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Vitamin B12 Status among Pregnant Women and Its Association with Obesity and Gestational Diabetes

Mohammed Mohammed Gebril, Tarek Ramadan Abbas,
Mohammad Saad Eldeen Radwan and Mustafa Mohammed Abd El-Kader *
Obstetrics and Gynecology Department, Faculty of Medicine, Al- Azhar University
***Corresponding Author:** Mustafa Mohammed Abd El-kader, **Phone No.:** (+2) 01018564599,
E-mail: mustafamohd123123@gmail.com

ABSTRACT

Background: pregnant women in resource-poor areas are at risk of multiple micronutrient deficiencies, and diets that are low in animal products place women at increased risk of vitamin B12 deficiency.

Aim of the Work: to investigate the vitamin B12 status of pregnant women in Egypt and its relationship with obesity and gestational diabetes mellitus (GDM).

Patients and Methods: this clinical observational prospective study has been conducted at Bab Al-Shaaria University Hospital Obstetrics and Gynecological Department from April 2018 to January 2019. To assess vitamin B12 status among pregnant woman and its association with obesity and gestational diabetes. 80 pregnant women attended to out-patient clinic were included in the study. The evaluation included data collection through: history taking, examination, anthropometric measurement, maternal blood vitamin B12 level estimation at second and third trimester and blood glucose level.

Results: vitamin B12 deficiency in pregnancy is common particularly in obese women and those with GDM.

Conclusion: vitamin B12 deficiency is silent and common in general population. Causes of vitamin B12 deficiencies are multifactorial and associated with many health problems. Also, obesity is common and its prevalence is increasing in the world. Therefore, both health problems have gained importance in family medicine practice in the last decades.

Keywords: Vitamin B12 - Pregnant Women - Obesity and Gestational Diabetes.

INTRODUCTION

Vitamin B12 also known as cobalamin, is a micronutrient essential for cellular growth, differentiation, and development [(1)]. Together with folic acid, vitamin B12 is necessary for the synthesis of DNA, RNA, lipids, and protein in the cellular cytoplasm [(2)].

More specifically, vitamin B12 and folate are necessary cofactors for the conversion of homocysteine to methionine, the latter being an important methyl donor required for the synthesis of neurotransmitters and phospholipids. Vitamin B12 is an essential water soluble vitamin that plays a vital role in the physiological dynamics of human body ranging from production of erythrocytes on one hand to optimal nervous system functioning on the other. Whenever there is depletion of vitamin B12 because of poor dietary source or increased cell turn over, the deficiency can manifest as wide range of symptoms. If not detected and treated timely, these medical symptoms can present within a short period of time. It has been suggested that vitamin B12 deficiency may be treated by parental or high dose oral cobalamin therapy [(3)].

Pregnant females are at an increased risk of B12 deficiencies as they are in a state of high cellular turnover and increased overall dietary requirements especially vitamin B12. The rapidly growing fetus consumes vitamin B12 from the mother's body, thus posing a threat of deficiency if dietary requirements are not met. In the mother classical cobalamin deficiency features may be produced including macrocytic red blood cells with or without anemia, ovalocytosis, hyper-segmented white blood cells, pancytopenia, atrophic glossitis, stomatitis, malabsorption due to villi atrophy and mucositis [(4)].

The fetus will also bear the consequences of low cobalamin levels. An uncorrected deficiency will not only lead to impaired fetal growth in utero but may also make the fetus susceptible to a multitude to chronic diseases including diabetes mellitus, fatty liver disease, cardiovascular diseases, depression and even cancer in future [(4)].

The burden of maternal obesity (defined as body mass index (BMI) greater than 30 kg/m^2) is rapidly increasing, affecting nearly 35.9% of pregnant women in UK [(3)].

High BMI is associated with adverse pregnancy outcomes including recurrent miscarriages and maternal deaths. In parallel, the incidence of gestational diabetes mellitus (GDM) has also risen affecting 5%–18% of all pregnancies depending on the diagnostic criteria applied [(1)].

