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# Serum homocysteine level and gestational diabetes mellitus: A meta-analysis

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## ABSTRACT

**Aims/Introduction:** Homocysteine levels during pregnancy in women with gestational diabetes mellitus (GDM) have been studied; however, it remains unclear whether hyperhomocysteinemia is a useful predictor of insulin resistance. The present study aimed to evaluate the relationship between homocysteine level and GDM.

**Materials and Methods:** PubMed, Elsevier, Web of Science and CNKI were searched for relevant studies published up to January 2015. Manual searches of references of the relevant original studies were carried out. Meta-analysis was used to assessed the relationship between homocysteine level and GDM using the STATA 12.0 software.

**Results:** Homocysteine levels were significantly elevated in women with GDM compared with those without GDM (weighted mean difference 0.77, 95% confidence interval 0.44–1.10). This evidence was more consistent during the second trimester measurement of homocysteine (weighted mean difference 0.95, 95% confidence interval 0.67–1.23) and for women aged older than 30 years (weighted mean difference 0.90, 95% confidence interval 0.63–1.17).

**Conclusions:** The present meta-analysis shows that homocysteine level is significantly elevated among women with GDM compared with women with normal glucose tolerance, and this finding persists more during the second trimester.

## INTRODUCTION

Gestational diabetes mellitus (GDM) is metabolic complication exhibited as glucose intolerance among women without diabetes history during pregnancy[1]. Gestational diabetes mellitus screening is a routine prenatal project nowadays, though few pregnant women have reported symptoms. GDM affects 3–10% pregnant women depending on the population and GDM diagnosis, which results in more than 200,000 cases each year[2].

Gestational diabetes mellitus could lead to adverse maternal and neonatal outcomes, which is largely as a result of elevated high blood glucose levels during 24–28 weeks[3]. Usually, women with gestational diabetes mellitus would restore normal glucose metabolism after pregnancy; however, they still have a 35–60% chance of developing type 2 diabetes over the next 10–20 years. This risk is sevenfold higher compared with women who never had a history of GDM. Meanwhile, the recurrence rate of GDM in subsequent pregnancies ranges from 30% to 84% depending on the population's ethnic background. Infants are at risk of being both large for gestational age (macrosomic) in unmanaged GDM, and small for gestational age and intrauterine growth retardation in managed GDM. Neonates born from women with consistently high blood glucose levels are also at an increased risk of hypoglycemia, jaundice, polycythemia, hypocalcemia and hypomagnesemia. Unmanaged GDM also interferes with maturation, causing dysmature babies prone to respiratory distress syndrome as a result of incomplete lung maturation and impaired surfactant synthesis[4–6]. Resent studies show that infants born to women with GDM have an increased risk of developing childhood and adult obesity, and type 2 diabetes later in life[7,8]. This risk also has a positive relationship with increased maternal glucose levels. Even in managed diabetic pregnancy, the congenital malformation incidence of offspring is still two- to sixfold higher than that of normal pregnancy[9,10].

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The pathophysiology of GDM is similar to type 2 diabetes, in which abnormal pancreatic insulin release and insulin resistance are involved[3]. The response of β-cell to glucose is abnormal, and manifested as inadequate insulin secretion to compensate for the pregnancy-related insulin resistance. The inflammatory makers, metabolic abnormalities and endothelial dysfunctions in GDM can make women with GDM susceptible to cardiovascular diseases[11]. Homocysteine is a non-protein α-amino acid formed by the demethylation of methionine. It exerts detrimental effects on a number of cell lineages including endothelial cells through production of reactive oxygen species. Both acute and chronic exposure to homocysteine shows detrimental effects on β-cell metabolism and insulin secretion[12]. Elevated homocysteine level has been reported as a major independent risk factor for vascular complications of diabetes, as well as being associated with clinical conditions of insulin resistance[13,14]. However, conflicting results exist, as in some other studies a negative relationship was found between hyperhomocysteinemia and insulin resistance[15].

