Association between vitamin D levels in early pregnancy and gestational diabetes mellitus: A systematic review and meta-analysis

Kaneez Fatima¹, Muqaddus Asif³, Kanwal Nihal¹, Hassan Ul Hussain¹, Ayeza Waseem Hasan¹, Marium Zahid², Muhammad Husban Burney², Fatima Asad¹, Sarah Fatima¹, Minahil Binte Saleem¹, Muhammad Abdullah Khalid¹

¹Department of Medicine, Dow University of Health Sciences, Baba-e-Urdu Road, Saddar, Karachi, ²Karachi Medical and Dental College, Karachi, Pakistan

Abstract

Background: This meta-analysis aimed to pool all the available data to provide a well-powered assessment of the role of maternal Vitamin D levels in developing gestational diabetes mellitus (GDM) because already published studies evaluating this association are small in sample size and yielded conflicting findings. Material and Methods: A systematic review and meta-analysis of observational studies was performed. We searched electronic databases (PubMed and Cochrane Central) from inception to April 2021 for published and unpublished observational studies that determined the association between the reduction of Vitamin D levels and the risk of developing GDM in pregnant women. Results from studies were pooled as mean ± standard deviation (SD) and odds ratios (OR) using the random-effects model. Results: Forty-four studies, consisting of 37,838 pregnant women were included in this meta-analysis. Dichotomous studies showed a significant association between maternal Vitamin D deficiency and increased risk of GDM (OR = 1.38; 95% confidence interval [CI] = 1.21-1.57; P < 0.00001). Studies with continuous data also showed a significant association between maternal Vitamin D deficiency and the risk of developing GDM (weighted mean difference (WMD): –5.14 nmol/L, 95% CI = –6.28 to –4.00; P < 0.00001). Moderate heterogeneity was also detected. Conclusion: In conclusion, all studies demonstrated that lower levels of maternal serum Vitamin D were associated with a higher risk of developing GDM in pregnancy.

Keywords: Early pregnancy, GDM, gestational diabetes, gravidity, meta-analysis, vitamin D

Introduction

Gestational diabetes mellitus (GDM) commonly defined as glucose intolerance or insulin resistance, continues to be the most common metabolic disorder among pregnant women caused by several modifiable (body mass index [BMI], diet, physical activity, smoking) and non-modifiable (maternal age > 35, type-II diabetes family history) risk factors.[9] According to a recent study, the global prevalence of GDM in pregnant women is reported to be 14.2%. [10] GDM can lead to several maternal (preeclampsia and cesarean section) and fetal (macrosomia and cesarean section) and fetal (macrosomia) complications. It is therefore essential to identify and curb risk factors of GDM because Vitamin D deficiency has been proposed to be a possible risk factor.

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The prevalence of Vitamin D deficiency in pregnant women ranges from 18 to 84%. Vitamin D has been shown to influence insulin sensitivity by affecting the metabolism of calcium and phosphorus and by upregulating the insulin receptor gene, resulting in the reduction of insulin resistance. Therefore, it has been suggested that its deficiency may predispose pregnant women to GDM. However, individual studies evaluating the association between Vitamin D deficiency and incidence of GDM are small in sample size and have yielded conflicting findings. Therefore, we conducted a systematic review and meta-analysis to provide a holistic, well-powered assessment of the association between Vitamin D levels and GDM.

Materials and Methods

This systematic review and meta-analysis was conducted in compliance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines. Because this is a compilation of publicly accessible results, no Institutional Review Board permission (IRB) or patient informed consent was required for this report.

Data sources and search strategy

PubMed and Cochrane CENTRAL were searched from their inception to April 2021, without any language and time restriction. The search string used for both databases was (Vitamin D OR 25 (OH) D OR 25-hydroxyvitamin D OR cholecalciferol OR calcitriol OR ergocalciferol OR calcifediol) AND (Pregnan* OR gravid OR Matern*) AND (Diabetes). A detailed search string has been provided in [Table S1] of Supplements. The reference list of retrieved trials, meta-analyses, and review articles were then manually screened to find any suitable studies.

Study selection

Articles were included based on the following eligibility criteria: (a) the target patient population was pregnant women; (b) the outcome was GDM, with a group of pregnant women with normal glucose tolerance being in the control group and with a group of pregnant women with deficient Vitamin D levels in the experimental group; (c) the relation between Vitamin D deficiency and the risk of GDM was investigated; (d) for comparisons of Vitamin D insufficiency and sufficiency, an effect estimate (odds ratios [OR]) with 95% confidence intervals (CI) was provided or could be calculated.

