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A Randomized Controlled Clinical Trial Investigating the Effect of Calcium Supplement Plus Low-dose Aspirin on hs-CRP, Oxidative Stress and Insulin Resistance in Pregnant Women at Risk for Pre-eclampsia

[1]Z. Asemi, [2]M. Samimi, [1]Z. Heidarzadeh, [1]H. Khorrammian and [2]Z. Tabassi
[1]Research Center for Biochemistry and Nutrition in Metabolic Diseases,
[2]Department of Gynecology and Obstetrics, School of Medicine,
Kashan University of Medical Sciences, Kashan, Islamic Republic of Iran

Abstract: Increased levels of pro-inflammatory factors, markers of oxidative stress and insulin resistance during pregnancy have been associated with the development of pre-eclampsia. There is some evidence to suggest that calcium supplement and aspirin can reduce the risk of the disorder. To our knowledge, no reports are available indicating the effects of consumed calcium supplement plus aspirin on high sensitivity C-reactive protein (hs-CRP), oxidative stress parameters and insulin resistance in pregnant women at risk for pre-eclampsia. This study was designed to investigate the effects of consumed calcium supplement plus low-dose aspirin on hs-CRP, oxidative stress parameters and insulin resistance among Iranian pregnant women at risk for pre-eclampsia. This randomized single-blind controlled clinical trial was carried out among 42 pregnant women at risk for pre-eclampsia, primigravida, aged 18-40 year old who were carrying singleton pregnancy at their third trimester. Subjects were randomly assigned to received either the placebo (n = 22) or calcium supplement plus low-dose aspirin (n = 20) for 9 weeks. Calcium supplement plus low-dose aspirin were containing 500 mg carbonate calcium plus 80 mg aspirin. Fasting blood samples were taken at baseline and after 9 weeks intervention to measure serum hs-CRP, oxidative stress parameters including plasma Total Antioxidant Capacity (TAC) and Total Glutathione (GSH), Fasting Plasma Glucose (FPG), serum insulin and HOMA-IR score. Consumption of calcium supplement plus low-dose aspirin resulted in a significant difference serum hs-CRP levels as compared to the placebo (102.87 vs. 3227.75 ng mL^{-1}, p = 0.01). Also, mean changes for plasma TAC (68.96 vs. -74.46 mmol L^{-1}, p = 0.04) and total GSH levels (304.33 vs. -39.33 μmol L^{-1}, p = 0.03) were significantly different between the two groups. No significant differences were found comparing calcium supplement plus low-dose aspirin and placebo in terms of their effects on FPG, serum insulin levels and HOMA-IR. Within-group differences in the placebo group revealed a significant increase in serum hs-CRP levels (3227.75 ng mL^{-1}, p = 0.008) and marginally significant increase in plasma total GSH levels (304.33 μmol L^{-1}, p = 0.07). In conclusion, consumption calcium supplement plus low-dose aspirin during pregnancy for 9 weeks in pregnant women at risk for pre-eclampsia resulted in a significant difference serum hs-CRP and increased levels of plasma TAC and total GSH as compared to the placebo group, but could not affect serum insulin levels and HOMA-IR score.

Key words: Calcium supplement, low-dose aspirin, hs-CRP, oxidative stress, pre-eclampsia

INTRODUCTION

Pre-eclampsia occurs in 2-3% of all pregnancies (Poston *et al*., 2006). Published incidence rates from 1987 to 2004 were shown an increase in pre-eclampsia and from 1987 to 1988 the age-adjusted incidence per 1000 deliveries was 23.6 as well as this increased to 29.4 by 2003-2004 (Hawfield and Freedman, 2009). It is a syndrome in which the placenta is implicated in the development of a generalised maternal inflammatory response and oxidative stress and also characterised by activation of maternal vascular endothelial cells and leucocytes (Redman and Sargent, 2005; Roberts and Gammill, 2005). Pre-eclampsia can lead to several complications in mother and fetal. In the mother, it may be cause premature cardiovascular disease, insulin resistance and Gestational Diabetes Mellitus (GDM) (Kirwan *et al*., 2002; Szarka *et al*., 2010) and long-term increased risk for cardiovascular and cerebrovascular events (Garovic and Hayman, 2007). In fetal, it can result in congenital malformations and low birth weight (Banhidy *et al*., 2011). Various strategies for the managing pre-eclampsia and decreased inflammatory factors, oxidative stress and insulin resistance during pregnancy have been suggested