Although some studies have investigated the relationship between serum homocysteine level and gestational diabetes mellitus, the results were inconsistent and inconclusive. It is unclear whether women with gestational diabetes mellitus have elevated homocysteine levels. We carried out a meta-analysis to provide a more comprehensive estimation of the association between serum homocysteine level and GDM.

## METHODS

### Data Sources and Study Selection

A systematic search of studies was carried out on the association of homocysteinemia level and GDM in the published databases in English of PubMed, Elsevier and Web of Science, and in Chinese of CNKI (Chinese National Knowledge Infrastructure) up to January 2015. The searching terms included "homocysteinemia," "HCY," "hyperhomocysteinemia" and "gestational diabetes mellitus" or "glucose intolerance." References from these relevant studies were manually searched. Inclusion criteria of the present meta-analysis were: (i) original studies evaluating the association between homocysteine level during pregnancy and GDM; (ii) homocysteine levels of GDM women were presented as a group mean with either standard deviation or standard error; (iii) all the GDM groups had a proper control group of healthy pregnant women; (iv) all participants did not have a previous history of diabetes or present pregnant complications; and (v) full-text articles were published in English or Chinese.

### Data Extraction

All identified studies were carefully reviewed independently by two investigators to determine whether an individual study was eligible for the inclusion criteria in the present meta-analysis. When there was a conflicting evaluation, a discussion was carried out to reach an agreement. If a consensus could not be reached, a third experienced investigator was consulted. The following information was extracted by two investigators independently from each publication: name of the first author, year of publication, original country, ethnicity, group mean with standard deviation of homocysteine level in both the case group and control group, group mean of body mass index of GDM women and trimester of homocysteinemia level measurement.

### Statistical Analysis

Homocysteine levels were all presented as mg/dL. Homocysteine levels among women with GDM and the healthy pregnant controls were compared by weighted mean differences (WMD) and 95% confidence interval (95% CI). The statistical heterogeneity was assessed using the $\chi^2$-based $Q$-statistic and the $I^2$-statistic. The heterogeneity was not considered significant if $I^2$ <50%, 50% < $I^2$ < 75% was considered moderate heterogeneity and $I^2$ > 75% was considered high heterogeneity. The fixed-effects model was applied if no significant heterogeneity was detected; otherwise, the random-effects model was carried out. Publication bias was evaluated using funnel plots and the Egger's test. Sensitivity analyses were carried out by sequentially omitting one single study each time to assess the contribution of each individual data set to the summary effect. Statistical analyses were carried out using STATA 9.0 (Stata Corp, College Station, TX, USA).

## RESULTS

### Characteristics of Studies

A flow diagram of the included and excluded studies is shown in Figure 1. A total of 172 studies were identified after an initial search from the selected electronic databases. After excluding the studies that not meet the inclusion criteria, 37 full-text articles were obtained. Among these, 26 articles were excluded for the following reasons: (i) no randomization in the study design ($n$ = 4); (ii) no appropriate healthy controls ($n$ = 8); and (iii) not enough participant information provided ($n$ = 14). Eventually, 11 articles relating to homocysteine and GDN in human subjects were identified[16–26]. Among them, one study assessed the homocysteine level in pregnant GDM women with subclinical atherosclerosis, which failed the inclusion criteria[17]. Finally, 10 studies met the eligible criteria for inclusion in the meta-analysis that examined a total 1,362 participants, which included 408 women with GDM and 954 healthy pregnant women. All the included studies carried out the 100-g oral glucose tolerance test to diagnose GDM following the guidelines of the American Diabetes Association. The characteristics of the studies included in the present meta-analysis are shown in Table 1.

