The Incidence of Preeclampsia through Malondialdehyde (MDA), Tumor Necrosis Factor-alpha (TNF-α), and Soluble fms-like Tyrosine Kinase-1 (sFlt-1) Pathways:, induce by high at diet: A Prospective Cohort Study

(Original Reaserch)

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KEYWORDS

Preeclampsia, High-fat diet, Malondialdehyde (MDA), Tumor Necrosis Factor-alpha (TNF-α), Soluble fms-like Tyrosine Kinase-1 (sFlt-1)

ABSTRACT:

Background: Diet, particularly high-fat intake, has been associated with increased oxidative stress and inflammation, key contributors to preeclampsia. However, the direct impact of a high-fat diet on preeclampsia and the underlying mechanisms involving biomarkers such as MDA, TNF-α, and sFlt-1 remain underexplored. This study aimed to investigate the effect of a high-fat diet on the incidence of preeclampsia in pregnant women through oxidative stress (MDA), inflammation (TNF-α), and angiogenic imbalance (sFlt-1) pathways.

Methods: This prospective cohort study included 50 pregnant women in first trimester, divided into two groups based on their dietary fat intake (high-fat vs. low- fat diet). Dietary intake was assessed using a 24-hour food recall and analyzed with NutriSurvey software. Blood samples were collected for measuring MDA, TNF-α, and sFlt-1 levels using ELISA. The incidence of early-onset preeclampsia was monitored, and statistical analysis was performed using T-tests, Mann-Whitney tests, and path analysis.

Results: The analysis revealed that participants following a high-fat diet had significantly elevated systolic and diastolic blood pressures both at baseline and upon the onset of preeclampsia (PE) compared to those not on a high-fat diet ($p < 0.05$). Additionally, Mean Arterial Pressure (MAP) was significantly higher in the high-fat diet group under both initial and PE conditions. Biomarker analysis showed that oxidative stress and inflammation markers (MDA, TNF-α, and sFlt-1) were notably higher in the high-fat diet group, indicating increased

cardiovascular stress. Regression analysis, however, indicated that while a high-fat diet was associated with these elevated biomarker levels, it did not exert a significant direct effect on each biomarker individually.

Conclusion: The study concludes that a high-fat diet has a significant impact on blood pressure and biomarkers related to Preeclampsia. Participants on a high-fat diet showed significantly higher systolic and diastolic blood pressures, both initially and at the onset of PE, compared to those not on a high-fat diet. MAP was also notably elevated in the high-fat diet group in both initial and PE conditions. Additionally, key biomarkers, including MDA, TNF-α, and sFlt-1, were significantly higher in the high-fat diet group, suggesting increased oxidative stress and inflammation

INTRODUCTION

Preeclampsia (PE) is a hypertensive disorder of pregnancy that poses a significant risk to both maternal and fetal health. It is characterized by the onset of hypertension and proteinuria after 20 weeks of gestation and can lead to severe complications, including eclampsia, HELLP syndrome, and preterm birth. Despite extensive research, the exact cause of preeclampsia remains unclear. Still, it is believed to involve maternal and placental factors, with oxidative stress, inflammation, and vascular endothelial dysfunction playing critical roles in its pathogenesis.[1–3]

Diet and lifestyle factors are emerging as essential determinants in the development of preeclampsia. High-fat diets, in particular, have been associated with increased oxidative stress and inflammation, which are known as contributors to the vascular dysfunction observed in preeclampsia.[4,5] A high intake of dietary fats can lead to an elevated production of reactive oxygen species (ROS) and increasing malondialdehyde (MDA) levels, a marker of lipid peroxidation and oxidative stress.[6,7] This oxidative imbalance can trigger a cascade of inflammatory responses, including the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), which are also implicated in the pathogenesis of preeclampsia.[8–10]

In addition to oxidative stress and inflammation, angiogenic imbalance, particularly involving soluble fms-like tyrosine kinase-1 (sFlt-1), has been identified as a critical factor in preeclampsia. Elevated levels of sFlt-1, an anti-angiogenic protein, lead to endothelial dysfunction by inhibiting vascular endothelial growth factor (VEGF), a key regulator of blood vessel formation and function. Studies have shown that women who develop preeclampsia have higher circulating levels of sFlt-1 compared to those with normotensive pregnancies.[11–14]

Given the growing body of evidence linking high-fat diets to increased oxidative stress, inflammation, and angiogenic imbalance, it is plausible that maternal dietary habits, particularly those involving excessive fat consumption, may influence the risk of developing preeclampsia. However, research exploring the direct relationship between a high-fat diet and preeclampsia, primarily through biomarkers like MDA, TNF-α, and sFlt-1, remains limited.[6,15,16] This study aims to investigate the effect of a high-fat diet on the incidence of preeclampsia in pregnant women through MDA, TNF-α, and sFlt-1 pathways.

METHODS

Study Design

This is a prospective cohort study aimed at investigating the effect of a high-fat diet on the incidence of preeclampsia through malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), and soluble fms-like tyrosine kinase-1 (sFlt-1) pathways. The study was conducted in RSD. Mangusada, one of a district hospital in Badung Regency, Bali, Indonesia.

Study Subjects

This study's subjects were pregnant women in first trimester. Participants were selected using purposive sampling. Inclusion criteria were pregnant women aged 21-35 years; normal BMI; no previous history of preeclampsia; no history of diabetes mellitus, kidney failure, or cardiovascular diseases such as chronic hypertension or coronary heart disease; no history of liver disorders such as hepatic cirrhosis, pancreatitis, or sepsis; non-cancer patients; no history of respiratory disorders; non-smokers;; and single pregnancy (singleton).
All pregnant women who match the inclusion criteria were divided into two groups: those with a high-fat diet and those without, based on 3 time collecting datas using 24-hour food recall assessment followed by food intake analysis using NutriSurvey 2007 for Windows software. The following criteria determine the classification of a high-fat diet: fat 30%, saturated fat intake of more than 10% of the total daily caloric needs (based on an adult daily requirement of 2000 kcal) and trans-fat intake of more than 1% of total caloric intake. During these sessions, the incidence of early-onset preeclampsia was also being monitored. Participants were excluded from the study if they had abortus or pregnancy termination before 34 weeks of gestation, withdrawal from the study at any time, and development of pregnancy complications before 34 weeks of gestation.

Data Collection

The collected data was maternal plasma levels of MDA, TNF-α, and sFlt-1 on baseline. All study subjects underwent peripheral blood collection, with 5 ml of blood being drawn and processed for plasma isolation. The plasma was stored in 2.5 ml Eppendorf tubes and preserved at -20°C until all samples were ready for analysis. The plasma samples was examined using the ELISA (Enzyme-Linked Immunosorbent Assay) method to measure the levels of MDA, TNF-α, and sFlt Additional clinical and demographic data (e.g., maternal age, BMI, medical history) was also being recorded at baseline.

