Serum Osteocalcin As A Predictive Marker Of Gestational Diabetes Mellitus - A Systematic Review And Meta-Analysis

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Abstract

Introduction: Osteocalcin (OC) and its effect on insulin resistance in animals have been studied and demonstrated. But, the same is yet to be established in humans. Moreover, the association of Gestational Diabetes Mellitus (GDM) with serum Osteocalcin (OC) levels hasn't been studied much and hence, is an area of scope for research. This study is aimed at assessing the difference between serum OC levels in non-diabetic pregnant women (Group I – Controls) and women diagnosed with GDM (Group II – Cases) and determine whether they can be used in early prediction of GDM. **Methods:** A thorough and systematic search of PUBMED and SCOPUS databases was done to identify relevant articles, using the keywords Osteocalcin, pregnancy, diabetes and gestational diabetes. Studies which had included atleast 10 study participants per group and had measured serum OC levels in various forms viz., undercarboxylated Osteocalcin (ucOC), N terminal Mid fragment Osteocalcin (N MID OC) and total Osteocalcin (tOC) were considered relevant. Out of 45 studies, 9 studies were selected for the present study. **Results:** There was no significant difference in the mean ucOC and N MID OC levels between the GDM and nondiabetic controls, whereas, a significant difference was found in the tOC levels between the two groups. **Conclusion:** Thus, this meta-analysis shows that tOC may be used as potential marker for GDM rather than ucOC and N MID OC.

KEYWORDS: Bone markers, Hyperglycemia, Pregnancy

INTRODUCTION

Gestational Diabetes Mellitus (GDM) is defined as any impairment in glucose tolerance with initial onset/ recognition during pregnancy[1]. GDM is a common and leading cause of morbidity in the mothers as well as their children[2]. Prevention of complications in GDM mothers and their children has been a challenge to the obstetricians because of the delay in diagnosis.

Osteocalcin (OC), a protein which is chiefly involved in bone metabolism[3] is primarily derived from the bones, 10 – 30% of which is released into blood stream and is subsequently eliminated by the liver and the kidneys[4,5,6]. It occurs in three primary forms - carboxylated (cOC), undercarboxylated (ucOC), and total osteocalcin (tOC)[7], the estimation of the blood levels of which can be done by different methods such as ELISA (enzyme-linked immunoassay), RIA (radioimmunoassay), electrochemiluminescence immunoassay (ECLIA), IRMA (immunoradiometric assay) etc. N terminal mid fragment of Osteocalcin (N MID-OC) accounts for the high proportion of circulating Osteocalcin.

Several authors in their studies have found the tOC and ucOC levels to be lower than the normal range in both Type I and II diabetics[8-24]. However, J. R. Villafán-Bernal et al has found high cOC levels in Type II diabetics[24]. ucOC has also been shown to be insulinogenic and lipolytic in many studies[25]. Pregnancy-induced physiological changes occuring in the maternal body lead to fasting hypoglycemia in the first trimester followed by a steady decline in the insulin sensitivity in the second and third trimesters[26,27,28]. High tOC levels were

demonstrated in first trimester in women who developed GDM subsequently[29]. Hence, a combination of the serum levels of proteins related to insulin resistance such as tOC and ucOC and maternal characteristics might go a long way in predicting the risk for developing GDM[30,31].

This meta-analysis is aimed at comparing the serum levels of tOC and ucOC in nondiabetic pregnant women and women diagnosed with GDM and to determine whether OC can be used a predictive marker for GDM.

MATERIALS AND METHODS

This study was done from August 2021 to August 2022. A systematic search of PubMed and SCOPUS databases was performed to identify relevant articles published in English. As approval by the Institutional review board is not mandated for meta-analysis, it wasn't obtained. This review was carried out adhering to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines[32] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews and meta-analysis[33,34]. The abstracts of the relevant articles identified were analysed and then the articles with the suitable abstracts were selected for review. Manual search and scrutiny of the references of the selected articles was done for identification of more related studies. The corresponding authors of identified articles were contacted for the inadequate details regarding the studies.

Inclusion criteria

Both prospective and retrospective observational studies done on nondiabetic pregnant women (controls) and women diagnosed with GDM (cases) and which included estimation of serum OC levels.

Exclusion criteria

Studies done on pregnant women with past history of Diabetes Mellitus.

Data collection was done based on Cochrane Consumers and Communication Review Group's data extraction template[35]. The quality of the selected articles was assessed using Newcastle Ottawa scale, which is based on three criteria – selection of groups, comparability of groups and exposure ascertainment. One star was assigned for each positive choice for the questions and articles scoring 7 or more stars were believed to be authentic[36]. The data collected were pooled and analysed based on Cochrane handbook. Mean tOC, N MID OC and ucOC among the nondiabetic pregnant women and those with GDM were compared by taking Mean Difference by Random Effects Model. Between-study variability was assessed using the $\tau 2$ and
Cochran's Q and I2 statistics. Forest plots were drawn to display the results obtained from both the controls and cases. For subgroup analysis, both fixed and random effects model were employed. Funnel plots were used to identify and quantify publication bias respectively. Statistical analyses were conducted using Revman software 5.4.1.

RESULTS

After a thorough data searching, a total of 45 articles were found appropriate. We had 25 articles after removal of duplicates. After screening of all their abstracts, 15 articles were excluded, the reasons being inclusion of women with past history of Diabetes and non-inclusion of women of all three trimesters. Finally, ten articles were selected for full-text review and 9 of them were selected for the systematic review and meta-analysis as full text article of one abstract could not be accessed. The authors Tabatabaei et al.[37], Saucedo et al.[38], and Srichomkwun et al.[39] were contacted for inadequate data on their respective studies. Table I show the characteristics of the included studies and Figure I shows the PRISMA flow diagram.

