gabbe, Persson, Lowe, Dyer, Oats et al., (2010).

The screening process typically involves an oral glucose tolerance test (OGTT), which can be performed as a one-step 75-g test or a two-step approach using a 50-g screening test followed by a 100-g, 3-hour diagnostic OGTT if the initial result exceeds cut-off values (American Diabetes Association, 2020). While effective, these methods present challenges; there is presently no accurate way to predict GDM prior to these confirmatory tests. Moreover, the procedure is time-consuming and invasive, and some pregnant women find it difficult to tolerate high-concentration glucose beverages after fasting for at least eight hours, highlighting the need for simpler screening alternatives. (Zhang, Catalano, 2021)

Although the precise pathophysiology of GDM remains not fully understood, it is established that inflammation, insulin resistance, immune system dysregulation, and environmental variables play critical roles in its development (Duo, Song, Qiao, Zhang, Xu, Zhang et al., 2024). Previous studies have explored immunological and biochemical factors, such as granulocyte, monocyte, and lymphocyte ratios, as potential biomarkers for early GDM prediction. For instance, the lymphocyte-to-HDL-C ratio and alterations in specific immune cell frequencies have shown predictive potential, suggesting a unique function for immune cells in the prognosis of this illness (Fagninou, Nekoua, Fiogbe, Moutairou, Yessoufou, 2022). Because persistent low-grade inflammation in GDM causes vascular damage and platelet activation, researchers have concentrated on inflammatory markers derived from a complete blood count (CBC), such as the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) (Yilmaz, Celik, Namuslu, Inan, Onrat, Bilgic et al., 2014).

In addition to inflammatory markers, quantitative and qualitative alterations in circulating leukocytes and other hematological parameters, such as hemoglobin levels, are common during pregnancy and may reflect the metabolic status of the mother. Elevated hemoglobin has been associated with increased blood viscosity and iron overload, which can induce oxidative stress and contribute to insulin resistance (Lao, Ho, Chan, So, 2002; Phaloprakarn, Tangjitgamol, 2008). While previous research has indicated that individuals with GDM exhibit variations in systemic inflammatory response indicators and hematological profiles, these results have not been consistently confirmed across different populations.

The complete blood count (CBC) is an affordable, practical approach that yields valuable data on both leukocyte parameters and hemoglobin levels. Given that single markers often yield inconsistent predictive accuracy, combining inflammatory markers with hematological indices may offer a superior predictive value. These results could be crucial in predicting and managing diabetic pregnant women, thus averting negative consequences such as macrosomia, preterm birth, and future T2DM (Kampmann, Madsen, Skajaa, Iversen, Moeller, Ovesen, 2015). Therefore, this study aims to evaluate the efficacy of a combined predictive model utilizing the Neutrophil-to-Lymphocyte Ratio (NLR) and Hemoglobin levels as a straightforward predictor of GDM in Southern Thai women.

Objectives

1. To compare the hematological parameters (specifically Neutrophil-to-Lymphocyte Ratio and hemoglobin levels) and maternal characteristics between pregnant women with Gestational Diabetes Mellitus (GDM) and those with normal glucose tolerance.

2. To evaluate the diagnostic performance of the Neutrophil-to-Lymphocyte Ratio (NLR), hemoglobin levels, and maternal age as independent predictors for GDM.

3. To develop and validate a combined predictive model and a simplified clinical risk score for early GDM screening among pregnant women in Southern Thailand.

Conceptual Framework

The conceptual framework of this study was developed to evaluate the predictive efficacy of hematological and maternal factors on the risk of Gestational Diabetes Mellitus (GDM). As illustrated in Figure 1, the independent variables consist of Maternal Characteristics (specifically Maternal Age) and Hematological Parameters derived from a routine complete blood count (CBC), including Hemoglobin (Hb) and the Neutrophil-to-Lymphocyte Ratio (NLR). The dependent variable is the diagnosis of Gestational Diabetes Mellitus (GDM) based on the standard 2-step screening approach. This framework hypothesizes that these selected parameters, both individually and in combination, serve as significant predictors for identifying GDM risk in pregnant women.