All studies, including case reports, meta-analyses, or not released as published reports and studies measuring prenatal and postnatal Vitamin D levels were excluded.

Data extraction and quality assessment

The selected articles and related reports from the systemic search were exported to the EndNote Reference Library Software (X7 v17.0.0.7072) where duplicate studies were assessed and then removed. The remaining articles were blind-screened by two reviewers and only those that met the above eligibility criteria were finalized. We searched gray and white literature. Assessment for relevancy was first done based on title and abstract, and then full text. The difference in opinion among reviewers was resolved by group discussion. The concordance rate between the reviewers was 97.5%. We also looked up the bibliographies of related review articles.

Statistical analysis

RevMan (version 5.4; Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) was used for all statistical analyses. The results of the report were calculated as OR with a 95% CI and pooled using a random-effects model. Sensitivity analysis was performed on all results to examine the individual impact of each study. A forest plot was created to visually verify the pooled results. P values are used to assess heterogeneity between studies, with 25% to 50% of P values being considered mild heterogeneity and 50% to 75% being considered moderate heterogeneity. A value greater than 75% is considered severe heterogeneity. A P value of <0.05 was considered significant in all cases. Funnel plots and Egger’s regression test were inspected to eliminate publication bias.

Results

Literature search results

An initial search of three electronic databases identified 575 potential studies. After exclusion, 44 studies remained for analysis. The results of our literature study are summarized in the PRISMA flowchart [Figure 1].

Study characteristics and patients’ baseline characteristics

Baseline characteristics of all included studies are summarized in the supplementary file [Table S2]. Forty-four studies were included in our meta-analysis, which consisted of 20 cohort studies, 8 nested case-control studies, 9 case-control studies, and 7 cross-sectional studies. The earliest study was published in 2008, whereas the latest one was published in 2021.

The sample size of participants in each study ranged from 76 to 4,984. The 44 studies showed a total of 37,838 pregnant women, with 6,694 GDM patients. Different criteria were used for the assessment of Vitamin D levels and the presence of GDM. There were different levels of 25 (OH) D in GDM cases in each study, ranging from 14.19 ± 4.46 to 80.0 ± 21.2 nmol/L. For the Vitamin D cut-off value, 34 studies used 50 nmol/L, 6 studies used 73.5–75 nmol/L, and the remaining 7 studies used 25–37.5 nmol/L.

Quality assessment and publication bias

All studies were of a markedly high methodological quality, with the Newcastle–Ottawa scale ranging from six to nine as depicted by the quality assessment table provided in [Table S3–S5] of supplements/appendix.
The publication bias was evaluated using Funnel plots, Begg's test, and Egger's test. The symmetrical funnel plots [Figure 2a and b] reveal that our analysis holds no small study or publication bias.

**Results of the meta-analysis**

Out of 44 selected studies, 33 of them in [Figure 3] showed that the maternal Vitamin D deficiency was significantly associated with an increased risk of GDM (OR = 1.28; 95% CI = 1.16–1.42; \( P < 0.00001 \)). Sensitivity analyses by removing two studies did not lead to a significant change in the results (OR = 1.38; 95% CI = 1.22–1.57; \( P < 0.00001 \)), but gave us a moderate heterogeneity across the included studies (\( I^2 = 49\% \), \( P = 0.001 \)).

On subgroup analyses by the type of study, case control studies (OR = 1.54; 95% CI = 1.12–2.10; \( P = 0.007 \)), nested case control studies (OR = 1.51; 95% CI = 1.35–1.69; \( P < 0.00001 \)), and cross-sectional studies (OR = 1.83; 95% CI = 1.35–2.48; \( P = 0.0001 \)) showed a significantly increased risk of GDM. No significant increased risk was noted in cohort studies (OR = 1.16; 95% CI = 0.92–1.47; \( P = 0.22 \)).

Out of 44 studies, there were 32 studies (25,760 participants, 5,136 GDM patients, and 20,132 control group) in [Figure 4], which demonstrated that Vitamin D deficiency was significantly associated with an increased risk of GDM. It was observed that the Vitamin D levels were significantly lower in the GDM group than in the control group. However, the mean difference for each study ranged from −33.57 to 11.00. The pooled effect was (WMD: −4.99 nmol/L; 95% CI = −6.73 to −3.26; \( P < 0.00001 \)). Sensitivity analyses by removing four studies led to a significant change in the results and the pooled effect changed to (WMD: −5.14 nmol/L; 95% CI = −6.28 to −4.00; \( P < 0.00001 \)), which shows that Vitamin D level in the experimental group decreased by 5.14 nmol/L when compared with the control group. Moderate heterogeneity was also observed (\( I^2 = 51\% \), \( P = 0.001 \), [Figure 4]).