Table I: Characteristics of the included studies

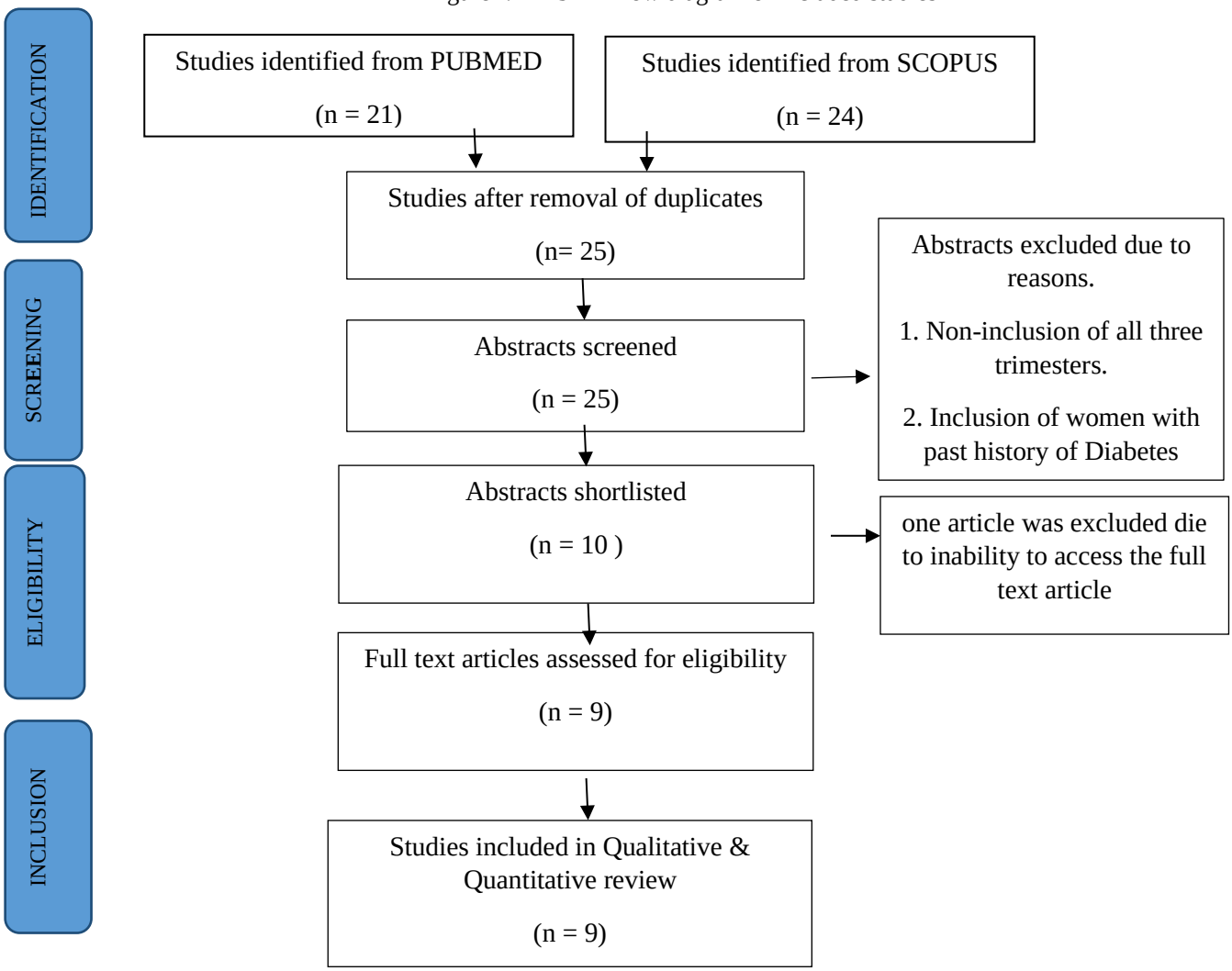
Name of the author	Year of study	Country of study	Type of study	Inclusion criteria	Exclusion criteria	Criteria for diagnosis of GDM	Form of Osteocalcin measured	Method of Assay	No. of study participants	No. of Cases	No. of Controls
Ioannis Papastefanou	2015	Greece	Prospective cohort study	Healthy pregnant women in first trimester	Previous history of Diabetes mellitus	2 hour OGTT 75g of glucose - (95	tOC	ECLIA	134	40	94

						mg/dl, 180 mg/dl and 155 mg/dl cutoff at 0, 1 & 2h)					
Yvonne Winhofer	2010	Austria	Prospective cohort study	Healthy pregnant women referred from the Dept of Obstetrics & Gynaecology (O&G) for Oral Glucose Tolerance Test (OGTT) between 24 – 28 weeks of gestation and followed up 3 months postpartum	History of GDM/ Impaired Glucose Tolerance/ previous obstetric complications/ fetal macrosomia/	2 hour OGTT 75g of glucose - (95 mg/dl, 180 mg/dl and 155 mg/dl cutoff at 0, 1 & 2h)	N-MIDOC	ECLIA	78	26	52
Negar Tabatabaei	2014	Canada	Cohort	Healthy pregnant Caucasian women and women with GDM throughout pregnancy	Past history of Diabetes mellitus	One-step: 75 g of glucose (5.5, 10.0, and 7.8 mmol/l cutoff at 0, 1, and 2h)	tOC	ELISA	96	48	48
Jing Zhang	2018	China	Case Control study	Healthy pregnant women and women	Previous history of GDM, bad obstetric history	One-step: 75 g of	N-MIDOC	ECLIA	151	105	46