Statistical Analysis

The collected data was analyzed using SPSS version 21. Descriptive statistics was used to summarize baseline characteristics. For inferential analysis, a T-test was used to compare continuous variables such as MDA, TNF-α, and sFlt-1

levels between groups with and without preeclampsia. Multivariate analysis with path analysis was conducted to control for potential confounders and to determine the independent effect of a high-fat diet on the incidence of preeclampsia. The cutoff value for MDA, TNF-α, and sFlt-1 as risk factors for preeclampsia was determined using receiver operating curve (ROC) analysis. A p-value < 0.05 will be considered statistically significant.

RESULTS

This study included 50 pregnant women aged less than 20 weeks. Table 1 shows the participants' baseline characteristics.

Table 1. Baseline Characteristic

Variables	Total (n=50)
High school Graduate. n (%)	
Yes	20 (40)
No	30 (60)
Occupation. n (%)	
Working	11 (22)
Non-working	39 (78)
Age (years)	31 (19-35)
Parity	2 (1-4)
Gestational age (weeks)	7 (5-8)
Weight (kg)	61.9 ± 0.9
Height (cm)	154.7 ± 3.9
BMI (kg/m2)	25.8 ± 3.9
Systolic BP (mmHg)	110.4 ± 7.5
Diastolic BP (mmHg)	79.0 ± 4.5
MAP	89.5 ± 3.9
Hb (g/dL)	11.0 (10.7-12.1)
SGOT(U/L)	19 (11-113)
SGPT (U/L)	19 (13-22)
Fat consumption (%)	24 (7-35)
TNF-α (pg/ml)	192.6 (104.4-495.2)
sFLT1 (pg/ml)	8.93 (6.3-22.8)
MDA (nmol/mL)	7.66 (4.67-20.88)

**BMI: Body Mass Index, Systolic BP: Systolic Blood Pressure, Diastolic BP: Diastolic Blood Pressure, MAP: Mean Arterial Pressure, Hb: Hemoglobin, SGOT: Serum Glutamic Oxaloacetic Transaminase, SGPT: Serum Glutamic Pyruvic Transaminase, Fat consumption: Fat consumption percentage, TNF-α: Tumor Necrosis Factor-alpha, sFLT1: Soluble fms-like Tyrosine Kinase-1, MDA: Malondialdehyde* Table 2. Normality Test Using Shapiro Wilk in Systole and Diastole in High Fat and in No High Fat Diet

Variable	Group	Statistic	Significant	Note
Systole (initial)	High fat det	0.900	0.018	Not normal
	No high fat diet	0.960	0.414	Normal
Diastole (Initial)	High fat det	0.954	0.305	Normal
	No high fat diet	0.920	0.051	Normal
Systole	High fat det	0.942	0.162	Normal

(Onset PE)	No high fat diet	0.926	0.069	Normal
Diastole	High fat det	0.882	0.007	Not normal
(Onset PE)	No high fat diet	0.951	0.270	Normal

From the normality test results, it is found that the data for variables such as Systolic (Initial) in the non-high fat diet group, Diastolic (Initial) in both groups, and Systolic (Onset of PE) in both groups, and Diastolic (Onset of PE) in the non-high fat diet group, all have a normal distribution, as the significance values are greater than α (0.05). However, the Systolic (Initial) in the high fat diet group and Diastolic (Onset of PE) in the high fat diet group do not have a normal distribution, as their significance values are less than α (0.05). For comparison of the Diastolic (Initial) and Systolic (Onset of PE) means, an independent t-test is used, while for Systolic (Initial) and Diastolic (Onset of PE), the Mann-Whitney test is used.

Table 3. Summary of Mean Difference Test Results Systole and Diastole in High Fat and in No High Fat Diet

GROUP	Systole (Initial)[b] s (Mean±SD)	Diastole (Initial) a (Mean±SD)	Systole (Onset PE)[a] (Mean±SD)	Diastole (Onset PE)[b] (Mean±SD)
High fat diet (n=25)	110.40±7.48	79.04±4.57	173.04±16.03	96.80±4.66
No high fat diet (n=25)	107.40±7.45	74.72±6.03	109.64±7.20	74.40±3.66
Statistic (p-value)	Z = –1.415 (0.157)	t = 2.855 (0.007)*	t = 18.037 (0.000)*	Z = –6.073 (0.000)*
Table	$Z_{0.05}$ = 1.960	t(0.05,45) = 2.014	t(0.05,33) = 2.035	$Z_{0.05}$ = 1.960

Nb. a=Independent sample t-test, b=Mann Whitney, *significant p<0.05.

The average Systole (initial) of the high-fat diet group was 110.40±7.48, higher than the average Systole (initial) of the non-high-fat diet group of 107.40±7.45. From the Mann Whitney test, the calculated Z value was greater than the -Z table (-1.405> -1.960), and the p-value was greater than α (0.157> 0.050), so the decision was taken that H0 was accepted, which means that there was an insignificant difference in the average Systole (initial) between the high-fat diet group and the non-high-fat diet group. The average Systole (initial) of the high-fat diet group was slightly higher than the non-high-fat diet group, and the difference was not significant.
The average Diastole (initial) of the high-fat diet group was 79.04±4.57, higher than the average Diastole (initial) of the non-high-fat diet group of 74.72±6.03. From the unpaired t-test, the value of |t count| was obtained which was greater than the t table (2.855> 2.014), and the p-value was smaller than α (0.007 <0.050), so the decision was taken to reject H0 which means there is a significant difference in the average Diastole (initial) between the high-fat diet group and the non-high-fat diet group. The average Diastole (initial) of the high-fat diet group was higher than the non-high-fat diet group, and the difference was significant.
The average Systole (PE appeared) of the high-fat diet group was 173.04±16.03 higher than the average Systole (PE appeared) of the non-high-fat diet group of 109.64±7.20. From the unpaired t-test, the value of |t count| which is greater than the t table (18.037 > 2.035), and the p-value is smaller than α (0.000 < 0.050), then the decision is taken to reject H0 which means there is a significant difference in the average Systole (PE appears) between the high-fat diet group and the non-high-fat diet group. The average Systole (PE appears) of the high-fat diet group is much higher than the non-high-fat diet group, and the difference is significant.
The average Diastole (PE appears) of the high-fat diet group is 96.80 ± 4.66 higher than the average Diastole (PE appears) of the non-high-fat diet group of 74.40 ± 3.66. From the Mann Whitney test, the calculated Z value is obtained which is smaller than the –Z table (-6.073 < -1.960), and the p-value is smaller than α (0.000 < 0.050), then the decision is taken to reject H0 which means there is a significant difference in the average Diastole (PE appears) between the high-fat diet group and the non-high-fat diet group. The average Diastole (PE appears) of the high-fat diet group is much higher than the non-high-fat diet group, and the difference is significant.

