# Study of Serum Levels of Homocysteine, Vitamin $B_{12}$ and Folic Acid in Women with a previous history of Gestational Diabetes Mellitus

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***Abstract***
**Background**: *Gestational Diabetes mellitus (GDM) is a common condition seen during the 24th to 28th week of pregnancy which resolves spontaneously with the birth of the baby. In the present study we have studied the serum levels of homocysteine (Hcy), vitamin B12 and folic acid in women with the history of GDM and tried to highlight the correlation especially of homocysteine with GDM.*
***Materials and Methods:*** *The present study was conducted on 100 women with GDM patients attending the Gynaecology & Obstetrics OPD, J.L.N. Medical College & Associated groups of Hospital, Ajmer. The results of patients were compared with 100 women without GDM subjects. Anthropometric measurements and biochemical estimations were performed, after taking approval from ethical committee.*
***Results:*** *The mean serum levels of homocysteine, vitamin B12, folic acid, fasting glucose and fasting insulin were elevated in women with GDM as compared to women without GDM (control) subjects. Serum Hcy, fasting glucose and fasting insulin were observed statistically significant in subjects studied when compared with controls.*
***Conclusion:*** *In women with a previous history of GDM a statistically significant rise in the serum levels of homocysteine, fasting glucose, fasting insulin, HOMA IR, total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol was seen. Further deeper studies into the matter need to be carried out to bring out the importance of using homocysteine level as a marker of impending diabetes in women who have had an earlier pregnancy with GDM.*

**Keywords:** *Gestational Diabetes Mellitus, Homocysteine, Vitamin B12, Folic acid, Insulin, HOMA IR, Lipid Profile.*

## I. INTRODUCTION

Gestational Diabetes Mellitus (GDM) is a common condition seen during pregnancy and is defined as a varying degree of glucose intolerance that is first detected during a pregnancy [1]. This condition is generally encountered between 24th to 28th weeks of gestation and usually resolves spontaneously after the delivery. GDM affects about 2% to 5% of total cases of pregnancy in United States. In India, it affects about 16.55% of total pregnancies [2]. The pathogenesis of GDM is very similar to that of type 2 diabetes, in which both pancreatic insulin release and chronic insulin resistance have roles. GDM and impaired glucose tolerance during pregnancy are shown to be associated with future metabolic dysfunction and diabetes independent of other clinical risk factors [3]. GDM develops due to defect in the insulin receptors during pregnancy. It may be because of the presence of various factors like human placental lactogen, a polypeptide hormone, which interferes with the proper working of insulin receptors leading to an increase in the serum glucose level during pregnancy. During the past three decades, the importance of serum homocysteine level as a marker of various diseases has been studied upon. Vitamin B12, folic acid and homocysteine are metabolically closely related entities. At the same time homocysteine is found to be offending factor for vascular pathology causing preeclampsia. Preconceptional nutritional status influences the vitamin B12 & folic acid level. Which further affect the homocysteine level thus may affect pregnancy outcome [4][5]. Homocysteine is a naturally occurring sulphur containing amino acid which is metabolised by remethylation and trans-sulphuration.[6] Re-methylation of homocysteine is directly dependent on the enzyme methionine synthetase for which vit. B12 acts as a co-factor and methyltetrahydrofolate (MTHF) as a substrate. Thus, vit.B12 and folic acid deficiency

leads to an increase in serum level of homocysteine. Therefore, it is equally important to determine these factors for evaluating hyperhomocysteinemia and its possible etiology [7,8]. During pregnancy, levels of homocysteine are generally low either due to a physiological response to pregnancy such as an increase in oestrogen, haemodilution owing to increased plasma volume or increased demand for methionine by both maternally and also by the fetus [9]. A further possible mechanism for the reduction in homocysteine level during pregnancy is utilization by the foetus. A decreasing plasma homocysteine concentration gradient exists from the maternal vein to the umbilical artery, suggesting incorporation of homocysteine into the foetal metabolic cycle. Previous folic acid administration has been shown to reduce homocysteine level in healthy subjects and patients with renal and vascular diseases [10]. It has been shown by many studies that serum homocysteine levels were significantly increased in women with GDM independently of other confounding variables and were related to 2hr OGTT plasma glucose [11]. Datas about serum homocysteine level and glucose tolerance in both diabetic and non diabetic pregnant women have demonstrated an association between insulin resistance and hyperhomocysteinemia [12]. As stated by many studies that GDM heralds the development of overt diabetes in future, we aimed to study the feasibility of using homocysteine level as a marker of the development of diabetes by studying its correlation with the other biochemical parameters such as fasting blood sugar, fasting insulin, HOMA IR, lipid profile , Uric acid and creatinine.