				with GDM with gestational period of 24-28 weeks.		glucose (5.5, 10.0, and 7.8 mmol/l cutoff at 0, 1, and 2h)					
Panudda Srichomkwun	2015	Thailand	Case Control study	Pregnant women with varying degrees of Insulin Resistance	Previous history of GDM	Two step GTT: 50g if >7.2 mmol/L, 100g	tOC and ucOC	ELISA	130	74	56
Jingjing Ma	2019-2020	China	Case-control study	Healthy pregnant women who visited the dept of O&G with a gestational period of 20 weeks, 20-35 years old, with singleton pregnancy	History of gestational diabetes/hypertension, preeclampsia, heart disease, thyroid disorders, mental diseases, infectious diseases.	One-step: 75 g of glucose (5.1, 10.0, and 8.5 mmol/l cutoff at 0, 1, and 2h)	tOC	ECLIA	100	31	69
Yujia Gong	2015-2020	China	Prospective cohort study	Pregnant women who underwent GTT at 6-8 weeks postpartum	History of diabetes, /Impaired Fasting Glucose (IFG)/ Impaired Glucose Tolerance (IGT) before pregnancy	One-step: 75 g of glucose (5.1, 10.0, and 8.5 mmol/l cutoff at 0, 1, and 2h)	N-MIDOC	ECLIA	721	255	466
Hossein-nezhad	2010	Iran	Case-control study	Pregnant women without previous history of diabetes in the first	None	Two step GTT: 50g if >7.2 mmol/	tOC	ELISA	695	51	644

				half of pregnancy		L, 100g					
Saucedo	2015	Mexico	Prospective cohort study	GDM and non-GDM pregnant women at 30 weeksof gestation and 6 weeks postpartum	None	2 hour OGTT 75g of glucose - (95 mg/dl, 180 mg/dl and 155 mg/dl cutoff at 0, 1 & 2h)	tOC and ucOC	IRA for tOC and ELISA for ucOC	120	60	60

Figure I: PRISMA flow diagram of included studies



Six authors [29,37,38,39,40,41] had analysed the tOC levels alone in GDM and two authors[38,39] had estimated both tOC and ucOC levels in GDM. N MID OC had been measured in three studies[42,43,44]. Two case control studies and three cohort studies scored 6 stars, two case control and one cohort study scored 7 stars and one cohort study scored 8 stars in New Castle Ottowa scale (Tables II & III). A statistically significant

mean difference of tOC between women with GDM and nondiabetic pregnant controls was observed (2.20; 95% CI: 0.54 to 3.87; p= 0.009). A Q value of 2.94 with 5 degrees of freedom and p = 0.001 provides evidence that the effect size varies across studies. I2 indicates that 75% of the variation can be attributed to true effect rather than random error (Figure II). Funnel plot for studies on tOC measurement showed no significant publication bias (Figure III). Formal assessment of heterogeneity by subgroup analysis (both fixed and random effects) models showed that the use of ECLIA as the method for tOC analysis depicted similar results between studies. No added variability was found in this subgroup (I2 = 0%) (Figures IV and V). There was no statistically significant mean difference between the ucOC (p=0.13) and N MID OC (p=0.55) levels of GDM and nondiabetic pregnant women (Figures VI & VII). Funnel plot drawn for studies on ucOC and N MID OC showed no publication bias (Figures VIII & IX). Subgroup Analysis for ucOC and N MID OC for heterogeneity was not performed since there was no statistically significant difference between the study groups.

Table II: Newcastle- Ottawa scale for Case-control studies

Author	Year	Is the case definition adequate?	Representativeness of cases	Selection of controls	Definition of controls	Study control for main outcome	Study controls for additional outcomes	Ascertainment of exposure	Same method of ascertainment for cases and controls	Nonresponse rate	Stars
Jing Zhang	2018	*	*	*	*	*		*	*		7
Panudda Srichomkwun	2015	*	*			*		*	*	*	6
Jing Jing Ma	2019-20	*	*	*	*	*		*	*		7
Hossein-nezhad	2010	*	*	*		*		*	*		6

Table III: Newcastle- Ottawa scale for Cohort studies

Author	Year	Representativeness of exposed cohort	Selection of non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Study controls for main outcome	Study controls for additional outcomes	Ascertainment of outcome	Was follow-up enough for outcomes to occur	Adequacy of follow-up cohorts	Stars
Ioannis Papastefanou	2015	*	*	*	*	*		*			6
Yvonne Winhofer	2010	*	*	*	*	*		*	*		7
Negar Tabatabaei	2014	*	*	*		*		*	*		6
Yujia Gong	2015-20	*	*	*	*	*		*	*	*	8
Saucedo	2015	*	*	*	*			*	*		6

Figure II: Forest plot on mean difference of tOC among GDM and nondiabetic pregnant women