Table 4. Normality Test Using Shapiro Wilk in Mean Arterial Pressure Between High Fat Diet and No High Fat Diet

Variable	Group	Statistic	Significant	Note
MAP (Initial)	High fat diet	0.940	0.146	Normal
	No high fat diet	0.846	0.001	Not normal
MAP (Onset PE)	High fat diet	0.930	0.087	Normal
	No high fat diet	0.943	0.173	Normal

From the results of the normality test, the MAP variable data (initial) of the high-fat diet group, and the MAP data (PE appeared) of the high-fat diet and non-high-fat diet groups, each have been normally distributed with a significance value greater than α (0.05). However, the MAP variable data (initial) of the non-high-fat diet group is not normally distributed with a significance value less than α (0.05). So for the comparison of the average MAP (PE appeared) using the unpaired t-test, and for the comparison of the average MAP (initial) using the Mann Whitney test.

Table 5. Summary of Mean Difference Test Results Mean Arterial Pressur Between High Fat Diet and No High Fat Diet

GROUP	MAP (initial)[b] (Mean±SD)	MAP (onset PE)[a] (Mean±SD)	Statistic (p-value)	Table
High fat diet (n=25)	89.49±3.94	122.21±7.79	**t = -20.647 (0.000)***	**t(0.05,24) = 2.064**
No high fat diet (n=25)	85.61±4.48	86.15±3.33	**Z = –0.520 (0.603)**	**Z0.05 = 1.960**
Statistic (p-value)	**Z = –3.352 (0.001)***	**t = 21.285 (0.000)***		
Table	**Z0.05 = 1.960**	**t(0.05,32) = 2.037**		

Nb. a=Independent sample t-test, b=Mann Whitney, *significant p<0.05.

The average MAP (initial) of the high-fat diet group was 89.49±3.94 higher than the average MAP (initial) of the non-high-fat diet group of 85.61±4.48. From the Mann Whitney test, the calculated Z value was obtained which was smaller than the -Z table (-3.352 <-1.960), and the p-value was smaller than α (0.001 <0.050), so the decision was taken to reject H0 which means there is a significant difference in the average MAP (initial) between the high-fat diet group and the non-high-fat diet group. The average MAP (initial) of the high-fat diet group was higher than the non-high-fat diet group, and the difference was significant.

The average MAP (PE emergence) of the high-fat diet group was 122.21±7.79 higher than the average MAP (PE emergence) of the non-high-fat diet group of 86.15±3.33. From the unpaired t-test, the value of |t count| was obtained which was greater than t table (21.285 > 2.037), and the p-value was smaller than α (0.000 < 0.050), so the decision was taken to reject H0 which means there is a significant difference in the average MAP (PE appears) between the high-fat diet group and the non-high-fat diet group. The average MAP (PE appears) of the high-fat diet group was higher than the non-high-fat diet group, and the difference was significant.

In the high-fat diet group, the average MAP (initial) was 89.49±3.94 lower than the average MAP (final (PE appeared)) of 122.21±7.79. From the paired t-test, the value of |t count| was greater than the t table (20.647> 2.064), and the p-value was smaller than α (0.000 <0.050), so the decision was taken to reject H0, which means there is a significant difference in the average MAP between the initial and PE appearance. There was an increase in the average MAP from the beginning to the

end in the high-fat diet group, and the difference was significant.
In the non-high-fat diet group, the average MAP (initial) was 85.61±4.48 lower than the average MAP (final (PE appeared)) of 86.15±3.33. From the Wilcoxon test, the calculated Z value was greater than the –Z table (–0.520 > –1.960), and the p-value was greater than α (0.603 > 0.050), so the decision was taken that H0 was accepted, which means that there was an insignificant difference in the average MAP between the beginning and the emergence of PE. There was a slight increase in the average MAP from the beginning to the end in the non-high-fat diet group, but the difference was not significant.

Table 6. Normality Test Using Shapiro Wilk in MDA, TNF- α, sFlt-1 Between High Fat Diet and No High Fat Diet

Variable	Group	Statistic	Significant	Notes
MDA	High fat diet	0.725	0.000	**Not normal**
	No high fat diet	0.929	0.081	Normal
TNF-α	High fat diet	0.820	0.000	**Not normal**
	No high fat diet	0.927	0.076	Normal
sFlt-1	High fat diet	0.808	0.000	**Not normal**
	No high fat diet	0.682	0.000	**Not normal**

From the results of the normality test, the MDA variable data of the non-high-fat diet group, and the TNF-α data of the non-high-fat diet group, were each normally distributed with a significance value greater than α (0.05). However, for the MDA variable data of the high-fat diet group, the TNF-α variable data of the high-fat diet group, and the sFlt-1 variable data of the high-fat diet and non-high-fat diet groups, each were not normally distributed with a significance value less than α (0.05). Therefore, for the comparison of the average MDA, TNF-α, and sFlt-1 using the Mann Whitney test.

Table 7. Summary of Mean Difference Test Results in MDA, TNF- α, sFlt-1 Between High Fat Diet and No High Fat Diet

GROUP	MDA[b] (Mean±SD)	TNF-α[b] (Mean±SD)	sFlt-1[b] (Mean±SD)
High fat diet (n=25)	8.73±3.87	217.68±109.88	10.66±4.68
No high fat diet (n=25)	2.16±0.49	65.38±16.05	3.14±2.00
Statistic (p-value)	**Z = –6.064 (0.000)***	**Z = –6.063 (0.000)***	**Z = –5.714 (0.000)***
Table	**$Z_{0.05}$ = 1.960**	**$Z_{0.05}$ = 1.960**	**$Z_{0.05}$ = 1.960**

Nb. a=Independent sample t-test, b=Mann Whitney, *significant p<0.05.

The average MDA of the high-fat diet group was 8.73±3.87, higher than the average MDA of the non-high-fat diet group of 2.16±0.49. From the Mann Whitney test, the

calculated Z value was obtained which was smaller than the -Z table (-6.064 <- 1.960), and the p-value was smaller than α (0.000 <0.050), so the decision was taken to reject H0, which means there is a significant difference in the average MDA between the high-fat diet group and the non-high-fat diet group. The average MDA of the high-fat diet group was much higher than the non-high-fat diet group, and the difference was significant.

The average TNF-α of the high-fat diet group was 217.68±109.88, higher than the average TNF alpha of the non-high-fat diet group of 65.38±16.05. From the Mann Whitney test, the calculated Z value was obtained which was smaller than the -Z table (-6.063 < -1.960), and the p-value was smaller than α (0.000 < 0.050), so the decision was taken to reject H0 which means there is a significant difference in the average TNF alpha between the high-fat diet group and the non-high-fat diet group. The average TNF alpha of the high-fat diet group was much higher than the non-high-fat diet group, and the difference was significant.

