ORIGINAL ARTICLE

Association between Lipid Peroxidation and Hypocalcemia in patients with Pregnancy Induced Hypertension (PIH) in a local population of Pakistan

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ABSTRACT

Background: The study was carried out to evaluate the effects of calcium on lipid peroxidation in normal pregnancy and pregnancy induced hypertensive females. It is suggested that calcium may possibly play a key role in regression of PIH by reducing the oxidative stress. The study of serum calcium level in PIH advocates the value of serum calcium as a marker of pregnancy complicated by hypertension. Calcium supplementation during pregnancy lowers blood pressure and epidemiological data suggests an inverse correlation between dietary calcium intake and incidence of PIH.

Methodology: It was a case control study that included 35 normal pregnant women (NP group), 35 pregnant females with pregnancy induced hypertension (PIH group) and 10 non pregnant women (control group) between the ages of 18-35 years attending Sharif Medical City Hospital, Lahore. Venous blood was withdrawn and collected from the three study groups. It was centrifuged and was used for the estimation of calcium and malondialdehyde (MDA) the marker of lipid peroxidation.

Results: The results obtained showed that the levels of MDA were significantly increased in pregnancy induced hypertensive women (PIH group) when compared with the NP group and control group (P = 0.000). On the other hand, the level of calcium was significantly reduced in PIH group when compared with NP group, with an even further decrease in control group (P = 0.000).

Conclusion: The present evidence supports the concept that calcium supplements during pregnancy can reduce PIH when given to women with deficient calcium intake or when they are at risk for PIH.

Keywords: lipid peroxidation, MDA, Calcium, PIH

INTRODUCTION

Pregnancy is confronted with many physiological and metabolic changes[1]. Throughout normal pregnancy, a lot of gestational changes occur in the mother's body like enlarged uterus with increase in blood supply, increase in cardiac output, and total blood volume. Instead of incredible physiological changes in the mother's body, pregnancy does not have any undesirable effect on woman's health throughout or after pregnancy. Pregnancy induced hypertension is a medical complication which occurs in 5-10% of the pregnancies. The frequency of hypertension among different hospitals, regions and countries is variable. Pregnancy induced hypertension (PIH) is defined as hypertension which develops as a result of pregnancy and subsides after the delivery. The starting event in pregnancy induced hypertension has been assumed to be reduced uteroplacental perfusion as a consequence of atypical cytotrophoblast invasion of spiral arterioles. Placental ischemia is supposed to be the main cause of extensive activation/dysfunction of the maternal vascular endothelium. This results in exaggerated production of endothelin and thromboxane and decreased production of vasodilators such as nitric oxide (NO) and prostacyclin. These endothelial derangements cause hypertension.

Lipid peroxidation occurs at low levels in all cells and tissues. In a healthy body, oxidation by free radicals and neutralization by antioxidants continue to be in balance. When the reactive oxygen species (ROS) are in great quantity, oxidative stress occurs which is considered to be the contributory factor in PIH. Recently, the oxygen derived free radicals have been reported to play a significant role in the pathogenesis of PIH. In addition, it has been shown that a biochemical imbalance in pregnancy induced hypertension occurs with an increase of oxidative stress and lipid peroxidation and a lack of antioxidants. Lipid peroxides, as a yield of a changed oxidative stress, are involved in endothelial cell damage, vasospasm and imbalance between thromboxanes and prostacyclins. Calcium, an important electrolyte, plays a critical role in the function of the cardiac and vascular smooth muscles. It is known that the deficiency of calcium may lead to irritable nervous muscular symptoms, even tetanic convulsions, bleeding diathesis, capillary hemorrhages, and tissue exudation and osteomalacia. These features have got some resemblance to the clinical manifestations and pathological findings in pregnancy induced hypertension

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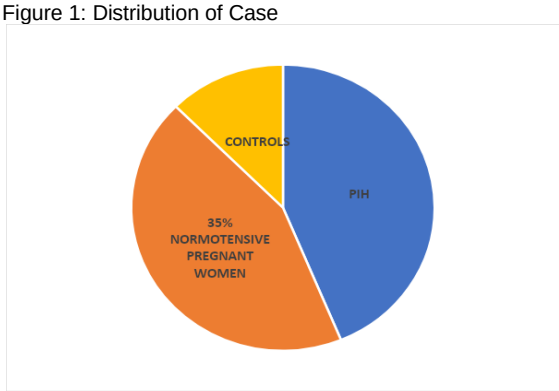
(PIH), particularly eclampsia. In contrast increase in the intracellular calcium causes vasoconstriction, increase in the peripheral resistance and therefore, an increase in the blood pressure. Pregnancy is a normal physiological phenomenon with many biochemical changes including alterations in calcium metabolism. The results of biochemical tests during pregnancy may therefore differ from the normal reference ranges. Over the years, researchers have suggested that calcium may be playing a role in the etiology of hypertensive disorders during pregnancy. Some studies have revealed changes in blood calcium level in women with hypertensive disorders during pregnancy[2-4]. So, prevention of hypertensive disorders during pregnancy and their severity are being attempted through calcium supplementation [5-12]. Researchers have reported the impairment of calcium metabolism with low circulating vitamin D concentration and/or inadequate dietary calcium which may be contributing to the risk of hypertensive disorders during pregnancy[13]. Clinical trials on healthy pregnant women have been performed to ascertain whether calcium supplementation during pregnancy would effectively lower blood pressure and whether such treatment would have an effect on the incidence of pregnancy induced hypertension. Regulation of intracellular calcium plays a key role in hypertension and calcium supplement has been hypothesized to reduce chances of pregnancy induced hypertension and preeclampsia[14,15,16].