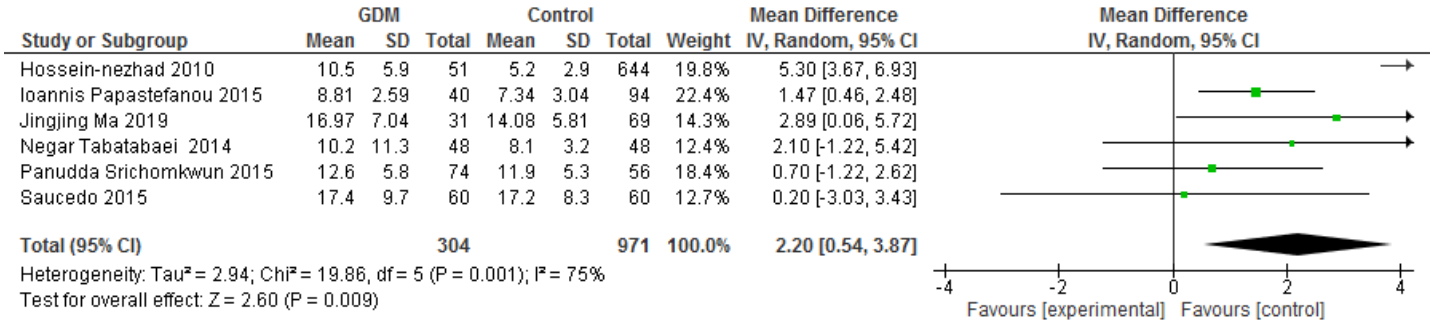


Figure III: Funnel plot for publication bias assessment in studies measuring tOC

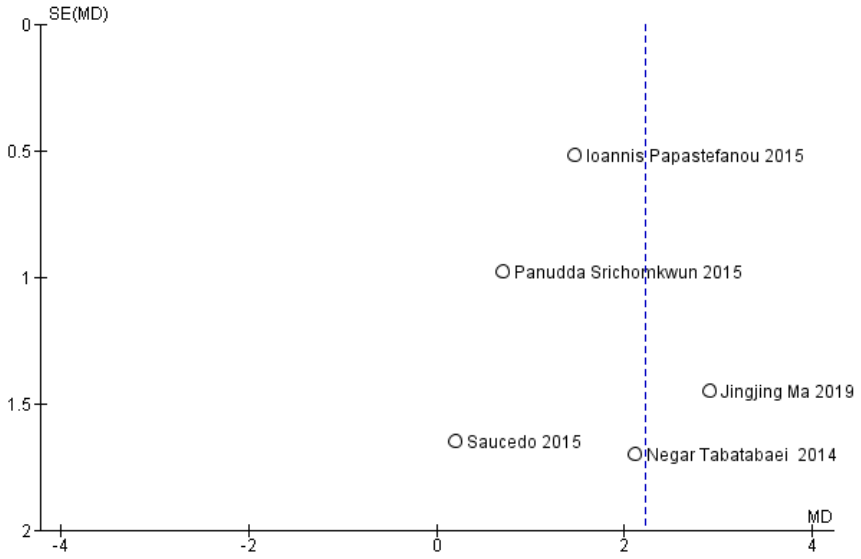


Figure IV: Subgroup analysis of studies measuring tOC by fixed effects model.

