

**Lipid profile and Vitamin B 12 levels in women having Gestational Diabetes Mellitus:****A Case Control Study****Abstract**

**Aim:** To assess the lipid profile and vitamin B12 levels in women having the gestational diabetes mellitus

**Study design:** Case Control study

**Place and Duration:** Department of Biochemistry and Gynecology/Obstetrics LUMHS Hospital Jamshoro from January 2018 to December 2018.

**Methodology:** A sample of 291 pregnant women was grouped as; 216 diagnosed gestational diabetes mellitus (GDM) and 75 non gestational diabetes mellitus were selected according to criteria. Sera were estimated of glucose (hexokinase method), blood lipids and Vitamin B12 (ECLIA assay method) by Cobas chemistry analyzer. Triglyceride/high density lipoprotein (TAGs/HDLc) ratio was calculated as log transformed of TAGs to HDLc. TAGs/HDLc ratio was labelled as low risk (0.3 – 0.1), medium risk (0.1 – 0.24) and high risk (>0.24). Statistical SPSS software 21.0 (IBM, Inc USA) analyzed the research variables at 95% CI ( $P \leq 0.05$ ).

**Results:** Age of cases was  $37.9 \pm 5.19$  years ( $P=0.051$ ). Fasting and random blood glucose levels were elevated significantly ( $P=0.0001$ ). Vitamin B12 in cases was low noted as  $155.6 \pm 71.3$  ng/mL compared to  $251.5 \pm 33.1$  in control ( $P=0.0001$ ). Any type of vitamin B12 deficiency was present in 153 (70.51%) in GDM cases compared to 37 (49.4%) in control ( $P=0.0001$ ). Low, medium and high atherogenicity risk was found in 56.01%, 37.5% and 6.4% GDM cases and 50.5%, 36.6% and 0% in control respectively ( $P=0.0001$ ).

**Conclusion:** The present study finds triglyceride to HDLc ratio was found elevated in Gestational Diabetes mellitus women with vitamin B12 deficiency.

**Key words:** Triglycerides, High density lipoprotein, Gestational Diabetes mellitus, Vitamin B12 deficiency

## **Introduction**

Gestational diabetes mellitus (GDM) is a metabolic disorder of high blood glucose levels first time diagnosed during pregnancy. It is a state of glucose intolerance noted during pregnancy without prior history of diabetes mellitus (DM).<sup>1</sup> Pregnant women develop hyperglycemia and glycosuria during initial period of gestation. True prevalence of GDM is unknown in developing countries including Pakistan because of lack of hospital registries and moreover by incomplete records. Global prevalence of GDM is reported as 1 – 14 %.<sup>2</sup> Geographical variations of GDM prevalence have been evident related to the ethnicity, dietary habits, racial factors, environmental factors and pollution. Approximately 7% of conceived women develop some degree of glucose intolerance that may be severe enough to develop the GDM. Global burden of GDM was proposed as >200,000 cases.<sup>2</sup>

Risk of maternal morbidity and mortality is increased by GDM beside risk to the fetus of developing congenital malformation and macrosomia.<sup>2, 3</sup> Currently, urbanization is on rise and similar rise of risk of developing GDM has been observed in modern community. This may be because of sedentary life style, modern life style, gourmet habits, obesity and intake of carbohydrate rich foods. Contaminated food, water and pollutants have put the community members at increased risk of DM and GDM.