The average sFlt-1 of the high-fat diet group was 10.66±4.68 higher than the average SFLT-1 of the non-high-fat diet group of 3.14±2.00. From the Mann Whitney test, the calculated Z value was obtained which was smaller than the –Z table (-5.714 < -1.960), and the p-value was smaller than α (0.000 < 0.050), so the decision was taken to reject H0 which means there is a significant difference in the average SFLT-1 between the high-fat diet group and the non-high-fat diet group. The average SFLT-1 of the high-fat diet group was higher than the non-high-fat diet group, and the difference was significant.

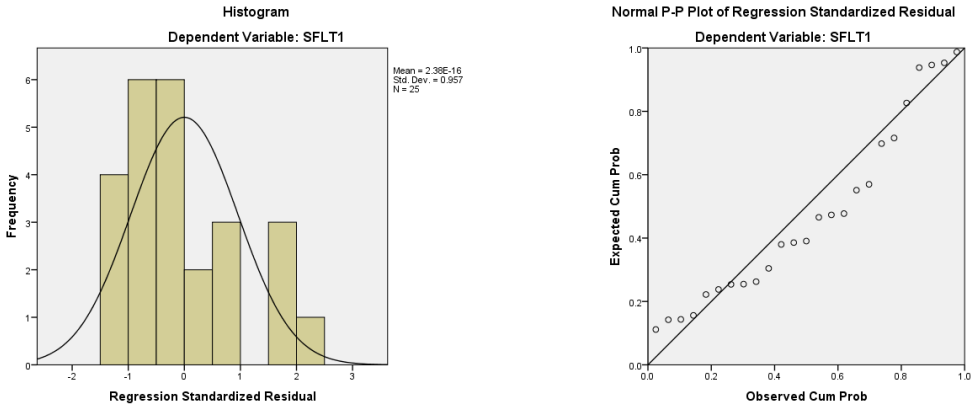

Figure 1 and 2. Histogram and Normal P-P Plot

Tabel 8. One Sample Test Kolmogorov-Smirnov

Residual	P-value
Model 1	0.082

Based on the histogram image, it shows that the bar chart follows the normal curve that is formed and from the normal P-P plot graph, the observation data is obtained around the diagonal line, and from the one sample Kolmogorov-Smirnov test, the p-value is 0.082 which is greater than α (0.05). Based on the three tests, the decision was taken to accept H0 which means that the residual distribution is normally distributed (assumptions are met).

Tabel 9. Multicollinearity Test with VIF

Variable	Tolerance	VIF
MDA	0.963	1.039
TNF-α	0.963	1.039

In the multicollinearity test, the VIF value of each independent variable is less than 10 with a tolerance value of more than 0.1, which means that there is no strong correlation between the independent variables or there is no multicollinearity (assumption met).

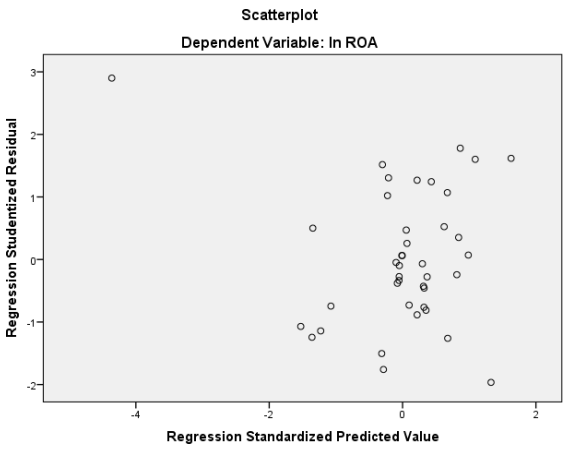

Figure 3. Heteroskedasticity Test with Scatterplot

From the scatterplot results, it shows that the points are randomly distributed (no pattern) both above and below the number 0 on the Y axis, which means that the assumption of heteroscedasticity is met (homogeneous residual variance). After all the classical regression assumptions are carried out, the analysis of the influence of independent variables on dependent variables is continued using multiple linear regression.

Furthermore, a regression analysis is carried out which is useful for obtaining the influence of independent variables (MDA, and TNF-α) on the dependent variable sFlt-1. In data processing using multiple linear regression analysis, several stages are carried out to find the influence between independent variables on the dependent. Based on the results of data processing using SPSS software, a summary is obtained as follows:

Table 10. Multiple Linear Regression Test Summary

Variable	*B*	t count	*P-value* t	Notes
Constant	12.930			
MDA	0.031	0.125	0.902	Not significant
TNF-alpha	-0.012	-1.317	0.202	Not significant
α	= 0.050			
Determinant coefficient (R^2)	= 0.079			
F-count	= 0.941			
F-table ($F_{2,22,0.05}$)	= 3.443			
P-value F	= 0.405			
t-table ($t_{22,0.05}$)	= 2.074			

Based on the results of the regression analysis, the following equation model was obtained: SFLT-1 = 12.930 + 0.031 MDA – 0.012 TNF-alpha + ei

a. The constant obtained of 12.930 indicates that before the influence of the independent variable, there was an initial increase in the sFlt-1 variable of 12.930 numbers.
b. The MDA variable has a positive and insignificant effect on the sFlt-1 variable. It can be seen from the t-test statistics with |t count| smaller than t table (0.125 < 2.074) and a p-value t greater than α (0.902 > 0.050). This test indicates that the decision that H0 is accepted. The coefficient obtained of 0.031 indicates that an increase of 1 number of the MDA variable can increase the SFLT-1 variable by 0.031 numbers but is not significant.
c. The TNF-α variable has a negative and insignificant effect on the sFlt-1 variable. It can be seen from the t-test statistics with |t count| smaller than t table (1.317 < 2.074) and a p-value t greater than α (0.202 > 0.050). This test indicates that the decision that H0 is accepted. The coefficient obtained of -0.012 indicates that an increase of 1 number of the TNF-alpha variable can decrease the SFLT-1 variable by 0.012 numbers but is not significant.

Based on table 11 above, it can be seen that the calculated F value is smaller than the F table (0.941 < 3.443) and has a p-value greater than α (0.405 > 0.050), so H0 is accepted. This means that simultaneously, the independent variables, namely MDA and TNF-α, have an effect but are not significant on the dependent variable sFlt-1.

The magnitude of the contribution of the influence of independent variables simultaneously on the dependent variable, based on the calculation results in table 11 with a determination coefficient value (R Square) of 0.079. These results explain the contribution or contribution of the influence of the independent variables (MDA, and TNF-α) included in the regression equation on the sFlt-1 variable is 7.9%, while the other 92.1% is contributed by other independent variables that are not included in this equation.

Tabel 11. One Sample Test with Kolmogorov-Smirnov

Residual	P-value
Model 1 (MDA)	0.000
Model 2 (TNF-α)	0.000
Model 3 (sFlt-1)	0.006

Based on the one sample Kolmogorov-Smirnov test with each p-value being smaller than α (0.05), the decision was taken to reject H0, which means that the residual distribution is not normally distributed. From the scatterplot results in the image above, it can be seen that the points are quite randomly spread (no pattern) both above and below the number 0 on the Y axis, which means that the assumption of heteroscedasticity is met (homogeneous residual variance). After the classical regression assumption has been carried out, the hypothesis test of the influence between variables is continued.