Corresponding Author: Z. Tabassi, Department of Gynecology and Obstetrics, Kashan University of Medical Sciences, Kashan, Iran Tel: +98-361-4460180 Fax: +98-361-4460181

including, but not limited to, the use of antioxidants, vitamins E and C (Kamiya *et al*., 2005; Rumbold and Crowther, 2005; Poston *et al*., 2006; Polyzos *et al*., 2007), magnesium supplementation (Winer and Tsasaris, 2008), low-dose aspirin (Caritis *et al*., 1998), consumption of constitutive androstane receptor (CAR) (Masuyama and Hiramatsu, 2012) and the use of anti-inflammatory agents (Khattab *et al*., 2011). Recently, few clinical trials in non-pregnant women have shown that calcium supplementation can decrease inflammation, oxidative stress and insulin resistance (Grey *et al*., 2006; Tian *et al*., 2011). However, these studies are limited with conflicting findings. Improvement of systemic inflammation, oxidative stress and insulin resistance by calcium and aspirin might be resulted from their effects on increased total glutathione (GSH) biosynthesis (Tian *et al*., 2011), catalase activity increase (Hajsadeghi *et al*., 2012), suppressing parathyroid hormone (PTH) production (Grey *et al*., 2006), inhibiting IKKbeta activity and suppressing inducible nitric oxide synthase production (Hundal *et al*., 2002; Carvalho-Filho *et al*., 2009).

We are aware of no study indicating the effects of received calcium supplement plus low-dose aspirin on hs-CRP, oxidative stress and insulin resistance among pregnant women at risk for pre-eclampsia. Therefore, the aim of current study was to investigate the beneficial effects of received calcium supplement plus low-dose aspirin on serum hs-CRP, oxidative stress parameters including plasma Total Antioxidant Capacity (TAC) and total GSH and insulin resistance in Iranian pregnant women.

MATERIALS AND METHODS

Participants: This randomized single-blind controlled clinical trial was conducted in Kashan, Iran, during April 2011 to October 2011. On the basis of sample size formula suggested for randomized clinical trials, we considered the type I error of 5% ($\alpha = 0.05$) and type II error of 20% ($\beta = 0.2$; Power = 80%) and plasma hs-CRP levels as a key variable (Hopkins *et al*., 2011), the sample size in the current study obtained 24 persons for each group. Subjects at risk for pre-eclampsia, primigravida, aged 18-40 year old who were carrying singleton pregnancy at their third trimester were recruited in this study. Gestational age was assessed from the date of last menstrual period and clinical assessment (Jehan *et al*., 2010). Individuals with the above-mentioned inclusion criteria were called for participation in the study from among those that attended maternity clinics affiliated to Kashan University of Medical Sciences, Kashan, Iran. A total of 60 women attended maternity clinics affiliated to Kashan University of Medical Sciences, Kashan, Iran, were enrolled for risk pre-eclampsia, of whom 48 met the inclusion criteria. We excluded those with maternal severe pre-eclampsia, Intra Uterine Fetal Death (IUFD), placenta abruption, Premature Preterm Rupture of Membrane (PPROM), Completed Bed Rest (CBR), preterm delivery and Gestational Diabetes Mellitus (GDM). A total of 48 pregnant women were recruited in the study and were randomly assigned to receive either the placebo (n = 24) or calcium supplement plus low-dose aspirin (n = 24) for 9 weeks. The study was carried out according to the guidelines laid down in the Declaration of Helsinki. The ethical committee of Kashan University of Medical Sciences approved the study and informed written consent was obtained from all participants.

Study design: Participants considered as high risk for pre-eclampsia which have the following status: young and nulliparous women, environmental, socioeconomic factors, race, ethnicity, overweight, obesity and seasonal influences (Sibai *et al*., 2000; Lawlor *et al*., 2005). At the baseline studies (25 weeks of gestation), pregnant women were randomly assigned to receive the placebo or calcium supplement plus low-dose aspirin for 9 weeks. Subjects were requested not to change their routine physical activity or usual diets and not to consume any supplement other than the one provided to them by the investigators. The placebo was provided by Share Darou Co, Tehran, Iran. Calcium supplement and aspirin were provided by Share Darou Co, Tehran, Iran. Calcium supplement plus aspirin were containing 500 mg carbonate calcium plus 80 mg aspirin and provided to subjects monthly. We kept all supplements in a cool temperature before using. Compliance with the consumption of supplement was monitored once a week through phone interviews.

Assessment of variables: Anthropometric measurements were assessed at baseline and after 9 weeks of intervention. Body weight was measured in an overnight fasting status, without shoes and in a minimal clothing state by the use of a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height was measured using a non-stretched tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm. BMI was calculated as weight in kg divided by height in meters squared. Fasting blood samples (10 mL) were taken at baseline and after 9-week intervention at Kashan reference laboratory in an early morning after an overnight fast (Asemi *et al*., 2011b). Serum hs-CRP concentration was quantified by ELISA kit

(LDN, Nordhorn, Germany). Plasma TAC was assessed by the use of FRAP method developed by Benzie and Strain (Asemi *et al*., 2012). The plasma total GSH was measured by the Beutler method (Asemi *et al*., 2012). Serum insulin levels were assayed by enzyme linked immunoassay kits (DiaMetra, Italy). Fating Plasma Glucose (FPG) levels were assayed by the use of glucose oxidase/peroxidase (GOD-POD) method with commercially available kits (Parsazmun Co., Iran). Serum insulin levels were quantified by enzyme linked immunoassay kits (DiaMetra, Italy). Insulin resistance was assessed using the homeostatic model assessment of insulin resistance (HOMA-IR) (Catalano *et al*., 2003).