Normally, the pregnancy is a state of change metabolism, hormones and altered internal milieu. Anti – insulin hormones are increased increasing the chances of insulin resistance. Insulin resistance and carbohydrate intolerance has been observed beginning at the gestation weeks 24–28 and then increase progressively.<sup>1-3</sup> GDM patients' shows altered primary

metabolic defect of glucose and secondarily the lipid metabolism in major. Pregnant women may develop enough insulin resistance resulting in hyperglycemia, hyperlipidemia, and dyslipidemia increasing the tendency of atherosclerosis.<sup>5,6</sup>

Vitamins are essential for the normal glucose and lipid metabolism and prevent against the complications of hyperglycemia and hyperlipidemia. Vitamin B12 deficiency<sup>4</sup> has been linked to the insulin resistance, glucose intolerance, hyperglycemia, hyperlipidemia, dyslipidemia, etc. Vitamin B12 maintains the normal lipid metabolism and endothelial functioning. Dyslipidemia<sup>5,6</sup> is grave complication of diabetes mellitus that is linked with many morbidities, hence there is need to analyze the blood lipid abnormalities in gestational diabetes mellitus patients.

The present prospective research study analyzed the triglyceride and high density lipoprotein levels in Gestational Diabetes mellitus patients having vitamin B12 deficiency.

**Methodology:**

The present case control study was planned to determine the triglyceride (TAG), high density lipoprotein (HDL) and vitamin B12 levels in Gestational Diabetes mellitus women. The study was approved by the ethics committee of institute. Duration of study was from Jan 2018 to December 2018. Place of study setting was the Department of Biochemistry Liaquat University of Medical and Health Sciences Jamshoro. The patients were selected from the Department of Gynecology/Obstetrics of the institute. Total 291 pregnant women were grouped as; 216 diagnosed GDM pregnant women and 75 pregnant controls had no GDM. Women were included according to the non-probability purposive technique. Diagnosis of GDM was diagnosed according to the standard criteria and confirmed by a consultant gynecologist. Diagnosed GDM patients were interviewed for voluntary inclusion in the study protocol. Patients were examined of Biodata, physical examination, and laboratory findings to confirm the diagnosis and fulfill the inclusion and exclusion criteria. Inclusion criteria were pregnant women, diagnosed GDM, 20 – 40 years of age, looking

healthy without systemic disease, and ultrasound reveals viable fetus of 2<sup>nd</sup> and 3<sup>rd</sup> trimester. Type 2 DM pregnant women, postpartum women, concomitant systemic diseases, insulin resistance cases, polycystic ovarian syndrome (PCOS) and those having family history of diabetes mellitus were excluded from the study. GDM diagnosed cases were examined physically by a consultant gynecologist and obstetrician of institute. The volunteers were informed the study will help future management of GDM cases for their better health, so the consent was taken purely on humanitarian grounds. Volunteers were asked for blood sampling. Study protocol strictly followed the “Helsinki’s Declaration” of human handling for research purpose. Patient’s data was secured in a pre – structured proforma and locked in lockers. Blood samples were collected and centrifuged (at x3000 rpm for 15 minutes) to get sera for biochemical analysis. Hemolyzed, lipemic and icteric samples were discarded. Sera were stored at – 20 °C. ELISA assay kit measured serum vitamin B12 levels (Abcam, USA) at 450nm “absorbance”. Vitamin B12 deficiency was defined as cited.<sup>7</sup> Patients showing serum total cholesterol  $\geq 200$  mg/dL, TAGs  $\geq 150$  mg/dL, LDLc  $\geq 100$  mg/dL and HDLc  $\leq 50$  mg/dl were taken as suffering from dyslipidemia.<sup>8</sup> TAGs/HDLc ratio was calculated as log transformation of triglyceride to HDLc.<sup>6</sup> TAGs/HDLc ratio was labelled as low risk (0.3 – 0.1), medium risk (0.1 – 0.24) and high risk ( $>0.24$ ).<sup>6</sup> Biochemical analyses were performed on chemistry Cobas analyzer at laboratory. Data variables were analyzed on SPSS 21.0 using Student’s t-test for continuous data and results presented as mean $\pm$  SD. Categorical data variables were analyzed by Chi-square ( $X^2$ ) test and results presented as frequency and %. Statistical significance of results of data variables was taken significant at 95% CI ( $P \leq 0.05$ ).

