Review Article

Maternal Chromium Levels in Gestational Diabetes: Systematic Review and Meta-Analysis

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Abstract

The topic of maternal Chromium (Cr) levels in Gestational Diabetes Mellitus (GDM) has remained controversial; some studies have found lower levels of Cr in GDM population, whereas others found no significant changes in Cr status in GDM. Therefore, this systematic review and meta-analysis was aimed at qualitatively and quantitatively synthesizing past studies to find the relationship of maternal Cr levels with GDM. The study protocol was registered at International prospective register for systematic reviews (PROSPERO) (ID CRD42021272979). Strict adherence to the Preferred Reporting Items for Systematic review and Meta-analysis checklist, 2009 was followed during the entire study. Random-effect model for calculation of distribution of true effect sizes was used for the meta-analysis with a confidence interval (CI) of 95%. The pooled Standard Mean Difference of control and GDM groups were compared using Z statistics with a *P* value of <.05 as significant. Six studies were included for the systematic review and four studies entered meta-analysis. The test of overall effect revealed that the pooled Cr values did not differ significantly between controls and GDM group (Z = 1.52, *P* =0.13). Heterogeneity between the studies was high (I^2 = 97%). A subgroup analysis revealed that results varied as per place of study, trimester of pregnancy, and Cr estimation technique. Results from meta regression analysis revealed that sample size of individual studies (Q = 0.003, *P* =0.67) and year of publication of studies (Q = 0.22, *P* =.48) had no significant effect on the overall Standard Mean Difference. Factors such as ethnicity, lack of history of infection, and diet history can influence the results of this study.

Keywords: Chromium, gestational diabetes mellitus, meta-analysis, systematic review

Introduction

Gestational Diabetes Mellitus (GDM) is a disturbance in carbohydrate metabolism which manifests during pregnancy leading to maternal hyperglycemia.[1] Although this condition subsides after pregnancy in some cases, it leads to glucose intolerance in women after delivery.[2]

Pregnancy is characterised by an increased demand for glucose as a source of fuel supply to the foetus.[3] The body meets this demand by undergoing a number of physiological and biochemical changes. One such change is an increase in insulin resistance (IR).[3] Increased IR increases circulating levels of glucose that can diffuse across placenta to the foetus.[3] IR gradually increases as pregnancy progresses from first to second and third trimester.[4,5] Failure to counter the growing IR due to inability to simultaneously increase insulin production can lead to GDM in pregnancy.[3]

IR in pregnancy occurs due to changes in insulin receptor functioning and signal transduction mechanisms.[5] Placental hormones such as progesterone, estrogen, and human placental lactogen are known to cause changes in IR.[4] Progesterone decreases insulin-mediated migration of GLUT 4 receptors to cell surface, whereas high estrogen concentration is characterized by decreased receptor sensitivity to insulin.[6]

Trivalent chromium (Cr) is considered an an essential element with a recommended daily allowance of 50–200 μg/day for adult men and women.[7] Once absorbed from the intestine, Cr binds to transferrin protein in blood. Transferrin when attached

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to transferrin receptors is endocytosed in the cell. Inside the acidic medium of endocytosed vesicle, Cr is detached from transferrin and binds to low–molecular weight Cr protein (LMWCr) converting it from an inactive or apo form to an active form.[7] A number of clinical studies suggests that use of parenteral Cr in Type 2 DM reduces glucose intolerance.[7] Molecular researchers have found that this beneficial effect of Cr on glucose metabolism is due to its ability to potentiate insulin action.[7]

Insulin receptor is a tyrosine-kinase receptor. Binding of insulin to receptor triggers dimerization and autophosphorylation of tyrosine residues of beta subunit of insulin receptor.[8] This causes phosphorylation of insulin receptor substrate IRS 1 and IRS 2. Phosphorylated IRS triggers a downstream of signal transduction pathways notably through phosphoinositol 3,4,5 tri phosphate kinase (PI3 kinase) and mitogen-activated protein kinase (MAP kinase).[8]

Studies by Wang *et al*.[9] demonstrated that Cr was involved in upregulating the phosphorylation of IRS, PKB, and PI3 kinase that enhances the insulin action, although these results were produced in animal models in a controlled laboratory setting and exact mechanism of action of Cr on insulin signalling in humans is still speculated.[8] Laboratory studies have also pointed out that the beneficial effect of Cr on glucose metabolism was absent in euglycemic animal models with normal IR.[10,11] This is consistent with clinical trials showing a negligible effect of Cr supplementation on insulin signalling in euglycemic subjects.[12] The action of Cr mostly manifests through two proteins: LMWCr and glucose tolerance factor (GTF).[4] LMWCr is an oligopeptide that has been found to bind to insulin receptor and enhance its tyrosine-kinase activity. However, this action is mediated only when LMWCr is attached to Cr.[7] GTF was first identified from brewer's yeast.[7] Cr is a component of GTF produced in vivo. GTF has been postulated to enhance an insulin signalling pathway in many studies.[7]

Pregnancy is characterized by hemodilution due to an increase in blood volume which increases the glomerular filtration rate.[4] An increased urinary output can lead to loss of Cr from maternal circulation.[13] If sufficient dietary replacement is not met, this can aggravate the growing IR that can predispose to GDM[4] [Figure 1].