## II. MATERIALS AND METHODS

The present study was performed in the Department of Biochemistry J.L.N. Medical College, Ajmer, Rajasthan. The participants were randomly selected from the list of non pregnant women who are beyond 6 weeks post partum and well within 2years from the indexed pregnancy (pregnancy with GDM), attending the Gynaecology & Obstetrics OPD, J.L.N. Medical College & Associated groups of Hospital, Ajmer. A total of 200 women were enrolled, of which 100 were with a history of GDM in the previous pregnancy within the aforesaid time period, another group of 100 women with any history of GDM serves as the controls. At the very onset informed consent was taken from every participant and questionnaire was given to find out the relevant informations. Anthropometric assessment was done. BMI was calculated by using the formula: [BMI= weight(Kgs)/height (metre)$^2$. Blood pressure was measured after 10 minutes of rest. Women with overt diabetes, family history of GDM, history of intake of Vitamin $B_{12}$, Folic acid , Phenytoin or any other medications which may influence the level of Hcy, women with hypertension or cardiovascular disease, smoking current or past, breast carcinoma, ovarian tumour, chronic renal failure and hypothyroidism were excluded from the study.

Blood samples were collected after an overnight fast (8-12 hrs) under strict aseptic condition and were subjected to the measurements of the biochemical parameters of concern. Serum homocysteine, VitaminB12, folic acid and fasting insulin levels were determined by ELISA method, fasting glucose, uric acid, total cholesterol, triglyceride and HDL cholesterol were estimated by enzymatic method. Creatinine level was estimated by colorimetric kinetic method. VLDL and LDL cholesterol were calculated using Friedewald's equation. Insulin resistance was calculated by using the formula, HOMA IR= Fasting glucose (mg/dl) X Fasting insulin (µIU) / 405. Statistical analysis was performed by using Statistical Package for the Social Sciences (SPSS, version 16) and Microsoft excel. T test was performed and P values were calculated for the biochemical and anthropometric parameters. P values less than 0.05 were considered statistically significant.

**Table:1 Comparision of Biochemical and Anthropometric Parameters Among the Study Groups**

| PARAMETERS | WOMEN WITHOUT GDM (CONTROLS) | WOMEN WITH GDM | P value |
|---|---|---|---|
| Serum homocysteine | 8.50 ± 2.97 | 11.62 ± 7.26 | P<0.0001 |
| Serum Vitamin B12 | 409.38 ±150.49 | 437.78 ± 130.90 | P>0.1561 |
| Serum Folic acid | 5.37 ±2.42 | 5.48 ± 2.62 | P>0.759 |
| Serum Fasting Glucose | 92.78±14.88 | 100.15 ±22.70 | P<0.0072 |
| Serum Fasting Insulin | 8.12±5.13 | 12.47±4.23 | P<0.0001 |

| | | | |
|---|---|---|---|
| HOMA IR | 2.04±1.56 | 3.31±1.80 | P<0.0001 |
| Serum Cholesterol | 175.27±34.66 | 195.70±32.53 | P<0.0001 |
| Serum Triglyceride | 109.14±43.63 | 132.25±64.35 | P<0.0033 |
| Serum HDL cholesterol | 50.70±10.07 | 50.44±10.80 | P>0.8604 |
| Serum VLDL cholesterol | 21.84±8.71 | 26.45±12.88 | P<0.0034 |
| Serum LDL cholesterol | 102.74±36.04 | 118.80±30.37 | P<0.0008 |
| Serum Creatinine | 0.81± 0.1 | 0.82±0.1 | P>0.4803 |
| Serum Uric acid | 4.68±1.00 | 4.86±1.11 | P>0.2158 |
| BMI | 27.88±5.34 | 28.15±3.65 | P>0.6768 |
| Age (yrs) | 32.28±6.02 | 32.07±5.00 | P>0.7887 |