### **Results:**

Table – 1 show the demographic and biochemical findings of cases and control. Age of cases was  $37.9 \pm 5.19$  years ( $P=0.051$ ). Fasting and random blood glucose levels were elevated significantly ( $P=0.0001$ ). Vitamin B12 in cases was low noted as  $155.6 \pm 71.3$

ng/mL compared to 251.5±33.1 in control (P=0.0001). Blood lipids were found elevated in GDM cases compared to control with low HDLc (P=0.0001).

Any type of vitamin B12 deficiency was present in 153 (70.51%) in GDM cases compared to 37 (49.4%) in control (P=0.0001) (Table 2). Dyslipidemia was found in 59 (27.3%) GDM cases compared to 9 (12.0%) control ( $X^2=351.0$ ) (P=0.0001). Table 3 shows the TAGs/HDLc ratio. Low, medium and high atherogenicity risk was found in 56.01%, 37.5% and 6.4% GDM cases and 50.5%, 36.6% and 0% in control respectively (P=0.0001). Correlation (Pearson's analysis) proved negative correlation of vitamin B12 with cholesterol (r= -0.12, P=0.02), TAGs (r= -0.11, P=0.01), LDLc (r= -0.19, P=0.03), TAGs/HDLc ratio (r= -0.67, P=0.0001) and positive correlation with HDLc (r= 0.77, P=0.0001). Scatter plots A – E show the correlation of cholesterol, TAGs, LDLc, HDLc, TAGs/HDLc ratio with vitamin B12 levels.

**Table -1. Findings in cases and control (n=291)**

|                                       | <b>GDM Cases<br/>n=216</b> | <b>Control<br/>n=75</b> | <b>P-value</b> |
|---------------------------------------|----------------------------|-------------------------|----------------|
| Age (years)                           | 37.2±9.3                   | 37.9±5.19               | 0.78           |
| Fasting Glucose (mg/dl)               | 135.1±19.3                 | 79.5±11.5               | 0.0001         |
| Random Glucose (mg/dl)                | 234.5±36.5                 | 149.1±11.3              | 0.0001         |
| Cholesterol (mg/dl)                   | 234.7±39.3                 | 123.4±16.3              | 0.0001         |
| Triglycerides (mg/dl)                 | 539.5±57.3                 | 497.2±23.5              | 0.0003         |
| LDL cholesterol (mg/dl)               | 139.7±12.3                 | 90.9±11.5               | 0.0001         |
| HDL cholesterol (mg/dl)               | 31.3±6.1                   | 39.5±3.6                | 0.0002         |
| Vitamin B <sub>12</sub> level (ng/mL) | 155.6±71.3                 | 251.5±33.1              | 0.0001         |