Assessment of Cr levels in GDM has been attempted by cross-sectional and longitudinal studies in the past. However, these studies are conducted in different regions, trimesters of pregnancy, and employ different methods of Cr assessment and are not sufficient to provide conclusive evidence. There is a need to synthesize and meta-analyse past studies to evidence the role of Cr in GDM.

In this study, we hypothesize that in pregnant women, the diagnosis of GDM is associated with a decrease in tissue/plasma Cr levels as compared with normoglycemic pregnant women.

Methodology

Study protocol and structure

The study protocol was registered in PROSPERO (ID CRD42021272979). Preferred Reporting Items for Systematic review and Meta-analysis (PRISMA) guidelines were used to make a protocol for the systematic review before its commencement.[14]

Screening and checking for eligibility of the studies was done following PRISMA guidelines.[15] Strict adherence to the PRISMA checklist 2009 was followed during the entire study.[16]

Data sources and search strategy

Electronic search was done using legitimate indexing services or search engines, namely, PubMed, Google scholar, Embase, and SCOPUS. The search was done using a combination of keywords or MeSH terms with Boolean operators which was predetermined and mentioned in the protocol. The combination was (chromium [MeSH] OR trace elements [MeSH]) AND (gestational diabetes [MeSH] OR pregnancy induced diabetes OR diabetes in pregnancy).

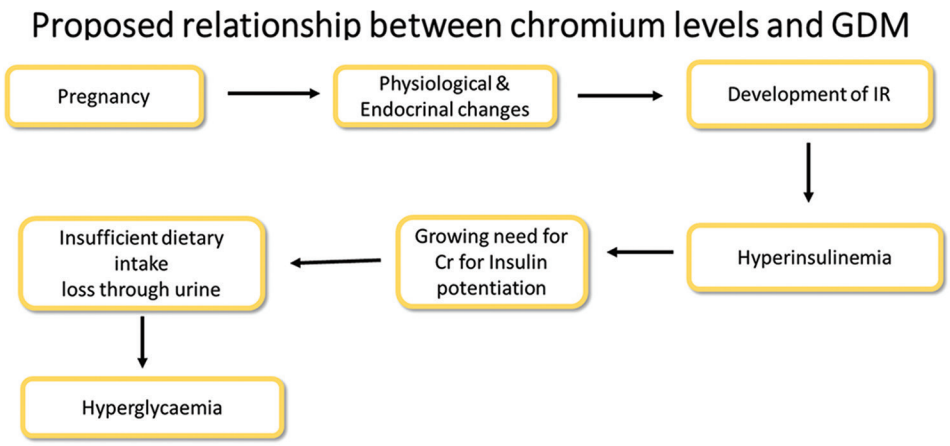

Figure 1: Figure depicting proposed relationship of Cr with GDM

Inclusion and exclusion criteria

The screening and checking of eligibility of the studies was performed using a set of inclusion and exclusion criteria that were predetermined in the study protocol. Studies involving pregnant women exposed to GDM measuring serum/tissue Cr levels were included in the study. Studies conducted from year 1991 to 2021, written in English language, and having cross-sectional/longitudinal case control or cohort design were included.

Studies with women diagnosed with diabetes before pregnancy, case reports/case series/review articles/commentaries based on Cr levels in GDM, animal-based studies, and studies involving giving Cr supplementation to GDM cases were excluded from the systematic review.

Screening and eligibility of the studies

Reference lists obtained from the mentioned sources were exported to Zotero software, version 5.0 in a compatible format. References obtained from all four databases were combined into a single folder. Duplicates and studies not written in English were removed using the Zotero software followed by a careful visual inspection. Screening of the studies using the inclusion and exclusion criteria was done independently by two reviewers. Initial screening was done using the title and abstract. After the initial screening, a careful inspection of the full texts of the remaining studies was carried out. Any conflict between the two reviewers was resolved by the remaining reviewers.

Data extraction

Data from the included studies were extracted on an excel sheet. Data extraction was done using the following headings: title, first author, year of publication, research design, country of research, sample size (sum of GDM and controls), number of GDM participants, number of control participants, and trimester of pregnancy. Body mass index (BMI), type of sample collected, mean with standard deviation (Std) of Cr levels, and other biochemical markers including fasting plasma glucose (FPG), glycated hemoglobin (HbA1C), homeostatic assessment for IR (HOMA-IR), and fasting insulin levels in specific units of measurements were recorded separately for GDM and control group (with CI of 95%).