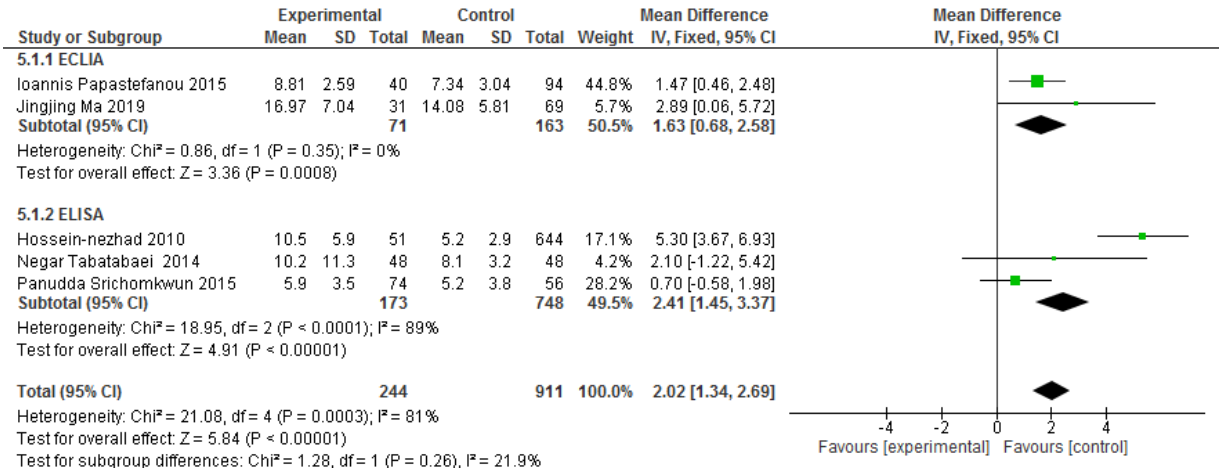


Figure V: Subgroup analysis of studies measuring tOC by random effects model

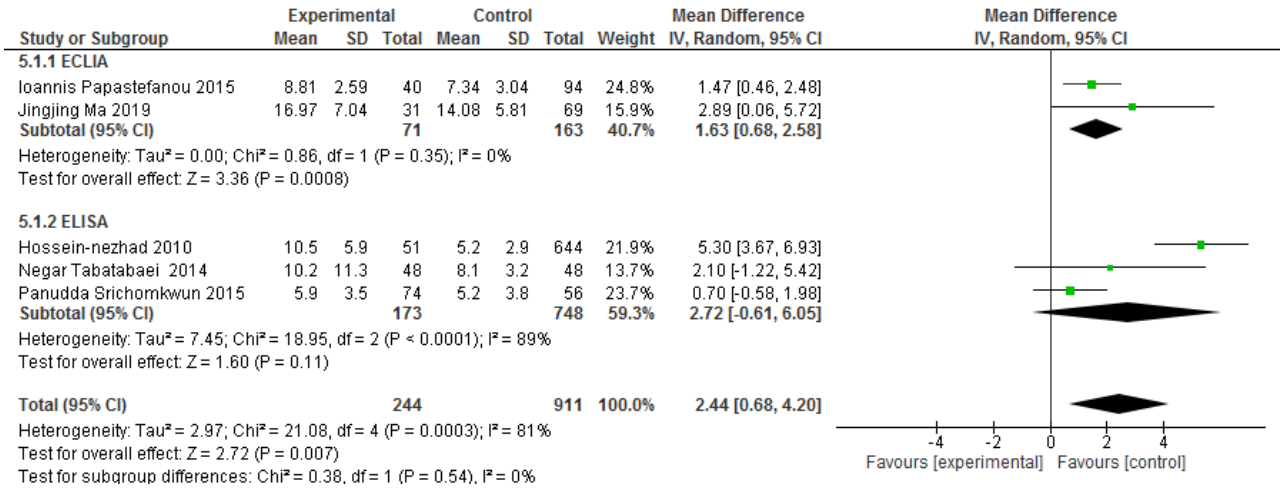


Figure VI: Forest plot on mean difference of ucOC among GDM and nondiabetic pregnant women

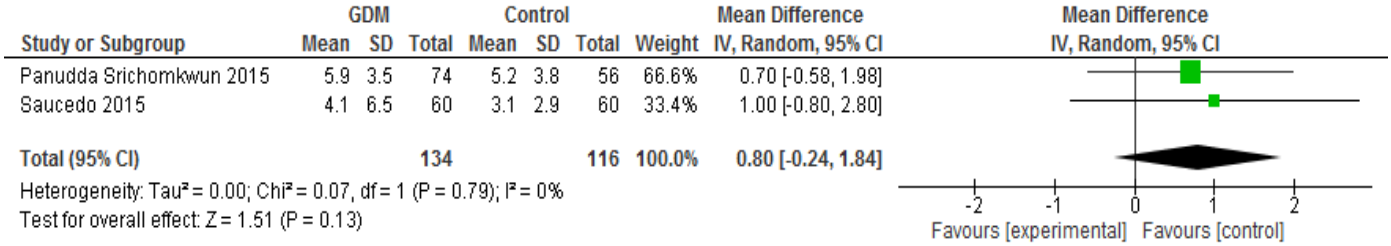


Figure VII: Forest plot on mean difference of N MID OC among GDM and nondiabetic pregnant women

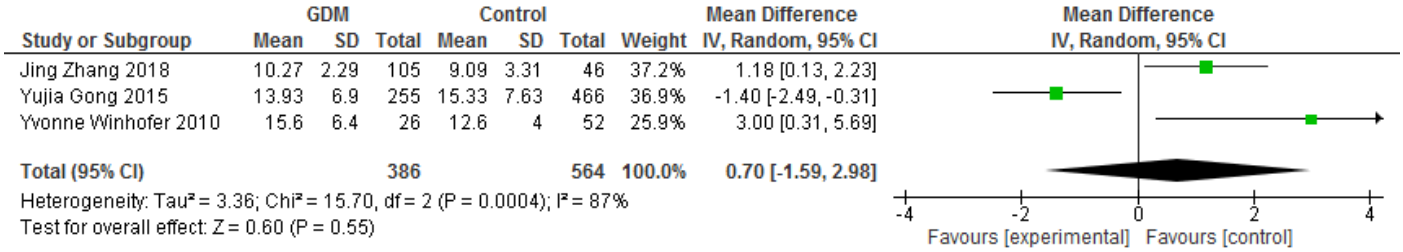


Figure VIII: Funnel plot for publication bias in studies measuring ucOC

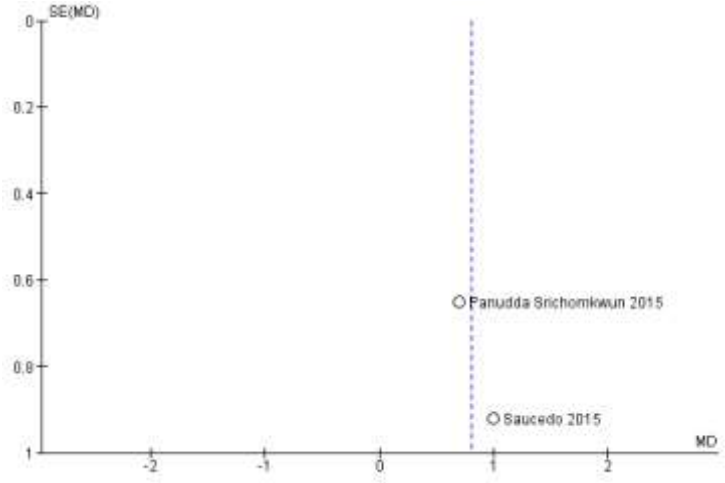
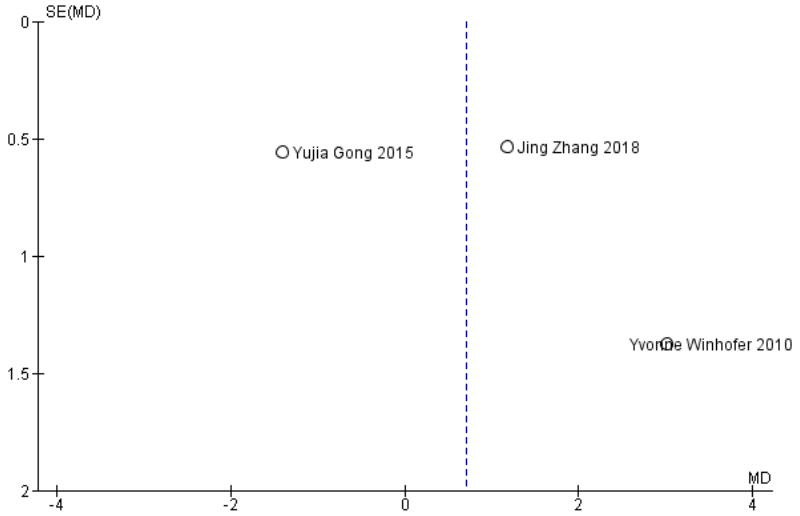