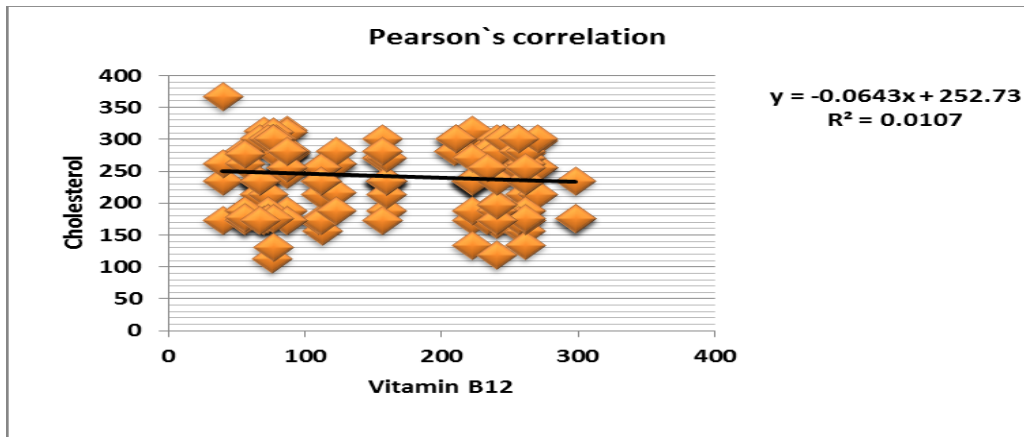
**GDM** – gestational diabetes mellitus

**Table -2. Cross tabulation of vitamin B<sub>12</sub> in cases and control**

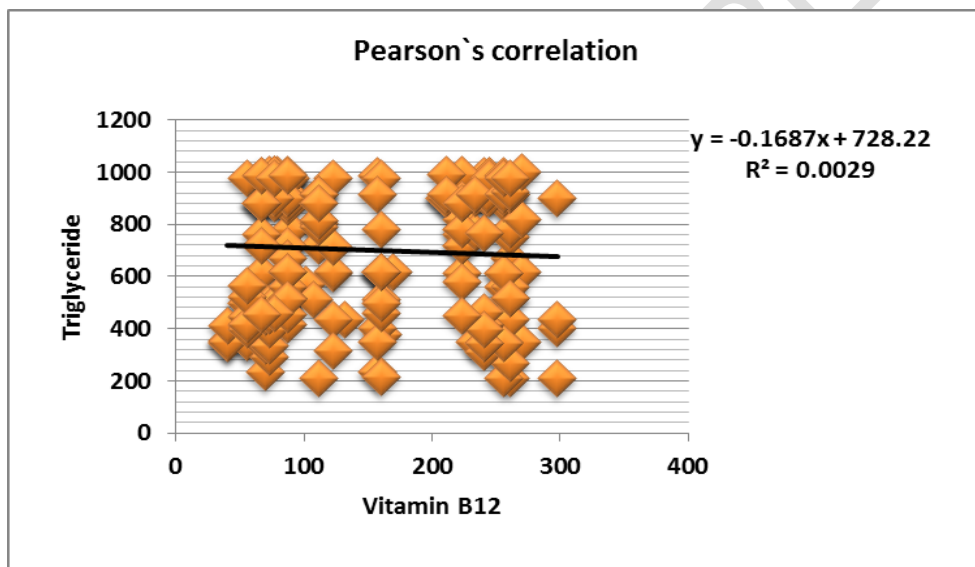
|                                   | Cases  |      | Control |      | P-value |
|-----------------------------------|--------|------|---------|------|---------|
|                                   | Number | %    | Number  | %    |         |
| Vit. B <sub>12</sub> – Normal     | 63     | 29.1 | 38      | 50.5 | 0.0001  |
| Vit. B <sub>12</sub> – Borderline | 37     | 16.9 | 26      | 36.6 |         |
| Vit. B <sub>12</sub> – Deficiency | 31     | 13.9 | 08      | 10.6 |         |
| Vit. B <sub>12</sub> – Severe     | 85     | 39.7 | 03      | 3.9  |         |
| Total                             | 216    | 100  | 75      | 100  |         |