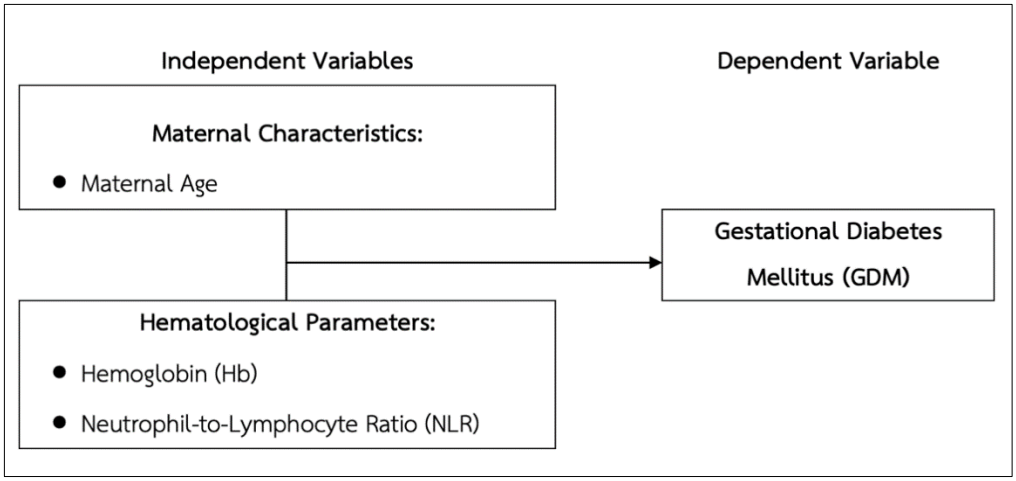


Figure 1 Conceptual Framework of the study

Note: This framework illustrates only the independent variables that demonstrated statistical significance in the initial univariate analysis and were subsequently included in the final combined predictive model. Other maternal characteristics, such as body mass index (BMI) and blood pressure, were evaluated but excluded from this conceptual model due to a lack of statistical significance.

Methods

This retrospective cohort study was conducted at the Department of Obstetrics and Gynecology, Thasala Hospital, Nakhon Si Thammarat, Thailand. Data were collected from the medical records of pregnant women who attended antenatal care (ANC) between January and July 2024.

Population and Sample

A total of 180 pregnant women were included in the study. A consecutive sampling method was employed to select the participants. Specifically, the medical records of all pregnant women who attended the antenatal care clinic between January and July 2024 and met the predefined inclusion and exclusion criteria were retrospectively reviewed. The study population was categorized into two groups based on their GDM status: the GDM group (n = 62) and the non-GDM control group (n = 118).

Inclusion Criteria: Pregnant women aged over 18 years who underwent GDM screening between the 24th and 28th gestational weeks and had complete medical records, including demographic data, body mass index (BMI), blood pressure, urinalysis, and a complete blood count (CBC) with specific data on hemoglobin levels and leukocyte differential counts.

Exclusion Criteria: Pregnant women with pre-existing type 1 or type 2 diabetes mellitus, chronic hypertension, renal failure, thyroid disorders (hypo-or hyperthyroidism), cardiovascular diseases, HIV infection, or malaria.

Screening and Diagnosis of Gestational Diabetes Mellitus

The diagnosis of GDM followed a standard two-step screening approach consistent with the American College of Obstetricians and Gynecologists (ACOG) guidelines. First, a 50-g Glucose Challenge Test (GCT) was performed. If the plasma glucose level at 1 hour exceeded 140 mg/dL, the patient underwent a diagnostic 100-g, 3-hour Oral Glucose Tolerance Test (OGTT). GDM was diagnosed if at least two of the four plasma glucose values met or exceeded the Carpenter and Coustan thresholds: fasting ≥ 95 mg/dL, 1-hour ≥ 180 mg/dL, 2-hour ≥ 155 mg/dL, and 3-hour ≥ 140 mg/dL.

Data Collection

Demographic and clinical data were extracted from electronic medical records. Hematological parameters, specifically hemoglobin concentration, neutrophil count, and lymphocyte count, were obtained from the CBC performed during the first ANC visit. The Neutrophil-to-Lymphocyte Ratio (NLR) was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count.

Statistical Analysis

Data were analyzed using SPSS version 28. Continuous variables (mean ± SD) and categorical variables (frequencies, percentages) were compared between GDM and non-GDM groups using independent t-tests and Chi-square tests, respectively.