Vitamin B12 and folate are essential micronutrients required for the synthesis of DNA, protein, and lipids, in a series of cellular reactions collectively known as one-carbon metabolism [(2)]. One step in this process is the conversion of homocysteine (Hcy) to a methyl donor, methionine, for which B12 and folate are necessary cofactors. Additionally, the

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mitochondrial conversion of methylmalonyl-CoA to succinyl-CoA requires B12 as a coenzyme and in its absence, accumulation of the former compound inhibits fatty acid oxidation, thereby promoting lipogenesis [5].

Therefore it can be postulated that low B12, at a cellular level, may be linked to adipocyte dysfunction and obesity-related complications by modulating lipid metabolism, cellular inflammation, and causing hypomethylation of cholesterol biosynthesis pathways [6].

A recent systematic review showed that B12 insufficiency among pregnant women across the world was common in all trimesters (20% – 30%) [7].

Low B12 during pregnancy has implications for materno-fetal health including maternal adiposity, maternal and offspring insulin resistance [8], and adverse lipid profile in neonates [6].

Low B12 can have an impact on fetal birthweight by influencing placental development, although evidence for association with low birthweight (LBW) is equivocal [7].

At the other end of the spectrum, maternal obesity and insulin resistance are well-known to be associated with higher fetal birthweight [6].

Since B12 may be inversely associated with maternal BMI [8], it is possible that B12 is an independent mediator or a confounder for high birthweight.

During pregnancy, low circulating levels of B12 or folate have been associated with complications such as neural tube defects (NTDs), spontaneous abortion, premature birth and possibly low birth weight [7].

Daily maternal vitamin B12 supplementation (50 µg/day) during pregnancy through 6 weeks postpartum substantially improved maternal vitamin B12 status and increased breast milk and infant vitamin B12 concentration [9].

AIM OF THE WORK

The objective of this study is to investigate the vitamin B12 status of pregnant women in Egypt and its relationship with obesity and gestational diabetes mellitus (GDM).

PATIENTS AND METHODS

This clinical observational prospective study has been conducted at Bab Al-Shaaria University Hospital, Obstetrics and Gynecological Department from April 2018 to January 2019. 80 pregnant women attended to out-patient clinic were included in this study. The evaluation included data collection through: history taking, examination, anthropometric measurement, maternal vitamin B12 level estimation at second and third trimester and blood glucose level.

Informed consent was taken from each woman before participation in the study. Also, An approval of the study obtained from Faculty of Medicine, Al- Azhar University academic and ethical committee.

Definitions:

A selective screening approach was used to screen high-risk women for GDM according to the National Institute for Health and Care Excellence guidelines. (i.e., BMI > 30 kg/m^2, previous GDM, previous macrosomia, first degree relative with diabetes, and ethnic minority race). The modified World Health Organization (WHO) 1999 criteria were used to diagnose GDM (fasting glucose ≥ 6.1 mmol/L or 2-h glucose ≥ 7.8 mmol/L) during the study period. The reference range for serum B12 was 150–489 pmol/L. Insufficiency of the B12 level was defined as <150 pmol/L [8].

Inclusion criteria:

- Pregnant woman with BMI > 30 kg/m^2.
- Having fasting blood glucose level > 126 mg/dl.
- 2 hours post prandial blood glucose level > 180 mg /dl.
- Gestational age between 24th and 36th week of pregnancy.

Exclusion criteria:

- Medical illnesses including other endocrine or metabolic diseases.
- Isolated postprandial hyperglycemia.
- Patients on vitamin B12 supplements.
- Major diabetic complications.
- Pre-gestational diabetes (Type 1 and 2).
- Multiple pregnancies.