**Table -3. Cross tabulation of TAGs/HDLc ratio in cases and control**

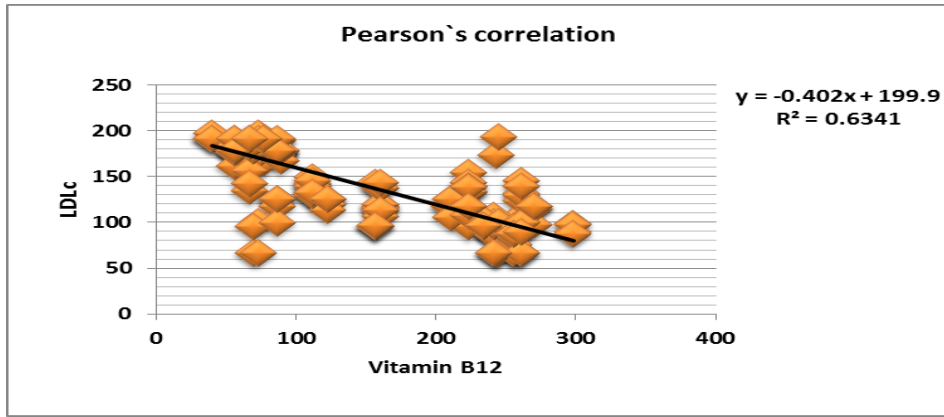
| Risk categories     | Cases  |       | Control |      | P-value |
|---------------------|--------|-------|---------|------|---------|
|                     | Number | %     | No.     | %    |         |
| No (<-0.3)          | 0      | 0     | 51      | 50.5 | 0.0001  |
| Low (-0.3 – 0.1)    | 121    | 56.01 | 23      | 50.5 |         |
| Medium (0.1 – 0.24) | 81     | 37.5  | 01      | 36.6 |         |
| High (>0.24)        | 14     | 6.4   | 0       | 0    |         |
| Total               | 216    | 100   | 75      | 100  |         |



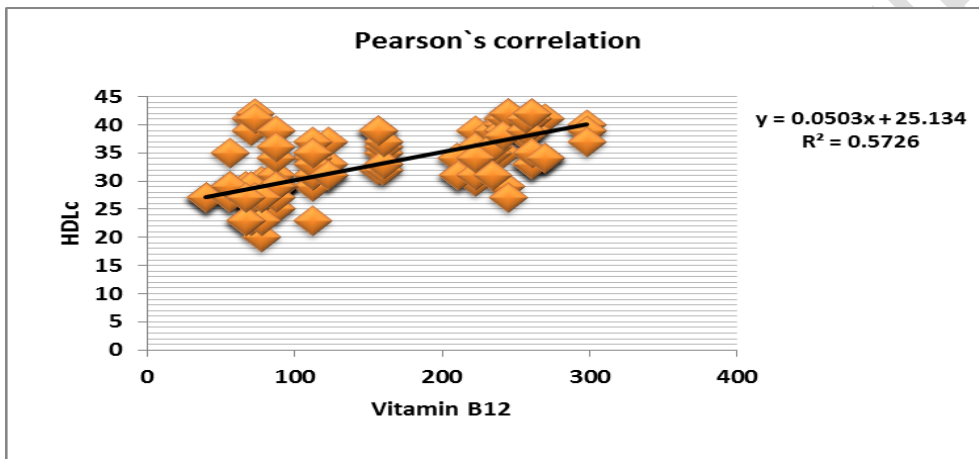
**Graph- A.** Scatter plot showing -ve correlation of cholesterol and vitamin B<sub>12</sub>



