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ORIGINAL ARTICLE

Correlation of Vitamin B12 Deficiency with Blood Glucose Level in Pregnant Females of Tertiary Care Hospital, KP

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ABSTRACT

Background: A developing fetus can be adversely affected by gestational diabetes mellitus (GDM). This condition is prevalent among South Asian women. A contributing factor to GDM is insulin resistance (IR). Only a few B12 and folate markers have been studied in association with GDM and IR, as studies have shown vitamin B12 (B12) and folate status to be associated with GDM.

Objective: The current study aimed to determine the association between Vitamin B12 and folate markers and insulin resistance in pregnant women with GDM and NGT.

Methods: Pakistani women (29 GDM and 41 NGT) with a mean age of 29 y, BMI, and gestational age of 33 weeks were included in the research. The serum total vitamin B12, and other parameters like folate, methylmalonic acid, plasma homocysteine and 5-methyl tetrachloro folic acid (RCF). The T-tests and chi-squared test and spearman's correlation test were used to determine whether there was any correlation between vitamin B12 and blood glucose levels in pregnant women with and without gestational diabetes. A simple multiple regression analysis was used to see whether B12 and folate status indicators predicted Insulin Resistance (IR).

Results: There was no statistically significant difference between NGT and GDM, demonstrating that both groups have the same features. Although a significant difference was found between BMI (p=0.037), Serum Fasting Glucose (p=0.001), HOMA_IR (p=0.001), Serum HDL (p=0.001), Serum TC (p=0.040), and Systolic BP (p=0.001). Women in both groups took iron and folic acid supplements in roughly equal numbers. Still, the GDM group had an excess of women taking multivitamin supplements (P = 0.039) and women taking iron supplements (P=0.001). In Spearman's analysis HOMA-IR correlated negatively with total serum B12 (P < 0.00). The regression model is statistically significant with (F=1.927, P=0.046) p-value is less than the alpha value. Coefficient of determination R^2=0,309, which indicated that 30.9% variation came in the response variable due to the explanatory variable and the rest due to other unknown factors.

Practical implication

Conclusions: it is concluded that there is a significant correlation between vitamin B12, folate and blood glucose level in Pakistani pregnant women in the third trimester. Moreover, vitamin B12 and folic acid have an impact on fetal health, i.e., weight, length and circumference of the head and chest.

Keywords: gestational diabetes Mellitus, HOMA-IR, normal glucose tolerance, Peshawar

INTRODUCTION

It is crucial for fetal development that the maternal environment is healthy. The most common pregnancy disorder is GDM, also known as glucose intolerance. The etiology of GDM is thought to involve insulin resistance (IR). An environment of physiological adaptation that is necessary to provide glucose to a growing fetus naturally predisposes pregnant women to IR. IR is reduced during early pregnancy, which promotes the accumulation of adipose tissue. Increasing maternal IR promotes the transfer of nutrients to the fetus during pregnancy [(1)]. The pancreatic beta cells produce more insulin to compensate for IR. The amount of insulin secreted during pregnancy tends to rise between 200% and 250% to maintain the mother's euglycemia during late pregnancy in normal pregnancies [(2)]. GDM is caused by faulty insulin action and secretion, which predisposes a woman to type 2 diabetes because she already has problems with insulin action and secretion [(3)]. A family history of the condition, a body mass index of more than 30, prior GDM, and a macrosomic newborn weighing more than 4.5 kg are all risk factors for gestational diabetes mellitus. Women of Pakistani heritage are more likely to develop gestational diabetes mellitus due to a combination of genetic, intrauterine, socioeconomic, and behavioural risk factors. GDM has a prevalence of 25% in some Asian countries, compared to 10% in North America [(4)]. Adverse consequences on the fetus from GDM include macrosomia, hypoglycemia, and obesity (because of prenatal programming). When it comes to muscle mass, Asians have less of it and a larger amount of body fat, which means they have a higher IR than other groups. Intrauterine malnutrition during pregnancy is one possible cause of this trait [(5)]. More obesity and insulin resistance were linked to a mother's higher levels of folate and her child's lower levels of vitamin B12. The youngsters with the highest amounts of folate but the lowest levels of B12 were the most insulin-resistant [(6)]. Pregnant women using folic acid supplements may also be at risk for developing gestational diabetes and insulin resistance, according to another research. There are currently no known mechanisms underlying this association, but it may depend on the methyl-trap effect, which leads to DNA hypomethylation and endothelial dysfunction [(4)]. It also impairs beta-oxidation when B12 is deficient because fatty acids cannot be transferred into mitochondria. Increasing adipogenesis and lipogenesis would increase the risk of IR [(7)]. Nonpregnancy-derived cutoffs for assessing B12 and folate levels in investigations of insulin resistance and gestational diabetes mellitus have been employed up to now. Because of its low sensitivity, serum total B12 has been questioned as a diagnostic for B12 insufficiency [(8)]. Due to less haptocorrin being made and more blood being diluted during pregnancy, this measure (the most common B12-binding protein) is hard to understand. A maternal methylmalonic acid deficiency, as measured by serum MMA and plasma homocysteine is also associated with B12 deficiency [(9)].