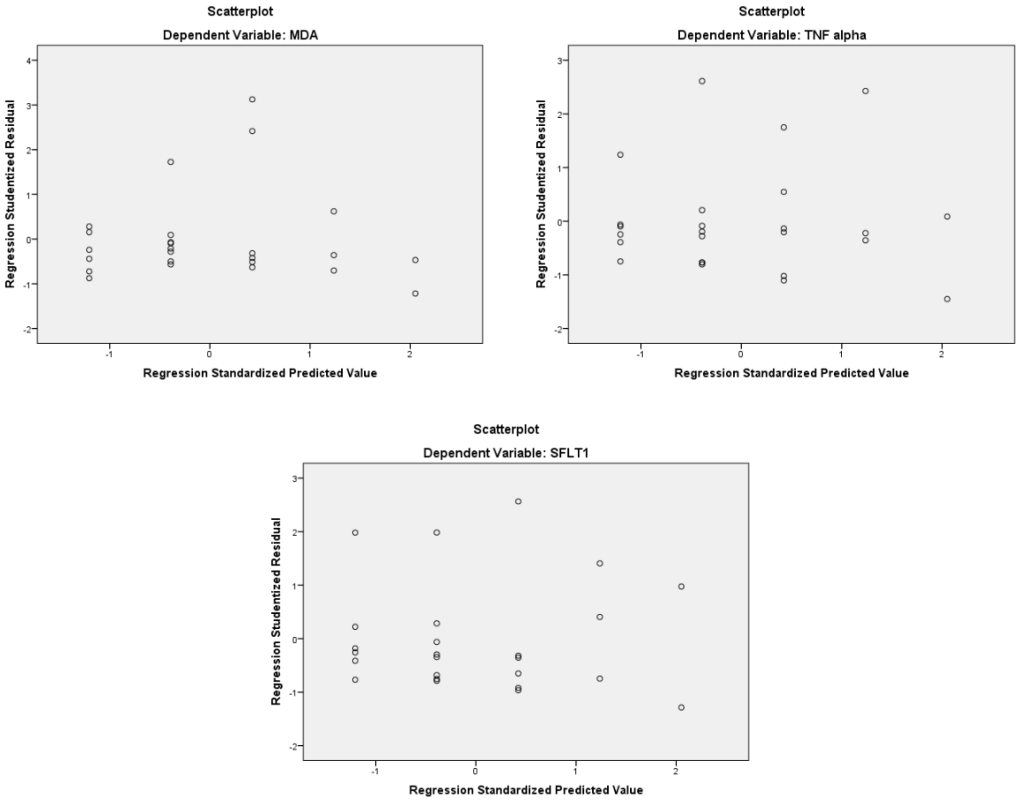

Figure 4, 5, and 6. Scatterplot Model 1, 2 and 3

To test whether there is a significant effect between the high-fat diet variable and the MDA variable, a simple linear regression analysis was performed. Based on the results of data processing, the following summary was obtained.

Table 12. Summary of Simple Linear Regression Test Model 1 (MDA)

Variable	*B*	t count	*p-value* (sig. t)	Note
Constant	-9.523			
HFD	0.562	0.870	0.393	Not significant
α	= 0.050			
R^2	= 0.032			
t-table (0.05,23)	= 2.069			

Based on the table above, the following regression model is obtained:
MDA = -9.523 + 0.562 HFD + ei
The high-fat diet variable has a positive and insignificant effect on the MDA variable. The value of |t count| is smaller than the t table (0.870 < 2.069), and the p-

value is greater than α (0.393 > 0.050). The regression coefficient obtained is positive, which means that an increase of 1 number in the high-fat diet variable can increase the MDA variable by 0.562 numbers but is not significant.
The magnitude of the contribution of the influence of the high-fat diet variable on the MDA variable with a coefficient of determination (R Square) of 0.032. The magnitude of the influence of the high-fat diet variable on the MDA variable is 3.2%, while the other 96.8% is contributed by other independent variables that are not included in this equation.
To test whether there is a significant effect between the high-fat diet variable and the TNF-α variable, a simple linear regression analysis was performed. Based on the results of data processing, the following summary was obtained.

Table 13. Summary of Simple Linear Regression Test Model 1 (TNF-alpha)

Variable	*B*	t count	*p-value* (sig. t)	Notes
Constant	-184.318			
HFD	12.377	0.670	0.509	Not significant
α	= 0.050			
R^2	= 0.019			
t-table (0.05,23)	= 2.069			

Based on the table above, the following regression model is obtained:
TNF-alpha = -184.318 + 12.377 HFD + ei
The high-fat diet variable has a positive and insignificant effect on the TNF-α variable. The value of |t count| is smaller than the t table (0.670 < 2.069), and the p-value is greater than α (0.509 > 0.050). The regression coefficient obtained is positive, which means that an increase of 1 number of the HFD variable can increase the TNF-α variable by 12.377 numbers but is not significant.
The magnitude of the contribution of the influence of the high-fat diet variable on the TNF-α variable with a coefficient of determination (R Square) value of 0.019. The magnitude of the influence of the high-fat diet variable on the TNF-α variable is 1.9%, while the other 98.1% is contributed by other independent variables that are not included in this equation.
To test whether there is a significant influence between the high-fat diet variable and the sFlt-1 variable, a simple linear regression analysis was performed. Based on the results of data processing, the following summary was obtained:

Table 13. Summary of Simple Linear Regression Test Model 1 (sFlt-1)

Variable	*B*	t count	*p-value* (sig. t)	Notes
Constant	-9.457			
HFD	0.619	0.790	0.438	Not significant
α	= 0.050			
Coefficient determinant (R^2)	= 0.026			
t-tabel (0.05,23)	= 2.069			

Based on the table above, the following regression model is obtained:
SFLT-1 = -9.457 + 0.619 HFD + ei
The high-fat diet variable has a positive and insignificant effect on the sFlt-1 variable. The value of |t count| is smaller than the t table (0.790 < 2.069), and the p-value is greater than α (0.438 > 0.050). The regression coefficient obtained is positive, which means that an increase of 1 number of the high-fat diet variable can increase the sFlt-1 variable by 0.619 numbers but is not significant.
The magnitude of the contribution of the influence of the high-fat diet variable on the sFlt-1 variable with a coefficient of determination (R Square) value of 0.026. The magnitude of the influence of the high-fat diet variable on the sFlt-1 variable is 2.6%, while the other 97.4% is contributed by other independent variables that are not included in this equation.