**Graph- B.** Scatter plot shows -ve correlation of triglycerides and vitamin B<sub>12</sub>

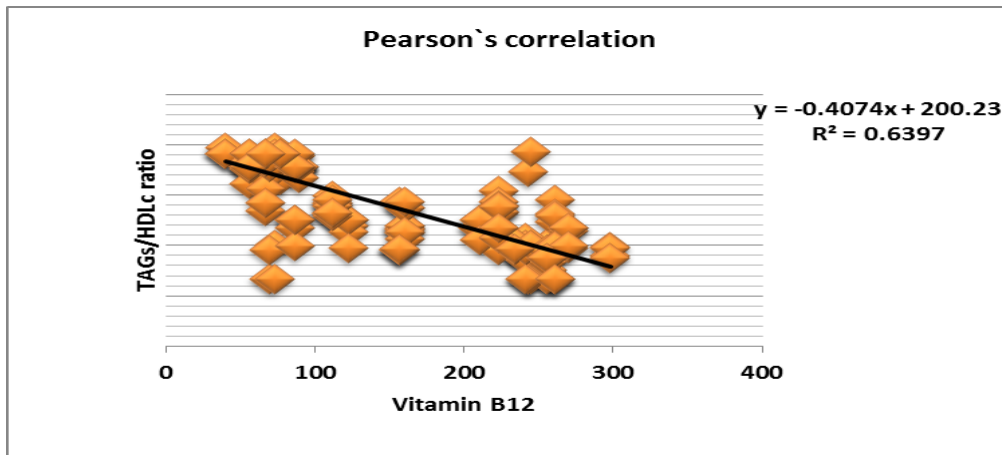


**Graph – C.** Scatter plot showing -ve correlation of LDLc and vitamin B<sub>12</sub>



**Graph – D.** Scatter plot showing +ve correlation of HDLc and vitamin B<sub>12</sub>





**Graph – E.** Scatter plot showing -ve correlation of TAGs/HDLc ratio and vitamin B<sub>12</sub>

### Discussion

The present study is first case control study probing into the problem of dyslipidemia and vitamin B12 deficiency in GDM women. GDM occurs as a result of imbalance of gestational hormones in women of particular ethnicity. The study is the first to determine the triglyceride to HDLc ratio (TAGs/HDLc) in Gestational Diabetes mellitus with vitamin B12 deficiency from our institute. We found any type of vitamin B12 deficiency was present in 153 (70.51%) in GDM cases compared to 37 (49.4%) in control (P=0.0001). High frequency of vitamin B12 deficiency is consistent with previous studies.<sup>9,10</sup> Previous studies<sup>11,12</sup> show high prevalence of vitamin B12 deficiency in general population of Pakistan. Prevalent vitamin B12 deficiency has negative impact on the health of both pregnant mothers and their babies.<sup>11, 13</sup> Dyslipidemia was found in 59 (27.3%) GDM cases compared to 9 (12.0%) control (P=0.0001). Low, medium and high atherogenicity risk was found in 56.01%, 37.5% and 6.4% GDM cases and 50.5%, 36.6% and 0% in control respectively (P=0.0001). Above findings of present study are in agreement with previous studies.<sup>14, 15</sup>

Findings of present study points to the gravity of health problem of GDM female suffering from the concomitant vitamin B12 deficiency leading to atherogenicity, related complications and danger to the fetus. The findings of present study are supported by

previous studies.<sup>16, 17</sup> Vitamin B12 deficiency in GDM cases has been reported by other previous studies.<sup>18, 19</sup> A previous study<sup>19</sup> reported pregnant women suffering from vitamin B12 deficiency were at high risk of insulin resistance and adiposity, and it was concluded the risk of GDM is increased twice in pregnant women with vitamin B12 deficiency. Findings of above study support the observations of present study. Vitamin B12 deficiency was noted 70.9% of study sample and is consistent with a previous study<sup>20</sup> that reported 70.6% deficiency in pregnant women. Previous studies<sup>11, 12</sup> had reported high prevalence of vitamin B12 deficiency concluding nutritional deficiencies is prevalent in the country. Vitamin B12 deficiency has also been reported in pregnant women of western country.<sup>21</sup> Dyslipidemia was very high in GDM cases found in 59 (27.3%) compared to 9 (12.0%) control (P=0.0001). Low, medium and high TAGs/HDLc atherogenicity risk was found in 56.01%, 37.5% and 6.4% GDM cases and 50.5%, 36.6% and 0% in control respectively (P=0.0001) in present study.

The present study noted inverse correlation of vitamin B12 with cholesterol (r= -0.12, P=0.02), TAGs (r= -0.11, P=0.01), LDLc (r= -0.19, P=0.03), TAGs/HDLc ratio (r= -0.67, P=0.0001) and positively with HDLc (r= 0.77, P=0.0001). The finding is supported by a previous studies.<sup>21, 22</sup> Another previous study<sup>23</sup> from UK reported vitamin B12 deficiency is common in pregnant women increasing the risk of GDM by 2.59 time in vitamin B12 deficient. In light of evidence based findings of elevated TAGs/HDLc ratio in vitamin B12 deficient pregnant women, it is worth to report the association of atherogenic tendency and vitamin deficiency in gestational diabetes mellitus pregnant women.