HoloTC, the physiologically active portion of B12, is less influenced by low B12 levels [(10)]. Results from this research found that B12 and folate status were not linked with IR among Bangladeshi-British women with normal glucose tolerance and gestational diabetes mellitus. We employed RCF and plasma 5-MTHF, and serum folate as indicators of vitamin B12 status (5-MTHF). Serum and red cell folate, (MTHF) were utilised to measure folic acid levels. In addition, we wanted to see how pregnant women in the Pakistan fared in terms of B12 and folate levels using standard non-pregnancy cutoffs. From research on maternal gestational diabetes and fetal epigenetic alterations, this study was proceeded.

METHODS

After the informed consent, the pregnant women in their third trimester of pregnancy were selected from different tertiary care hospital at Peshawar, Pakistan. Moreover, these women may or may not have the GDM. For the purpose to screen out the GDM, in these pregnant women entered in their 28 week of gestation, oral glucose tolerance test was used. Before and after 2 hours of glucose ingestion blood samples were collected. Screening for GDM was based on conventional cutoffs of GDM screening (0-min serum glucose was more than 5.8 mmol/L and/or 120-min was greater than 7.8). Participants who had a history of diabetes or were using insulin were ruled out of the trial. The participants taking folic acid, iron or multivitamin supplements were categorize into different groups. In order to find out the vitamin B12, status blood samples were taken to determine Serum Total Cholesterol, Triglyceride, Low Density Lipid and High-Density Lipoprotein. Furthermore, systolic and diastolic blood pressure were measured.

From each woman exposed to eight hours of fast and to standard dietary management, fasting blood samples of 30 ml was collected (22). After two hours when the blood gets cooled, a standard procedure was used to separate serum from the whole blood. Plasma/Serum were analyzed after being stored in -80 degree centigrade. For determination of fasting insulin and serum glucose, sandwich electrochemical immunoassay (Roche) and a hexokinase assay were used respectively. For TG, LDL- and HDL-cholesterol, and Serum Total Cholesterol, measurement Cobas 6000 (Roche) was used. Blood folate and B12 levels were determined using Roche electrochemiluminescent competitive immunoassays, whereas RCF was assessed using the Beckman Coulter competitive binding paramagnetic particle method. HOMA-IR is calculated using fasting glucose and insulin concentrations, which are obtained from a mathematical analysis of the balance between hepatic glucose production and insulin secretion (27, 28). The HOMA-IR value increases in an IR situation.

To determine the normal distribution of the data, the Shapiro-wilk test was used. To detect women on very high levels of B12 or folate supplements, we examined serum total B12, HoloTC, and folate, as well as plasma 5-MTHF and RCF results for outliers using the outlying labeling rule, g = 2.2 (29). In this study, one serum B12 result, four HoloTC, one RCF, and one 5-MTHF result were excluded. Logarithmic transformations were applied to results that were not normally distributed. To determine the difference between NGT and GDM women, descriptive statistics and independent sample t-tests and chi square tests were utilised. spearman's range test was performed to examine the relationship between HOMA-IR and vitamin B12 and Folate indicators. In order to find out the independent predictor of HOMA- insulin resistance, multiple regression analyses were performed based on the following: age, gestational diabetes and age, serum HoloTC, Body mass index, serum MMA, Blood pressure, Serum B12, plasma Hcy, plasma 5-MTHF, RCF, and serum folate. Multicollinearity exists among B12 and folate markers, therefore we constructed separate models with serum folate, 5-MTHF, serum B12, RCF, HoloTC and Hcy. SPSS 26 was used to conduct statistical analyses.