To develop the predictive model, univariate analysis first identified significant candidate parameters (maternal age, hemoglobin, and NLR; *p-value* < .05). These predictors were then evaluated using a multivariable binary logistic regression model to adjust for confounders. The final model retained only independently significant predictors (*p-value* < .05), and predicted probabilities were calculated to assess combined efficacy.

Diagnostic performance comparing NLR alone versus the combined model was assessed via Receiver Operating Characteristic (ROC) curves, reporting AUC, sensitivity, specificity, PPV, and NPV. Optimal cutoffs were established using Youden's index. Statistical significance was set at *p-value* < .05.

Ethical Consideration

All methods in the present study were conducted in accordance with the Helsinki Declaration for research involving human subjects. The requirement for written informed consent was waived by the Ethics Committee due to the retrospective nature of the study and the use of anonymized medical records. This research protocol has been approved by the Ethics Committee in Human Research at Walailak University (WUEC-23-151-01).

Results

1. Socio-demographic and Biochemical Parameters in Pregnant Women

A total of 180 pregnant women were included in the study, stratified into 62 (34.40%) participants with GDM and 118 (65.60%) non-GDM controls. The baseline demographic and clinical characteristics are summarized in Table 1. Women in the GDM group were significantly older than those in the control group (31.65 ± 6.28 vs. 29.25 ± 5.56 years; *p-value* = .013). Conversely, no significant differences were observed between the cohorts regarding BMI, systolic and diastolic blood pressure, or gravidity (*p-value* > .05)

Hematological analysis revealed significantly elevated hemoglobin levels in the GDM group compared to controls (12.10 ± 1.01 vs. 11.55 ± 1.05 g/dL; $p < 0.001$). As anticipated, all glycemic indices including, fasting plasma glucose and 1-, 2-, and 3-hour OGTT were markedly higher in the GDM group (*p-value* < .001). Furthermore, the prevalence of glucosuria was significantly greater in the GDM group (38.7% vs. 11.9%; *p-value* < .001), whereas proteinuria rates showed no statistically significant difference.

Table 1 Comparison of socio-demographic, clinical, and biochemical characteristics between GDM and Non-GDM groups

Parameters	GDM (n = 62)		Non-GDM (n = 118)		*p-value*
	M/N	*SD*/%	*M/N*	*SD*/%	*(2-tailed)*
Maternal Characteristics					
Age (years)	31.65	6.28	29.25	5.56	.013
BMI (kg/m²)	27.22	5.36	26.21	4.88	.220
Systolic BP (mmHg)	119.81	11.72	117.19	12.26	.163
Diastolic BP (mmHg)	77.71	8.68	75.68	9.61	.153
Glycemic Parameters (mg/dL)					
FPG	103.02	33.76	83.25	8.76	< .001
OGTT 1-hour	199.13	55.85	142.23	22.96	< .001
OGTT 2-hour	178.84	63.34	122.69	19.17	< .001
OGTT 3-hour	151.94	61.48	110.36	17.62	< .001
Biochemical & Urinalysis					
Hemoglobin (g/dL)	12.10	1.01	11.55	1.05	< .001
Urine Glucose, n (%)					< .001
Negative	38	61.3	104	88.1	
Positive	24	38.7	14	11.9	
Urine Protein, n (%)					.479
Negative	38	61.3	80	67.8	
Positive	24	38.7	38	32.2	

BMI; Body Mass Index, BP; Blood Pressure, FPG; Fasting Plasma Glucose, OGTT; Oral Glucose Tolerance Test

2. Comparative Analysis of Hematological Parameters

The analysis of maternal hematological profiles is detailed in Table 2. While the total White Blood Cell (WBC) count was comparable between the GDM and non-GDM groups (*p-value* = .404), significant variations were identified in specific leukocyte subsets. Women with GDM exhibited a

significantly lower percentage of neutrophils compared to the control group (69.11 ± 5.66% vs. 71.49 ± 6.91%; *p-value* = .014). In contrast, lymphocyte percentage was significantly higher in the GDM group (23.17 ± 5.28% vs. 21.08 ± 5.67%; *p-value* = .015). These opposing trends resulted in a significantly lower Neutrophil-to-Lymphocyte Ratio (NLR) in the GDM group compared to non-GDM controls (3.16 ± 0.88 vs. 3.79 ± 1.80; *p-value* = .002).

