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Serum ferritin, insulin, and c-reactive protein as early biochemical indicators for gestational diabetes mellitus among a Filipino population in Manila

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ABSTRACT

Gestational diabetes mellitus (GDM) is one of the metabolic diseases that affects pregnant women. GDM diagnosis is done during the late second or early third trimester, which may be too late to prevent complications. **Objective**: The study aimed to determine the association between serum ferritin, insulin, and CRP measurements, alone or in combination, and GDM. **Methods:** A prospective cohort study was done longitudinally over the three trimesters of gestation on 118 Filipino pregnant women who were 20-45 years old, had no recognizable diabetes, and were free from cardiovascular and other inflammatory disorders- thirty-six (36) with GDM and 82 without GDM. Ferritin, insulin, and CRP were measured in the first, second, and third trimesters of pregnancy. GDM diagnosis was done by OGTT at 28-32 weeks of gestation. Serum ferritin and insulin were determined by sandwich ELISA and CRP by solid-phase sandwich-format immunometric assay. **Results**: Women with GDM had higher ferritin levels in the first, second, and third trimesters at 30.47, 20.76, and 13.68 (ng/ml), respectively than in the non-GDM group at 27.41, 13.32, and 13.02 (ng/ml), respectively. Differences in the means of CRP and ferritin levels among women with GDM across the three trimesters were significant at $p < 0.01$. When tested individually, CRP and ferritin were significantly associated with GDM in the first at $p<0.05$ and second at $p<0.01$ trimester. In combination, the effects of ferritin and CRP (R-value=0.280>) were significant in the first trimester. Insulin in the GDM group was higher in the second trimester at 7.36 (µIU/ml) than in the non-GDM group at 7.15 (µIU/ml). Differences in means of insulin in the three trimesters were not significant. CRP was higher in the GDM group in the first trimester at 5.25 (mg/L), and the second

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trimester at 6.11 (mg/L), than in the non-GDM group at 5.00 (mg/L), and 5.82 (mg/L) in the first and second trimesters, respectively. **Conclusions**: In the first trimester of pregnancy, the combined effects of ferritin and CRP can enhance early detection of GDM risk. In the second trimester, the combination of ferritin and CRP, and the combination of ferritin and insulin can serve as potential biochemical indicators of GDM.

INTRODUCTION

Gestational diabetes mellitus (GDM) is a form of hyperglycemia that is first diagnosed during pregnancy, especially in its second or third trimester in the absence of Type 1 or Type 2 diabetes mellitus (McIntyre et al. 2019). This hyperglycemia is caused by glucose intolerance, which occurs when the beta cells of the pancreas can no longer offset the rise in insulin resistance that typically occurs during pregnancy. Although the exact pathophysiology is not yet clear, GDM is found to result from the interaction of genetic and environmental factors. Although not given much attention as a public health burden, studies have shown a rise in the incidence of GDM from 3.86% in 2007 to 11.83% in 2010 (Cho et al. 2015). On the other hand, in 2007, the incidence of patients requiring insulin treatment decreased significantly from 13.87% to 5.94% by 2010. In the Philippines, the prevalence rate of GDM is 14% in 1,203 pregnant women who were surveyed, according to the Asian Federation of Endocrine Societies Study Group on Diabetes in Pregnancy (Tan 2015). The prevalence rate of GDM based on the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria in 919 Filipino women was 29.27% (Pineda-Cortel 2018). The increasing prevalence of GDM worldwide has caused maternal and child morbidity and mortality, a major health problem that confronts healthcare professionals. Currently, the Oral Glucose Tolerance Test (OGTT) is used to diagnose GDM. Unfortunately, glucose elevation typically occurs late in pregnancy; thus, the utility of an OGTT is limited to the late second or early third trimester of pregnancy, which exposes both the mother and the baby to GDM complications. Possible interventions that are done early in pregnant women who are at risk for GDM can prevent GDM or the occurrence of its complications. The delay in detecting GDM negates the beneficial outcome of early intervention and management of those at risk. Tests that detect the early development of GDM are not yet available, although studies have been conducted to identify analytes with potential applications as biochemical indicators in GDM. It is essential to identify a test associated with the early development of GDM, allowing for prompt intervention and management of cases, such as diet modification, exercise, and pharmacological therapy, thereby reducing costs and improving outcomes (Kumru et al. 2016). This study examined the association of serum ferritin, insulin, and CRP levels alone or in combination with other parameters in the early development of GDM among pregnant Filipino women. It has been noted that in pregnant women with GDM serum ferritin is increased compared to the serum ferritin levels in women who did not develop GDM (Sun et al. 2019).