Randomization: Random assignment was performed by the use of computer-generated random numbers. Randomized allocation sequence, enrolled participants and assigned participants to intervention or placebo were performed by a trained midwife at maternity clinic. The study was single blind for receiving groups of the placebo or calcium supplement plus aspirin. That is, with the exception of the study investigators, all study participants were blinded to consumption of the placebo or calcium supplement plus aspirin.

Statistical analysis: To ensure the normal distribution of variables, Histogram and Kolmogorov-Smirnov tests were applied. We used paired-samples t-test to identify within group differences. Independent samples Student's t-test was used to detect differences between groups. This test was applied for comparison of changes between the two groups. The p-value <0.05 was considered as statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 17 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Among subjects in the placebo group, 2 persons [CBR (n = 1) and severe pre-eclampsia (n = 1)] were excluded. The exclusions in the calcium plus low-dose aspirin group was 4 women [PPROM (n = 1), severe pre-eclampsia (n = 1), CBR (n = 1) and placenta abruption (n = 1)]. Finally, 42 participants [placebo (n = 22) and calcium plus low-dose aspirin (n = 20) completed the trial (Fig. 1).

Mean age of study participants were not statistically different between calcium supplement plus aspirin and placebo groups. Baseline pre-pregnancy weight and BMI as well as their means before and after intervention were not significantly different between individuals received calcium supplement plus aspirin and placebo (Table 1).

Consumption of calcium supplement plus low-dose aspirin resulted in a significant difference serum

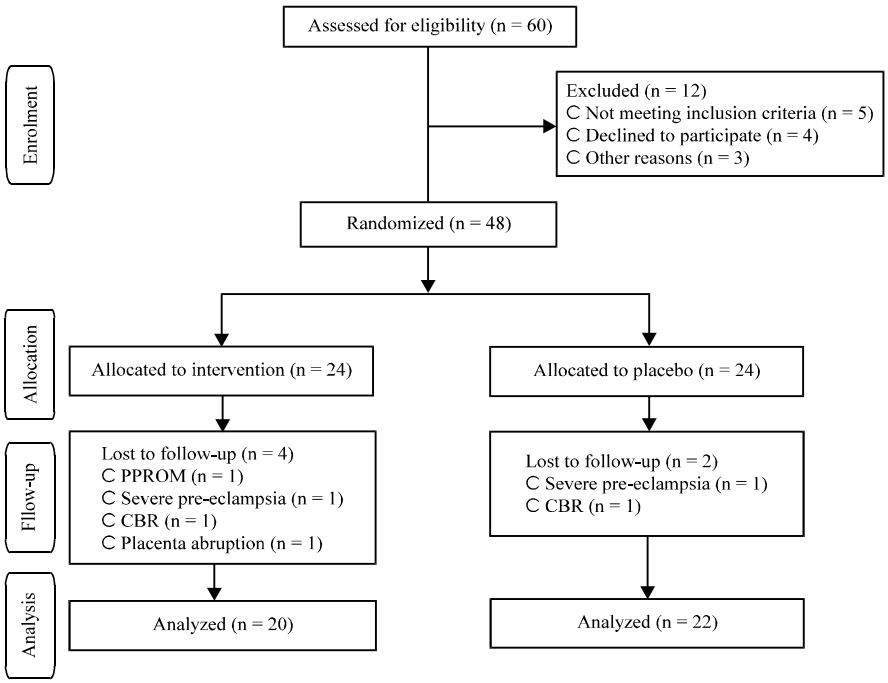

Fig. 1: Summary of patients participated in the study

Table 1: General characteristics of the study participants

Parameters	Calcium plus aspirin group (n = 20)	Placebo group (n = 22)	p-value
Maternal age (y)	26.6±5.5	24.4±3.7	0.13
Height (cm)	160.2±5.1	158.6±6.2	0.37
Pre-pregnancy weight (kg)	67.0±11.2	63.3±9.7	0.26
Weight at study baseline (kg)	74.5±11.0	69.1±9.6	0.10
Weight at end-of-trial (kg)	77.9±10.8	72.8±9.4	0.11
Pre-pregnancy BMI (kg m^{-2})	26.1±3.8	25.1±3.5	0.40
BMI at study baseline (kg m^{-2})	29.0±3.6	27.5±3.4	0.17
BMI at end-of-trial (kg m^{-2})	30.3±3.6	28.9±3.3	0.20

Values are as Mean±SD, p-values were determined by independent t-test, BMI: Body mass index

Table 2: The hs-CRP, oxidative stress parameters and insulin resistance at baseline and after the intervention