METHODOLOGY

The study was conducted at the Institute of Molecular Biology and Biotechnology, University of Lahore, Defense Road Campus Lahore. Blood samples were collected from the patients attending the antenatal OPD in Gynecology & Obstetrics Department of Sharif Medical City Hospital, Lahore. The present study was approved by the Ethical Review Committee of University of Lahore. Medical history of all patients was taken in accordance with the criteria provided by WHO prior to blood collection. Blood was drawn from forearm vein and left at room temperature to separate the serum from blood cells. Blood samples were centrifuged at 30,000rmp for 10 minutes to obtain clear serum. Samples were kept in the freezer maintained at -20ºC. Pregnant women and controls completed a pre-coded questionnaire after formal consent. The questionnaire included personal information (age, medical history, number of pregnancies, and level of education). A total of 80 subjects were enrolled in the current study, out of which were 10 non-pregnant controls (control group), 35 were normal pregnancies (NP group) and 35 pregnant females with diagnosed pregnancy induced hypertension (PIH group). Serum calcium and MDA levels were measured in the three groups. The age group of the enrolled patients and controls was between 18-35 years. Serum calcium measurement was performed with enzymatic method using automated spectrophotometer (Biosystems 30 Spain). MDA was measured by spectrophotometric method of Okawa *et al.* (1979). All values were expressed as mean±standard deviation. All the data obtained was analyzed statistically by using Univariate Analysis of Variance. For differences in level of serum calcium t- test was performed. Level of statistical significance was set at P value < 0.05.

Figure 1: Distribution of Case



RESULTS

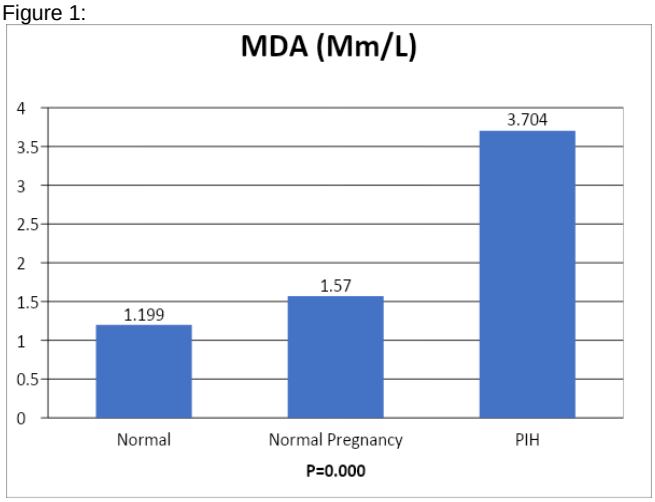
This case-control study analyzed the role of calcium, a micronutrient in PIH compared with normal pregnant females and further with the non-pregnant females taken as controls. Malondialdehyde (MDA), an indicator of lipid peroxidation and oxidative stress was calculated in all the enrolled subjects and mean was calculated. There was a statistically significantly increase in MDA levels in normal pregnancy (NP group) (1.571±0.146) compared to non-pregnant females (control group) (1.1990±0.12982). The MDA levels further showed a highly significant increase in pregnancy induced hypertension (PIH group) (3.704±0.138) than in NP and control groups. (Table 1, Figure 1)

Table 1: Deviation of antioxidant MDA levels in normal pregnancy, PIH and controls.