**Discussion**

This meta-analysis consists of data pooled from 44 studies to evaluate the association between Vitamin D levels and the risk of GDM. These studies comprise a total of 37,838 pregnant women,
of which, 6,694 were GDM patients. Our results suggested a significant association depicting that Vitamin D deficiency can cause GDM in pregnant women, as 25 (OH) D levels in GDM patients dropped by 5.14 nmol/L when compared with the control group. This result corresponds with those of previous meta-analyses on prospective studies that indicated a significantly lower risk of GDM concerning higher levels of 25 (OH) D.[9‑11]

Previous meta-analyses have suggested the association between Vitamin D insufficiency and increased risk of GDM; however, those studies missed some vital observational studies and did not assess the association in terms of study designs. Our meta-analysis includes data from all the important observational studies conducted up till now determining the association between decreased Vitamin D levels and risk of GDM in pregnant women considering study designs as well.

Significant heterogeneity was observed in 33 included studies, which is evident in the different study designs. We performed subgroup analysis and sensitivity analysis was performed to reduce overall heterogeneity. Sub-grouping was done on the basis of the types of study designs present. Sub-group analysis portrays that maternal Vitamin D deficiency and an inclined risk of having GDM are significantly associated only if the following study designs were chosen: Cross-sectional, case–control studies, and nested case–control studies as depicted in [Figure 3]. The OR in the subgroup having only cohort studies was much lesser than ORs in other study designs, hence making cohort studies for this association less reliable in our study, which marks the novelty in our study.

Apart from Vitamin D levels, numerous variables increase the risk of GDM including BMI, age, ethnicity, maternal age, physical activity, and socioeconomic status, which were adjusted for [Table S2].

The positive association suggested by this meta-analysis is reasonable. According to McIntyre et al.[12] pregnancy is a condition that promotes physiological insulin resistance and GDM is the most prevalent medical condition during pregnancy as the pervasiveness of undiagnosed hyperglycemia and overt diabetes in young women is escalating. Maternal overweight and obesity, history of GDM, familial T2DM, and ethnicity are major risk factors involved. Vitamin D has been known to sway glucose by propelling the recovery of physiological insulin secretion through anti-inflammatory properties, increasing duodenal and renal absorption of calcium, which is then available for intracellular signaling activated by insulin, acting on insulin...
receptors assisting in insulin sensitivity, and indirectly by reducing obesity.[13]

Worldwide, 21.3 million pregnancies are associated with hyperglycemia and of these, 18.4 million pregnancies are associated with GDM.[14] It has been documented that low Vitamin D levels not only cause GDM but primary caesarian section, periodic pregnancy loss, high blood pressure in diabetic pregnancy, preterm labor, and postpartum depression can be the major consequences.[15] Our meta-analysis highlights the importance of prenatal management and routine screening of pregnant women for early recognition and suitable commencement of treatment and supplementations. Such attentiveness can potentially counteract the remarkable morbidity associated with GDM.

Our findings suggest that screening should be performed by primary care physicians or obstetricians in women of childbearing age and those in the early stages of pregnancy for Vitamin D deficiency. Vitamin D supplementation should be prescribed in early pregnancy in deficient women.

Limitations
This meta-analysis has certain limitations. Firstly, the diagnostic criteria adopted by different studies were broad as it was determined by different health organizations. Secondly, there were contrasting approaches for methods of assessment of Vitamin D, and cutoff values varied overall. Moreover, there is a confounding bias as some adjusted models were found to differ, whereas some of them could not get adjusted for in the studies. Furthermore, our study does not report any data regarding exposure to sunlight and long-term detrimental outcomes in mothers and their children.

Conclusion
In conclusion, all studies consisting of dichotomous or continuous variables demonstrated that lower levels of maternal serum Vitamin D were associated with a higher risk of developing GDM in pregnancy. We suggest further studies be conducted to assess the usefulness of Vitamin D supplementation in women who are deficient before pregnancy or during pregnancy.

Keypoints
- The meta-analysis was performed to clear the prevailing confusion regarding the association between Vitamin D deficiency in early pregnancy and GDM.

-Lack of locally performed research on this issue and an inadequate number of observational studies included in

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Figure 4: Forest plot comparing Vitamin D levels for the occurrence of GDM. CI, confidence interval; GDM, Gestational Diabetes Mellitus
international meta-analyses are the two factors that pushed us to perform this meta-analysis, making it the most comprehensive and conclusive study on this topic to date.