Table 2 Comparison of maternal hematological parameters between GDM and non-GDM groups.

Hematological Parameters	GDM (n = 62)		Non-GDM (n = 118)		Mean Difference (95% CI)	*p-value (1-tailed)*
	M	*SD*	*M*	*SD*		
WBC Count ($10^3/\mu L$)	10.08	2.17	10.39	2.63	-0.31 (-1.08, 0.46)	.404
Neutrophils (%)	69.11	5.66	71.49	6.91	-2.38 (-4.29, -0.47)	.014
Lymphocytes (%)	23.17	5.28	21.08	5.67	2.09 (0.41, 3.77)	.015
Monocytes (%)	4.43	1.24	4.55	1.31	-0.12 (-0.51, 0.27)	.541
Eosinophils (%)	3.02	2.89	2.59	2.73	0.43 (-0.44, 1.30)	.331
Basophils (%)	0.26	0.13	0.27	0.17	-0.01 (-0.05, 0.04)	.859
NLR	3.16	0.88	3.79	1.80	-0.63 (-1.03, -0.23)	.002

NLR; Neutrophils-to-Lymphocytes Ratio

To visually illustrate the significant disparities in key hematological parameters identified in Table 2, the distributions of neutrophil percentage, lymphocyte percentage, and the Neutrophil-to-Lymphocyte Ratio (NLR) are depicted in Figure 2.

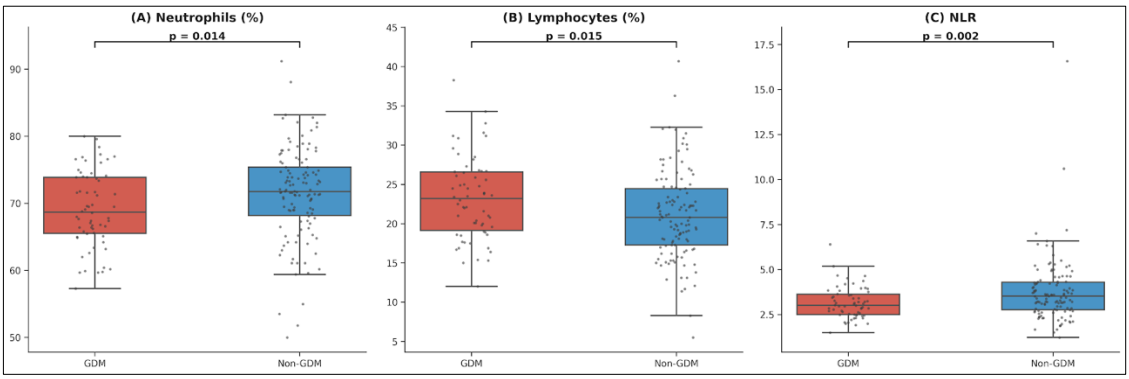


Figure 2 Comparison of hematological parameters between GDM and Non-GDM groups. Box plots showing the distribution of (A) Neutrophils (%), (B) Lymphocytes (%), and (C) Neutrophil-to-Lymphocyte Ratio (NLR).

As shown in Figure 2A and Figure 2B, distinct patterns were observed between the two groups. Women with GDM displayed a significantly lower distribution of neutrophils (*p-value* = .014) but a significantly higher distribution of lymphocytes (*p-value* = .015) compared to the non-GDM control group. These inverse alterations in leukocyte subpopulations contributed directly to the significant reduction in NLR observed in the GDM group, as illustrated in Figure 2C (*p-value* = .002).

3. Predictive Factors and Diagnostic Performance of the Combined Model

To evaluate the independent predictive value of the identified markers, Multivariable logistic regression analysis was performed (Table 3). After adjusting for maternal age, both NLR (aOR = 0.632, p=0.007) and hemoglobin (aOR = 1.710, *p-value* = .002) remained significantly associated with GDM.

Table 3 Univariate and Multivariable Logistic Regression Analysis for predicting GDM.