	Calcium plus aspirin group (n = 20)				Placebo group (n = 22)				
Parameter	Week 0	Week 9	Change	p-value[a]	Week 0	Week 9	Change	p-value[a]	p-value[b]
hs-CRP (ng mL^{-1})	7713.78±3338.46	7816.65±3287.69	102.87±1828.52	0.80	7938.68±6059.97	11166.43±9141.42	3227.75±4760.70	0.008*	0.01*
TAC (mmol L^{-1})	669.41±243.78	738.37±263.33	68.96±236.39	0.20	697.84±160.25	623.38±177.71	-74.46±199.07	0.100	0.04*
GSH (μmol L^{-1})	957.05±495.17	1261.38±848.30	304.33±709.32	0.07	788.60±373.35	749.27±291.50	-39.33±174.43	0.310	0.03*
FPG (mg dL^{-1})	73.30±11.80	74.20±18.90	0.90±17.70	0.82	75.60±12.80	75.70±14.50	0.10±19.20	0.830	0.88
Insulin (μIU mL^{-1})	8.76±3.13	12.23±11.18	3.47±11.08	0.18	5.27±3.14	9.14±9.03	3.87±9.19	0.130	0.91
HOMA-IR	1.62±0.77	2.12±1.81	0.50±1.78	0.23	1.01±0.58	2.02±2.79	1.01±2.86	0.190	0.55

Values are as Mean±SD, [a]indicates within-group differences (paired samples t-test),[b] indicates between group differences (independent samples t-test), hs-CRP: High sensitivity C-reactive protein, TAC: Total antioxidant capacity, GSH: Total glutathione, FPG: Fasting plasma glucose, Week 0: baseline intervention, HOMA-IR: Homeostatic model assessment of insulin resistance, *Significant difference ($p<0.05$) between both groups

hs-CRP levels as compared to the placebo (102.87 vs. 3227.75 ng mL^{-1}, p = 0.01). Also, mean changes for plasma TAC (68.96 vs. -74.46 mmol L^{-1}, p = 0.04) and total GSH levels (304.33 vs. -39.33 μmol L^{-1}, p = 0.03) were significantly different between the two groups. No significant differences were found comparing calcium supplement plus low-dose aspirin and placebo in terms of their effects on FPG, serum insulin and HOMA-IR. Within-group differences in the placebo group revealed a significant increase in serum hs-CRP levels (3227.75 ng mL^{-1}, p = 0.008) and marginally significant increase in plasma total GSH levels (304.33 μmol L^{-1}, p = 0.07) (Table 2).

DISCUSSION

The current study showed that consumption of calcium supplement plus low-dose aspirin for 9 weeks among pregnant women at risk for pre-eclampsia resulted in a significant difference serum hs-CRP and increased plasma TAC and total GSH levels. We did not find any significant effect of received calcium supplement plus aspirin on FPG, serum insulin levels and HOMA-IR score as compared to the placebo.

Pregnant women at risk for pre-eclampsia are susceptible to increased levels of inflammatory factors, oxidative stress (Asemi *et al*., 2011a) and insulin resistance (Jahromi *et al*., 2011). Elevated inflammatory factors, oxidative stress and insulin resistance during pregnancy would result in the development of severe pre-eclampsia and eclampsia (Walsh, 2009), congenital malformations, low birth weight in neonates (Min *et al*., 2006; Banhidy *et al*., 2011) and premature delivery (Mericq, 2011).

This study showed that consumption of calcium supplement plus low-dose aspirin for 9 weeks during pregnancy resulted in a significant difference serum hs-CRP levels between two groups. To our knowledge, this data represent the first report of the effect of received calcium supplement plus low-dose aspirin on serum hs-CRP among pregnant women at risk for pre-eclampsia. In consistent to this study findings, ribonuclease-enriched lactoferrin supplementation [(ribonuclease-enriched lactoferrin (2×125 mg day^{-1}) and carbonate calcium (100% RDA)], showed beneficial effects towards improvement of inflammatory status CRP among postmenopausal women (Bharadwaj *et al*., 2010). In peritoneal dialysis patients, inadequate dietary intakes of iron, zinc, calcium and vitamins A, B6, C, niacin and folic acid have also been resulted in increased serum inflammatory factor hs-CRP (Martin-del-Campo *et al*., 2012). However, Peake *et al*. (2011) has failed to find any significant effect on serum hs-CRP levels with daily consumption of 400 mL milk containing 1000 mg calcium plus 800 IU vitamin D3 in healthy men aged 50-79 years. Similar findings have also been reported among healthy postmenopausal women after 1 year of calcium supplementation (Grey *et al*., 2006). On the other hand, the findings of present study were consistent with other studies in relation to the effect of aspirin on serum hs-CRP levels. Serum hs-CRP, TNF-alpha, IL-6 and TXB2 levels were significantly decreased after 2 weeks of treatment with 300 mg day^{-1} of aspirin. The same findings have also