-The data from 44 studies comprising 37,838 pregnant women were analyzed to reveal a significant association between Vitamin D deficiency and GDM.

-Previously conducted research has pointed out inconsistencies in the screening and management practice of GDM, that is, studies analyzed in our meta-analysis aim to fix by providing primary care physicians with clinically relevant and evidence-based data, which they can incorporate into their daily practice for an overall more streamlined diagnosis and management of GDM.

**Key take-home message**

Pregnant women with Vitamin D deficiency are more prone to suffer from GDM.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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Table S1: Search strategy used in each database

| Database          | Search strategy                                                                 | Obtained articles |
|-------------------|---------------------------------------------------------------------------------|-------------------|
| Medline           | ((("vitamin d"[MeSH Terms] OR "vitamin d"[All Fields] OR "ergocalciferols"[MeSH Terms] OR "ergocalciferols"[All Fields] OR 25[UID]) AND ("hydroxide ion"[Supplementary Concept] OR "hydroxide ion"[All Fields] OR "oh"[All Fields] OR "hydroxyvitamin d"[MeSH Terms] OR "25 hydroxyvitamin d"[Supplementary Concept] OR "25 hydroxyvitamin d"[All Fields] OR "25 hydroxyvitamin d"[All Fields] OR "calcifediol"[MeSH Terms] OR "calcifediol"[All Fields]) OR ("cholecalciferol"[MeSH Terms] OR "cholecalciferol"[All Fields] OR "cholecalciferols"[All Fields] OR "colecalciferol"[All Fields]) OR ("calcitriol"[MeSH Terms] OR "calcitriol"[All Fields] OR "calcitriols"[All Fields]) OR ("ergocalciferols"[MeSH Terms] OR "ergocalciferols"[All Fields] OR "ergocalciferol"[All Fields])) AND ("pregnan*"[All Fields] OR ("gravid"[All Fields] OR "gravids"[All Fields] OR "matern*"[All Fields] OR ("diabetes mellitus"[MeSH Terms] OR "diabetes mellitus"[All Fields] AND "mellitus"[All Fields] OR "diabetes"[All Fields] OR "diabetes insipidus"[MeSH Terms] OR ("diabetes insipidus"[All Fields] OR "diabetes insipidus"[All Fields] OR "diabetes"[All Fields] OR "diabetics"[All Fields] OR "diabetes"[All Fields]))) | 414    |
| Cochrane          | (Vitamin D OR 25(OH)D OR 25-hydroxyvitamin D OR cholecalciferol OR calcitriol OR ergocalciferol OR Calcifediol) AND (Pregnant* OR gravid OR matern*) AND (Diabetes)                                                                 | 155    |
| Study (study year) | Population  | Study Design | Participants | GDM (n) | GDM Criteria* | Assessment of Vitamin D* | Gestational Age | 25(OH)D nmol/L Mean (SD) | Significant† | Cut Off value (nmol/L) | Adjustments‡ |
|-------------------|-------------|--------------|--------------|---------|---------------|--------------------------|----------------|----------------------------|--------------|------------------------|-------------|
| Clifton-Bligh (2008) | Australia | Cohort | 307 | 81 | ADPS | LC-MS | second/third trimester | 48.6 (24.9) | 53.3 (23.3) | yes | 50 | (1), (2), (3) |
| Zhang (2008) | US | Nested case control | 171 | 57 | ADA | ELISA | 24-28 weeks | 60.5 (21.2) | 75.3 (24.3) | yes | 50 | (1), (2), (3), (4) |
| Maghboli (2008) | Iran | Cross sectional | 579 | 52 | C&C | RIA | 24-28 weeks | 16.5 (10.4) | 23.0 (18.3) | yes | 35 | (1), (2) |