Critical appraisal of studies

Critical appraisal or risk of bias assessment of studies was done using the Joanna Briggs Institute (JBI) critical appraisal checklist for observational studies, University of Adelaide, Australia.[17] Two reviewers conducted the critical appraisal to check for internal and external validity of the studies and assess how each study has attempted to reduce risk of bias in design, sampling, data collection, analysis, and publication. The JBI critical appraisal checklist consists of questions with three predefined answers: Yes, No, or Maybe. One score will be given for each answer Yes. The appraisal was done separately by the two reviewers. Average of the two scores for each study was calculated. If the average was 50% or more of the total score, the study was included for review synthesis and meta-analysis.

Outcome measurement

The main outcome measurement is in the form of a pooled standard mean difference (SMD) of Cr levels in control and GDM groups to analyse if there is any statistical difference between the two. A meta-analysis was run to find changes in Cr levels in GDM. A subgroup analysis was performed for geographical location, method of Cr estimation, and trimester of pregnancy. A meta-regression analysis was done to decipher the influence of BMI and parity on Cr levels in the GDM group.

Data analysis

For a meta-analysis, subgroup analysis, and calculation of publication bias, RevMan 5.3 Software (Cochrane Organization, England) was used. The meta-analysis results were presented in the form of a Forrest plot, whereas publication bias was viewed through Begg's Funnel Plot. Random-effect model for calculation of the distribution of true effect sizes was used for the meta-analysis with a CI of 95%. Through Hedge's adjusted G statistics, pooled SMD was calculated as the main outcome measure for both the control and GDM groups. Statistical heterogeneity was calculated between studies using I^2 statistics. The pooled SMD of control and GDM groups were compared using Z statistics with a *P* value of <.05 as significant. STATA software was used for meta regression, meta-influence analysis, and forming the funnel plot.

Results

Search results

One thousand three hundred seventy four studies were retrieved for screening and checking eligibility through web search on legitimate indexing websites and search engines (355 from PubMed, 263 from Google Scholar, 33 from Embase, and 723 from SCOPUS). Of 1,374 studies, 93 duplicate studies and 23 studies not written in English were removed with Zotero software and manual inspection. One thousand two hundred fifty eight studies entered record screening by checking titles and abstracts. Using the inclusion and exclusion criteria, 1,243 studies were removed. The full texts of 27 studies were carefully read and nine studies were removed for various reasons (four studies were removed because they involved type 2 diabetic patients, one was removed because there was no control group, one provided ambiguous results, and three were removed because they measured trace elements other than Cr). Studies by Roverso *et al*.[18] and Aharoni *et al*.[19] were excluded from the meta analysis because they measured Cr levels from placenta and hair in GDM population, which differed qualitatively from the other studies that used serum for Cr estimation [Figure 2].

Study characteristics

Six studies measuring Cr levels in GDM were included in the systematic review [Table 1]. The studies were published

in years ranging from 1992 to 2018. The studies belonged to different geographical locations—two from Europe,[4,18] three from Asia,[19-21] and one from America.[22] Five studies were case control studies[4,18-21] and one was a prospective cohort study.[22] One study recruited subjects in the third trimester,[4] one study recruited subjects in the second and third trimesters,[21] two studies in the second trimester,[19,20] one prospective cohort study obtained the first sample in the first trimester and the second sample in the second trimester,[22] and one study obtained samples at the time of parturition.[18] To measure Cr levels, four studies obtained serum samples,[4,20-22] one study hair samples,[19] and one study placenta samples.[18] For Cr estimation, one study used graphite furnace atomic absorption spectrometry,[4] two studies used atomic absorption spectrometry,[19,21] and three studies used inductive couple plasma emission spectrometry.[18,20,22] Four studies entered meta-analysis for pooled SMD estimation of Cr levels between GDM and controls.

Results from critical appraisal

Rigorous appraisal of included observational studies (case-control and cross-sectional studies with 10-point and 9-point scales, respectively) resulted in average quality scores ranging between 5 and 10. Fortunately, all included studies fulfilled the minimum criteria and retained for systematic review and meta-analysis [Table 2].

Results from meta-analysis

One hundred fifty six GDM cases and 938 controls were recruited in the meta-analysis for pooled Cr SMD estimation [Figure 3]. The studies are identified by the first author and are arranged as per the total weight contribution in the pooled SMD, with the study having the lowest weight present at the bottom of the plot. The mean and standard deviation of Cr levels in GDM and control groups were used to calculate the SMD and weight of the study. In the graph, the weight of each study is represented in the form of boxes with horizontal lines representing the extent of 95% CI. The peaks of the diamond in the bottom represent the pooled SMD estimate, whereas the size of the diamond is proportional to the CI of the pooled SMD. The central line denotes the line of no effect and the demarcations in the horizontal line represent point estimates. Shift to the left represents Cr levels higher in the GDM group, whereas shift to the right represents Cr levels higher in the control group of studies. The pooled SMD of the studies was -0.99 with 95% CI (-2.26, 0.29). The test of overall effect denotes that the pooled Cr values did not differ significantly between controls and the GDM group (Z = 1.52, *P* =0.13) [Figure 3]. Although not commonly practised in publications, we compared the two groups using the pooled SMD as a measure of comparison. Approximately less than 50% of the control group samples were below the mean of the GDM group, indicating a small effect.[23] The CI of pooled SMD varied from -2.26 to 0.29 and the wide range of CI indicates lower precision and more sample size is needed for meta-analysis to provide a concise CI.[23] Heterogeneity between the studies was high (I^2 = 97%)