Increased serum ferritin concentration has been associated with chronic inflammatory conditions, diabetes, cardiovascular diseases, and pregnancy. A significant relationship between increased serum ferritin and insulin resistance which causes inflammation and other metabolic complications is shown (Powe 2017). An association between elevated serum ferritin and the risk of GDM early in gestation is noted in the study of (Chen et al. 2006). However, there are inconsistencies in findings that show whether serum ferritin can be an independent risk factor for GDM, an indication of inflammation, or a reflection of iron stores in the body. Furthermore, findings that correlate serum ferritin and insulin levels early in pregnancy to establish its potential to determine GDM risks were scarce. Most studies determined the correlation of serum ferritin and insulin with GDM during the last trimester of gestation but were not done early in pregnancy. In the pathophysiology of GDM, insulin sensitivity is decreased as pregnancy progresses. The utilization of lipids leads to the production of fatty acids which is responsible for the increase in insulin resistance of the cells. Insulin resistance and diminished pancreatic function to synthesize insulin led to GDM (Baz et al. 2016). CRP is an indicator of inflammation. Increased CRP and ferritin levels reflect the pathophysiological mechanism of an inflammation associated with GDM (Sun et al. 2019). CRP is increased in GDM but is found to be affected by BMI (Alyas et al. 2019). Also, very few studies on GDM were conducted among Filipino women. It has been pointed out that maternal characteristics and other circulating biomarkers can be useful in assessing GDM risk and that more studies are needed to establish this. Universal screening for GDM among Filipinos, more efforts to monitor this trend, and establishing biochemical indicators were recommended.

MATERIALS AND METHODS

This study utilized the prospective cohort study design. At the time the baseline characteristics were taken, none of the participants had developed GDM. After baseline information was gathered, which included the age, body mass index (BMI), and HgbA1c, the participants' blood samples were collected in the first, second, and third trimesters of pregnancy to determine changes in ferritin, insulin, and C-reactive protein in the development of GDM. The participants were grouped based on their GDM or non-GDM status which was diagnosed in the late second or third trimester of pregnancy by Oral Glucose Tolerance Test (OGTT) using the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria: GDM is indicated if the fasting blood sugar (FBS) was ≥5.1 mmol/L, 1st hour after oral administration of 75 g glucose was ≥10.0 mmol/L, 2nd hour was ≥8.5 mmol/L. The groups were identified as those who developed gestational diabetes mellitus (GDM) and those who did not (non-GDM). The data gathered over the gestation period were used to determine the risk factors associated with the development of GDM.

Participants

One hundred eighteen (118) healthy Filipino women were recruited to participate in the study. None should be using hormonal contraception, tobacco, or other medications that might affect carbohydrate metabolism or energy expenditure. Each participant was instructed on a standard diet regimen two weeks prior to the study period. Serum ferritin, insulin, and CRP levels were determined in the first, second, and third trimesters of pregnancy. OGTT was done either in the second or third trimester of pregnancy. The participants were selected based on the following criteria: a.) pregnant women, b.) 20-45 years old, c.) no recognizable diabetes, and d.) free from cardiovascular and other inflammatory diseases.