RESULTS

Table 1: Characteristics Features of the participants and Comparison of Women with GDM[1] and NGT[1]

Variables	NGT (41 Individuals)	GDM (29 Individuals)	P-value
Age	27.51 ± 2.4	29.03 ± 3.15	0.089
Height	1.52 ± 0.09	1.51 ± 0.1	0.44
Weight	58.7 ± 4.7	65.27 ± 5.41	0.377
BMI	25.38 ± 2.96	28.93 ± 5.2	0.037
Serum OGTT[2] after 0 hours	4.44 ± 0.099	4.63 ± 0.22	0.001
Serum OGTT after 2 hours	5.29 ± 0.28	8.9 ±1.01	0.001
Serum Fasting Glucose	4.02 ± 0.10	4.3 ± 0.4	0.001
Serum Fasting Insulin	11.2 ± 0.52	11.2 ± 0.45	0.644
HOMA-IR	1.95 ± 1.51	2.09 ± .22	0.001
Serum LDL-C3	3.4 ± 0.06	3.7 ± 0.07	0.091
Serum TC[4]	2.22 ± 0.28	2.06 ± 0.123	0.040
Serum TG[4]	5.9 ± 0.06	5.93 ± 0.07	0.421
Serum HDL3	1.4 ± 0.03	1.5 ± 0.09	0.001
Diastolic BP	70.48 ± 1.9	71.48 ± 2.3	0.757
Systolic BP	110 ± 0.83	106.9 ± 2.7	0.001
Gestational Age at Sampling	30.7 ± 1.6	31.6 ± 1.5	0.679

1 GDM; Gestational Diabetes Mellitus, NGT; Normal Glucose Tolerance, 2 OGTT; Oral Glucose Tolerance Test, 3 LDL; Low-Density Lipid, HDL;High-Density Lipid, 4 TC; Total Cholesterol, TG; Triglyceride,

Both the NGT and GDM have the same characteristics features and there is no statistically significant difference between both groups were detected (Table. 1). Although a significant difference was found between BMI (p=0.037), Serum Fasting Glucose (p=0.001), HOMA_IR (p=0.001), Serum HDL (p=0.001), Serum TC (p=0.040), and Systolic BP (p=0.001) (Table. 01).

Table 2: Participants using vitamins and association of markers concentration

Variables	NGT	GDM	P-value
Multivitamin users	31	26	0.002
Folic Acid	33	25	0.210
Iron Supplements	30	26	0.001
Serum Total B12	190±1.5	169.3±2.3	0.111
Serum HoloTC[1]	50.0±1.4	50.3±1.5	0.935
Serum Folate	16.7±0.22	16.3±0.46	0.001
Serum- 5-MTHF[1]	15.7±0.2	15.1±0.46	0.004
RCF[2]	678.9±5.2	713.6±20	0.001
Serum Hcy[2]	4.7±0.37	5.6±0.40	0.452
Serum MMA[3]	164.5±2.0	240.4±10.6	0.001
cB12[3]	0.135±0.008	0.1502±0.12	0.008

1 Holo TC; Holo Transcobalamin; 5-MTHF; 5-Methytetrahydrofolate; 2 RCF; Red Cell Folate; Hcy; Plasma Homocysteine, 3 MMA; Methylmalonic Acid, cB12; Combined indicator of Vitamin B12 status

NGT and GDM women had the same levels of B12 and folate, as seen in Table 2. Contrary to expectations, the GDM group had significantly more women using multivitamins (P = 0.039) and iron supplements (P = 0.001) than the control group.

Table 3: Vitamin B12 and folate markers with HOMA-IR, serum fasting insulin, and serum glucose were correlated using Spearman's correlation and coefficient.

Variables	HOMA-IR		Serum Fasting Insulin		Serum Glucose	
	Coefficient ρ^2	P-Value	Coefficient ρ^2	P-Value	Coefficient ρ^2	P-Value
Age	.304	.010	-0.035	0.774	0.376	0.001
Height	0.048	0.695	0.014	0.908	-0.002	0.986
Weight	0.265	0.027	0.089	0.463	0.102	0.197
BMI	0.150	0.190	0.043	0.725	0.167	0.168
Serum OGTT After 0 hours	-0.121	0.139	0.020	0.870	-0.131	0.279
Serum OGTT After 2 hours	0.293	0.014	-0.004	0.977	0.315	0.008
Gestational Age at Sampling	0.407	0.001	0.235	0.050	0.365	0.002
Serum Fasting Glucose	0.731	0.001	0.203	0.092	-	-
Serum Fasting Insulin	0.698	0.001	-	-	0.203	0.092
HOMA-IR	-	-	0.698	0.001	0.731	0.001
Serum LDL-C	0.275	0.021	0.014	0.906	0.389	0.001
Serum TG	0.079	0.517	-0.199	0.098	0.219	0.068
Serum TC	-0.136	0.263	-0.127	0.293	-0.041	0.735