Table 1: Study characteristics of GDM and controls of included studies

First author	Year of publication	Country	Study design	Cases/ control	Trimester of pregnancy	Type of sample	Method of Cr estimation	Cr controls (Mean±Std or Mean (95% CI)	Cr cases (Mean±Std or Mean (95% CI)
Houldsworth A *et al.*[4]	2017	Britain	Cross sectional Case control	8/26	Third trimester	Serum	GFAAS[#]	6.41±1.24	8.20±2054
PG Sunderraman *et al.*[20]	2018	India	Cross sectional Case control	30/60	Second trimester	Serum	ICP MS*	4.58±0.62	1.59±0.02
Reza Dididar *et al.*[21]	2016	Iran	Cross sectional Case control	60/60	Second & third	Serum	AAS^	7.76±2.83	6.52±2.74
Aharoni *et al.*[19]	1992	Israel	Prospective case control	68/42	Second	Hair	AAS^	472±61	734±155
Woods *et al.*[22]	2008	USA	Cohort study	29/396	1st visit- First trimester 2nd visit- Second trimester	Serum	ICP MS*	0.15±0.06, 0.14±0.06	0.14±0.03, 0.14±0.04
Roverso *et al.*[18]	2015	Italy	Cross sectional case control	28/19	At parturition	Placental	ICP MS	0.61 (0.020–11)	NA

[#]GFAAS- Graphite furnace atomic adsorption spectrometry. *ICP MS- Inductive coupled plasma mass spectrometry. ^AAS- Atomic adsorption spectrometry

as a result of which subgroup analysis and meta-regression analysis were performed to assess the sources of heterogeneity between the studies.

Results from sub group analysis

Subgroup analysis on the basis of the continent of the study showed that Cr concentration between controls and GDM group did not differ for continents America (SMD = -0.09, Z score = 1.16, *P* =0.53) and Asia (SMD = -3.12, Z Score = 1.16, *P* =0.40) [Table 3]. Test for subgroup differences revealed that results varied as per the place of study (X^2 = 9.53, *P* =.01*). Subgroup analysis on the basis of trimester of pregnancy revealed that Cr levels were different in GDM and control group in studies conducted in the third trimester (SMD = 1.21 Z Score = 2.80, *P* =.005) and during both second and third trimester (SMD = -0.44, Z score = 2.39, *P* =.02*). Test for subgroup differences revealed that results varied as per the trimester of pregnancy in which samples were taken (X^2 Score = 13.21, *P* =.005*). Subgroup analysis on the basis of method for Cr estimation revealed that test for overall effect was significant when samples were assessed using graphite furnace spectrometry (Z score = 2.80, *P* =.005*) [Table 3]. Test for subgroup differences revealed that the test results varied with the technique of Cr estimation (X^2 = 14.56, *P* =.03*).

Results from meta regression analysis

Univariate meta regression analysis was performed to find the influence of factors, sample sizes of individual studies, and year of publication on overall SMD of Cr levels [Table 4]. Meta regression analysis with sample size was done to avoid the small sample size effect in meta-analysis. Year of publication can also affect the SMD as with time the criteria for diagnosis of GDM have changed, also change in methods of Cr estimation with time can also affect the results.

Results from meta regression analysis reveal that year of publication of individual studies (Q = 0.22, *P* =0.48) and the sample size of studies (Q = 0.003, *P* =0.67) had no significant effect on the overall SMD of Cr estimation. Bubble plots of meta regression analysis were derived, Y-axis determines the log of SMD, whereas X-axis denotes the sample size of individual studies [Figure 4a], and year of publication [Figure 4b]. The size of study points or 'bubbles' is directly proportional to the precision or inversely proportional to the variance of log of SMD of Cr levels of each study.

Result from meta influence analysis

Metainfluence analysis was carried out using the 'leave-me-out' method.[23] Each study was omitted turn by turn k number of times, where k is the total number of studies. The graph shows point estimate and CI after removal of each study and