been reported with received 100 mg day^{-1} of aspirin among patients with metabolic syndrome (Gao *et al*., 2009). Aspirin treatment with 300 mg day^{-1} has also been resulted in decreased serum CRP levels in patients with chronic coronary artery disease (Ikonomidis *et al*., 2006). However, low-dose aspirin has no significant effect on decreasing serum CRP levels among patients with controlled hypertension which had low inflammatory burden (Kim *et al*., 2011). A 6-week course of aspirin could also not improve low-grade inflammation and serum CRP levels in patients with type 2 diabetes without cardiovascular disease (Hovens *et al*., 2008). The exact mechanisms by which calcium supplement might affect inflammatory factors are unknown. The findings of current study suggest that calcium supplement may be have an effect lesser of parathyroid hormone (PTH) secretion, which in turn resulted in decreased production of inflammatory factors including CRP. Earlier studies shown that serum PTH was significantly correlated with serum CRP levels (Senturk *et al*., 2008; Emam *et al*., 2012). Furthermore, the beneficial effects of aspirin in decreasing inflammatory factors may be result from suppressing PTH production (Grey *et al*., 2006), inhibit IKKbeta activity and decrease inflammatory cytokine production (Hundal *et al*., 2002) and inhibiting inducible nitric oxide synthase production (Carvalho-Filho *et al*., 2009).

The current study showed that the use of calcium supplement plus low-dose aspirin for 9 weeks among pregnant women at risk for pre-eclampsia resulted in a significant increase plasma TAC and total GSH levels as compared to the placebo group. Calcium supplementation (0.9%) in nutrition of High-fat Diet (HFD) mice after 9 weeks was shown that duodenal glutathione and oxidized glutathione (GSH/GSSG) ratios were strongly positively correlated with the apparent calcium absorption rate and the expression of PMCA (1b) and Calbindin-D (9K), whereas reactive oxygen species levels were negatively correlated with them, as well as not affect TAC levels (Xiao *et al*., 2010). Consumption of calcium and vitamin D3 also decreased oxidative DNA damage in the normal human colorectal mucosa (Fedirko *et al*., 2010). Furthermore, in consistent with current study, aspirin administration increased liver tissue GSH levels in rats with hepatic damage induced by Bile Duct Ligation (BDL) (Emre *et al*., 2008). In a study by Gibson *et al*. (2011) aspirin 10-100 μM has also been resulted in reduced thrombotic propensity in type 2 diabetes patients by increasing platelet antioxidant status due to elevated GSH synthesis and lowering platelet-derived ROS. The impact of aspirin on human platelets has also been resulted in elevated GSH synthesis *in vitro* (Gibson *et al*., 2009). However, aspirin administration has been resulted in decreased GSH levels in animal models (Panneerselvam and Arumugam, 2011; Sun *et al*., 2011; Tuluce *et al*., 2011). It appears that consumption of calcium supplement plus low-dose aspirin can increase plasma TAC and total GSH levels by several ways. The administration of calcium supplement may be affect oxidative stress parameters particularly by calcium transport and signaling line (Ermak and Davies, 2002). Calcium might also be act as antioxidant and DNA damage decreasing agents (Fedirko *et al*., 2010) and resulted in the free radical scavenging and then increase plasma TAC levels. Furthermore, aspirin can affect oxidative stress parameters by reduced both thrombin-induced and adenosine diphosphate-induced platelet aggregation (Gibson *et al*., 2009), elevated antioxidant synthesis including GSH and reduced ROS generation (Gibson *et al*., 2011) and increased NO bioavailability (Martina *et al*., 2008). In addition, calcium supplementation plus low-dose aspirin tended to be stronger in those with higher baseline anti-oxidant relative to pro-oxidant exposures.

The present study revealed that consumption of calcium supplement plus low-dose aspirin for 9 weeks among pregnant women at risk for pre-eclampsia could not affect FPG, serum insulin levels and HOMA-IR. The results of this study are consistent with previous studies showing supplementation with 1000 mg calcium/day in obese adults after 24 weeks (Shalileh *et al*., 2010) and 1000 mg calcium plus 400 IU vitamin D3 in health women after 7 years (De Boer *et al*., 2008) did not have any effect on serum insulin and insulin resistance. Effects of aspirin on serum insulin levels and insulin resistance were previously addressed in several studies (Hundal *et al*., 2002; Carvalho-Filho *et al*., 2009). In consistent to current study, consumption of high-dose aspirin (120 mg kg day^{-1}) could not affect insulin action for 2 days in diet-induced obese rats (Carvalho-Filho *et al*., 2009). Administration of rosiglitazone, low-dose aspirin or high-dose aspirin to diabetic rats has also been resulted in nonsignificant effect in insulin levels (Abdin *et al*., 2010). However, improved insulin resistance and signaling line were seen with consumption of high-dose aspirin in diet-induced obese rats (Carvalho-Filho *et al*., 2009). A significant reduction in fasting plasma glucose and insulin resistance was also seen with consumption of high-dose aspirin (approximately 7 g day^{-1}) among type 2 diabetic after 2 weeks (Hundal *et al*., 2002). Discrepancies between present study and different studies may be related to different doses of calcium supplement and aspirin used, study designs and period of supplementation.