Serum HDL-C	0.116	0.339	-0.028	0.819	0.181	0.134
Systolic BP	-0.230	0.055	0.089	0.465	-0.317	0.007
Diastolic BP	0.282	0.018	0.105	0.387	0.281	0.019
Serum Total B12	-0.321	0.007	-0.063	0.602	-0.339	0.004
Serum Holo TC	0.161	0.181	0.170	0.160	0.214	0.076
Serum Folate	-0.157	0.194	0.211	0.080	-0.229	0.056
Serum 5-MTHF	0.076	0.533	0.342	0.004	-0.022	0.855
Plasma Hcy	0.328	0.006	-0.039	0.749	0.370	0.002
RCF	0.104	0.393	-0.200	0.097	0.260	0.030
Serum MMA	0.334	0.005	0.102	0.403	0.358	0.002
cB12	0.313	0.009	0.153	0.210	0.258	0.032

In the above table, the spearmen correlation coefficient was applied to check the association. The correlation's coefficient for HOMA-IR with age is ρ =.304, which indicates the positive linear relationship between age and HOMA-IR with p=.010 which is greater than the alpha value which indicated that is statistically insignificant. HOMA-IR correlated with height (ρ =0.048, p=0.695), weak negative associations were found HOMA-IR with Serum OGTT After 0 hours (ρ =-0.121, p=0.139) which is statistically insignificant. Strong positive associations were found HOMA-IR with Serum Fasting Glucose (ρ= 0.731, p=0.001) which is statistically significant. Strong positive associations were also found HOMA- IR with Serum Fasting Insulin (ρ= 0.698, p=0.001) which is statistically significant. Association was found HOMA-IR with cB12, Serum MMA, Plasma Hcy, Serum Total B12 and Serum LDL-C, their p-value is less alpha value all are statistically significant, which indicated that there is the association between them (table 3).

The correlation's coefficient for Serum Fasting Insulin with age (ρ =-0.035, p=0.774), the p-value is greater than the alpha value, which is statistically insignificant, weak positive association were found Serum Fasting Insulin with height, weight, BMI, Serum OGTT After 0 hours, Gestational Age at Sampling, Serum Fasting Glucose and Serum LDL-C but their p-value is greater than alpha value all are statistically insignificant. Strong positive associations were found between Serum Fasting Insulin and HOMA-IR with (ρ =0.698, p=0.001) p-value is less than the alpha value which is statistically significant. Weak Negative associations were found in Serum Fasting Insulin with Serum OGTT After 2 hours, Serum TG, Serum TC, Serum HDL-C, Plasma Hcy and RCF but their p-value is greater than the alpha value all is statistically insignificant (Table 3).

Table 4: Multiple Regression of HOMA-IR With Different Vitamin B12 and Folate Markers

HOMA-IR	β	95% CI for β		P-Value	Anova	R^2
Age	.344	.006	.041	.010	P=0.046 Or F=1.927	0.309
Height	-1.299	-1.385	.374	.254		
Weight	-.111	-3.030	2.580	.873		
BMI	.230	-.025	.040	.652		
Gestational Age at Sampling	-.238	-.082	.061	.769		
Systolic BP	.036	-.031	.039	.807		
Diastolic BP	.177	-.024	.049	.499		
Serum Total B12	.156	-.012	.040	.281		
Serum Holo TC	-.566	-.037	.016	.428		
Serum Folate	.144	-.015	.053	.272		
RCF	-.188	-.246	.062	.238		
Serum MMA	-.162	-.006	.003	.503		

The correlation coefficient for Serum Glucose with age is ρ =0.376, which indicates the positive linear relationship between age and HOMA-IR with p=.001 which is less than the alpha value which indicated that is statistically significant. Strong positive associations were found with Serum In the HOMA-IR model, glucose has a p-value smaller than the alpha value of 0.73, indicating statistical significance (p=0.001). Serum Glucose and Serum Fasting Insulin were shown to have weak negative correlations, however their p-values are bigger than their alpha values, all of which are statistically insignificant (Table 3).

The above table shows the analysis of multiple regression. HOMA-IR is the response variable and age, weight, height and BMI etc are explanatory variables all regression coefficients are individually statistically insignificant because the p-value is greater than the alpha value. But the overall model is statistically significant with (F=1.927, P=0.046) p-value is less than the alpha value. Coefficient of determination R^2=0,309 which indicated that 30.9% variation came in the response variable is due to explanatory variable the rest due to others factor which is unknown (Table 4).