Of the 10 included studies, four were carried out in Turkey, two in China, one in Italy, one in Spain, one in Poland and one in India. The sample size of these investigations ranged from 60 to 243 pregnant women. Half of the included studies measured homocysteine levels during the second trimester, and another half carried out homocysteine measurements during

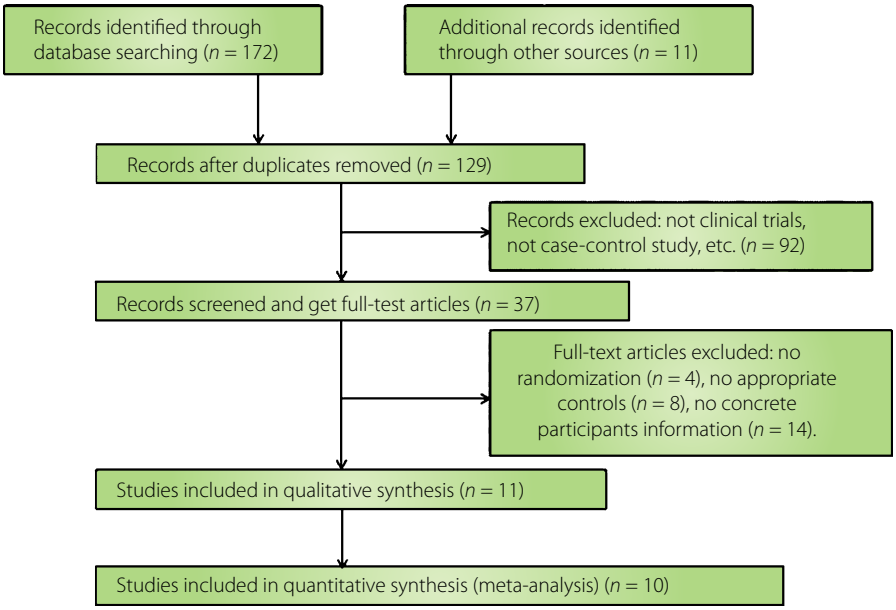


**Figure 1** | Flow chart of selection process of reviewed studies.

**Table 1** | Characteristics of included studies

| Author | Country | Year | Case group | | Control group | | HCY Measurement trimester | Average age of GDM women | Average BMI of GDM women (kg/m$^2$) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Sample size | Serum HCY (mol/L) | Sample size | Serum HCY (mol/L) | | | |
| Seghieri | Italy | 2003 | 15 | 5.88 + 2.26 | 78 | 4.45 + 1.52 | Third | 34.60 | 26.70 |
| Lopez-Quesada | Spain | 2005 | 17 | 6.80 + 2.70 | 190 | 6.60 + 2.00 | Third | 30.00 | 25.30 |
| Tarima | Turkey | 2006 | 30 | 5.96 + 1.70 | 40 | 5.03 + 0.91 | Second | 30.53 | 28.65 |
| Guven | Turkey | 2006 | 30 | 9.00 + 3.10 | 147 | 7.40 + 1.60 | Second | 30.00 | 29.17 |
| Idzior-Walum | Poland | 2008 | 44 | 8.00 + 2.00 | 17 | 7.40 + 1.10 | Third | 30.50 | 27.80 |
| Mujde Akturk | Turkey | 2010 | 54 | 5.20 + 2.20 | 69 | 5.62 + 2.82 | Third | 29.70 | 29.59 |
| Ebru Tarim | Turkey | 2004 | 28 | 5.70 + 0.90 | 210 | 4.80 + 0.98 | Second | 32.00 | 27.12 |
| Mascarenhas | India | 2014 | 7 | 15.66 + 7.61 | 83 | 14.41 + 7.98 | Third | 24.76 | 21.70 |
| Ji | China | 2013 | 30 | 14.90 + 7.10 | 30 | 15.20 + 6.20 | Second | 30.90 | 28.00 |
| Li | China | 2012 | 153 | 9.81 + 2.97 | 90 | 8.86 + 2.56 | Second | 28.16 | 28.10 |

BMI, body mass index; GDM, gestational diabetes mellitus; HCY, homocysteine.

the third trimester. Except for two Chinese studies that used the International Association for Diabetes in Pregnancy Study Group Criteria to carry out GDM diagnosis, all studies used the criteria suggested by the American Diabetes Association to diagnose GDM. The range of the mean homocysteine levels among women with GDM was 5.20–15.66 mg/dL. In women with normal glucose tolerance, the range of the mean homocysteine levels ranged from 4.45 to 15.20 mg/mL.