Variables	Crude OR (95% CI)	*p-value*	Adjusted OR (95% CI)	*p-value (1-tailed)*
NLR	0.661 (0.485 - 0.900)	.008*	0.632 (0.454 - 0.880)	.007
Hemoglobin (g/dL)	1.678 (1.219 - 2.310)	.002*	1.710 (1.218 - 2.400)	.002
Age (years)	1.073 (1.016 - 1.133)	.011*	1.090 (1.028 - 1.156)	.004

NLR; Neutrophils-to-Lymphocytes Ratio

The diagnostic performance of the markers was assessed using ROC curve analysis (Figure 3). The NLR alone yielded an Area Under the Curve (AUC) of 0.623. However, the Combined Model, incorporating NLR, hemoglobin, and maternal age, significantly improved the predictive accuracy, achieving an AUC of 0.734. This demonstrates that integrating hematological markers with maternal characteristics offers a superior screening capability compared to using NLR alone.

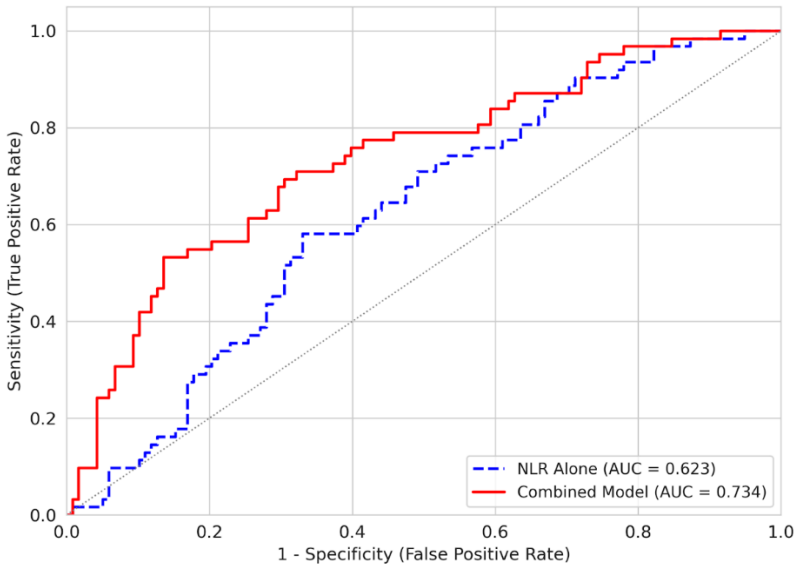


Figure 3 Receiver Operating Characteristic (ROC) curves comparing the diagnostic performance of the Neutrophil-to-Lymphocyte Ratio (NLR) alone versus the Combined Predictive Model. The blue dashed line represents the NLR alone model (AUC = 0.623), while the red solid line represents the Combined Model incorporating NLR, Hemoglobin, and Maternal Age (AUC = 0.734). The combined model demonstrates superior predictive accuracy, as indicated by the larger area under the curve and the shift towards the upper-left corner.

4. Development of a Simplified Risk Score

To develop a practical clinical tool, continuous variables were categorized using optimal cut-offs (maternal age ≥ 30 years, hemoglobin ≥ 11.8 g/dL, NLR < 3.15) and analyzed via multivariable logistic regression. Item scores were mathematically derived by dividing each adjusted coefficient (β) by the smallest β (maternal age, β = 0.453) and rounding the quotients to the nearest integer. This standard weighting assigned 2 points each to high hemoglobin (β = 0.766) and low NLR (β = 1.071), and 1 point to maternal age, yielding a cumulative score range of 0 to 5.

Table 4 Simplified GDM Risk Assessment Score

Risk Factors	Criteria	β coefficient	aOR (95% CI)	Calculation (β / 0.453)	Score
NLR	< 3.15	0.453	1.57 (0.82 – 3.01)	0.453 / 0.453 = 1.00	1
Hemoglobin	≥ 11.8 g/dL	0.766	2.15 (1.11 – 4.16)	0.766 / 0.453 = 1.69	2
Maternal Age	≥ 30 years	1.071	2.92 (1.52 – 5.60)	1.071 / 0.453 = 2.36	2
Total Score					**(0 - 5)**