Several limitations must be considered in the interpretation of findings. First of all, due to budget

limitations, investigators of this study were unable to assess other markers of inflammatory and oxidative stress. Secondly, the beneficial effects of calcium supplement plus aspirin on the biochemical indicators of newborn infants were not assessed. Thirdly, duration of interventions relatively was short. Application of a longer duration of intervention might result in greater changes. This study concludes that consumption of calcium supplement plus low-dose aspirin for 9 weeks during pregnancy in pregnant women at risk for pre-eclampsia resulted in a significant difference serum hs-CRP and increased levels of plasma TAC and total GSH as compared to placebo group, but could not affect FPG, serum insulin levels and HOMA-IR score.

CONCLUSION

Pregnant women at risk for pre-eclampsia are very susceptible to increased levels of inflammatory factors, insulin resistance and oxidative stress especially in the third trimester. It seems that calcium deficiency during pregnancy may be an important risk factor for insulin resistance and oxidative stress. Considering the above cases, it is recommended that pregnant women at risk for pre-eclampsia for managing inflammation and oxidative stress receive routinely calcium supplement plus low-dose aspirin.

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REFERENCES

Abdin, A.A., A.A. Baalash and H.E. Hamouda, 2010. Effects of rosiglitazone and aspirin on experimental model of induced type 2 diabetes in rats: Focus on insulin resistance and inflammatory markers. J. Diabetes Complications, 24: 168-178.

Asemi, Z., M. Samimi, Z. Tabasi, P. Talebian, Z. Azarbad, Z. Hydarzadeh and A. Esmaillzadeh, 2011a. Effect of daily consumption of probiotic yoghurt on lipid profiles in pregnant women: A randomized controlled clinical trial. J. Matern. Fetal. Neonatal. Med. 10.3109/14767058.2011.640372.

Asemi, Z., S. Jazayeri, M. Najafi, M. Samimi and V. Mofid *et al*., 2011b. Effects of daily consumption of probiotic yoghurt on inflammatory factors in pregnant women: A randomized controlled trial. Pak. J. Biol. Sci., 14: 476-482.

Asemi, Z., S. Jazayeri, M. Najafi, M. Samimi and V. Mofid *et al*., 2012. Effect of daily consumption of probiotic yogurt on oxidative stress in pregnant women: A randomized controlled clinical trial. Ann. Nutr. Metab., 60: 62-68.

Banhidy, F., A. Dakhlaoui, I. Dudas and A.E. Czeizel, 2011. Birth outcomes of newborns after folic Acid supplementation in pregnant women with early and late pre-eclampsia: A population-based study. Adv. Prev. Med., 10.4061/2011/127369.

Bharadwaj, S., T.A. Naidu, G.V. Betageri, N.V. Prasadarao and A.S. Naidu, 2010. Inflammatory responses improve with milk ribonuclease-enriched lactoferrin supplementation in postmenopausal women. Inflamm Res., 59: 971-978.

Caritis, S., B. Sibai, J. Hauth, M.D. Lindheimer and M. Klebanoff *et al*., 1998. Low-dose aspirin to prevent preeclampsia in women at high risk. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. N. Engl. J. Med., 338: 701-705.

Carvalho-Filho, M.A., E.R. Ropelle, R.J. Pauli, D.E. Cintra and D.M. Tsukumo *et al*., 2009. Aspirin attenuates insulin resistance in muscle of diet-induced obese rats by inhibiting inducible nitric oxide synthase production and S-nitrosylation of IRbeta/IRS-1 and Akt. Diabetologia, 52: 2425-2434.

Catalano, P.M., J.P. Kirwan, S. Haugel-de Mouzon and J. King, 2003. Gestational diabetes and insulin resistance: Role in short and long-term implications for mother and fetus. J. Nutr., 133: 1674S-1683S.

De Boer, I.H., L.F. Tinker, S. Connelly, J.D. Curb and B.V. Howard *et al*., 2008. Calcium plus vitamin D supplementation and the risk of incident diabetes in the women's health initiative. Diabetes Care, 31: 701-707.

Emam, A.A., S.G. Mousa, K.Y. Ahmed and A.A. Al-Azab, 2012. Inflammatory biomarkers in patients with asymptomatic primary hyperparathyroidism. Med. Princ. Pract., 21: 249-253.

Emre, M.H., A. Polat, M. Esrefoglu, A.B. Karabulut and M. Gul, 2008. Effects of melatonin and acetylsalicylic acid against hepatic oxidative stress after bile duct ligation in rat. Acta Physiol. Hung., 95: 349-363.

Ermak, G. and K.J. Davies, 2002. Calcium and oxidative stress: From cell signaling to cell death. Mol. Immunol., 38: 713-721.

Fedirko, V., R.M. Bostick, Q. Long, W.D. Flanders and M.L. McCullough *et al*., 2010. Effects of supplemental vitamin D and calcium on oxidative DNA damage marker in normal colorectal mucosa: A randomized clinical trial. Cancer Epidemiol. Biomarkers Prev., 19: 280-291.