| Farrant (2009) | India | Cohort | 599 | 39 | C&C | RIA | <32 weeks | 38.8 (NR) | 37.8 (NR) | no | 50 | (1), (2), (5) |
| Soheilykhan (2010) | Iran | Case control | 165 | 54 | C&C | ELISA | 24-28 weeks | 24.1 (20.7) | 32.3 (35.8) | NR | 50 | NR |
| Savvidou (2011) | UK | Case control | 320 | 100 | WHO | LC-MS | 11-13 weeks | NR | NR | NR | 75 | (1), (2), (3), (6), (7), (8) |
| Makgoba (2011) | UK | Case control | 248 | 90 | WHO | LC-MS | first trimester | 47.2 (26.7) | 47.6 (26.7) | no | 50 | (1), (2), (3), (4), (5), (6) |
| Burriss (2012) | US | Cohort | 1155 | 68 | ADA | CLIA | 26-28 weeks | NR | NR | NR | 25 | (1), (2), (3), (6), (7), (9), (12), (13), (14), (15), (16), (17) |
| Perez ferre (2012) | Spain | Cohort | 266 | 49 | ADA | CLIA | 24-28 weeks | NR | NR | NR | 50 | (1), (3), (4), (11) |
| Baker (2012) | US | Nested case control | 180 | 60 | NDDG | LC-MS | first trimester | 97.0 (29.0) | 86.0 (22.0) | yes | 50 | (1), (2), (6), (9) |
| Wang (2012) | China | Nested case control | 400 | 200 | ADA | ELISA | 26-28 weeks | 22.4 (11.7) | 25.9 (15.8) | yes | 25 | (1), (4), (11) |
| Parkea (2012) | Canada | Nested case control | 335 | 116 | NDDG | CLIA | 15-18 weeks | 56.3 (19.4) | 62.0 (21.6) | yes | 73.5 | (9), (10) |
| Bener (2013) | Qatar | Cohort | 1873 | 260 | WHO | RIA | >24 weeks | NR | NR | NR | 75 | NR |
| Parikdar (2013) | Turkey | Case control | 122 | 44 | IADPSG | CLIA | 24-32 weeks | 48.8 (23.3) | 57.3 (25.0) | no | 50 | NR |
| Zuhur (2013) | Turkey | Cross sectional | 402 | 234 | IADPSG | ECLIA | 24-28 weeks | 30.8 (16.3) | 36.0 (16.2) | yes | 50 | (1), (2), (4), (5) |
| Lacroix (2014) | Canada | Cohort | 655 | 54 | IADPSG | LC-MS | 6-13 weeks | 57.5 (17.2) | 63.5 (18.9) | yes | 50 | (1), (2), (4), (5), (17), (21), (22), (23) |
| Park (2014) | Korea | Cohort | 523 | 23 | C&C | ECLIA | 24-28 weeks | 49.4 (19.4) | 48.0 (24.8) | no | 50 | (1), (2), (5), (6), (9), (20) |
| Kramer (2014) | Canada | Cohort | 524 | 142 | NDDG | ECLIA | Second/third trimester | NR | NR | NR | 50 | (1), (2), (3), (4), (6), (14), (15), (20) |
| Zhou (2014) | China | Cohort | 1953 | 31 | IADPSG | ECLIA | 16-20 weeks | NR | NR | NR | 50 | (1), (2), (24), (25) |
| Schneuer (2014) | Australia | Nested case control | 4900 | 376 | ADPS | AIA | first trimester | 52.1 (22.1) | 56.9 (26.9) | yes | 37.5 | (1), (3), (5), (6), (7), (10), (17), (18), (19) |
| Nobles (2015) | US | Cohort | 237 | 31 | ADA | CLIA | 15.2 weeks | NR | NR | NR | 75 | (1), (2), (3), (6), (9), (14) |
| Loy (2015) | Asian | Cohort | 940 | 155 | WHO | LC-MS | 26-28 weeks | NR | NR | NR | 75 | (1), (2), (3), (5), (7), (12), (15), (17), (19), (26), (27) |
| Rodriguez (2015) | Spain | Cohort | 2382 | 93 | NDDG | HPLC | 13.5 weeks | 71.1 (NR) | 71.0 (NR) | no | 50 | (1), (2), (3), (7), (9), (12), (17), (19), (26), (27) |
| Pleskačová (2015) | Czech | Case control | 76 | 47 | WHO | ELISA | 24-30 weeks | 28.5 (13.0) | 31.7 (16.0) | no | 50 | (2) |
| Arnóld (2015) | US | Nested case control | 652 | 135 | ADA | LC-MS | <20 weeks | 68.3 (21.8) | 73.3 (20.8) | yes | 50 | (1), (2), (3), (4), (6) |
| Boyle (2016) | New Zealand | Cohort | 1544 | 32 | ADHB | LC-MS | 15 weeks | 61.6 (23.9) | 72.9 (27.0) | yes | 50 | (2), (3) |
| Dodds (2016) | Canada | Nested case control | 2320 | 395 | CDA | AIA | before 20 weeks | 45.5 (20.8) | 51.9 (21.8) | NR | 50 | (1), (2), (3), (6), (9), (28), (29) |
Table S2: Contd...