### Meta-Analysis

Results from the WMD meta-analysis of homocysteine measurements during pregnancy and GDM are presented in Figure 2. Overall, women with GDM had higher serum homocysteine levels than women with normal glucose tolerance (WMD 0.77, 95% CI 0.44–1.10). The WMDs from the individual studies were analyzed using fixed-effects models, as the heterogeneity was not considered significant with $I^2$ of 27.0% and Tau-square of 0.068. Sensitivity analysis was carried out by omitting studies one by one, which showed that none of these studies alone had a significant effect on the final meta-analysis results. No significant publication bias was found in our meta-analysis by Begg rank correlation analysis ($P = 0.929$) and Egger weighted regression analysis ($P = 0.552$). The Begg's funnel plot with pseudo 95% confidence limits is shown in Figure 3.

To obtain thorough information from this meta-analysis, subgroup analysis was further carried out. Subgroup analysis was carried out by geographic site, the average age of the GDM

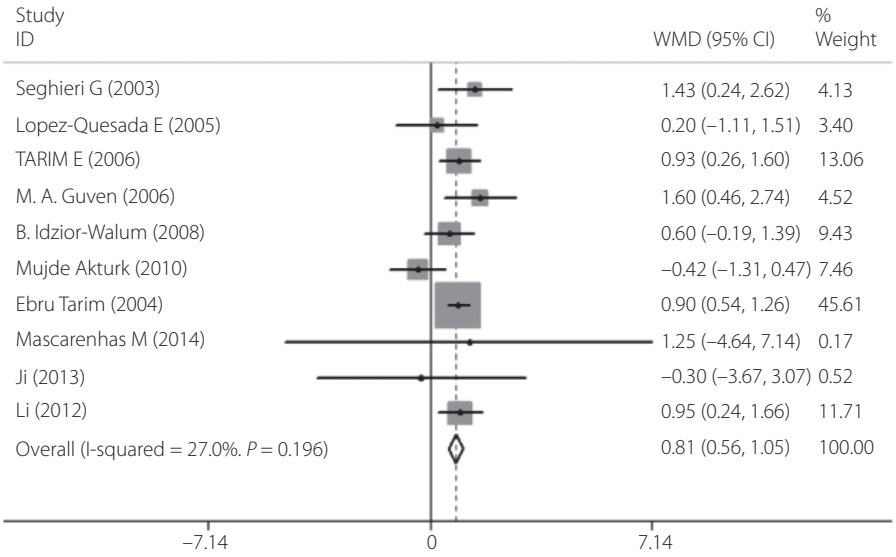


**Figure 2** | Overall meta-analysis of included studies. CI, confidence interval.

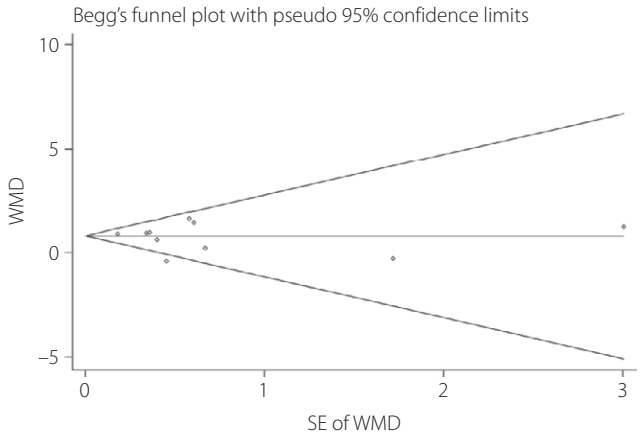


**Figure 3** | Begg's funnel plot with pseudo 95% confidence limits. WMD, weighted mean difference.

group, trimester of serum homocysteine measurement and group mean of body mass index in women with GDM. The comprehensive results are shown in Table 2. When stratifying by geographic site, these studies were classified as the European group and Asian group. The results indicated that both the European group (WMD 0.74, 95% CI 0.32–1.17) and Asian group (WMD 0.90, 95% CI 0.21–1.59) showed higher homocysteine levels among women with GDM. When stratifying by the average age of the GDM group, the 10 studies were classified as average age ≥30 years and <30 years. For women aged older than 30 years, the serum homocysteine level increased among the GDM group (WMD 0.90, 95% CI 0.63–1.17); however, for women aged younger than 30 years, the difference was not significant (WMD 0.33, 95% CI –0.85–1.52). In the subgroup analysis of trimester of serum homocysteine measurement, the difference of homocysteine level between the GDM group and controls was not significant for the measurement of homocysteine during the third trimester (WMD 0.42, 95% CI –0.27–1.10); however, the difference was considered significant for the measurement of homocysteine during the second trimester (WMD 0.95, 95% CI 0.67–1.23). The studies were classified to two subgroups according to the mean body mass index cut-off of 28 kg/m$^2$, both groups showed higher serum homocysteine levels among women with GDM than women with normal glucose tolerance.