Based on the scoring criteria defined in Table 4, the cumulative GDM risk for the study population was stratified. The effectiveness of this scoring system is visually demonstrated in Figure 4, which illustrates the relationship between the total risk score and the probability of GDM diagnosis. A clear stepwise increase in GDM prevalence was observed as the total score increased. Notably, participants in the highest risk category (total score of 5) exhibited a GDM probability of 61.1%, representing a three-fold increase compared to those in the lowest risk categories (scores 0–1), where the probability was approximately 8–20%. This distinction highlights the potential of the combined score to effectively identify high-risk pregnancies. The probability of GDM for each risk score category was empirically calculated by dividing the number of confirmed GDM cases by the total number of participants within that specific score group, expressed as a percentage.

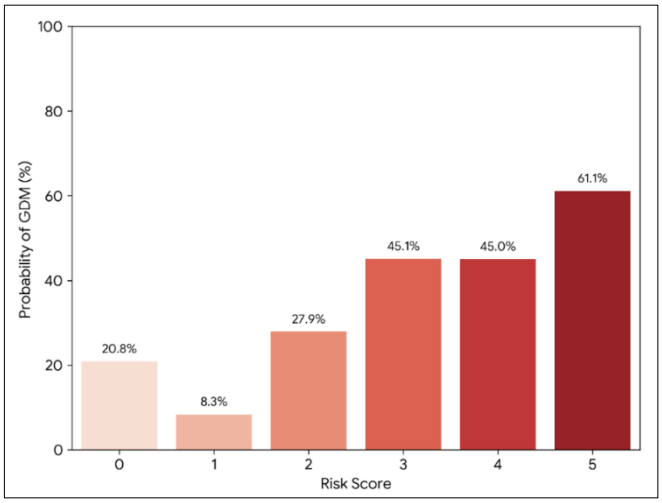


Figure 4 Risk stratification for Gestational Diabetes Mellitus based on the simplified risk score. The bar chart illustrates the prevalence of GDM across different risk score categories. Patients with a total score of 5 (High Risk) have a >60% probability of being diagnosed with GDM,

compared to ~20% in the lowest risk group.

Discussion

This study elucidates the predictive value of hematological markers, specifically the Neutrophil-to-Lymphocyte Ratio (NLR) and hemoglobin levels, in the screening of Gestational Diabetes Mellitus (GDM) among pregnant women in Southern Thailand. The principal finding of this research is that a combined predictive model integrating NLR, hemoglobin, and maternal age offers superior diagnostic performance (AUC = 0.734) compared to NLR alone. Furthermore, we successfully developed a simplified clinical risk score that effectively stratifies pregnant women into low, moderate, and high-risk categories for GDM.

A notable observation in our cohort was the significant inverse association between NLR and GDM, driven primarily by an elevated percentage of lymphocytes in the GDM group. This finding contrasts with several recent studies, such as Sahin, Oguz, Tüzün, Işiktaş, Işiktaş, Ülgen et al., (2022). which reported elevated NLR in GDM attributed to neutrophilia associated with systemic inflammation. However, our results align with the physiological immune modulation characteristic of the second trimester of pregnancy. During this period, maternal immunity naturally shifts towards a Th2-dominant response to maintain tolerance to the fetus, often resulting in relative lymphocytosis (Abu-Raya, Michalski, Sadarangani, Lavoie, 2020). It is plausible that in our specific population, the inflammatory dysregulation associated with GDM manifests as an exaggeration of this adaptive immune response, leading to sustained lymphocyte levels rather than neutrophil dominance. This suggests that the interplay between pregnancy-induced immune adaptation and metabolic inflammation may vary across different populations and gestational stages (Bozbay, Medinaeva, Akyürek, Orgul, 2024).

In addition to leukocyte alterations, our study identified high hemoglobin levels as a robust independent risk factor for GDM. Women with GDM exhibited significantly higher hemoglobin concentrations compared to controls, a finding consistent with a recent systematic review by Tiongco, Arceo, Clemente, Pineda-Cortel (2019). This association may be explained by two mechanisms: first, iron overload and excess hemoglobin can induce oxidative stress, which impairs insulin signaling and beta-cell function (Georgieff, 2020); second, higher hemoglobin levels may reflect increased blood viscosity, potentially affecting microcirculatory perfusion and glucose metabolism (Yang, Yang, Pan, Fu, Cheng, Liu, 2022). These findings reinforce the utility of routine complete blood count (CBC) parameters beyond anemia screening, serving as potential metabolic indicators.