| Study (study year) | Population | Study Design | Participants | GDM (n) | GDM Criteria | Assessment of Vitamin D | Gestational Age | 25(OH)D nmol/L Mean (SD) | Significant | Cut Off value (nmol/L) | Adjustments |
|--------------------|------------|--------------|--------------|---------|--------------|------------------------|----------------|--------------------------|-------------|------------------------|-------------|
| Wen (2017)         | China      | Nested case control | 4718         | 1280    | ADPS        | ELISA                  | Second/third trimester | 42.4 (19.5) | 44.3 (22.8) | yes | 50 | (1), (2), (3), (4), (5), (6), (17), (30), (31), (32), (33), (34) |
| Hauta‑alus (2017)  | Finland    | Cross sectional | 723          | 81      | ADA         | CLIA                   | 7-25 week           | 80.0±22.1 | 81.9±19.5 | no | 50 | NR |
| Eggemoen (2018)    | Oslo, Norway | Multi-ethnic cohort | 745          | 235    | WHO         | RIA                    | First-second trimester | 47.7 (44.0, 51.3) | 51.4 (49.2, 53.7) | no | 50 | (1), (3), (6), (12), (17), (35), (36) |
| Al ajlan (2018)    | Saudi Arabia | Cohort       | 419          | 116    | IADPSG      | ECLIA                  | first trimester     | 26.3 (14.59) | 28.23 (18.22) | yes | 50 | (1), (2), (4), (6), (11), (14), (15), (17), (20), (37), (38), (39) |
| Raiput (2019)      | India      | Cross sectional | 100          | 50     | NR          | ELISA                  | second trimester    | 32.6±24.33 | 39.9±21.86 | yes | 50 | NR |
| Dwarkanath (2019)  | India      | Cohort       | 392          | 40     | IADPSG      | LC‑MS/MS               | ~12 weeks           | 34±17.4     | 37.5±19.2 | no | 50 | (1), (6), (10), (12), (15), (17) |
| Shao (2019)        | Chinese    | Cohort       | 3318         | 718    | IADPSG      | LC‑MS/MS               | first-second trimester | 48.75±21.25 (T1) | 45.75±21.5 (T1) | yes | 50 | (1), (6) |
| Ede (2019)         | Turkey     | Cross sectional | 80           | 40     | C&C         | HPLC                   | second trimester    | 42±24.75   | 52.25±20.40 | yes | 35 | (1), (2), (14), (17) |
| Fernando (2020)    | Australia  | Cohort       | 304          | 55     | ADIPS       | CLIA                   | <20 week            | 55±19.2     | 50.7±24.4 | yes | 75 | (1), (2), (3) |
| Wang (2020)        | China      | Case control  | 81           | 41     | IADPSG      | ECLIA                  | 24-28 weeks         | 14.19±4.46 | 19.16±7.97 | yes | NR | NR |
| Salakos (2020)     | France & Belgium | Nested case control | 1191       | 250    | WHO         | NR                     | first trimester (11-15 weeks) | 52.75±25   | 56.75±25 | no | 25 | NR |
| Iqbal (2020)       | India      | Cohort       | 290          | 45     | IADPSG      | LC‑MS/MS               | first trimester     | 33±16.3     | 38.2±18.5 | no | 50 | (1), (6), (10), (12), (15), (17) |
| Cabrera (2020)     | Philippines | Cross sectional | 211         | 56     | ADPSG       | ECLIA                  | NR                  | 21.0±8.1   | 18.7±5.3 | no | 50 | (1), (2), (5), (17) |
| Ren (2020)         | China      | Case control  | 99           | 51     | NR          | ELISA                  | NR                  | 50.85±12.8 | 61±13.55 | NR | 57.7 | NR |
| Alahlhol (2020)    | Saudi Arabia | Cross sectional case control | 322       | 82     | NR          | ELISA                  | 22-37              | 17.7±4.6   | 28.46±22.77 | yes | 50 | NR |
| Yasqong (2020)     | China      | Cross sectional case control | 210       | 110    | NR          | ELISA                  | after 24 weeks     | 48.07 (24.6) | 81.64 (35.16) | yes | 25 | (1), (2), (4), (5), (12) |
| Magnusdottir (2021)| Iceland    | Cohort       | 837          | 126    | IADPSG      | ECLIA                  | 11-14 weeks        | 60±24       | 63±24       | no | 50 | (1), (2), (7), (17) |