Methodology:

All patients enrolled in this study were subjected to the following:

1) **History**: including obstetric history with determination of gestational age from 1st trimesteric ultrasound and asking about family history of DM and diabetic complications.
2) **Physical examination:** including general examination, maternal weight, blood pressure, assessment of maternal health, obstetric abdominal examination for: fundal level, fetal presentation, estimating fetal weight and scars of previous operations.
3) **Investigations:**

A. Ultrasound was done for gestational age confirmation through fetal biometry, detection of fetal presentation and exclusion of major congenital malformations, or

placenta previa, estimation of the amniotic fluid index (AFI) and amniotic fluid volume (AFV).

B. Blood-glucose level as random blood sugar (RBS), fasting blood glucose (FBS), 2-hour postprandial (2HPP) levels and glycosylated hemoglobin (HbA1c).

C. For the quantitative determination of vitamin B12 concentrations:-

This ELISA kit used the method of Sandwich-ELISA. The stop solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of vitamin B12 in the sample, this vitamin B12 ELISA Kit included a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of optical density (O.D.) versus vitamin B12 concentration. The concentration of vitamin B12 in the samples was then determined by comparing the O.D. of the samples to the standard curve.

Sample collection and storages

Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at approximately 3000×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles

Assay procedure

1. Prepare all reagents before starting assay procedure.
2. Add standard: Set standard wells, testing sample wells. Add standard 50 µl to standard well.
3. Add Sample: Add testing sample 10 µl then add sample diluent 40 µl to testing sample well.
4. Add 100 µl of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37°C.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with wash solution (400 µl) using a squirt bottle.
6. Add chromogen solution A 50 µl and chromogen solution B 50 µl to each well.
7. Add 50 µl stop solution to each well. The color in the wells should change from blue to yellow.
8. Read the optical density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

Diet:

All patients were maintained on their usual dietary pattern while limiting their consumption of vitamin B12 rich foods throughout the study.

As patients were on self-selected diet, each patient was instructed by a dietician to use comprehensive food list that containing food items by type, portion size, method of preparation, and vitamin B12 content. This had enabled patient to substitute foods with low vitamin content for those patients who normally consume higher levels of vitamin B12 and also to ensure that their daily intake from dietary sources remained the same.

4) The outcome measures:

1ry outcome:

- Measuring random blood sugar, fasting blood glucose, 2 hours postprandial levels and glycosylated hemoglobin (HbA1c) at Bab Al-Shaaria University Hospital laps.
- Measuring serum vitamin B12 level.

Statistical Analysis

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 18.0, IBM Corp., Chicago, USA, 2009.

Descriptive statistics were done for quantitative data as minimum and maximum of the range as well as mean±SD (standard deviation) for quantitative normally distributed data, while it was done for qualitative data as number and percentage.

Inferential analyses were done for quantitative variables using Shapiro-Wilk test for normality testing, independent t-test in cases of two independent groups with normally distributed data. In qualitative data, inferential analyses for independent variables were done using Chi square test for differences between proportions and Fisher's Exact test for variables with small expected numbers. The level of significance was taken at:

- P value > 0.05 is insignificant.
- P value < 0.05 is significant.
- P value < 0.001 is highly significant.

RESULTS

Table (1): Demographic data of the patient

Variable		Total no. = 80
Age	Mean ± SD	30.70 ± 4.33
	Range	25 – 40
Gravidity	Two	24 (30.0%)
	3 or more	56 (70.0%)
	Mean ± SD	3.49 ± 1.44
	Range	2 – 8
Parity	One	37 (46.3%)
	Two	25 (31.3%)
	3 or more	18 (22.5%)
	Mean ± SD	1.85 ± 0.98
	Range	1 – 5
Abortion	0	39 (48.8%)
	One	30 (37.5%)
	Two	11 (13.8%)
	Mean ± SD	0.65 ± 0.71
	Range	0 – 2
GA	Mean ± SD	31.18 ± 2.22
	Range	28 – 35
BMI	Mean ± SD	33.73 ± 1.90
	Range	31 – 37