Gao, X.R., C.M. Adhikari, L.Y. Peng, X.G. Guo and Y.S. Zhai *et al*., 2009. Efficacy of different doses of aspirin in decreasing blood levels of inflammatory markers in patients with cardiovascular metabolic syndrome. J. Pharm. Pharmacol., 61: 1505-1510.

Garovic, V.D. and S.R. Hayman, 2007. Hypertension in pregnancy: An emerging risk factor for cardiovascular disease. Nat. Clin. Pract. Nephrol., 3: 613-622.

Gibson, K.R., I.L. Neilson, F. Barrett, T.J. Winterburn, S. Sharma, S.M. MacRury and I.L. Megson, 2009. Evaluation of the antioxidant properties of N-acetylcysteine in human platelets: Prerequisite for bioconversion to glutathione for antioxidant and antiplatelet activity. J. Cardiovasc Pharmacol., 54: 319-326.

Gibson, K.R., T.J. Winterburn, F. Barrett, S. Sharma, S.M. MacRury and I.L. Megson, 2011. Therapeutic potential of N-acetylcysteine as an antiplatelet agent in patients with type-2 diabetes. Cardiovasc. Diabetol., Vol. 10.

Grey, A., G. Gamble, R. Ames, A. Horne, B. Mason and I.R. Reid, 2006. Calcium supplementation does not affect CRP levels in postmenopausal women-a randomized controlled trial. Osteoporos Int., 17: 1141-1145.

Hajsadeghi, S., M. Hejrati, S. Moghadami, S. Rismantab and P. Namiranian, 2012. Dilated cardiomyopathy in two patients with xeroderma pigmentosum disease: A case report. Acta Med. Iran, 50: 147-150.

Hawfield, A. and B.I. Freedman, 2009. Pre-eclampsia: the pivotal role of the placenta in its pathophysiology and markers for early detection. Ther. Adv. Cardiovasc. Dis., 3: 65-73.

Hopkins, M.H., J. Owen, T. Ahearn, V. Fedirko and W.D. Flanders *et al*., 2011. Effects of supplemental vitamin D and calcium on biomarkers of inflammation in colorectal adenoma patients: A randomized, controlled clinical trial. Cancer Prev. Res., 4: 1645-1654.

Hovens, M.M., J.D. Snoep, Y. Groeneveld, M. Frolich, J.T. Tamsma and M.V. Huisman, 2008. Effects of aspirin on serum C-reactive protein and interleukin-6 levels in patients with type 2 diabetes without cardiovascular disease: A randomized placebo-controlled crossover trial. Diabetes. Obes. Metab., 10: 668-674.

Hundal, R.S., K.F. Petersen, A.B. Mayerson, P.S. Randhawa, S. Inzucchi, S.E. Schoelsen and G.I. Schulman, 2002. Mechanism by which high dose aspirin improves glucose metabolism in type 2 diabetes. J. Clin. Invest., 109: 1321-1326.

Ikonomidis, I., J. Lekakis, G. Vamvakou, S. Loizou and I. Revela *et al*., 2006. Aspirin reduces anticardiolipin antibodies in patients with coronary artery disease. Eur. J. Clin. Invest., 36: 839-843.

Jahromi, A.S., P. Zareian and A. Madani, 2011. Insulin resistance and interleukin-1β during normal pregnancy. Asian J. Biochem., 6: 366-372.

Jehan, I., S. Zaidi, S. Rizvi, N. Mobeen and E.M. McClure *et al*., 2010. Dating gestational age by last menstrual period, symphysis-fundal height and ultrasound in urban Pakistan. Int J. Gynaecol. Obstet., 110: 231-234.

Kamiya, K., M. Wang, S. Uchida, S. Amano, T. Oshika, N. Sakuragawa and J. Hori, 2005. Topical application of culture supernatant from human amniotic epithelial cells suppresses inflammatory reactions in cornea. Exp. Eye Res., 80: 671-679.

Khattab, S., I.A. Mohsen, I. Aboul foutouh, H.S. Ashmawi and M.N. Mohsen *et al*., 2011. Can metformin reduce the incidence of gestational diabetes mellitus in pregnant women with polycystic ovary syndrome? Prospective cohort study. Gynecol. Endocrinol., 27: 789-793.

Kim, M.A., C.J. Kim, J.B. Seo, W.Y. Chung and S.H. Kim *et al*., 2011. The effect of aspirin on C-reactive protein in hypertensive patients. Clin. Exp. Hypertens., 33: 47-52.

Kirwan, J.P., S. Hauguel-De Mouzon, J. Lepercq, J.C. Challier and L. Huston-Presley *et al*., 2002. TNF-α is a predictor of insulin resistance in human pregnancy. Diabetes, 51: 2207-2213.

Lawlor, D.A., S.M. Morton, D. Nitsch and D.A. Leon, 2005. Association between childhood and adulthood socioeconomic position and pregnancy induced hypertension: Results from the Aberdeen children of the 1950s cohort study. J. Epidemiol. Community Health, 59: 49-55.