Table 5: Spearman correlation between Vitamin B12 and Folate Markers

Variables	Markers	ρ	P-Value
Serum B12	Serum Holo-TC	.012	0.922
	Plasma Hcy	-.700	.001
	RCF	-.523	.001
	Serum MMA	-.730	.001
Serum Holo-TC	Plasma Hcy	-.013	.916
	RCF	.073	.548
	Serum MMA	.059	.626
Plasma Hcy	RCF	.676	.001
	Serum MMA	.753	.000
RCF	Serum MMA	.607	.000

In the above table, spearmen correlation coefficients were applied to check the association. weak positive associations were found between Serum B12 and Serum Holo-TC (ρ =.012, p=0.922), the p-value is greater than the alpha value which is statistically insignificant. strong negative associations were found Serum B12 with Plasma Hcy, RCF and Serum MMA but their p-value is less than the alpha value which is statistically significant. A weak positive association was found Serum Holo-TC with RCF and Serum MMA the alpha value is greater than the p-value which indicated that is statistically insignificant. A strong positive association was found between Plasma Hcy with RCF and Serum MMA with a p-value less than the alpha value which is statistically significant. A strong positive association was found between RCF and serum MMA ρ =.012 and the value of p is less than the alpha value which is statistically significant (Table 5).

DISCUSSION

During the third trimester of Pakistani women's conceptions, vitamin B12, HoloTC, and red cell folate had a strong connection (by using the homeostatic model HOMA-IR). Both the bivariate and multivariate analyses of this study's results (negative connections between blood total B12 and HoloTC) were in agreement (negative associations for serum total B12, positive for RCF). According to previous research, a higher HOMA-IR score is connected to lower blood total B12 levels in the third trimester of pregnancy [11, 12]. As opposed to earlier research, this study evaluated biochemical markers of B12 and folate status rather than total blood B12 and folate levels in assessing GDM risk. In previous studies, serum total B12 only was quantified, and this particular test, which involves B12 binding proteins, has been shown to be greatly affected by pregnancy [13, 14] as well as declining B12 stores due to fetal consumption [9].

During pregnancy, total B12 is less useful as a diagnostic test. Our correlation and regression analyses revealed a negative relationship between HOMA-IR and serum total B12 and HoloTC. These findings support the hypothesis that B12 may increase the incidence of IR/GDM. Aside from these findings, it's worth noting that the predominance of B12 deficiency significantly different when serum total B12 and HoloTC indicators were considered separately (21 percent compared to 3 percent), which indicates that the commonly used cutoffs for pregnant women do not have any useful information [15]. There was a significant increase in plasma HoloTC levels during the third trimester in Fernandez-Costa and Metz studies [16] whereas Murphy et al. [15] found a plateau in plasma HoloTC levels after the initial decrease in early pregnancy. There seems to be a compensatory mechanism during pregnancy for increasing the mother's B12 supply to meet the fetus's increased requirements [16]. In this way, pregnancy related B12 deficiency may be underestimated when using total serum B12. During the 3rd trimester, we found that holoTC levels were correlated with homocysteine levels, indicating that it may be a superior diagnostic tool than blood total B12 for assessing the B12 status. It is critical to note that the HOMA-IR test did not correspond with MMA, which is used to determine B12 status.

31 percent of the individuals had high MMA levels, although there were no relationships with blood total B12, HoloTC or Hcy levels in this investigation. A cofactor of B12 converts succinyl-CoA, a key intermediary in the citric acid cycle, into succinyl-CoA in only 3 (17 percent) of the women with MMA >280 nmol/L. CPT-1, a key enzyme in the process of reducing body fat and developing insulin resistance, is blocked by MMA-CoA buildup in B12 deficiency [17, 18]. MMA-CoA may be converted into MMA with or without B12 deficiency if lipids are used for energy instead of carbs at the end of pregnancy. In contrast to HoloTC but unrelated to HOMAIR, serum total B12, or MMA, another functional sign of B12 insufficiency, Hcy, emerged. Another mechanism of GDM risk is the increase in HCY levels and the resulting impairment of endothelial function [19]. A number of studies have found that there is an association between Hcy concentrations in women with GDM [19-21], but others have failed to observe the same [22]. During pregnancy, Hcy levels are usually quite low, which may explain this phenomenon. Hcy concentrations in pregnancy are usually low due to hemodilution, hormonal changes, and folic acid supplementation. In addition, subnormal Hcy has been reported in prediabetic hyperinsulinemic non-diabetics [23].