Table (2): The state of blood glucose of the study group

Variable		Total no. = 80
RBS(mg/dl)	Mean ± SD	230.06 ± 12.51
FBS(mg/dl)	Mean ± SD	128.13 ± 1.33
2HPP(mg/dl)	Mean ± SD	200.03 ± 9.92
HbA1C%	Mean ± SD	6.54 ± 0.31

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Table (3): Vitamin B12 status in the study group of the patients

Vitamin B12	Total no. = 80
Mean ± SD	153.36 ± 30.29
Insufficient vitamin B12	55 (68.8%)
Sufficient vitamin B12	25 (31.2%)

As seen in table (3), patient with obesity and GDM were more liable to have insufficient vitamin B12.

Table (4): The relation between vitamin B12 and the study parameters among study group

Variable		Insufficient vitamin B12	Sufficient vitamin B12	Test value	P-value	Sig.
		No. = 55	No. = 25			
Age (Year)	Mean ± SD Range	30.15 ± 4.66 25 – 40	31.92 ± 3.24 25 – 38	-1.720•	0.089	NS
Gravidity	Two 3 or more	21 (38.2%) 34 (61.8%)	3 (12.0%) 22 (88.0%)	5.610*	0.018	S
	Mean ± SD Range	3.27 ± 1.43 2 – 8	3.96 ± 1.37 2 – 7	-2.016•	0.047	S
Parity	One Two 3 or more	30 (54.5%) 14 (25.5%) 11 (20.0%)	7 (28.0%) 11 (44.0%) 7 (28.0%)	4.999*	0.082	NS
	Mean ± SD Range	1.73 ± 0.97 1 – 5	2.12 ± 0.97 1 – 4	-1.677•	0.098	NS
Abortion	0 One Two	30 (54.5%) 19 (34.5%) 6 (10.9%)	9 (36.0%) 11 (44.0%) 5 (20.0%)	2.655*	0.265	NS
	Mean ± SD Range	0.56 ± 0.69 0 – 2	0.84 ± 0.75 0 – 2	-1.623•	0.109	NS

P-value > 0.05: Non significant; P-value < 0.05: Significant, *: Chi-square test; •: Independent t-test

Table (5): Relation between vitamin B12 and diabetic state of the patient including body mass index of the patient

Variable		Insufficient vitamin B12	Sufficient vitamin B12	Test value	P-value	Sig.
		No. = 55	No. = 25			
GA (week)	Mean ± SD Range	30.84 ± 2.23 28 – 35	31.92 ± 2.06 28 – 35	-2.064	0.042	S
BMI	Mean ± SD Range	33.71 ± 1.86 31 – 37	33.76 ± 2.03 31 – 37	-0.110	0.913	NS
RBS (mg/dl)	Mean ± SD Range	230.71 ± 12.20 205 – 250	228.64 ± 13.29 208 – 260	0.684	0.496	NS
FBS (mg/dl)	Mean ± SD Range	128.13 ± 1.35 126 – 130	128.12 ± 1.30 126 – 130	0.023	0.982	NS
2HPP (mg/dl)	Mean ± SD Range	199.27 ± 10.02 185 – 220	201.68 ± 9.71 185 – 215	-1.006	0.318	NS
HbA1C %	Mean ± SD Range	6.56 ± 0.32 6 – 7	6.48 ± 0.29 6 – 7	1.140	0.258	NS

P-value > 0.05: Non significant; P-value < 0.05: Significant, •: Independent t-test

Table (6): Relation between vitamin B12 and gravidity, parity and abortion

Variable		Vitamin B12		Test value	P-value	Sig.
		Mean ± SD	Range			
Gravidity	Two 3 or more	145.79 ± 25.8 156.61 ± 31.68	118 – 230 111 – 220	-1.474•	0.144	NS
Parity	One Two 3 or more	147.38 ± 26.41 158.44 ± 34.65 158.61 ± 30.77	118 – 230 111 – 220 120 – 214	1.356••	0.264	NS
Abortion	0 One Two	151.26 ± 30.05 155.43 ± 31.3 155.18 ± 30.72	118 – 230 111 – 220 120 – 214	0.180••	0.835	NS