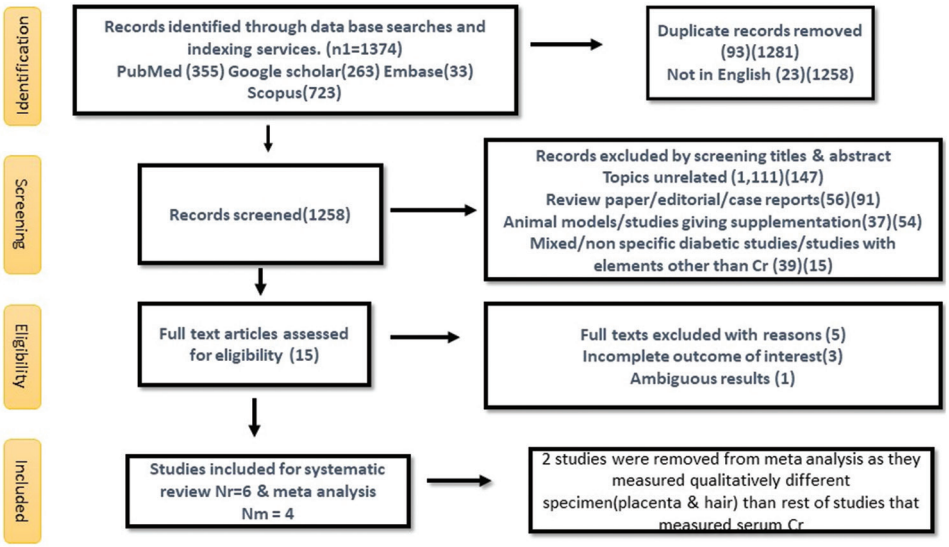

Figure 2: PRISMA flow diagram showing selection process of included studies

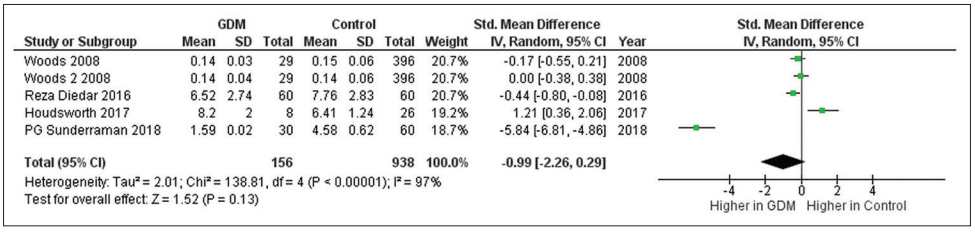

Figure 3: Results from meta analysis for pooled Cr estimates in controls and GDM

Table 2: Critical appraisal of included studies

Study	JBI Critical appraisal tools questions																				Total marks		Average marks
Case Control	Q.1		Q.2		Q.3		Q.4		Q.5		Q.6		Q.7		Q.8		Q.9		Q.10				
Houldsworth A *et al*. 2017[4]	U	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	U	Y	Y	Y	Y	Y	Y		8	10	9
PG Sunderamam *et al*., 2018[20]	Y	Y	Y	Y	U	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	U		7	10	8
Reza Didedar *et al*., 2016[21]	Y	Y	U	Y	Y	Y	Y	Y	U	Y	N	N	U	N	Y	N	U	Y	Y		5	8	5.8
Aharoni *et al*., 1992[19]	Y	Y	U	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	Y	Y	Y	U		8	8	8.8
Marco Roverso *et al*., 2015[18]	Y	Y	Y	Y	U	U	Y	Y	Y	Y	U	U	U	Y	Y	Y	Y	Y	Y		7	8	7.8
Cohort Study																							
Scott E. Woods *et al*., 2008[22]	Y	N	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	U	Y	N	N	Q.10-U Q.11-Y Q.12 Y	Q.10 N Q.11 Y Q 12 Y	9/12	7/12	9.5/12

*The two columns below each question contains the answers of the two authors during critical appraisal. Y denotes Yes, N denotes No and U denotes Unclear.

its influence on the overall SMD [Figure 5]. As is evident from the graph, a study by Sunderraman *et al*.,[20] showing a significant decrease in Cr levels in GDM population greatly influences the overall SMD. This could be a major source of high heterogeneity. We performed meta analysis after excluding the study by Sunderaman *et al.* to check for changes in SMD and heterogeneity [Figure 6]. After the removal of the study, the SMD between control and GDM group was still nonsignificant (P =0.94); however, there was a substantial decrease in the heterogeneity from 98% to 77%.

Results from assesment of publication bias

Publication bias was assesed graphically using Begg's Funnel plot and statistsically by using Egger's regression test and Begg's test.[24,25] No significant publication bias was found in our meta analysis (Egger's test P value =0.076, Begg's test P value =0.086) [Figure 7].

Discussion

The association of Cr with GDM has remained controversial. Some studies have supported the argument of Cr deficiency in GDM, whereas others have shown inconclusive results. A qualitative and quantitative synthesis of these studies was required.

A number of clinical trials on the effect of Cr supplementation on metabolic profile of type 2 diabetic patients have been done in the past.[12,26] A systematic review and meta-analysis by Balk *et al.*,[12] including randomized controlled trials giving Cr monotherapy to type 2 diabetic patients for duration of at least three weeks, found that Cr supplementation improved glycosylated hemoglobin (HbA1C) by 0.6% and FPG by 1.0 mM in diabetic patients. No effect of Cr was seen on lipid profile of diabetic group. Another systematic review and meta-analysis conducted in 2014 suggests a positive influence of Cr supplementation on metabolic profile of diabetic group including improvement in HbA1C, FPG, and triglycerides levels.[26] As per this study, an average Cr supplementation of 200 µg to 1000 µg daily improved both HbA1c and FPG in diabetic cases.[26] However, the efficacy of long-term supplementation of Cr in diabetic patients still has to be determined for clinical use.[26] A beneficial effect of Cr on glucose metabolism is explained on the basis of three possible mechanisms including an increase in the number of insulin receptors, increased binding of insulin to its receptors, and enhanced mobility of GLUT 4 receptors to cell surface by enhancing insulin-signalling cascade.[26]