High insulin levels and glomerular hyperfiltration seen in early diabetes have been discussed as possible causes of low Hcy [24]. Because of these changes in metabolism and hormone levels, Hcy levels may not be reliable indicators of B12 or folate status during the third trimester. To demonstrate the link between higher homocysteine and IR and GDM, more evidence is needed. B12 status can be evaluated more comprehensively with cB12 estimation since all B12 markers are considered, as well as age and folate status. The use of cB12 in pregnancy is not yet validated. Because other variables than B12 levels in pregnancy may alter serum total B12, MMA, and Hcy levels, further research on cB12 in pregnancy is needed. In the study, it was hypothesised that all B12 indicators would be correlated with cB12. Between cB12 and HoloTC patients, there were no significant changes in B12 status. HOMAIR has been associated to low B12 levels during the third trimester of pregnancy for reasons that are yet unknown. In addition to its role in lipid metabolism, B12 inhibits fatty acid oxidation, which may explain why insulin resistance occurs [6]. Hypomethylation was seen in the promoter regions of key cholesterol synthesising genes in adipocytes that did not get enough B12 to maintain normal cholesterol and homocysteine levels [25], The expression of triglyceride production genes rose whereas the expression of oxidation genes dropped. The accumulation of lipids by adipocytes under low B12 conditions has also been observed in other in vitro studies [26].

BMI and HOMA-IR seem to be linked, however more research is needed to identify whether BMI is a determining factor or whether the other aspects, such as socioeconomic status and vitamin supplementation use, are confounding variables. It is not feasible to do such an investigation in a cross-sectional research. Neither NGT nor GDM women had significantly different levels of B12. Both cB12 and other B12 markers revealed this. These findings differ from those of Krishnaveni and colleagues [11] and Sukumar and colleagues, who reported higher rates of GDM than NGT among pregnant women with South Indian or European descent. The risk of gestational diabetes is almost double for pregnant women who are low in vitamin B12, according to a new systematic review and meta-analysis [27]. Although two studies are discussed above, this meta-analysis only included two studies [11]. As a result of the small number of participants, we included women who used supplements in our study, which may have influenced the findings. NGT and GDM-associated women had the same B12 status, according to previous investigations [27]. There should be further investigation into the impact of GDM treatment on B12 and folate levels in the future. Carbohydrate limitation may lead to the replacement of foods that are higher in B12 and/or folate because of current dietary guidelines.

Despite the lack of research examining the impact of GDM diagnosis on supplementation/medication use, the discovery that four GDM patients had extraordinarily high HoloTC levels (extreme outliers) and the higher percentage of women using vitamin supplements may corroborate this theory. The HoloTC results also represent a more recent B12 consumption, and this trial was undertaken prior to the discovery of GDM's occurrence. The folate markers used in this study, in contrast to B12 markers, were highly correlated ($\rho \geq 0.8$, $P < 0.001$). As a measure of folate status, 5-MTHF was found to be more sensitive than serum folate and RCF to detecting low and high folate status. Several factors might account for this finding, including methodological differences, cutoff variations, and the small sample size. As the study size was too small to construct subgroups with low/normal folate status concentrations, it was not possible to determine if GDM women with low folate and low B12 status were more prevalent than women with normal folate status. RCF and HOMA-IR variance were shown to have a strong positive correlation. HOMA-IR variance was not substantially predicted by independent models that incorporated plasma 5-MTHF and serum folate. Because blood folate levels are altered by recent use of folic acid, RCF is a good indicator of long-term folate status [28]. Folate and 5-MTHF levels may be affected by dietary changes. Low and high folic acid levels have been linked to a variety of health problems [29], in GDM development, many routes have been discovered in which folate may have a role [19]. To determine the best and safest folate supplementation regime to use during pregnancy, more research is needed. In both multivariate and bivariate analyses, this research found a strong correlation between HOMA-IR and both BMI and newborn length [30].

CONCLUSIONS

Third-trimester IR is more common in Pakistani women who are deficient in B12 and folate. Pregnancy-related variations in B12 and folate levels need careful interpretation of indicators of status in light of these nutrients' relevance to mother and child. B12 markers should be used according to pregnancy-specific reference ranges. It may be worthwhile to replicate these findings in larger cohorts to gain a more thorough understanding of the mechanisms underlying B12 and folate status in IR and GDM.

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