Assays

The body mass index (BMI) was calculated as the pre-pregnancy weight (kilograms) divided by the height (meters) squared. A BMI of <25 kg/m2 was considered lean, 25-30 was considered overweight, and >30 kg/m2 was considered obese22. For the glucose determination, the FlUOstar Omega (BMG Labtech Allmendgrun 8 77799 Ortenberg, Germany) microplate reader was used and in the determination of HgbA1c and CRP, the NycocardTM Reader II (Abbott Nycocard Reader, Biocare Inc.) was used. The procedure and reagents were used according to the manufacturer's instructions. Serum ferritin and insulin were determined by enzyme-linked immunosorbent assay (ELISA)

using the *Raybio Human ELISA kit* (RayBiotech 3607 Parkway Lane Suite 100 Norcross, GA 30092) and CRP by immunometric assay in samples taken on the first, second, and third trimesters of pregnancy. Blood glucose was measured by the glucose oxidase method, and HbA1c by boronate affinity assay.

Ethical Considerations

Ethical clearance was obtained from the University of Santo Tomas Graduate School Research Ethics Committee. The research protocol was approved by the Institutional Review Board (E-2016-02-R3) and by the Scientific Review Committee of the Graduate School. Written informed consent was obtained from each participant after the purpose and nature of the study were thoroughly explained as part of ethical principles in conducting research. All information and data were kept confidential. This study adhered to the Helsinki Declaration of Ethics in Research.

Ethics Approval and Consent to Participate

After obtaining approval from the UST Institutional Review Board, a written informed consent was secured from each participant. All data were kept confidential.

Data Analysis

All variables measured were reported as mean (M) and standard deviation (SD). Differences in continuous variables were assessed using t-tests. Inferential statistics was used to analyze the null hypotheses. An F-test using ANOVA was used to determine the significant differences in mean for GDM and non-GDM between trimesters. To determine the relationship or association of serum ferritin, insulin, and CRP alone and in combination with the different analytes in GDM and non-GDM groups, multiple correlations of determination with two independent variables and one dependent variable were utilized. A p-value of ‹ 0.05 was considered significant for all tests.

RESULTS AND DISCUSSION

Demographic Profile of Participants

The participants' demographic profile included their age, body mass index (BMI), occupation, and family history. **Table 1** shows the mean distribution of participants' profile in terms of age and BMI, and **Table 2** shows the frequency of the participants in terms of family history of diabetes mellitus and GDM diagnosis. The GDM group was older (mean 29.61 years) than the non-GDM group (mean 26.36 years). This is consistent with the findings of (Li et al. 2020) that the GDM group has a higher mean age (30 years) compared to the non-GDM group (28 years). Age has been considered an important factor in the development of GDM in Southeast Asian countries, including the Philippines. In the study of (Kunasegaran 2021), pregnant women below 16 years old are also exposed to risks of having GDM, anemia, and other obstetric complications. In this study, the BMI of the GDM group (mean=25.26) is also higher compared to the non-GDM group (mean 22.82). This is consistent with the findings of (Gavish 2020), that BMI is higher in the GDM group than in those without GDM. The association of BMI with GDM has been confirmed in previous studies (Gavish 2020). Obesity has been known to be a risk factor for GDM. As pregnancy progresses, several physiological changes happen, such as hormonal changes, an increase in adipose tissues, and the occurrence of inflammation; thus, obesity could further contribute to the development of GDM.

Table 1: Mean Distribution of Participants in Terms of Age and BMI

Profile	GDM		Non GDM	
	Mean	SD	Mean	SD
Age	29.61	5.94	26.36	5.36
BMI	25.26	6.76	22.82	3.67

GDM- with Gestational Diabetes Mellitus; Non-GDM- without Gestational Diabetes Mellitus; BMI- Body Mass Index

One had diabetes in the family history. Out of 118 participants, 36 developed GDM, and 82 did not develop GDM as seen in **Table 2**.