Path Analysis

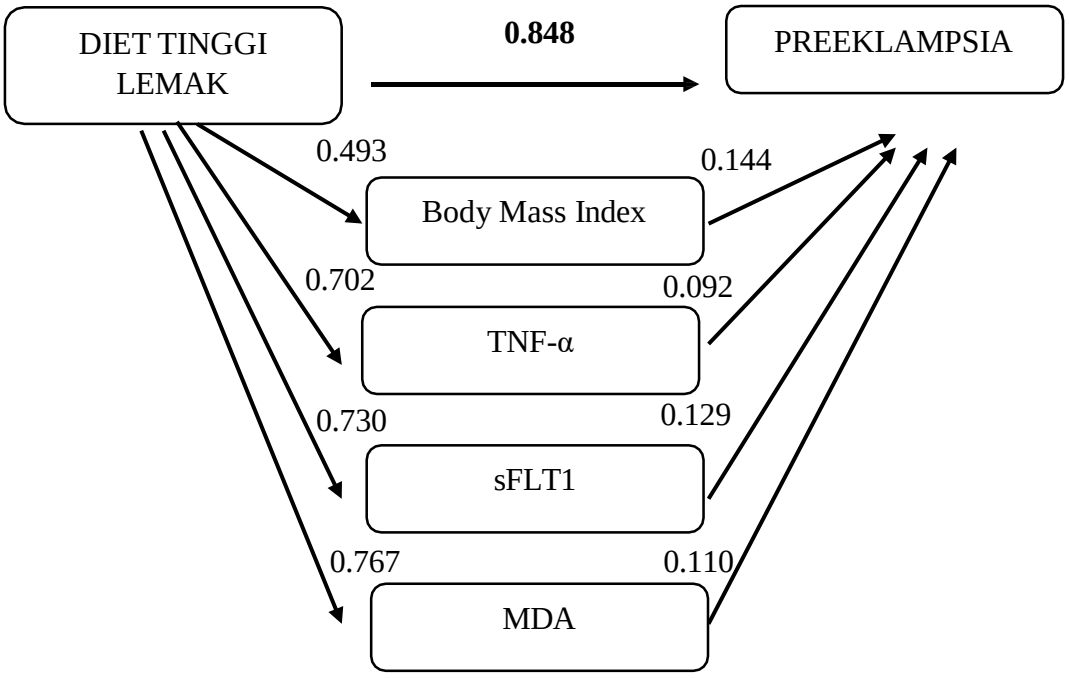

Figure 7. Path analysis diagram showing the relationship between a high-fat diet and preeclampsia through various mediating factors (BMI, TNF-α, sFLT1, MDA).

The path analysis diagram illustrates the relationship between a high-fat diet, preeclampsia, and various mediating factors (BMI, TNF-α, sFLT1, MDA). The direct path between a high-fat diet and preeclampsia is shown with a coefficient of 0.848, indicating a strong positive relationship. The diagram also shows several intermediary pathways that connect the high-fat diet to preeclampsia through variables such as BMI, TNF-α (a pro-inflammatory cytokine), sFLT1 (a soluble receptor involved in preeclampsia), and MDA (malondialdehyde, a marker of oxidative stress). Each of these mediators has different path coefficients, showing how they contribute to the overall relationship. The high-fat diet influences BMI (0.493), which then has a smaller effect on preeclampsia (0.144). Similarly, the high-fat diet affects TNF-α (0.702), sFLT1 (0.730), and MDA (0.767), which in turn have varying impacts on preeclampsia. The indirect effects through TNF-α, sFLT1,

and MDA are also represented, with their respective contributions shown as 0.092, 0.129, and 0.110. This model suggests that while a high-fat diet directly influences preeclampsia, its impact is also mediated by increases in BMI, inflammatory cytokines, and oxidative stress markers, all of which further contribute to the risk of preeclampsia.

DISCUSSION

The findings of this study highlight a strong association between high-fat dietary intake during pregnancy and the development of preeclampsia, as well as elevated levels of key biomarkers such as TNF-α, sFlt-1, and MDA. Our results provide compelling evidence that pregnant women with high-fat diets are significantly more likely to develop preeclampsia compared to those consuming a low-fat diet. Furthermore, elevated levels of TNF-α, sFlt-1, and MDA were correlated with the incidence of preeclampsia, reinforcing the hypothesis that oxidative stress and inflammatory pathways play critical roles in the pathogenesis of this condition.[17,18]

In this study, 69.4% of women with a high-fat diet developed preeclampsia, whereas none of those with a low-fat diet developed the condition. A high fat diet is strongly correlated with preeclampsia. These findings align with previous research that showed higher fat intake in women with preeclampsia and high trans-fat intake increase the risk of preeclampsia in pregnancy.[16,19–23] High dietary fat intake, especially in the form of saturated and trans fats, is strongly linked to adverse cardiovascular and metabolic effects, which can play a crucial role in the development of preeclampsia. Saturated fats are known to elevate low-density lipoprotein (LDL) cholesterol levels, which can contribute to the buildup of atherosclerotic plaques in blood vessels.[24,25] This process leads to vascular dysfunction, characterized by a reduced ability of the blood vessels to dilate properly, impaired blood flow, and increased vascular resistance. Trans fats, in particular, have been shown to increase inflammation and oxidative stress, further contributing to endothelial damage.[4,26]

In the context of pregnancy, this vascular dysfunction becomes especially critical, as the placenta requires optimal blood flow and vascular health to support fetal development. A high-fat diet can disrupt these processes by increasing the production of reactive oxygen species (ROS), which causes oxidative stress. Oxidative stress damages the endothelial lining of blood vessels and impairs nitric oxide production, a molecule crucial for vasodilation. The resulting endothelial dysfunction is a hallmark of preeclampsia, leading to increased blood pressure, reduced placental perfusion, and the release of pro-inflammatory cytokines.[4,7]

Our study's elevated TNF-α, sFlt-1, and MDA levels in women with preeclampsia underscore the involvement of inflammatory and angiogenic dysregulation in the disease process. TNF-α, a pro-inflammatory cytokine, was significantly higher in these women, and all cases of preeclampsia occurred in women with TNF-α levels above the cutoff of 100.3 pg/mL. This finding is consistent with previous studies that have identified TNF-α as a key mediator of inflammation and endothelial dysfunction in preeclampsia.[10,27] A meta-analysis reported that severe preeclampsia was associated with elevated TNF-α.[9] Elevated TNF-α levels contribute to vascular inflammation and oxidative stress, further impairing placental function and leading to the clinical manifestations of

preeclampsia.[27]