P-value > 0.05: Non significant; •: Independent t-test; ••: One Way ANOVA test

Table (7): Shows relation between vitamin B12 among study parameters of the patient

Variable	Vitamin B12	
	R	P-value
Age	**0.279***	**0.012**
Gravidity	0.166	0.142
Parity	0.148	0.191
Abortion	0.105	0.355
GA	**0.375****	**0.001**
BMI	0.093	0.412
RBS	0.011	0.924
FBS	0.018	0.877
2HPP	0.039	0.730
HbA1C	-0.011	0.924

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
Spearman correlation coefficient

As seen in table (7) there was a significant correlation between age and gestational age and vitamin B12 of patient in the study group.

DISCUSSION

Vitamin B12 deficiency is silent and common in general population. Causes of vitamin B12 deficiencies are multifactorial and associated with many health problems. Also, obesity is common and its prevalence is increasing in the world. Therefore, both health problems have gained importance in family medicine practice in the last decades [(10)].

It must be noted that a fall in B12 during pregnancy may be physiological due to a decrease in the fraction bound to inactive haptocorrin but the evidence is equivocal with regards to whether there is also a fall in the active form, holotranscobalamin [(11)]**.** In the absence of specific cutoff values to define B12 deficiency in pregnancy, we used the non-pregnant reference range (<150 pmol/L). It is noteworthy that associations with adverse maternal metabolic outcomes [(8)] and elevation in Hcy during pregnancy were found by other authors at B12 thresholds similar to this.

Vitamin B12 and folic acid supplement in patients with gestational diabetes improved insulin resistance and endothelial function. Therefore, it was suggested that vitamin B12 supplements may be better for patients with metabolic syndrome who have deficient or marginal level of vitamin B12 [(12)]**.**

It was reassuring to see that folate deficiency was rare. However, the combination of low B12 and high folate has been shown to be associated with lower neonatal birthweight as well as central adiposity and insulin resistance in 6-year old offspring [(13)]**.**

It is possible that the women with such a B12-folate imbalance are particularly at high risk of having larger babies. This phenomenon (high folate/low B12), is increasingly common in populations with mandatory folic acid fortification such as in the USA and Canada, and is related to adverse clinical outcomes in the elderly population [(14)]**.** Whilst our sample size was not large enough to perform a detailed subgroup analysis, we observed that women with gestational diabetes and obesity have insufficient B12.

Analysis of the current study results demonstrated that:

Absence of any statistically significant difference between sufficient vitamin B12 and insufficient vitamin B12 as regards data including age, parity and abortion, which agrees with other study conducted by **Nithya *et al.*** [(15)]**.**

It also showed that there was a statistical difference between sufficient vitamin B12 and insufficient vitamin B12 as regards gravidity of those patients. Patients with two gravidity with sufficient vitamin B12 were (12.0%) and with insufficient vitamin B12 were (38.2%). Patients with three or more gravidity with sufficient vitamin B12 were (88.0%) and with insufficient B12 were (61.8%), which showed significant difference, which disagrees with study conducted by **Nithya *et al.*** [(15)]**.**

As regard main outcome measures in current study of 80 pregnant women who were diabetic and obese we found that (68.8%) of these patients had insufficient B12 and about (31.2%) had sufficient B12. This means that in patient with obesity and gestational diabetes vitamin B12 was low.

The first study was done in the UK was retrospective in nature, showed three key findings. Firstly, it is the first study to show that low B12

status in pregnancy is associated with a higher risk of GDM in a UK population. Secondly, higher first trimester BMI was an independent predictor of later B12 insufficiency. Thirdly, low B12 levels were associated with macrosomia in the subgroup of no-GDM women, which seems to be partly mediated by maternal BMI [15].