Table 2: Frequency Distribution of Participants in terms of Family History and GDM status

Profile	Frequency	Percent (%)
With a family history of diabetes	1	0.2
Without a family history of diabetes	117	99.2
With GDM	36	30.5
Without GDM	82	69.5

HbA1c and OGTT Results of Pregnant Women with or without GDM

Pregnant women with GDM had higher HbA1c (mean=5.46%) than the non-GDM group (mean=5.17%) as shown in **Table 3.** Glycated hemoglobin (HbA1c) reflects the average blood glucose level within 3 months, the life span of the red blood cell. It has been used to diagnose diabetes and hyperglycemic control24. In this study, the difference in HbA1c between the GDM group and the non-GDM group is statistically significant. However, (Osmundson et al. 2016) found that women who have HbA1c below 6.5% have an increased risk for GDM, and some with HbA1c below 5.7% developed GDM. Thus, it is not possible to use HbA1c alone in predicting GDM. On the other hand, (Bozkurt et al. 2020) found that HbA1c ≥5.7% (39mmol/mol) indicates an effect on the β-cell function of the pancreas and uptake of glucose by the cells and reflects the inability of the pancreatic cells to compensate for insulin resistance, an interesting event in the development of GDM. These underlying biological mechanisms support the conclusion that HbA1c can be a useful early diagnostic tool in GDM. In OGTT, the GDM group showed higher FBS (mean=5.58), 1-hour blood glucose (mean=9.42), and 2-hour blood glucose (mean=8.70) than the non-GDM group (means= FBS, 4.50; 1-hour, 6.78; 2-hour, 6.17). These findings are consistent with the study of (Wang et al. 2018) that pregnant women with GDM are older, and have higher fasting blood glucose, 1-hour, and 2-hour blood glucose levels, and BMI compared to the non-GDM women.

Table 3: Mean Results of HbA1c and OGTT of Participants with and without GDM

	GDM		Non-GDM	
Parameters	Mean	SD	Mean	SD
HbA1c (%)	5.46	0.74	5.17	0.45
OGTT (mmol/L)				
FBS	5.58	0.18	4.50	0.05
1-hour blood glucose	9.42	2.25	6.78	1.24
2-Hour blood glucose	8.70	2.21	6.17	1.05

HbA1c- Hemoglobin A1c; OGTT- Oral Glucose Tolerance Test; FBS- Fasting Blood Sugar

Significant Difference between Means in HbA1c and OGTT for the Non-GDM and the GDM Groups

To determine if the difference in means between the non-GDM and the GDM groups is significant, a t-test was used. The 2-tailed value for HbA1c is less than the p=0.05 which means the difference is significant. The differences between the two groups in OGTT in terms of FBS, 1-hour, and 2-hour blood glucose levels are less than the p-value <0.01. This indicates that the differences in OGTT results between the two groups are significant (**Table 4**). These results with OGTT are consistent with the findings of (Wang et al. 2018) that FBS and OGTT results are higher in the GDM group than in the non-GDM group.

However, (Osmundson 2016) found that some women who had HbA1c below 5.7% developed GDM. Thus, the use of HbA1c, as an early predictor of GDM is unlikely. Furthermore, the practicality of an early OGTT and fasting blood glucose in determining GDM risk needs to be confirmed. An early abnormal glucose test does not sufficiently replace a later OGTT for the diagnosis of GDM.

Table 4: Significant Differences between Means for the Non-GDM and the GDM Participants

Parameters	t-value	Sig. (2-tailed)
HbA1c	2.508*	0.014
OGTT		
FBS	7.583**	0.000
Blood Glucose 1-hour	8.117**	0.000
Blood Glucose 2-hour	8.362**	0.000

* Significant at p<.05 level
** Significant at p<.01 level

Relationship between the Demographic Profile and the HbA1c and OGTT of the Women with or without GDM