Timely screening of blood lipids, TAGs/HDLc ratio and vitamin B12 may prevent maternal and fetal complications of dyslipidemia and atherogenicity. Confounding factor of prevalent nutritional and vitamin deficiencies cannot be ruled out. The findings of present study cannot be generalized to other settings as this study participants belonged to peculiar ethnicity with small sample size of different dietary resources.

**Conclusion:**

The present study finds triglyceride to HDLc ratio was found elevated in Gestational Diabetes mellitus with vitamin B12 deficiency. Any type of vitamin B12 deficiency was present in 153 (70.51%) in GDM cases compared to 37 (49.4%) in control. Dyslipidemia was found in 59 (27.3%) GDM cases compared to 9 (12.0%) control. It is concluded, the elevated TAGs/HDLc ratio may be corrected by vitamin B12 supplement to improve maternal and fetal outcome in gestational diabetes mellitus pregnant women. Further studies with large sample size are recommended to analyze the triglyceride to HDLc ratio in vitamin B12 deficient GDM pregnant women.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

**References:**

1. Morikawa M, Yamada T, Saito Y, Noshiro K, Mayama M, Nakagawa-Akabane K, et al. Predictors of recurrent gestational diabetes mellitus: A Japanese multicenter cohort study and literature review. *J Obst Gynaecol Res* 2021; 47 (4):1292-1304.
2. Hua J, Gilliesc CL, Lina S, Stewartb ZA, Melfordb SE, Abramsd KR, et al. Association of maternal lipid profile and gestational diabetes mellitus: A systematic review and meta-analysis of 292 studies and 97,880 women. *Eclin Med* 2021; 34 (100830):1-7.
3. Wang N, Peng Y, Wang L, Song L, Sun B, Wei J, Wang T, Mi Y, Cui W. Risk Factors Screening for Gestational Diabetes Mellitus Heterogeneity in Chinese Pregnant Women: A Case–Control Study. *Diabetes Metab Syndr Obes* 2021; 14:951-961.

4. Chen X, Zhang Y, Chen H, Jiang Y, Wang Y, Wang D, et al Association of Maternal Folate and Vitamin B<sub>12</sub> in Early Pregnancy with Gestational Diabetes Mellitus: A Prospective Cohort Study. *Diabetes Care* 2021 Jan; 44(1): 217-223.
5. Adaikalakoteswari A, Manu M, Alam MT, Ott S, Kumar S, Saravanan P. Low Vitamin B<sub>12</sub> in Pregnancy Is Associated With Adipose-Derived Circulating miRs Targeting PPAR $\gamma$  and Insulin Resistance. *J Clin Endocrinol Metab* 2017; 102: 4200–9.
6. Shim YS, Baek JW, Kang MJ, Oh YJ, Yang S, Hwang IT. Reference Values for the Triglyceride to High-Density Lipoprotein Cholesterol Ratio and Non-High-Density Lipoprotein Cholesterol in Korean Children and Adolescents: The Korean National Health and Nutrition Examination Surveys 2007-2013. *J Atheroscler Thromb* 2016; 23(12):1334-44.
7. Shaikh S, Memon A, Ata MA, Koharo HK. Cobalamin deficiency; helicobacter pylori infected patients: a myth or reality. *Professional Med J* 2016; 23(2):176-181.
8. Narindrarakura P, William Bosl W, Ram Rangsin R, Panadda Hatthachote P. Prevalence of dyslipidemia associated with complications in diabetic patients: a nationwide study in Thailand. *Lipids Health Dis* 2019; 18 (90): 1-8.
9. Lai JS, Pang WW, Cai S, Lee YS, Chan JKY, Shek LPC, et al. High folate and low vitamin B<sub>12</sub> status during pregnancy is associated with gestational diabetes mellitus. *Clin Nutrition* 2018; 37(3):940-947.
10. Gebril MM, Abbas TR, Saad M, Radwan E, Abd El-Kader MM. Vitamin B<sub>12</sub> status among pregnant women and its association with obesity and gestational diabetes. *European J Hospital Med* 2019; 75 (3): 2381- 88.
11. Fatima SS, Rehman R, Alam F, Madhani S, Chaudhry B, Khan TA. Gestational diabetes mellitus and predisposing factors. *J Pak Med Assoc* 2017; 67: 261-5.