Significant difference in serum 25(OH)D between gestational diabetes and controls. *Assay method of 25(OH)D. AD: radioimmunoassay; LC: liquid chromatography; MS: mass spectrometry; ELISA: enzyme-linked immunosorbent assay; CLIA: chemiluminescence immunoassay; ECLIA: electrochemiluminescence immunoassay; AIA: automated immunoassay; HPLC: High Performance Liquid Chromatography. †Diagnostic criteria of gestational diabetes: C&C: Carpenter and Coustan; ADPS: Australasian Diabetes in Pregnancy Society; ADA: American Diabetes Association; WHO: World Health Organization; NDDG: National Diabetes Data Group; IADPSG: International Association of the Diabetes and Pregnancy Study Groups; ADHB: Auckland District Health Board; CDA: Canadian Diabetes Association. | Adjustments (1) age; (2) body mass index (BMI); (3) ethnicity; (4) family history of diabetes; (5) previous history of diabetes; (6) season; (7) smoking; (8) method of conception; (9) gestational age; (10) maternal weight; (11) triglyceride (TG); (12) education; (13) marital status; (14) pregnancy weight gain; (15) physical activity; (16) dietary intake of fish and calcium; (17) parity; (18) previously diagnosed hypertension; (19) socio-economic status; (20) vitamin D intake; (21) vitamin D lifestyle score; (22) parathyroid hormone (PTH); (23) waist circumference; (24) systolic/diastolic pressure; (25) serum calcium; (26) no smoking; (27) alcohol consumption; (28) study site; (29) year of blood collection; (30) maternal age; (31) trimester; (32) abnormal pregnancy history; (33) history of uterine fibroids; (34) sum of skin folds at visit 1; (35) change in skin folds from visit 1 to visit 2; (36) sun exposure; (37) HbA1c; (38) waist to hip ratio; (39) fasting blood glucose. NR, not reported.
| Study                        | Selection | Representativeness of exposed cohort | Selection of non-exposed cohort | Ascertainment of exposure | Demonstration that outcome of interest was present or not at start | Adjustment for the most important risk factors | Adjustment for other risk factors | Assessment of outcome | Follow-up length | Loss of follow-up length | Total Quality Score |
|-----------------------------|-----------|-------------------------------------|---------------------------------|---------------------------|-------------------------------------------------------------------|---------------------------------------------|--------------------------------|----------------------|---------------------|------------------------|---------------------|
| Clifton-Bligh (2008)        | 0         | 1                                   | 0                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 0                   | 6                     |
| Farrant (2009)              | 1         | 1                                   | 0                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 1                   | 7                     |
| Burris (2012)               | 1         | 1                                   | 0                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 1                   | 7                     |
| Perez ferre (2012)          | 1         | 1                                   | 0                               | 0                         | 1                                                                 | 1                                           | 1                              | 1                    | 1                   | 6                     |
| Bener (2013)                | 1         | 1                                   | 1                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 0                   | 8                     |
| Lacroix (2014)              | 1         | 0                                   | 0                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 0                   | 6                     |
| Park (2014)                 | 1         | 1                                   | 0                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 0                   | 6                     |
| Knmer (2014)                | 1         | 1                                   | 0                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 0                   | 7                     |
| Zhou (2014)                 | 1         | 1                                   | 1                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 0                   | 8                     |
| Nobles (2015)               | 1         | 0                                   | 1                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 1                   | 8                     |
| Loy (2015)                  | 1         | 1                                   | 0                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 1                   | 8                     |
| Rodriguez (2015)            | 1         | 0                                   | 1                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 1                   | 8                     |
| Boyle (2016)                | 1         | 0                                   | 1                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 1                   | 8                     |
| Eggemoen (2018)             | 1         | 1                                   | 1                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 1                   | 9                     |
| Al ajlan (2018)             | 1         | 1                                   | 1                               | 1                         | 0                                                                 | 1                                           | 1                              | 1                    | 1                   | 8                     |
| Dwarkanath (2019)           | 1         | 1                                   | 1                               | 0                         | 1                                                                 | 1                                           | 1                              | 1                    | 0                   | 7                     |
| Shao (2019)                 | 1         | 1                                   | 0                               | 0                         | 1                                                                 | 1                                           | 1                              | 1                    | 1                   | 7                     |
| Fernando (2020)             | 1         | 1                                   | 1                               | 0                         | 1                                                                 | 1                                           | 1                              | 1                    | 1                   | 8                     |
| Iqbal (2020)                | 0         | 1                                   | 0                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 1                   | 7                     |
| Magnusdottir (2021)         | 1         | 1                                   | 0                               | 1                         | 1                                                                 | 1                                           | 1                              | 1                    | 0                   | 7                     |
Table S4: A detailed Newcastle-Ottawa Scale of each included case control study:

| Study            | Case definition adequate | Representativeness of the cases | Selection of control | Definition of controls | Adjustment for the most important risk factors | Adjustment for additional risk factors | Ascertainment of exposure | Same method of ascertainment for cases and controls | Non-response rate | Total Quality Score |
|------------------|---------------------------|-------------------------------|----------------------|------------------------|-----------------------------------------------|---------------------------------------|--------------------------|------------------------------------------------------|-----------------|---------------------|
| Zhang (2008)     | 1                         | 1                             | 1                    | 1                      | 1                                             | 1                                     | 0                        | 1                                                   | 0               | 7                   |
| Soheilykhak (2010) | 1                     | 1                             | 1                    | 1                      | 1                                             | 0                                     | 1                        | 1                                                   | 0               | 7                   |
| Savvidou (2011)  | 1                         | 1                             | 1                    | 1                      | 1                                             | 1                                     | 1                        | 1                                                   | 0               | 8                   |
| Makgoba (2011)   | 1                         | 1                             | 1                    | 1                      | 1                                             | 1                                     | 1                        | 1                                                   | 0               | 7                   |
| Baker (2012)     | 1                         | 1                             | 1                    | 1                      | 1                                             | 1                                     | 1                        | 0                                                   | 0               | 7                   |
| Wang (2012)      | 1                         | 0                             | 1                    | 1                      | 1                                             | 1                                     | 0                        | 1                                                   | 0               | 6                   |
| Parlea (2012)    | 1                         | 1                             | 1                    | 1                      | 1                                             | 1                                     | 0                        | 1                                                   | 0               | 7                   |
| Parildar (2013)  | 1                         | 0                             | 1                    | 1                      | 1                                             | 0                                     | 1                        | 1                                                   | 0               | 6                   |
| Schneuer (2014)  | 1                         | 1                             | 1                    | 1                      | 1                                             | 1                                     | 1                        | 1                                                   | 0               | 8                   |
| Pleskacová (2015)| 1                        | 1                             | 1                    | 1                      | 1                                             | 1                                     | 1                        | 1                                                   | 0               | 7                   |
| Arnold (2015)    | 1                         | 1                             | 1                    | 1                      | 1                                             | 1                                     | 1                        | 1                                                   | 0               | 8                   |
| Dodds (2016)     | 1                         | 1                             | 1                    | 1                      | 1                                             | 1                                     | 1                        | 1                                                   | 0               | 8                   |
| Wen (2017)       | 1                         | 1                             | 1                    | 1                      | 1                                             | 0                                     | 1                        | 1                                                   | 0               | 7                   |
| Wang (2020)      | 1                         | 0                             | 1                    | 1                      | 1                                             | 1                                     | 0                        | 1                                                   | 0               | 6                   |
| Salakos (2020)   | 1                         | 1                             | 1                    | 1                      | 1                                             | 1                                     | 1                        | 1                                                   | 0               | 8                   |
| Ren (2020)       | 0                         | 1                             | 1                    | 1                      | 1                                             | 0                                     | 0                        | 1                                                   | 1               | 6                   |
| Albahlool (2020) | 1                         | 1                             | 1                    | 1                      | 1                                             | 0                                     | 1                        | 1                                                   | 0               | 7                   |
Table S5: A detailed Newcastle-Ottawa Scale of each included cross-sectional study:

| Study              | Selection                  | Comparability           | Outcome         | Total Quality Score |
|--------------------|-----------------------------|-------------------------|-----------------|---------------------|
|                    | Representativeness of the sample | Ascertainment of the exposure (risk factor) | Confounding factors controlled | Assessment of outcome | Statistical test     |
| Maghbooli (2008)   | 1                           | 1                       | 2               | 1                   | 1                    | 7                   |
| Zuhur (2013)       | 1                           | 1                       | 2               | 1                   | 1                    | 7                   |
| Hauta-alus (2017)  | 1                           | 0                       | 1               | 1                   | 1                    | 6                   |
| Rajput (2019)      | 0                           | 1                       | 1               | 1                   | 1                    | 6                   |
| Ede (2019)         | 1                           | 0                       | 1               | 1                   | 1                    | 7                   |
| Cabrera (2020)     | 1                           | 1                       | 2               | 1                   | 1                    | 7                   |
| Yaqiong (2020)     | 1                           | 0                       | 2               | 1                   | 1                    | 7                   |