Recently, the focus of research has shifted to understanding the influence of GDM on maternal serum trace elements, especially Cr.[27] A study found the effect of pregnancy on Cr stores in the body by comparing the hair Cr levels of multiparous and nulliparous women. In this research, hair Cr levels of multiparous women were significantly decreased. The authors hypothesize that this could play a significant role in glucose intolerance in subsequent pregnancies in multiparous women.[28] Other studies also found decreased Cr stores in multiparous women, highlighting the depletion of body Cr stores in pregnancy.[29-31] A study by Morris *et al*.[32] tries to evaluate the effect of pregnancy on urinary excretion of Cr and if there is any subsequent alteration in insulin sensitivity. Twenty two percent of pregnant participants displayed an increased excretion of urinary Cr above the established reference range. In the subgroup of women that provided fasting insulin samples, women with an increased excretion of Cr showed 65% higher IR. Thus, enhanced excretion of Cr could be a risk factor for the development of GDM.[32] In another study by Saner *et al.*,[13] a decrease in urinary Cr to creatinine ratio was observed after glucose loading during glucose tolerance test in the third trimester of pregnancy which is different from a normal increase in the ratio in nonpregnant adults. As per the study, this indicates

Table 3: Subgroup analysis based on continent of study, Method of Cr estimation and trimester of pregnancy of the study subjects

Variables and subgroups	Effect size			Heterogeneity		
	SMD [95% CI] IV	Z-statistic/Chi²	*P*	X²	I²	*P*
Continent						
Asia	-3.12 [-8.41, 2.17]	1.16	0.25	103.17	99%	< .001*
America	-0.09 [-0.35, 0.18]	0.63	0.53	---	----	-----
Europe	1.21 [0.36, 2.06]	2.80	0.005*	-----	-----	------
Test for Subgroup difference		9.53	0.009*	138.81	97%	< .0001*
Method of Cr estimation						
Atomic absorption	-0.44 [-0.80, -0.08]	2.39	0.02*	------	-------	-------
Graphite furnace	1.21 [0.36, 2.06]	2.80	0.0005	----	-----	-----
ICP-MS	-1.93 [-4.16, 2.09]	1.71	0.09	124.56	98%	< .0001*
Test for subgroup difference		14.65	0.0007*	138.81	97%	< .0001*
Trimester of pregnancy						
First trimester	-0.17 [-0.55, 021]	0.89	0.38	---	---	-----
Second trimester	-2.90 [-8.62, 2.82]	0.99	0.32	119.58	99%	< .0001*
Second-third trimester	-0.44 [-0.80, -0.08]	2.39	0.02*	------	-------	--------
Third trimester	1.21 [0.36, -2.06]	2.80	0.005*	------	-------	--------

Table 4: Table for meta regression analysis

	Regression coefficient (Q)	Standard Error (SE)	*P*	95% confidence interval of Q	
				Lower bound	Upper bound
With year of publication as independent variable	-0.2267	0.2883	0.489	-1.144	0.6909
With sample size as independent variable	0.0037	0.0079	0.678	-0.2179	-0.2911

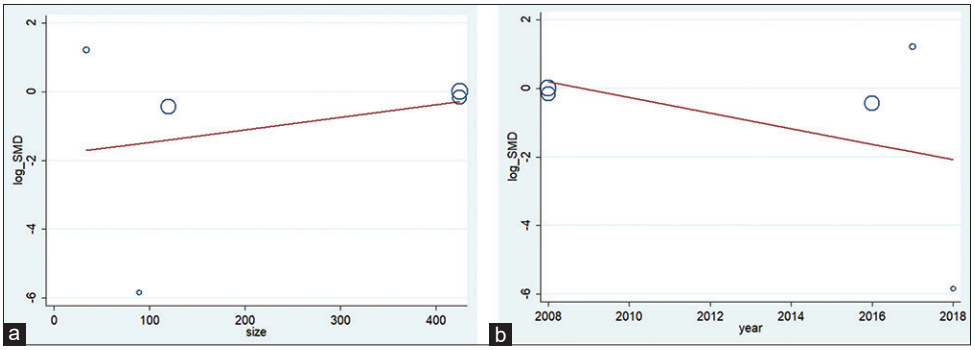

Figure 4: (a) Bubble plot showing relationship of sample size of indivdual studies with Log of SMD on each study. (b) Bubble plot showing the relationship of year of publication of each study with SMD

that pregnancy is characterized by an increased demand for Cr to meet the growing IR and this demand was not replenished through diet in the sampled pregnant women.[13] The study by Jovanovic *et al*.[33] is a randomized control trial to study the effect of Cr supplementation on GDM. After eight weeks of supplementation, the group of GDM women who received Cr supplementation had lower levels of glucose and insulin than the baseline levels and the placebo group.[33]