## DISCUSSION

GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Women with GDM generally have few symptoms, and it is most commonly recognized by screening during pregnancy. GDM affects a increasing number of pregnant women, different ethnic groups present different incidences of GDM[27]. The precise mechanisms of insulin resistance underlying gestational diabetes remain unknown. It is likely as a result of pregnancy-related factors, such as the presence of human placental lactogen that interferes with susceptible insulin receptors. GDM usually occurs in the second or third trimester, as insulin antagonist hormones reach their peak during these periods[28]. Insulin promotes the entry of glucose into cells, and insulin resistance leads to inappropriately elevated blood glucose levels. It is unclear why some women are unable to generate enough insulin and develop GDM. Autoimmunity, single gene mutations and obesity are the possible explanations[29,30].

Recently, more and more studies have shown that an elevated level of homocysteine in the blood is an independent risk factor for diabetic complications and cardiovascular disease. Microangiopathy has a high incidence among diabetic patients

**Table 2** | Subgroup meta-analysis of the included studies

| Stratification | No. studies | WMD | 95% CI | *P* | Heterogeneity | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | $\chi^2$ | *P* | $\tau^2$ | $I^2$ |
| Geographic site | | | | | | | | |
| European | 7 | 0.74 | 0.32–1.17 | <0.01 | 11.72 | 0.07 | 0.15 | 48.8% |
| Asian | 3 | 0.90 | 0.21–1.59 | 0.01 | 0.52 | 0.77 | 0.00 | 0.00% |
| Average age of GDM group | | | | | | | | |
| <30 years | 3 | 0.33 | –0.85–1.52 | 0.58 | 5.67 | 0.06 | 0.59 | 64.7% |
| ≥30 years | 7 | 0.90 | 0.63–1.17 | <0.01 | 4.35 | 0.63 | 0.00 | 0.00% |
| HCY trimester measurement | | | | | | | | |
| Second | 5 | 0.95 | 0.67–1.23 | 0.76 | 1.86 | 0.76 | 0.00 | 0.00% |
| Third | 5 | 0.42 | –0.27–1.10 | 0.16 | 6.56 | 0.16 | 0.22 | 39.0% |
| BMI of GDM women | | | | | | | | |
| <28 kg/m$^2$ | 5 | 0.85 | 0.55–1.16 | <0.01 | 2.33 | 0.68 | 0.00 | 0.00% |
| ≥28 kg/m$^2$ | 5 | 0.70 | 0.01–1.39 | 0.04 | 9.77 | 0.04 | 0.33 | 59.0% |

BMI, body mass index; CI, confidence interval; GDM, gestational diabetes mellitus; HCY, homocysteine; WMD, weighted mean difference.