Similarly, sFlt-1, an anti-angiogenic factor, was significantly elevated in women with preeclampsia compared to normotensive women. This finding is in line with the previous studies.[12,13,28,29] High sFlt-1 levels have been widely implicated in the pathogenesis of preeclampsia due to their role in inhibiting vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), which are essential for promoting angiogenesis and maintaining the health of the endothelium. In a normal pregnancy, VEGF and PlGF bind to their receptors on the surface of endothelial cells to stimulate blood vessel formation, promote proper placental development, and ensure adequate blood supply to the growing fetus. These pro-angiogenic factors help maintain endothelial function, allowing the maternal blood vessels to adapt to the increased circulatory demands of pregnancy. However, in women with preeclampsia, elevated levels of sFlt-1 bind to VEGF and PlGF, sequestering these critical factors and preventing them from interacting with their receptors. This inhibition of VEGF and PlGF signalling leads to endothelial dysfunction, a hallmark of preeclampsia.[30,31]

Our findings also revealed the role of oxidative stress in preeclampsia, as all women with MDA levels ≥3.815 nmol/mL developed preeclampsia. MDA is a well-established marker of lipid peroxidation, indicating oxidative damage to cell membranes. Elevated MDA levels in women with preeclampsia suggest that increased oxidative stress may contribute to the endothelial dysfunction seen in the disease. Our results align with existing literature that has shown increased oxidative stress markers in preeclamptic women, further implicating oxidative stress in the pathophysiology of the disorder.[32–40]

The results of this study have important clinical implications, as they suggest that dietary interventions aimed at reducing fat intake during pregnancy could potentially lower the risk of preeclampsia. Furthermore, monitoring the level of inflammatory and oxidative stress markers such as TNF-α, sFlt-1, and MDA could serve as valuable tools for early detection and risk stratification of preeclampsia in pregnant women, particularly those with high-fat diets.

While this study provides valuable insights, it is not without limitations. The relatively small sample size and the fact that it was conducted in a single geographic area may limit the generalizability of the findings. Further research involving larger, more diverse populations is needed to confirm these associations. The study's observational nature also precludes establishing a direct causal relationship between a high-fat diet and preeclampsia.

CONCLUSION

The study concludes that a high-fat diet has a significant impact on blood pressure and biomarkers related to preeclampsia. Participants on a high-fat diet showed significantly higher systolic and diastolic blood pressures, both initially and at the onset of preeclampsia (PE), compared to those not on a high-fat diet. Mean Arterial Pressure (MAP) was also notably elevated in the high-fat diet group in both initial and PE conditions. Additionally, key biomarkers, including MDA, TNF-α, and sFlt-1, were significantly higher in the high-fat diet group, suggesting increased oxidative stress and inflammation. High fat diet also releated to the risk of

preeclampsia directly or through MDA, TNF-α, sFlt-1 as mediators. However, despite these elevations, the study finds that the high-fat diet does not significantly influence the biomarkers MDA, TNF-α, and sFlt-1 in isolation, as determined through regression analysis.

REFERENCES

1. Dimitriadis E, Rolnik DL, Zhou W, et al. Pre-eclampsia. *Nat Rev Dis Prim*. 2023;9(1):8. doi:10.1038/s41572-023-00417-6
2. Rana S, Lemoine E, Granger J, Karumanchi SA. Preeclampsia: Pathophysiology, Challenges, and Perspectives. *Circ Res*. 2019;124(7):1094-1112. doi:10.1161/CIRCRESAHA.118.313276
3. Jung E, Romero R, Yeo L, et al. The etiology of preeclampsia. *Am J Obstet Gynecol*. 2022;226(2S):S844-S866. doi:10.1016/j.ajog.2021.11.1356
4. Tan BL, Norhaizan ME. Effect of High-Fat Diets on Oxidative Stress, Cellular Inflammatory Response and Cognitive Function. *Nutrients*. 2019;11(11). doi:10.3390/nu11112579
5. Ofosu-Boateng M, Shaik F, Choi S, et al. High-fat diet induced obesity promotes inflammation, oxidative stress, and hepatotoxicity in female FVB/N mice. *Biofactors*. 2024;50(3):572-591. doi:10.1002/biof.2028
6. Chandranita Manuaba IA, Andarini S, Soeharto S, Rahardjo B. High-fat diet in pregnant women may induce preeclampsia through lipid peroxidation pathways. *J Med Pharm Chem Res*. 2024;6(12):1877-1887. doi:10.48309/jmpcr.2024.455763.1214
7. Vial G, Dubouchaud H, Couturier K, et al. Effects of a high-fat diet on energy metabolism and ROS production in rat liver. *J Hepatol*. 2011;54(2):348-356. doi:10.1016/j.jhep.2010.06.044
8. Öcal E, Toprak V, Akalin SA, Aşir F, Deveci E. Investigation of Beclin 1 and TNF-α expressions in preeclampsia placentas: Immunohistochemical study. *Medicine (Baltimore)*. 2023;102(33):e34757. doi:10.1097/MD.0000000000034757
9. Lau SY, Guild SJ, Barrett CJ, et al. Tumor necrosis factor-alpha, interleukin-6, and interleukin-10 levels are altered in preeclampsia: a systematic review and meta-analysis. *Am J Reprod Immunol*. 2013;70(5):412-427. doi:10.1111/aji.12138
10. Adenekan MA, Oluwole AA, Olorunfemi G, Sekumade AI, Ajepe AA, Okunade KS. Maternal tumour necrosis factor-alpha levels in preeclamptic pregnancies in Lagos, South-West Nigeria. *Pregnancy Hypertens*. 2022;30:198-203. doi:10.1016/j.preghy.2022.10.011
11. Miller JJ, Higgins V, Melamed N, et al. Clinical Validation of the sFlt-1:PlGF Ratio as a Biomarker for Preeclampsia Diagnosis in a High-Risk Obstetrics Unit. *J Appl Lab Med*. 2023;8(3):457-468. doi:10.1093/jalm/jfad003
12. Dathan-Stumpf A, Rieger A, Verlohren S, Wolf C, Stepan H. sFlt-1/PlGF ratio for prediction of preeclampsia in clinical routine: A pragmatic real-world analysis of healthcare resource utilisation. *PLoS One*. 2022;17(2):e0263443. doi:10.1371/journal.pone.0263443
13. Verlohren S, Brennecke SP, Galindo A, et al. Clinical interpretation and implementation of the sFlt-1/PlGF ratio in the prediction, diagnosis and

management of preeclampsia. *Pregnancy Hypertens*. 2022;27:42-50. doi:10.1016/j.preghy.2021.12.003