Figure IX: Funnel plot for publication bias in studies measuring N MID OC



DISCUSSION

The main Findings are a) Serum concentrations of tOC are significantly higher in women with GDM when compared to nondiabetic controls b) There is no significant difference in ucOC concentrations among women with GDM and nondiabetic pregnant controls and finally, c) ECLIA as a method for measuring tOC was the only one comparable among studies, showing no heterogeneity and yielding similar results. The molecular mechanisms underlying OC differences among normal pregnant and GDM women are yet to be elucidated. One of the attributable factors might be the fact that placental-induced insulin resistance reaches its peak between 24 and 28 weeks of gestation. This insulin resistance leads to an increase in insulin secretion by pancreatic B-cells as a compensatory mechanism that stimulates an increased anabolic feature on bone metabolism via IGF-1, therefore, influencing OC concentrations especially during the second trimester of pregnancy as described by Winhofer et al.[42]. Similar to our results, the study of Telejko et al.[45] showed no significant difference in tOC levels among women with and without GDM. This study was excluded due to the inclusion of women after 28 weeks of gestation as defined previously. The study conducted by Martinez et al.[46] showed an increase of tOC concentrations among women with diabetes, which was also discarded from our metaanalysis due to the inclusion of prediabetic patients.

Early diagnosis and treatment of GDM can go a long way in preventing perinatal maternal and fetal complications. This, to a certain extent has been achieved by life style modifications[47,48] and various predictive models[49,50] though with a limited capacity. Our study suggests that serum ucOC level may be a useful predictive marker of GDM, when used in combination with the existing predictive models.

Strengths and Limitations

The main strengths of our study are that an extensive and organised review done by multiple reviewers and blinding of the reviewers for the authors and the institutions of study to minimise bias. The main limitation of this study is the small number of publications found in the literature, which was due to the limited research done on this particular topic and not due to the search strategy. Moreover, the impact of other possible coexistent conditions like thyroid disorders on serum Osteocalcin were not studied due to lack of available literature.

CONCLUSION

This meta-analysis has found evidence favouring the use of tOC as a potential marker for GDM rather than ucOC, in view of its reduced variability, irrespective of the analytical assay methods used, especially with ECLIA.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

1. B. E. Metzger, D. R. Coustan, and Organizing Committee, "Summary and recommendations of the fourth international workshop-conference on gestational diabetes mellitus," Diabetes Care, vol. 21, pp. B161–B167, 1998.
2. J. Djelmis, M. Pavic, V. Mulliqi Kotori, I. Pavlic Renar, M. Ivanisevic, and S. Oreskovic, "Prevalence of gestational diabetes mellitus according to IADPSG and NICE criteria," International Journal of Gynecology & Obstetrics, vol. 135, no. 3, pp. 250–254, 2016.
3. P. V. Hauschka, J. B. Lian, D. E. Cole, and C. M. Gundberg, "Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone," Physiological Reviews, vol. 69, no. 3, pp. 990–1047, 1989.
4. M. J. Seibel, "Biochemical markers of bone turnover part I: biochemistry and variability," Clinical Biochemist Reviews, vol. 26, no. 4, pp. 97–122, 2005.
5. P. D. Delmas, D. M. Wilson, K. G. Mann, and B. L. Riggs, "Effect of renal function on plasma levels of bone Gla-protein," The Journal of Clinical Endocrinology & Metabolism, vol. 57, no. 5, pp. 1028–1030, 1983.
6. W. Farrugia and R. A. Melick, "Metabolism of osteocalcin," Calcified Tissue International, vol. 39, no. 4, pp. 234–238, 1986.
7. J. R. Villafán-Bernal, S. Sánchez-Enriquez, and M.-V. J. Francisco, "Molecular modulation of osteocalcin and its relevance in diabetes (review)," International Journal of Molecular Medicine, vol. 28, no. 3, pp. 283–293, 201
8. Z. Berberoglu, A. Gursoy, N. Bayraktar, A. C. Yazici, N. Bascil Tutuncu, and N. Guvener Demirag, "Rosiglitazone decreases serum bone-specific alkaline phosphatase activity in postmenopausal diabetic women," The Journal of Clinical Endocrinology & Metabolism, vol. 92, no. 9, pp. 3523–3530, 2007.
9. A. B. Choudhury, P. D. Sarkar, D. K. Sakalley, and S. B. Petkar, "Role of adiponectin in mediating the association of osteocalcin with insulin resistance and type 2 diabetes: a cross sectional study in pre- and post-menopausal women," Archives of Physiology and Biochemistry, vol. 120, no. 2, pp. 73–79, 2014.
10. D. M. S. L. Cutrim, F. A. Pereira, F. J. A. Paula, and M. C. Foss, "Lack of relationship between glycemic control and bone mineral density in type 2 diabetes mellitus," Brazilian Journal of Medical and Biological Research, vol. 40, no. 2, pp. 221–227, 2007.
11. K. K. Danielson, M. E. Elliott, T. LeCaire, N. Binkley, and M. Palta, "Poor glycemic control is associated with low BMD detected in premenopausal women with type 1 diabetes," Osteoporosis International, vol. 20, no. 6, pp. 923–933, 2009.
12. D. Gogas Yavuz, L. Keskin, S. Kıyıcı et al., "Vitamin D receptor gene BsmI, FokI, ApaI, TaqI polymorphisms and bone mineral density in a group of Turkish type 1 diabetic patients," Acta Diabetologica, vol. 48, no. 4, pp. 329–336, 2011.
13. R. M. Hussein, "Biochemical relationships between bone turnover markers and blood glucose in patients with type 2 diabetes mellitus," Diabetes & Metabolic Syndrome: Clinical Research & Reviews, vol. 11, pp. S369–S372, 2017.
14. E. Y. Liu, J. Wactawski-Wende, R. P. Donahue, J. Dmochowski, K. M. Hovey, and T. Quattrin, "Does low bone mineral density start in post-teenage years in women with type 1 diabetes?" Diabetes Care, vol. 26, no. 8, pp. 2365–2369, 2003.
15. L. D. Mastrandrea, J. Wactawski-Wende, R. P. Donahue, K. M. Hovey, A. Clark, and T. Quattrin, "Young women with type 1 diabetes have lower bone mineral density that persists over time," Diabetes Care, vol. 31, no. 9, pp. 1729–1735, 2008.
16. T. Neumann, S. Lodes, B. Kästner et al., "Osteocalcin, adipokines and their associations with glucose metabolism in type 1 diabetes," Bone, vol. 82, pp. 50–55, 2016.
17. J. M. Olmos, J. L. Pérez-Castrillón, M. T. García, J. C. Garrido, J. A. Amado, and J. González-Macías, "Bone densitometry and biochemical bone remodeling markers in type 1 diabetes mellitus," Bone and Mineral, vol. 26, no. 1, pp. 1–8, 1994.
18. S. Sanchez-Enriquez, I. T. Ballesteros-Gonzalez, J. R. Villafán-Bernal et al., "Serum levels of undercarboxylated osteocalcin are related to cardiovascular risk factors in patients with type 2 diabetes mellitus and healthy subjects," World Journal of Diabetes, vol. 8, no. 1, pp. 11–17, 2017.