There is a significant relationship between the demographic profile of the GDM and the non-GDM groups in terms of their age, BMI, HbA1c, and OGTT results in **Table 5**. The age and OGTT results are significant at p<.01 level while BMI and HbA1c are significant at p<.05 level. Based on the WHO criteria in the diagnosis of GDM, age is an independent risk factor in GDM. This supports the study of (Li 2020) that there is a direct relationship between age and GDM. Age has been found a risk factor in women of 25 years and above especially among Asian women. Furthermore, the findings of (Soheilykhah et al. 2017) indicated that women who developed GDM are older and have higher BMI compared to those who did not develop GDM. BMI is an important risk factor for GDM. A BMI of <25 kg/m2 was considered lean, 25-30 was considered overweight, and >30 kg/m2 was considered obese (Petry et al. 2022). Thus, obesity may play an important role in the development of GDM (Retnakaran et al. 2010). In a metabolomics study, some molecules are associated with inflammation. These molecules stimulate the xanthurenic acid synthesis. At the same time, high blood sugar promotes the production of uric acid and superoxide radicals which contribute to the development of GDM (Law and Hua 2017). In this study, the BMI of the GDM group is 25.26 kg/m2 which is considered overweight based on Petry's classification (Petry et al. 2017).

Table 5: Relationship between the Demographic Profile and the HbA1c and OGTT of Women with or without GDM

Profile Variable	R	Sig. (2-tailed)
Age	0.263**	0.004
BMI	0.228*	0.019
GDM history	0.140	0.132
HbA1c	0.237*	0.014
OGTT		
FBS	0.576**	0.000
Glucose 1-hour	0.604**	0.000
Glucose 2-hour	0617**	0.000

**Significant at p<.01
*Significant at p<.05

Ferritin, Insulin and CRP in the First, Second, and Third Trimesters of Pregnancy of Women with or without GDM

The ferritin concentration is higher in women with GDM in the first, second, and third trimesters (Mean=30.47, 20.76,13.68, respectively) than those without GDM (mean=27.41, 13.32, 13.02, respectively). There is a decreasing level of ferritin in the first, second, and third trimesters of gestation with a higher level in the first trimester in both groups as seen in **Table 6**. These results agree with the findings of (Soheilykhah et al. 2017) that ferritin level is increased in GDM although their sampling was done only once in the second trimester of pregnancy while in this study the samples were taken in the first, second, and third trimesters. It is also in agreement with the findings of (Sun et al. 2019) which suggest that increased ferritin levels are associated with GDM. It is also consistent with (Naureen et al. 2020) findings19 that ferritin levels are higher in pregnant women with GDM compared to women without GDM and thus, it can be a risk factor for GDM.

Ferritin is said to be indicative of iron stored in the body (Sun et al. 2019). It may also indicate inflammation aside from being a storage form of iron (Hernshenfeld et al. 2019). However, conflicting studies show whether ferritin is an independent risk factor for GDM or a reflection of iron stores or inflammation in the body. This raises a concern about pregnant women's iron supplementation, which may lead to insulin resistance and inflammation. Thus, ferritin alone may not be a useful risk factor for GDM.

Insulin level is lower in the GDM group in the first and third trimester of gestation (mean 5.92, 7.36, 4.00, respectively) in **Table 6** but higher in the second trimester at 7.36. The non-GDM group has a higher insulin level than the GDM group in the first and third trimesters (mean=7.07, 7.15, 6.84, respectively) but lower in the second trimester at 7.15. This is also not in line with the observation of (Buchanan et al. 1990) who demonstrated an increase in insulin secretion by preptin whose effects on GDM are unknown. This does not also agree with (Gavish et al. 2020) who observed that insulin is elevated in women with GDM in their study determining the first trimester biomarkers in GDM. Insulin secretion is stimulated in the presence of insulin resistance (Powe 2017) to compensate for decreased sensitivity. Thus, increased insulin may precede the occurrence of Type 2 diabetes. However, increased fasting insulin in the first trimester may only be observed in a particular group of cases (Powe 2017). Combining the effects of biomarkers can enhance their utility in determining risk for GDM (Nanda et al. 2011, Ravnsborg et al. 2016, Powe 2017).