Martin-del-Campo, F., C. Batis-Ruvalcaba, L. Gonzalez-Espinoza, E. Rojas-Campos and J.R. Angel *et al*., 2012. Dietary micronutrient intake in peritoneal dialysis patients: Relationship with nutrition and inflammation status. Perit Dial Int., 32: 183-191.

Martina, V., A. Masha, V.R. Gigliardi, L. Brocato and E. Manzato *et al*., 2008. Long-term N-acetylcysteine and L-arginine administration reduces endothelial activation and systolic blood pressure in hypertensive patients with type 2 diabetes. Diabetes Care, 31: 940-944.

Masuyama, H. and Y. Hiramatsu, 2012. Treatment with a constitutive androstane receptor ligand ameliorates the signs of preeclampsia in high-fat diet-induced obese pregnant mice. Mol. Cell Endocrinol., 48: 120-127.

Mericq, V., 2011. Prematurity and insulin sensitivity. J. Endocrinol. Invest., 34: 145-149.

Min, J., H. Park, B. Park, Y.J. Kim and J. Park *et al*., 2006. Paraoxonase gene polymorphism and vitamin levels during pregnancy: Relationship with maternal oxidative stress and neonatal birthweights. Reprod. Toxicol., 22: 418-424.

Panneerselvam, S. and G. Arumugam, 2011. A biochemical study on the gastroprotective effect of hydroalcoholic extract of *Andrographis paniculata* in rats. Indian J. Pharmacol., 43: 402-408.

Peake, J.M., S. Kukuljan, C.A. Nowson, K. Sanders and R.M. Daly, 2011. Inflammatory cytokine responses to progressive resistance training and supplementation with fortified milk in men aged 50+years: An 18-month randomized controlled trial. Eur. J. Appl. Physiol., 111: 3079-3088.

Polyzos, N.P., D. Mauri, M. Tsappi, S. Tzioras, K. Kamposioras, I. Cortinovis and G. Casazza, 2007. Combined vitamin C and E supplementation during pregnancy for preeclampsia prevention: A systematic review. Obstet. Gynecol. Survey, 62: 202-206.

Poston, L., A.L. Briley, P.T. Seed, F.J. Kelly and A.H. Shennan, 2006. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): Randomized placebo-controlled trial. Lancet, 367: 1145-1154.

Redman, C.W. and I.L. Sargent, 2005. Latest advances in understanding preeclampsia. Sci., 308: 1592-1594.

Roberts, J.M. and H.S. Gammill, 2005. Preeclampsia: Recent insights. Hypertension, 46: 1243-1249.

Rumbold, A. and C.A. Crowther, 2005. Vitamin C supplementation in pregnancy. Cochrane Database Syst. Rev., Vol. 18.

Senturk, N., O. Ozkaya, S. Aytekin, K. Bek and Y. Acikgoz *et al*., 2008. Characteristics of pruritus in children on peritoneal dialysis. Nephron Clin. Pract., 109: c168-172.

Shalileh, M., F. Shidfar, H. Haghani, S. Eghtesadi and I. Heydari, 2010. The influence of calcium supplement on body composition, weight loss and insulin resistance in obese adults receiving low calorie diet. J. Res. Med. Sci., 15: 191-201.

Sibai, B.M., J. Hauth, S. Caritis, M.D. Lindheimer and C. MacPherson *et al*., 2000. Hypertensive disorders in twin versus singleton gestations. Am. J. Obstetrics Gynecol., 182: 938-942.

Sun, Y., L. Huang, G.G. Mackenzie and B. Rigas, 2011. Oxidative stress mediates through apoptosis the anticancer effect of phospho-nonsteroidal anti-inflammatory drugs: Implications for the role of oxidative stress in the action of anticancer agents. J. Pharmacol. Exp. Ther., 338: 775-783.

Szarka, A., J. Rigo, L. Lazar, G. Beko and A. Molvarec, 2010. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. BMC Immunol., Vol. 11.

Tian, S., L. Lu, J. Zhang, K. Wang, P. Brown, Z. He, J. Liang and X. Yang, 2011. Calcium protects roots of *Sedum alfredii* H. against cadmium-induced oxidative stress. Chemosphere, 84: 63-69.

Tuluce, Y., H. Ozkol, I. Koyuncu and H. Ine, 2011. Gastroprotective effect of small centaury (*Centaurium erythraea* L.) on aspirin-induced gastric damage in rats. Toxicol. Ind. Health, 27: 760-768.

Walsh, S.W., 2009. Plasma from preeclamptic women stimulates transendothelial migration of neutrophils. Reprod. Sci., 16: 320-325.

Winer, N. and V. Tsasaris, 2008. Latest developments: Management and treatment of preeclampsia. J. Gynecol. Obstet. Biol. Reprod., 37: 5-15.

Xiao, Y., J. Cui, Y.H. Shi, J. Sun, Z.P. Wang and G.W. Le, 2010. Effects of duodenal redox status on calcium absorption and related genes expression in high-fat diet-fed mice. Nut., 26: 1188-1194.