19. P. D. Sarkar and A. B. Choudhury, “Relationship of serum osteocalcin levels with blood glucose, insulin resistance and lipid profile in central Indian men with type 2 diabetes,” Archives of Physiology and Biochemistry, vol. 118, no. 5, pp. 260–264, 2012.

20. P. D. Sarkar and A. B. Choudhury, “Relationships between serum osteocalcin levels versus blood glucose, insulin resistance and markers of systemic inflammation in central Indian type 2 diabetic patients,” European Review for Medical and Pharmacological Sciences, vol. 17, no. 12, pp. 1631–1635, 2013.

21. K. Suzuki, T. Kurose, M. Takizawa et al., “Osteoclastic function is accelerated in male patients with type 2 diabetes mellitus: the preventive role of osteoclastogenesis inhibitory factor/osteoprotegerin (OCIF/OPG) on the decrease of bone mineral density,” Diabetes Research and Clinical Practice, vol. 68, no. 2, pp. 117–125, 2005.

22. M. Takizawa, K. Suzuki, T. Matsubayashi et al., “Increased bone resorption may play a crucial role in the occurrence of osteopenia in patients with type 2 diabetes: possible involvement of accelerated polyol pathway in its pathogenesis,” Diabetes Research and Clinical Practice, vol. 82, no. 1, pp. 119–126, 2008.

23. A. Verrotti, F. Basciani, F. Carle, G. Morgese, and F. Chiarelli, “Calcium metabolism in adolescents and young adults with type 1 diabetes mellitus without and with persistent microalbuminuria,” Journal of Endocrinological Investigation, vol. 22, no. 3, pp. 198–202, 1999.

24. B. Yeap, H. Alfonso, S. A. P. Chubb et al., “Higher serum undercarboxylated osteocalcin and other bone turnover markers are associated with reduced diabetes risk and lower estradiol concentrations in older men,” The Journal of Clinical Endocrinology & Metabolism, vol. 100, no. 1, pp. 63–71, 2015.

25. J. R. Villafán-Bernal, M. A. Llamas-Covarrubias, J. F. Muñoz-Valle et al., “A cut-point value of uncarboxylated to carboxylated index is associated with glycemic status markers in type 2 diabetes,” Journal of Investigative Medicine, vol. 62, no. 1, pp. 33–36, 2014.

26. N. K. Lee, H. Sowa, E. Hinoi et al., “Endocrine regulation of energy metabolism by the skeleton,” Cell, vol. 130, no. 3, pp. 456–469, 2007.

27. P. M. Catalano, E. D. Tyzbir, N. M. Roman, S. B. Amini, and E. A. H. Sims, “Longitudinal changes in insulin release and insulin resistance in nonobese pregnant women,” American Journal of Obstetrics & Gynecology, vol. 165, no. 6, pp. 1667–1672, 1991.

28. J. L. Mills, L. Jovanovic, R. Knopp et al., “Physiological reduction in fasting plasma glucose concentration in the first trimester of normal pregnancy: the diabetes in early pregnancy study,” Metabolism, vol. 47, no. 9, pp. 1140–1144, 1998.

29. I. Papastefanou, M. Eleftheriades, D. Kappou et al., “Maternal serum osteocalcin at 11-14 weeks of gestation in gestational diabetes mellitus,” European Journal of Clinical Investigation, vol. 45, no. 10, pp. 1025–1031, 2015.