12. Subhadra S, Bhagwan SJ, Manisha Y, Usha BR, Sunesh K, Mukhopadhyay AK. Cross-sectional study of nutritional markers in pregnancy. *Indian J Endo Metab* 2016; 20 (6) 825-830.
13. Baltaci D, Kutlucan A, Turker Y, Yilmaz A, Karacam S, Deler H, et al. Association of vitamin B12 with obesity, overweight, insulin resistance and metabolic syndrome, and body fat composition; primary care-based study. *Med Glas (Zenica)* 2013; 10(2):203-210.
14. Adaikalakoteswari A, Ramamurthy Jayashri<sup>2</sup>, Nithya Sukumar N, Hema Venkataraman H, Rajendra Pradeepa R, Kuppan Gokulakrishnan K, et al. Vitamin B12 deficiency is associated with adverse lipid profile in Europeans and Indians with type 2 diabetes. *Cardiovasc Diabetol* 2014; 13:129.
15. Weikert C, Dierkes J, Hoffmann K, Berger K, Drogan D, Klipstein-Grobusch K, Spranger J, Mohlig M, Luley C, Boeing H: B vitamin plasma levels and the risk of ischemic stroke and transient ischemic attack in a German cohort. *Stroke* 2007; 38(11):2912–2918
16. Radzicka S, Ziolkowska K, Zaborowski MP, Brazert J, Pietryga M. Serum homocysteine and vitamin B12 levels in women with gestational diabetes mellitus. *Ginekologia Polska* 2019; 90 (7): 381–387.
17. Riaz M, Shaikh F, Fawwa A, Hakeem R, Shera AS, Hitman GA, et al. Maternal Nutrition during Early Pregnancy and Cardiometabolic Status of Neonates at Birth. *J Diabet Res* 2018; Article ID 7382946:1- 8.
18. Finkelstein JL, Guillet G, Pressman EK, Fothergill A, Guetterman HM, Kent TR, O'Brien KO. Vitamin B12 Status in Pregnant Adolescents and Their Infants. *Nutrients* 2019; 11 (397): 1-16
19. Krishnaveni G, Hill J, Veena S, Bhat D, Wills A, Karat C, et al. Low plasma vitamin B12 in pregnancy is associated with gestational ‘diabesity’ and later diabetes. *Diabetologia* 2009; 52: 2350-8.

20. Farren M, Daly N, O'Higgins AC, McKeating A, Maguire PJ, Turner MJ. The interplay between maternal obesity and gestational diabetes mellitus. *J Perinat Med* 2018; 43(3):311-7.
21. Li B, Yang H, Zhang W, Shi Y, Qin S, Wei Y, et al. Fatty acid-binding protein 4 predicts gestational hypertension and preeclampsia in women with gestational diabetes mellitus. *PLoS ONE* 2018; 13(2): e0192347.
22. Chung S, Sawyer JK, Gebre AK, Maeda N, Parks JS. Adipose Tissue ATP Binding Cassette Transporter A1 Contributes to High-Density Lipoprotein Biogenesis In Vivo/Clinical Perspective. *Circulation* 2011;124(15):1663–72.17
23. Kaya C, Cengiz SD, Satiroğlu H. Obesity and insulin resistance associated with lower plasma vitamin B12 in PCOS. *Reprod Biomed Online* 2009; 19:721-6.