compared with non-diabetic patients. Homocysteine could be a determining risk factor for the development of microangiopathy among diabetic patients. These could further lead to elevated blood pressure or even pre-eclampsia in women with GDM. Their relationship should be thoroughly investigated in the future. Serum homocysteine level is influenced by multiple factors, such as age, sex, duration of diabetes, smoking habits, body mass index, impaired renal function, vitamin status and blood pressure, as well as environmental and genetic factors[31–33]. Homocysteine levels decline to their lowest during the second trimester of pregnancy, and increase in the second half of the third trimester of pregnancy[22]. A large number of studies have focused on the relationship between homocysteine and diabetes; however, the investigations between homocysteine and GDM were much fewer, and their results were inconsistent. These inconsistencies might be as a result of the differences in the study design, investigation sample, GDM diagnosis strategy and failure in some studies to make proper adjustments for risk factors. A comprehensive summary was lacking on the relationship between homocysteine and gestational diabetes mellitus. Therefore, we carried out a meta-analysis to assess the relationship between homocysteine and gestational diabetes mellitus. Based on the results of this meta-analysis, serum homocysteine concentration was higher among women with GDM than that of normal controls. The evidence was more consistent among women with homocysteine blood draw during the second trimester and for women aged older than 30 years. There is a natural decrease of serum concentrations of homocysteine during pregnancy. It is associated with the physiological fall in albumin during pregnancy, as well as with folic acid supplementation[34]. Generally, pregnant women would have extra folic acid supplementation during the first trimester, thus there is a high peak of homocysteine level during the second trimester, then a persistent decrease. This could account for the significance of higher homocysteine levels for women with GDM during the second trimester measurement. Other possible reasons were the lack of enough related studies and so on. Furthermore, serum homocysteine levels are also influenced by environmental and genetic factors, as well as by age, duration of diabetes and body mass index[35–37]. Meanwhile, there have been no investigations about the relationship between homocysteine level during the first trimester and GDM. Further studies should be carried out.

After adding the study-assessed homocysteine level in pregnant gestational diabetes women with subclinical atherosclerosis into our meta-analysis, a random-effects model was carried out and the overall result still showed statistical significance (WMD 1.17, 95% CI 0.60–1.74). This finding could provide more credibility to our interpretation of results, still more investigation is required to strengthen this evidence. The hyperglycemic status during pregnancy not only affects the fetus, but also placental development. The underlying mechanisms are not totally unclear, some evidence indicates proper coordination of trophoblast proliferation and apoptosis are required for placental development. Increased expression of villous cytotrophoblast, syncytiotrophoblast, stromal cells and fetal endothelial cells in the human placenta is reported among women with diabetes. Furthermore, reduced apoptotic index and expression of some apoptotic genes are also reported in the placenta of women with GDM[38,39]. More investigations are required to explore the balance between proliferation, apoptosis and differentiation in trophoblast cells among women with diabetes mellitus in order to ensure their relationship.

Women with GDM generally have few symptoms; homocysteine might be a potential predictor for assessing microvascular change in women with GDM. This result could help clinical staff to instruct women with GDM to prevent the progression of GDM. Clinical practice shows that serum homocysteine level can be substantially lowered with folic acid supplementation, which is also a public strategy to prevent congenital neural tube defects.

As far as we know, this is the first meta-analysis evaluating the relationship between serum homocysteine and GDM. The

results of the present meta-analysis could provide evidence for GDM management. However, several limitations of the present study should be addressed. First, only published studies in English and Chinese in the selected databases were included, which might bias the results. Second, because the original data from each study were not all available, we could not carry out a thorough analysis, such as of the human genetic background, pregnancy weight gain, lifestyle and so on. Therefore, the results should be interpreted with caution.

Despite these limitations, the summary of the present meta-analysis suggests that elevated homocysteine level is significantly associated with GDM development, especially for homocysteine measurement during the second trimester. For future studies, well-designed epidemiological studies with large sample sizes among different ethnicities are required. The interaction between genetic polymorphism and serum homocysteine level should also be considered in future studies.

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## DISCLOSURE

The authors declare no conflict of interest.

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