30. A. M. Maged, G. A. F. Moety, W. A. Mostafa, and D. A. Hamed, “Comparative study between different biomarkers for early prediction of gestational diabetes mellitus,” The Journal of Maternal-Fetal & Neonatal Medicine, vol. 27, no. 11, pp. 1108–1112, 2014.

31. C. E. Powe, “Early pregnancy biochemical predictors of gestational diabetes mellitus,” Current Diabetes Reports, vol. 17, no. 2, p. 12, 2017.

32. D. F. Stroup, J. A. Berlin, S. C. Morton et al., “Meta-analysis of observational studies in epidemiology: a proposal for reporting,” JAMA, vol. 283, no. 15, pp. 2008–2012, 2000.

33. D. Moher, A. Liberati, J. Tetzlaff, D. G. Altman, and The PRISMA Group, “Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement,” PLoSMedicine, vol. 6, no. 7, article e1000097, 2009.

34. E. Stovold, D. Beecher, R. Foxlee, and A. Noel-Storr, “Study flow diagrams in Cochrane systematic review updates: an adapted PRISMA flow diagram,” Systematic Reviews, vol. 3,no. 1, p. 54, 2014.

35. J. P. T. Higgins and S. Green, Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [Updated March 2011], The Cochrane Collaboration, 2011.

36. A. Wells, B. Shea, D. O'Connell et al., “The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses,” http://www.ohri.ca/programs/clinical epidemiology/oxford.asp. https://youtu.be/TmQCJF9B6PU.

37. N. Tabatabaei, Y. Giguere, J. C. Forest, C. J. Rodd, R. Kremer and H. A. Weiler, “Osteocalcin is higher across pregnancy in Caucasian women with gestational diabetes mellitus,” Canadian Journal of Diabetes, vol. 38, no. 5, pp. 307–313, 2014.

38. R. Saucedo, G. Rico, G. Vega et al., “Osteocalcin, undercarboxylated osteocalcin and osteopontin are not associated with gestational diabetes mellitus but are inversely associated with leptin in non-diabetic women,” Journal of Endocrinological Investigation, vol. 38, no. 5, pp. 519–526, 2015.

39. P. Srichomkwun, N. Houngngam, S. Pasatrat, T. Tharavanij, L. Wattanachanya, and W. Khovidhunkit, “Undercarboxylated osteocalcin is associated with insulin resistance, but not adiponectin, during pregnancy,” Endocrine, vol. 53, no. 1, pp. 129–135, 2016.

40. Jingjing Ma , Lulu Han , Xue Zhou , Zhihong Li Clinical significance of Vitamin-D and other bone turnover markers on bone mineral density in patients wiTH gestational diabetes mellitus PaKISTAN Journal of Medical Sciences2022 Jan-Feb;38(1):23-27. doi: 10.12669/pjms.38.1.4461.

41. A. Hossein-nezhad, Z. Maghbooli, K. Mirzaei, M. Rahmani, and B. Larijani, “Osteocalcin and cross laps status among women with gestational diabetes mellitus during pregnancy,” Journal of Diabetes and Metabolic Disorders, vol. 9, pp. 1– 7, 2010.

42. Y. Winhofer, F. W. Kiefer, A. Handisurya et al., “CTX (crosslaps) rather than osteopontin is associated with disturbed glucose metabolism in gestational diabetes,” PLoS One, vol. 7, no. 7, article e40947, 2012.

43. Jing Zhang , Yiduo Zhang , Jing Wang , Fan Yu Characteristics of bone turnover markers in women with gestational diabetes mellitus2020 Mar;77:36-40. Clinical Biochemistry doi: 10.1016/j.clinbiochem.2019.12.013. Epub 2019 Dec 30.

44. Yujia Gong , Na Li , Mengyu Lai , Fang Fang , Jiaying Yang , Mei Kang et al., Consistently Low Levels of Osteocalcin From Late Pregnancy to Postpartum Are Related to Postpartum Abnormal Glucose Metabolism in GDM Patients Frontiers in Endocrinology 2022 Mar 7;13:803624 doi: 10.3389/fendo.2022.803624. eCollection 2022

45. B. Telejko, K. Kalejta, M. Kuzmicki et al., "The association of bone turnover markers with pro- and anti-inflammatory adipokines in patients with gestational diabetes," Annals of Agricultural and Environmental Medicine, vol. 22, no. 2, pp. 307–312, 2015.

46. M. Martinez, P. Catalan, A. Lisbona et al., "Serum osteocalcin concentrations in diabetic pregnant women and their newborns, "Hormone and Metabolic Research, vol. 26, no. 7, pp. 338–342, 1994.

47. N. Oostdam, M. N. M. van Poppel, M. G. A. J. Wouters, and W. van Mechelen, "Interventions for preventing gestational diabetes mellitus: a systematic review and meta-analysis," Journal of Women's Health, vol. 20, no. 10, pp. 1551–1563, 2011.

48. S. Thangaratinam, E. Rogozinska, K. Jolly et al., "Effects of interventions in pregnancy on maternal weight and obstetric outcomes: meta-analysis of randomised evidence," BMJ, vol. 344, no. may16 4, p. e2088, 2012.

49. M. B. Landon, C. Y. Spong, E. Thom et al., "A multicenter, randomized trial of treatment for mild gestational diabetes," The New England Journal of Medicine, vol. 361, no. 14, pp. 1339–1348, 2009. [55]

50. C. A. Crowther, J. E. Hiller, J. R. Moss et al., "Effect of treatment of gestational diabetes mellitus on pregnancy outcomes," The New England Journal of Medicine, vol. 352, no. 24, pp. 2477–2486, 2005.