The other study that examined the link between B12 and GDM by **Krishnaveni *et al.*** [8] was in an Indian cohort. The magnitude of association found in that study was similar to the other study, but the significance was lost after adjusting for maternal BMI. The recent finding by **Knight *et al.*** [16], in no-GDM women, also supports the inverse link between B12 levels and gestational diabetes in pregnant White Caucasian women.

Vitamin B12 deficiency in obesity was previously studied in several investigations among Turkish population. **Guven *et al.*** [17] reported that vitamin B12 level was significantly lower in patients with metabolic syndrome than those without metabolic syndrome.

Kaya et al. conducted a study on association between obesity and vitamin B12 in patients with gestational diabetes. They concluded that obesity and gestational diabetes were associated with vitamin B12 deficiency [18] and this association was significant.

Previously reported study on the association between vitamin B12 and obesity in middle-aged women have shown a significantly lower vitamin B12 in obese women and negative correlation with BMI [19].

Also in current study we found that there was no statistically significantly difference between sufficient vitamin B12 and insufficient vitamin B12 as regards data including maternal BMI, RBS, FBS, 2HPP and HbA1C, which agrees with the study done by **Adaikalakoteswari *et al.*** [5].

It also showed that there was a statistical significance difference between sufficient vitamin B12 and insufficient vitamin B12 as regards gestational age of these patient, gestational age ranged from (28 week – 35 week). In patient with sufficient vitamin B12 it was (31.92 ± 2.06) and with insufficient vitamin B12 it was (30.84 ± 2.23), which showed significantly difference. In other studies conducted by **Nithya *et al.*** [15] **and Baltaci *et al.*** [19], gestational age was not demonstrated.

Indeed, gestational diabetes in the context of low B12 was shown by other authors in obese adolescents, non-pregnant adults, as well as in women with polycystic ovarian syndrome [18]. Prospective longitudinal studies are needed to investigate whether the presence of low B12 status in early pregnancy independently increases the risk of incident GDM.

The etiology of the inverse relationship between B12 and BMI found in our study is an intriguing one. While confounding factors such as dietary habits, socioeconomic status, and hemodilution may be present, other studies that have corrected for these still show an independent link between B12 and BMI [8]. Interestingly, the frying and roasting of meat products reduces the bioavailability of B12 by 20%–40% [18], so higher consumption of processed foods may increase the risk of vitamin B12 insufficiency.

There is also another study that demonstrated a relationship between maternal B12 and maternal obesity, the rates of B12 insufficiency observed in the study in no-GDM population was similar to that observed by **Knight *et al.*** [16] (22% vs. 20%), suggesting that such higher rates of insufficiency are not limited to Indian populations [7].

We had identified associations between B12, maternal obesity, GDM, age and GA of the patient. Current study proved that there is a positive correlation between age, gestational age and vitamin B12 in that with increasing age and gestational age of the patient vitamin B12 will increase. The direction of the relationship between these factors was obtained from a small sample size so to confirm that relationship big sample size is needed.

The study indicated that vitamin B12 deficiency is more common in pregnant woman with obesity and gestational diabetes compared to healthy individuals. The study has also shown that vitamin B12 level was correlated with BMI, gestational diabetes, age and gestational age of the patient.

CONCLUSION

Vitamin B12 deficiency is silent and common in general population. Causes of vitamin B12 deficiencies are multifactorial and associated with many health problems. Also, obesity is common and its prevalence is increasing in the world. Therefore, both health problems have gained importance in family medicine practice in the last decades.

Obesity and overweight are risk factors for vitamin B12 deficiency. It was also noticed that maternal gestational diabetes is an additional risk factor for vitamin B12 deficiency, the reason for vitamin B12 deficiency is multifactorial and modifiable in both conditions.

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