Although maternal age and hemoglobin levels independently predicted GDM, their mean values remained within standard clinical ranges (i.e., age < 35 years and normal hemoglobin). While this raises questions regarding direct clinical significance, predictive biomarkers do not need to cross pathological thresholds. Variations within the normal physiological spectrum can reflect early metabolic shifts. For instance, high-normal hemoglobin (≥ 11.8 g/dL) may indicate blunted hemodilution or marginally increased blood viscosity, which can subtly exacerbate insulin resistance (Lao, Ho, Chan, So, 2002; Yang, Yang, Pan, Fu, Cheng, Liu, 2022). Furthermore, GDM risk increases linearly with age well before the traditional 35-year mark (Li, Ren, He, Li, Zhang, Chen, 2020); thus, a cut-off of ≥ 30 years captures the inflection point of elevated metabolic risk. Therefore, while

these modest physiological shifts are not independently diagnostic, their combination provides a clinically meaningful tool for early GDM risk stratification.

To translate these findings into clinical practice, we developed a simplified GDM Risk Score (Table 4). This tool utilizes readily available parameters age, hemoglobin, and NLR to calculate a risk score ranging from 0 to 5. Our validation demonstrated that women with a high score (5 points) had a three-fold increased risk of GDM compared to those with low scores. This approach aligns with the American Diabetes Association recommendations for identifying risk factors to guide screening. In resource-limited settings where universal OGTT screening may be logistically challenging or costly, this scoring system could serve as an effective triage tool to prioritize high-risk women for definitive diagnostic testing.

This study has certain limitations. First, the retrospective, single-center design may limit the generalizability of the findings to other populations with different demographic or nutritional profiles. Second, while the sample size was sufficient for statistical validity, larger multi-center prospective studies are warranted to validate the proposed risk score. Finally, data regarding other potential confounders, such as dietary habits and physical activity, were not available for analysis.

Conclusion

In conclusion, the combination of low NLR, high hemoglobin, and advanced maternal age significantly enhances the prediction of GDM. The physiological decrease in NLR observed in this population highlights the complexity of immune adaptation in GDM. The developed Simplified GDM Risk Score offers a practical, cost-effective, and accessible tool for early risk stratification, potentially improving antenatal care management and maternal health outcomes.

Implication of the Results

Clinical Implications: Based on the findings of this study, we propose the integration of the "Simplified GDM Risk Score" into routine antenatal care, particularly in primary care settings and resource-limited environments. Since the score utilizes standard parameters obtained from a routine complete blood count (CBC) specifically Hemoglobin and the Neutrophil-to-Lymphocyte Ratio (NLR) it incurs no additional cost. We recommend that healthcare providers calculate this risk score during the first trimester or early second trimester. Pregnant women identified as High Risk (Total Score ≥ 4) should be prioritized for early diagnostic testing (OGTT) and receive intensified nutritional counseling, rather than waiting for universal screening between 24–28 weeks. This targeted approach could facilitate early detection and timely intervention, potentially reducing adverse maternal and fetal outcomes.

Recommendation for Further Study

While this study establishes a strong predictive model, further research is warranted to validate these findings.

1. Validation Studies: Large-scale, prospective, multi-center studies are needed to validate the accuracy and cut-off values of the proposed risk score across different Thai and Asian populations.

2. Mechanistic Investigation: Given the inverse association observed between NLR and GDM, future studies should investigate specific immunological markers (e.g., Th1/Th2 cytokines) to elucidate the underlying pathophysiological mechanisms of immune adaptation in this specific population.

3. Longitudinal Analysis: Longitudinal studies tracking changes in NLR and hemoglobin throughout all trimesters would provide deeper insights into the temporal relationship between hematological adaptations and the onset of insulin resistance.

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Declaration of Use of AI in Academic Writing

During the preparation of this work, the authors used Grammarly to check and improve the English language, grammar, and readability of the manuscript. After using this tool, the authors thoroughly reviewed and edited the content as needed and take full responsibility for the final content of the publication.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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