Table 6: Ferritin, Insulin, and CRP in the First, Second, and Third Trimester of Pregnancy of Women with or without GDM

Parameter	GDM		Non-GDM	
	Mean	SD	Mean	SD
Ferritin (ng/mL)	30.47	18.42	27.41	20.18
1st Trimester	20.76	14.99	13.32	10.05
2nd Trimester	13.68	10.88	13.02	9.63
3rd Trimester				
Insulin (µIU/mL)	5.92	4.26	7.07	4.91
1st Trimester	7.36	4.93	7.15	5.47
2nd Trimester	4.00	3.21	6.84	5.37
3rd Trimester				
CRP (mg/L)				
1st Trimester	5.25	0.716	5.00	.0000
2nd Trimester	6.11	1.672	5.82	1.240
3rd Trimester	5.00	0.000	5.00	.0000

CRP- C-Reactive Protein

C-reactive protein (CRP) in the GDM group is higher than the non-GDM group in the first, second, and third trimesters of pregnancy (mean=5.25, 6.11, 5.0, respectively). The CRP of the non-GDM group is lower in the first, second, and third trimesters (mean 5.0, 5.82, and 5.0, respectively). This is in concordance with the study of (Retnakaran 2010) that C-reactive protein (CRP) is elevated in GDM during pregnancy. C-reactive protein (CRP) is increased in GDM but is found to be affected by BMI. It may also reflect inflammation that may be brought about in pregnancy. Thus, CRP's usefulness as a GDM predictor is unlikely. Combining the effects of biomarkers can enhance their

utility in determining risk for GDM (Nanda et al. 2011, Ravnsborg et al. 2016, Powe 2017). C-reactive protein is said to be associated with inflammatory conditions. It is used to determine the presence and extent of inflammation (Dasanayake et al. 2008). In this study, the increased CRP level in the GDM group indicates the presence of inflammation that may be due to iron oxidative stress on the B-cells of the pancreas.

Both groups show declining levels of ferritin. Ferritin is said to be associated with iron stored in the body or inflammation and there are conflicting reports about its role in the development of GDM. The decline in ferritin in both groups may be attributed to an increasing demand for iron as pregnancy progresses (Rawal et al. 2017). Insulin is higher in the non-GDM group in the first and third trimesters compared to the GDM group. This may be due to decreased secretion of insulin caused by β-cell dysfunction of the pancreas in the GDM group. CRP is higher in the GDM group compared to the non-GDM group. CRP is associated with inflammation attributed to hormonal and metabolic changes brought about by pregnancy.

Significant Differences in Means of Ferritin, Insulin, and CRP

Based on **Table 7**, there are significant differences in the means of ferritin (p<.01) in the three trimesters among women with GDM. The differences in means of insulin in the three trimesters are not significant. In the non-GDM group, there are significant differences in means of ferritin and CRP (p<.01) and insulin (<p.05). In both groups, there is a decreasing concentration of ferritin in the first, second, and third trimesters. Although there are no significant differences in means of insulin, the second-trimester level of insulin in the GDM group is higher than the non-GDM group, which suggests increased insulin secretion and insulin resistance and decreased insulin sensitivity. Insulin secretion is stimulated in the presence of insulin resistance (Powe 2017) to compensate for decreased sensitivity.

As regards the C-reactive protein, the GDM group has a higher level in the first and second trimesters than the non-GDM group, and the difference is significant (p<0.01). (Bossick et al. 2016) reported that among African-American women, there was an association of C-reactive protein with GDM risk. In a review of 31 studies by (Azam et al. 2020), C-reactive protein is significantly associated with GDM and concluded that CRP or hs-CRP can be used as a diagnostic tool for GDM. The increase in CRP suggests the presence of inflammation that characterizes the development of GDM (Nanda 2011, Ravnsborg 2016, Powe 2017).

Table 7: Significant Differences of Means of Ferritin, Insulin, and CRP between GDM and Non-GDM on the Three Trimesters using Analysis of Variance (ANOVA)

Parameters	GDM	Non-GDM
Ferritin		
df	2	2
Mean Square	12.639	2052.3
F-test	20.348**	9.345
Sig. (2-tailed)	0.000	
Insulin		
df	2	2
Mean Square	1.674	72.08
F-test	0.060	4.009*
Sig. (2-tailed)	0.942	0.023
CRP		
df	2	2
Mean Square	12.639	8.54
F-test	20.348**	8.944**
Sig. (2-tailed)	0.000	0.002

**Significant at p<.01
*Significant at p<.05

Pairwise Comparison of Means for the 3 trimesters

To determine in which trimester differences in ferritin, insulin, and CRP are significant, the pair-wise comparison of means for the 3 trimesters using Post-hoc Scheffe was made for the GDM and the non-GDM groups in **Table 8**. When the means were paired by trimester, the first-trimester difference in means of ferritin of the GDM group was significant while the differences in means of insulin and CRP were significant in the second trimester. In the non-GDM group, CRP is significant in the second trimester, while ferritin is significant in the first trimester. Insulin, in the non-GDM group in the three trimesters, is not significantly different.