Studies evaluating maternal Cr levels in GDM have conflicting results. Didedar *et al*.[21] confirmed these findings in an Iranian study but the differences were not statistically significant. Some studies measured maternal tissue Cr stores in GDM. There were no significant differences in tissue Cr levels between the GDM and normoglycemic groups in either study. A prospective study by Woods *et al*.[22] did not show any change in serum Cr levels in the GDM population during two visits. The study results from Woods *et al*. were seconded by Houldsworth *et al*.,[4] who did not find any changes in serum Cr levels in the diabetic group. However, Houldsworth *et al.* pointed out that Cr levels increased with an increase in fasting insulin levels in the whole patient group and controls and nonsignificant changes in the GDM group could be attributed to low sample size.

We included six studies for our systematic review, whereas four studies entered the meta-analysis. In our

study, meta-analysis revealed that pooled Cr estimates did not significantly differ in GDM and controls. Interstudy heterogeneity was high. After the removal of a study (20) in the meta analysis, the heterogeneity reduced from 98% to 77% but was still high [Figure 6]. A lack of diet history or history of mental stress in pregnancy and small sample size could be some of the factors limiting the study and leading to heterogeneity. Subgroup analysis revealed that results varied as per study location, pregnancy trimester, and Cr estimation technique.

Subgroup analysis based on trimester of pregnancy revealed that studies recruiting subjects in the third trimester of pregnancy showed significant differences in Cr values between the control and GDM groups. This could be attributed to enhanced urinary excretion of Cr with pregnancy.[32]

Some of our included studies could be limited by inadequate representation of all racial ethnicities in the test population. Two studies conducted in Asian countries had lower mean serum Cr levels in the GDM population than controls,[20,21] whereas studies conducted in western populations did not show similar results.[4,18,22] These differences could be attributed to changes in the dietary patterns of Asian and western populations. More mixed population studies are required to come up with conclusive data.

The type of sample used, tissue or serum, can also influence the results of the study. Scientists have debated if hair Cr (H-Cr) or plasma Cr (P-Cr) is an accurate measure of Cr metabolism in the body.[34-37] In our meta-analysis, although most of the studies measuring serum Cr levels showed either decreased Cr levels or no significant changes, the study by Aharoni *et al*.[19] showed increased Cr levels in hair samples of the GDM population. The authors point out that an underlying defect in GDM could be underutilization of tissue Cr stores, which can cause an imbalance in glucose metabolism. This difference could be attributed to the fact that the metabolism of Cr is different in different tissues. We conducted a subgroup analysis based on the type of sample, which showed a significant difference among studies as per the type of sample used (Z = 0.73). Thus, we decided to include studies using a single type of sample (serum) for Cr analysis. Results of studies measuring H-Cr and P-Cr levels in type 2 diabetes mellitus have shown inconsistent results. Average results from all the previous literature show that mean P-Cr and H-Cr values in the diabetic population did not differ from controls.[37] More experimental studies are required to establish the relationship between H-Cr and P-Cr levels with Cr metabolism in the body.

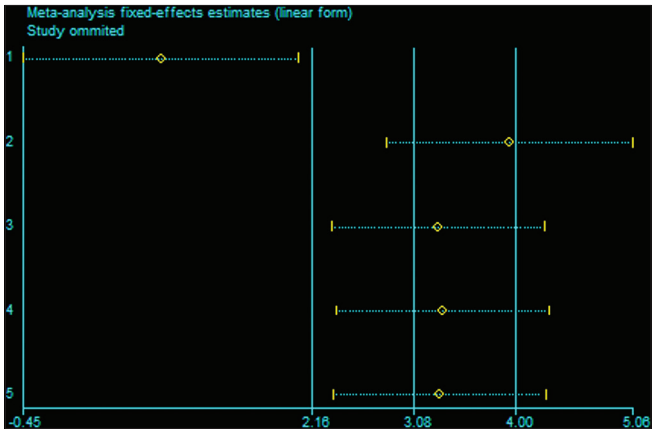

Figure 5: Results from Influence analysis. **The studies have been designated by numbers on the extreme left-hand side. 1st study- Sunderraman et al, 2018, 2nd Study- Houldsworth et al, 2017, 3rd study- Reza Didedar et al, 2016, 4th and 5th Study- Woods et al, 2008 1st and 2nd follow up respectively