## III. RESULTS

The biochemical and anthropometric assessment are given in table 1.The mean ages of the study groups were 32.07±5.00 and 32.28±6.02 for the cases and the controls. There were no significant difference between the BMI, Vitamin $B_{12}$, Folic acid, Creatinine, Uric acid and HDL cholesterol of the two study groups. Statistically significant difference were observed for serum homocysteine, Fasting glucose, Fasting insulin, total cholesterol, Triglyceride, VLDL cholesterol and LDL cholesterol between the study groups. Serum homocysteine and fasting insulin level were studied for any possible correlation between the two groups and their relation with insulin resistance by using basic statistical tests. The evaluation yields a positive correlation between them. We have observed that the serum homocysteine level increases correspondingly with the increased in the glucose, insulin and HOMA IR values thereby suggesting a possibility of using serum homocysteine levels as a predictor of development of overt diabetes in future.

## IV. DISCUSSION

In the present study, we have observed that the levels of serum homocysteine were elevated in the women with a previous history of GDM as compared to the controls. It is in concordance with the previous studies which also stated that hyperhomocysteinemia could seen in the women who had a previous history of GDM [13][14]. The difference in the levels of serum homocysteine between the study groups was significant statistically. It was also observed that the cases had slightly higher levels of Vitamin $B_{12}$ and Folic acid as compared to the controls, although their differences were not significant statistically. As homocysteine metabolism requires Vitamin $B_{12}$ and Folic acid as cofactor, their deficiency may produce an elevation in the level of homocysteine. Initial stage of insulin resistance was seen in the case group who also had higher levels of homocysteine, fasting glucose, insulin and HOMA IR. Studies have proven that impaired secretion of insulin from β cells of pancreas is responsible for development of impaired OGTT from normal OGTT and eventually the development of overt diabetes [15]. It has been observed from our study that in the cases (GDM) there were increased in the levels of fasting glucose in concordance with the increase in the levels of homocysteine. A proper assessment of the β cell function was not done as it was not the objective of the study. Nonetheless our study pointed out that, women with a previous history of GDM had more chances of having impaired OGTT as compared to the controls as shown by the increase in the levels of fasting glucose. HOMA IR value alone provides a rough knowledge of insulin resistance. According to a study by Bonora et al [16] a HOMA IR value of ≤ 2.06 was found in normal non diabetic population. In our study we have classified insulin resistance as, normal insulin resistance (HOMA IR < 3), moderate insulin resistance (HOMA IR= 3-5) and severe insulin resistance (HOMA IR> 5). The mean HOMA IR in our study was 3.31 for the cases and 2.04 for the controls, showing an early stage of insulin resistance in the cases. As for the lipid profile, serum Cholesterol, triglyceride, LDL and VLDL were found to be increased correspondingly with homocysteine levels, in the cases as compared to the controls. The rise was significant statistically. HDL, creatinine, uric acid, BMI and age bear no correlation and were found to be statistically insignificant between the two study groups. Ultimately our study pointed out that GDM is associated with hyperhomocysteinemia as shown by other studies as

well [17][18]. The limitation of our study is that we did not perform a proper assessment of the β cell functions and insulin sensitivity.

## V. CONCLUSION

It is concluded from our case control study that there is an increase in level of the serum homocysteine in women with a previous history of GDM. Other metabolic changes can also be seen in all such women as shown by the measurements of biochemical parameters pertaining to lipid profile, creatinine and uric acid. The correlation between serum homocysteine and previous history of GDM as established by our study indicates that homocysteine can be used as a good method of screening for the development of overt diabetes mellitus in women who had a previous pregnancy affected by GDM. Further studies need to be conducted on this matter to prove the usefulness and constraints in using serum homocysteine as a marker of development of overt diabetes in later part of life in women with a history of GDM.

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