Table 8: Pairwise Comparison of Means for the 3 Trimesters Using Post-hoc Scheffe

Serum	Pair (Means) Trimester	Sig. (2-tailed)	Comparison	Interpretation
GDM				
Ferritin	1,2 (30.47, 20.76)	.052	1=2	
	1,3 (30.47, 13.68)	.000**	1>3	Significant in 1st trimester
	2,3 (20.76, 13.68)	.166	2=3	
Insulin	1,2 (5.92, 7.36)	.508	1=2	
	1,3 (5.92, 4.00)	.322	1=3	Significant in 2nd trimester
	2,3 (7.36, 4.00)	.023*	2>3	
CRP	1,2 (5.25, 6.11)	.037*	2>1	Significant in 2nd trimester
	1,3 (5.75, 5.00)	.763	1=3	
	2,3 (6.11, 5.00)	.003**	2>3	
NGDM				
Ferritin	1,2 (27.41, 13.32)	.000**	1>2	Significant in 1st trimester
	1,3 (27.41, 13.02)	.000**	1>3	
	2,3 (13.32,13.02)	.992	2=3	
Insulin	1,2 (7.07, 7.15)	.996	1=2	Not significant
	1,3 (7.07, 6.84)	.994	1=3	
	2,3 (7.15, 6.84)	.945	2=3	
CRP	1,2 (5.00, 5.82)	.000	2>1	Significant in 2nd trimester
	1,3 (5.00, 5.00)	1.00	1=3	
	2,3 (5.82, 5.00	.000	2>3	

**Significant at p<.01
*Significant at p<.05

Association of Ferritin, Insulin, and CRP alone or in Combination in the First, Second, and Third Trimesters of Pregnancy in Women with and without GDM

Tables 9 and **10** show the association of CRP, ferritin, and insulin with GDM when used singly or in combination. CRP in the first trimester is significant. CRP is associated with inflammatory conditions. GDM is characterized by inflammation, but it can also be affected by BMI. The area under the Receiver Operating Curve (ROC) in the first trimester is 0.575 indicating overlapping values between the GDM and the non-GDM groups. The same observation is seen in CRP (AUROC=.522 and 0.5) in the second and third trimesters respectively, when used alone.

Table 9: Association of Ferritin, Insulin, and CRP Singly in Pregnant Women with and without GDM for the Three Trimesters

Blood Analytes Singly	Trimester	R	Sig. (2-tailed)	Interpretation	R sq (effect)
Ferritin	1	0.075	0.530	Not sig.	
	2	0.286**	0.006	Sig. at .01 level	0.081 (8.1%)
	3	0.031	0.759	Not sig.	
Insulin	1	-0.111	0.356	Not sig.	
	2	0.018	0.865	Not sig.	
	3	-0.249*	0.017	Sig. at .05 level	0.062 (6.2%)
CRP	1	0.281*	0.030	Sig at .05 level	0.078 (7.8%)
	2	0.096	0.370	Not sig.	
	3				

**Significant at p<.01
*Significant at p<.05

Thus, CRP may not be enough to detect risk for GDM. The effect of the second-trimester level of ferritin is significantly related to GDM. This supports the findings of (Sun et al. 2019) that higher ferritin levels in the first and second trimesters can be used as early biomarkers for GDM. However, the area under the ROC is 0.654 which also shows some overlapping values that fairly distinguish between the GDM and the non-GDM groups. Hence, ferritin alone may not be sufficient to detect the occurrence of GDM early. Ferritin reflects iron stored in the body or an indication of inflammation. However, the exact role of ferritin in the development of GDM has not been clearly explained. On the other hand, the findings of (Sun et al. 2019) suggest that increased ferritin levels are associated with GDM. In this study, insulin, when used alone, is not significantly related to GDM in the first and second trimesters but is significant in the third trimester.