Houldsworth *et al*.[4] pointed out that there was a positive relationship between fasting insulin levels and serum Cr in the whole patient group and controls. The authors proposed a pathophysiological mechanism wherein growing IR in pregnancy is counteracted by growing insulin secretion and a subsequent increase in the utilization of body Cr stores. This continues until Cr stores in the body are depleted, which can lead to a decrease in insulin action and subsequent hyperglycemia if not replenished through diet.[4] We looked into other studies to find relevant data to support this proposed mechanism. However, some of the included studies failed to mention the relationship between fasting insulin levels and HOMA IR with maternal Cr levels in GDM and the test population as a whole.[4,20-22] As Cr plays an important role in potentiation of insulin action, there is a higher possibility that its influence over insulin levels is more than just overall hyperglycemia. Small sample size, lack of history of infection in pregnancy that can alter Cr excretion, lack of diet history, particularly history of sugar intake as complex sugars are known to alter Cr metabolism, and influence of mental stress on Cr levels are all factors that can contribute to disparities in the results of included studies. The exact mechanism of association of acute infections with reduced serum Cr levels is not known.[38] It is speculated that infections can either lead to increased urinary excretion of Cr or as infections can lead to a state of impaired glucose tolerance due to a decrease in

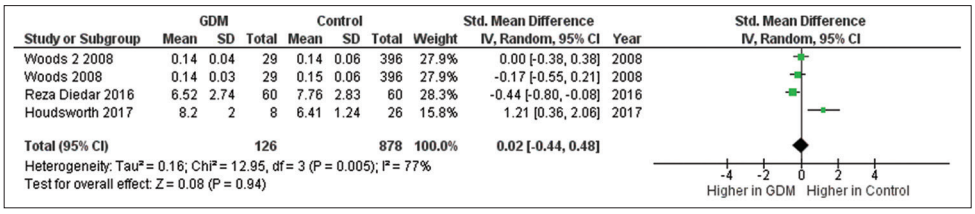

Figure 6: Meta analysis after removal of one study

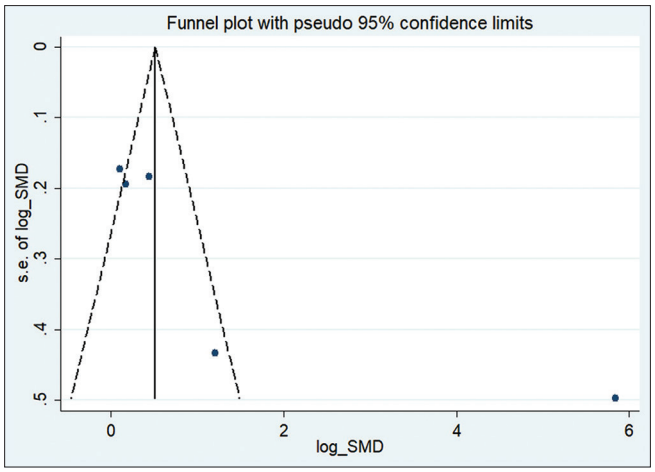

Figure 7: Results from assessment of Publication bias

circulating glucoregulatory hormones; redistribution of Cr from blood to tissues can occur to counter reduced glucose tolerance leading to low blood Cr levels.[38] With this said there is a possibility that changes in Cr levels associated with IR could become measurable only with chronic conditions like type 2 diabetes mellitus and not with acute conditions like GDM, which last for a few weeks.[22]

As there is limited research in the field of measuring maternal Cr levels in GDM, we suggest a larger prospective study measuring both tissue and plasma Cr levels along with urinary excretion of Cr to compare with fasting insulin levels and glucose intolerance in different trimesters of pregnancy. This, along with consideration of ethnicity, diet pattern, and parity on maternal Cr levels, could lead us to a better understanding of the role of Cr in GDM.

Conclusion

In our study, meta-analysis reveals that pooled Cr estimates did not significantly differ in GDM and controls. Interstudy heterogeneity was high. Subgroup analysis based on trimester of pregnancy reveals that studies recruiting subjects in the third trimester of pregnancy showed significant differences in Cr values between control and GDM group; this could be attributed to enhanced urinary excretion of Cr with proceeding pregnancy. Results also varied as per place of study and technique used for Cr estimation. Factors such as ethnicity, lack of history of occurrence of infection in pregnancy, lack of diet history, and influence of mental stress on Cr levels in pregnancy can contribute to disparity in results of included studies. There could be minute changes in Cr levels associated with IR that could become measurable only with chronic conditions like type 2 diabetes mellitus than acute condition like GDM. As there is limited research in the field of measuring maternal Cr levels in GDM, we suggest a larger prospective study measuring both tissue and plasma Cr levels along with urinary excretion of Cr to compare with fasting insulin levels and glucose intolerance in different trimesters of pregnancy. This along with consideration of ethnicity, diet pattern, and parity on maternal Cr levels could lead us to a better understanding of role of Cr in GDM.

Statement of significance

Although a number of experimental studies in the past have studied the role of Cr to counter growing IR in diabetic models, its effect and role in GDM remained controversial, with different clinical cross-sectional and cohort studies dispersed studying the effect. This study is an attempt to qualitatively and quantitatively synthesize past studies to assess the role of Cr in GDM. It is important to study maternal Cr changes and its effect on IR as it can direct future studies on Cr supplementation in GDM.

Data availability

The datasets generated during and/or analysed during the current study are available in the figshare repository. Please find below the private link or DOI to dataset and additional data on figshare.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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