14. Tataru-Copos AF, Popescu MI, Murvai R, El Kharoubi A. Efficiency of sFlt-1/PlGF Ratio in Preeclampsia Diagnosis. *Medicina (Kaunas)*. 2022;58(9). doi:10.3390/medicina58091196
15. Li S na, Liu Y hua, Luo Z yan, et al. The association between dietary fatty acid intake and the risk of developing preeclampsia: a matched case–control study. *Sci Rep*. 2021;11(1):1-10. doi:10.1038/s41598-021-83674-3
16. Arvizu M, Gaskins A, Stuart J, Rich-Edwards J, Chavarro JE. Intakes of Major Types of Fat Before Pregnancy and Hypertensive Disorders of Pregnancy. *Curr Dev Nutr*. 2020;4:nzaa054_008. doi:https://doi.org/10.1093/cdn/nzaa054_008
17. Aouache R, Biquard L, Vaiman D, Miralles F. Oxidative stress in preeclampsia and placental diseases. *Int J Mol Sci*. 2018;19(5). doi:10.3390/ijms19051496
18. Tenório MB, Ferreira RC, Moura FA, Bueno NB, de Oliveira ACM, Goulart MOF. Cross-Talk between Oxidative Stress and Inflammation in Preeclampsia. *Oxid Med Cell Longev*. 2019;2019:8238727. doi:10.1155/2019/8238727
19. Yusuf H, Subih HS, Obeidat BS, Sharkas G. Associations of macro and micronutrients and antioxidants intakes with preeclampsia: A case-control study in Jordanian pregnant women. *Nutr Metab Cardiovasc Dis*. 2019;29(5):458-466. doi:https://doi.org/10.1016/j.numecd.2019.01.008
20. Nahla K, Ibrahim BA, Bahaa-Eldin EAR. Association between dietary patterns and pregnancy induced hypertension: a case control study from Sudan. *Afr Health Sci*. 2022;22(4):368-374. doi:10.4314/ahs.v22i4.42
21. Alamolhoda SH, Simbar M, Mirmiran P, Mirabi P. Effect of low trans-fatty acid intakes on preeclampsia: A randomized controlled trial. *J Res Med Sci Off J Isfahan Univ Med Sci*. 2020;25:112. doi:10.4103/jrms.JRMS_149_19
22. Chavarro JE, Halldorsson TI, Leth T, Bysted A, Olsen SF. A prospective study of trans fat intake and risk of preeclampsia in Denmark. *Eur J Clin Nutr*. 2011;65(8):944-951. doi:10.1038/ejcn.2011.66
23. Brantsaeter AL, Haugen M, Samuelsen SO, et al. A dietary pattern characterized by high intake of vegetables, fruits, and vegetable oils is associated with reduced risk of preeclampsia in nulliparous pregnant Norwegian women. *J Nutr*. 2009;139(6):1162-1168. doi:10.3945/jn.109.104968
24. Guijarro C, Cosín-Sales J. LDL cholesterol and atherosclerosis: The evidence. *Clin e Investig en Arterioscler Publ Of la Soc Esp Arterioscler*. 2021;33 Suppl 1:25-32. doi:10.1016/j.arteri.2020.12.004
25. Ference BA, Ginsberg HN, Graham I, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J*. 2017;38(32):2459-2472. doi:10.1093/eurheartj/ehx144
26. Monguchi T, Hara T, Hasokawa M, et al. Excessive intake of trans fatty acid accelerates atherosclerosis through promoting inflammation and oxidative stress in a mouse model of hyperlipidemia. *J Cardiol*. 2017;70(2):121-127. doi:10.1016/j.jjcc.2016.12.012
27. Schipper EJI, Bolte AC, Schalkwijk CG, Van Geijn HP, Dekker GA. TNF-

receptor levels in preeclampsia--results of a longitudinal study in high-risk women. *J Matern neonatal Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet*. 2005;18(5):283-287. doi:10.1080/14767050500246466

28. Stepan H, Galindo A, Hund M, et al. Clinical utility of sFlt-1 and PlGF in screening, prediction, diagnosis and monitoring of pre-eclampsia and fetal growth restriction. *Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol*. 2023;61(2):168-180. doi:10.1002/uog.26032
29. Kifle MM, Dahal P, Vatish M, Cerdeira AS, Ohuma EO. The prognostic utility of soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PIGF) biomarkers for predicting preeclampsia: a secondary analysis of data from the INSPIRE trial. *BMC Pregnancy Childbirth*. 2022;22(1):520. doi:10.1186/s12884-022-04817-6
30. Jena MK, Sharma NR, Petitt M, Maulik D, Nayak NR. Pathogenesis of Preeclampsia and Therapeutic Approaches Targeting the Placenta. *Biomolecules*. 2020;10(6). doi:10.3390/biom10060953
31. Liu Y, Ren M, Bi X, et al. A systematic review on the application of vascular endothelial growth factors in preeclampsia. *Ann Palliat Med*.2021;10(8):9259-9266. doi:10.21037/apm-21-2109
32. Afrose D, Chen H, Ranashinghe A, et al. The diagnostic potential of oxidative stress biomarkers for preeclampsia: systematic review and meta-analysis. *Biol Sex Differ*. 2022;13(1):1-15. doi:10.1186/s13293-022-00436-0
33. Taravati A, Tohidi F. Comprehensive analysis of oxidative stress markers and antioxidants status in preeclampsia. *Taiwan J Obstet Gynecol*. 2018;57(6):779-790. doi:10.1016/j.tjog.2018.10.002
34. Asiltas B, Surmen-Gur E, Uncu G. Prediction of first-trimester preeclampsia: Relevance of the oxidative stress marker MDA in a combination model with PP-13, PAPP-A and beta-HCG. *Pathophysiology*. 2018;25(2):131-135. doi:10.1016/j.pathophys.2018.02.006
35. Rumopa H, Wagey FW, Suparman E. Malondialdehyde Levels in Preeclampsia before and after Delivery. *Indones J Obstet Gynecol*. 1970;6(3):143. doi:10.32771/inajog.v6i3.777
36. Gohil JT, Patel PK, Gupta P. Evaluation of oxidative stress and antioxidant defence in subjects of preeclampsia. *J Obstet Gynaecol India*. 2011;61(6):638-640. doi:10.1007/s13224-011-0094-8
37. Bambrana V, Dayanand CD, Kotur P. Relationship Between Xanthine Oxidase, Ischemia Modified Albumin, Nitric Oxide with Antioxidants in Non Pregnants, Pre and Post-delivery of Normal Pregnants and Preeclampsia. *Indian J Clin Biochem*. 2017;32(2):171-178. doi:10.1007/s12291-016-0599-0
38. Rani N, Dhingra R, Arya DS, Kalaivani M, Bhatla N, Kumar R. Role of oxidative stress markers and antioxidants in the placenta of preeclamptic patients. *J Obstet Gynaecol Res*. 2010;36(6):1189-1194. doi:10.1111/j.1447-0756.2010.01303.x
39. Shaker OG, Sadik NAH. Pathogenesis of preeclampsia: Implications of apoptotic markers and oxidative stress. *Hum Exp Toxicol*. 2013;32(11):1170-1178. doi:10.1177/0960327112472998

40. Loukidi B, Merzouk H, Merzouk SA, et al. Thrombosis factors and oxidant/antioxidant markers in obese and hypertensive women during pregnancy. *Blood Press*. 2015;24(4):242-249. doi:10.3109/08037051.2015.1032517