Table 10: Association of Ferritin, Insulin, and CRP in Combination in Pregnant Women with GDM for the Three Trimesters

Combination of 2 blood analytes on GDM	Rxy.z	R sq. (Effect)	R tabular value (N-2=98) Rtv=267	Sig. (2-tailed)
1st Trimester				
Ferritin and CRP on GDM	0.280	0.078 (7.8%)	.280>	Significant
Insulin and CRP on GDM	0.262	0.068 (6.8%)	.262<	Not significant
Ferritin and Insulin	0.126	0.015 (1.5%)	.126<	Not significant
2nd Trimester				
Ferritin and CRP on GDM	0.317	0.1005 (10.05%)	.317>	Significant
Insulin and CRP on GDM	0.096	0.0092 (0.92%)	.096<	Not significant
Ferritin and Insulin on GDM	0.285	0.0812 (8.12%)	.285>	Significant

The combined effect of ferritin and CRP in the development of GDM in the first trimester is significant. This suggests that the results of ferritin and CRP can help determine the risk for GDM.

Ferritin, an iron-containing protein, is increased in GDM, which is characterized by inflammation. This can be attributed to the iron's capacity to induce oxidative stress on the pancreatic cells, which leads to inflammation with increased insulin resistance and decreased insulin sensitivity (Sun et al. 2019). CRP, according to studies, is an indicator of inflammation and is associated with GDM. Thus, studies considered that the underlying mechanism in the development of GDM is the ferritin inflammatory effect on the pancreatic cells. High levels of ferritin, an acute-phase reactant, can cause inflammation that characterizes GDM. Therefore, inflammation may be related to the development of diabetes mellitus.

In the study of (O'Malley et al. 2020) using fasting plasma collected in the second trimester, increased levels of insulin, C-peptide, and leptin were found to be associated with GDM. However, obesity and a large overlap of values make a diagnosis of GDM difficult. They concluded that insulin, C-peptide, and leptin cannot be used alone or in combination as early biomarkers for GDM.

In this study, the combination of CRP and insulin and the combination of ferritin and insulin in the first trimester are not significantly associated with GDM. This may indirectly imply that the combinations of CRP and insulin, and ferritin and insulin, when used, will not be a useful indicator of GDM risk. In the second trimester, the combination of ferritin and CRP and the combination of ferritin and insulin are significantly associated with GDM. This indicates that in the second trimester, the combination of ferritin and CRP and the combination of ferritin and insulin may be useful in detecting GDM risk. However, the combination of insulin and CRP is not significantly associated with the early occurrence of GDM.

Hence, the combination of ferritin and CRP in the first trimester may be potentially useful to detect GDM risk. In the second trimester, the combinations of CRP and ferritin, and ferritin and insulin, may likely detect the occurrence of GDM. These findings support the conclusion of Ravnsborg et al. (2016) that the combined effect of biomarkers will likely enhance the tests' potential power to early detect women at risk for GDM (Powe 2017).

CONCLUSION

When used alone, ferritin, insulin, and CRP are not enough to predict the risk of GDM. A, combination of ferritin and CRP can be used to detect the risk of developing GDM early among Filipino pregnant women. A combination of ferritin and CRP, as well as ferritin and insulin, can serve as potential biochemical indicators for the occurrence of GDM in the second trimester of pregnancy. These findings align with those in similar studies on pregnant women of diverse ethnicities and races.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

This study is an extension of the project on Gestational Diabetes Mellitus initiated and headed by Dr. Maria Ruth Pineda-Cortel. Dr. Nini F. Lim conceptualized and wrote the manuscript and supervised the data analysis. Both authors supervised the implementation of the research and edited the manuscript.

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