Groups	mean ±SD	P-value
Normal Pregnancy (NP)	1.571±0.146	0.000*
Pregnancy induced hypertension (PIH)	3.704 ±0.138	0.000*
Non- pregnant controls (C)	1.1990±0.12982	0.000*

P-value ≤ 0.05

Figure 1:



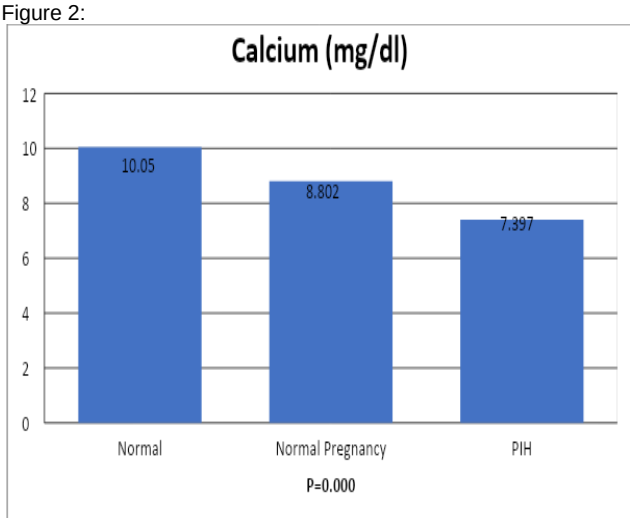
There was a significant fall in calcium levels in normal pregnancy (8.802±0.112) as compared to controls (10.050±0.246) with a further decrease in levels in pregnancy induced hypertensive patients (7.397±0.106) as

compared to normal pregnant women. The results obtained were statistically significant. (Table 2, Figure 2)

Table 2: Deviation of calcium levels in Normal pregnancy, PIH and Controls

Groups	Mean ±SD	P-value
Normal Pregnancy (NP)	8.802±0.112	0.000
Pregnancy induced hypertension (PIH)	7.397±0.106	0.000
Non-pregnant controls	10.050± 0.246	0.000

Figure 2:



The present study shows a statistically significant increase in MDA and a fall in calcium reflecting increased oxidative stress in pregnancy. PIH pregnant women shows imbalance between the prooxidants and non enzymatic antioxidants as compared to the healthy pregnant women so care should be taken during this period to minimize the pathogenesis caused by free radical damage. In this study, a significant decrease in serum calcium levels was seen in PIH as compared to normal pregnant women and the levels of normotensive pregnant were decreased than non-pregnant controls. The values indicate an association between calcium deficiency and PIH. There was no significant difference in serum calcium when compared among three trimesters of pregnancy. In the present study, the mean serum calcium levels of normal pregnant women (NP) (8.802 ± 0.112 mg/dl) were less than the non-pregnant controls (10.050± 0.246 mg/dl) and the calcium levels of PIH pregnant females were (7.397±0.16). The results were significant and supported the hypothesis that calcium might be a cause in the development of PIH. The effect of serum calcium on changes in blood pressure could be explained by the level of intracellular concentration of calcium. The increase of intracellular calcium concentration when serum calcium went lower led to constriction of smooth muscles in blood vessels and increase of vascular resistance.

DISCUSSION

Pregnancy induced hypertension (Gestational hypertension) is the hypertension detected for the first time after 20 weeks of gestation in the absence of proteinuria and measured systolic blood pressure greater or equal to 140 mmHg or diastolic blood pressure greater than or equal to 90 mmHg Hawfield, A et al (2009)[17]. The primary cause of PIH is endothelial dysfunction which plays a major role in the underlying pathophysiological mechanism of the disease. Marcoux et al. in their case-control study assessed the relation of calcium intake in the first 20 weeks of pregnancy to the risk of preeclampsia, gestational hypertension and reported that calcium intake during pregnancy may be inversely related to the risk of gestational hypertension.

In the present study the lipid peroxidation stress marker i.e. malondialdehyde (MDA) levels have been increased significantly in serum of the patients with pregnancy-induced hypertension. Rise in MDA could be due to increased production of reactive oxygen species (ROS) due to the enormous amount of oxidative damage produced in these patients. Lipid peroxides and free radicals may be important causative factor in the pathophysiology of PIH. The results were consistent with the past study of Sharma et al (2006) found raised oxidative stress and low antioxidant status in PIH[18]. During normal physiological processes ROS are continuously produced and they are removed by antioxidant defense mechanisms. In pathological conditions there is an imbalance, resulting in lipid peroxidation and oxidative damage. There is increasing evidence that oxidative stress is an important factor in the pathogenesis of pregnancy induced hypertension. Pregnancy induced hypertension is a condition of pregnancy in which antioxidant defenses system fail and tissues are injured. The current study shows a significant decrease in the levels of calcium in patients with PIH when compared to controls. Comparison among the trimester showed no significant change. The results are statistically insignificant. In the present study, the mean serum calcium levels of normal pregnant women (8.99 + 0.31 mg/dl) were less than the range (9.34-9.66 mg/dl) given by the previous report. The data supported the hypothesis that calcium might be a cause in the development of PIH. The effect of serum calcium on changes in blood pressure could be explained by the level of intracellular concentration of calcium. The increase of intracellular calcium concentration when serum calcium went lower led to constriction of smooth muscles in blood vessels and increase of vascular resistance. The results are consistent with the past study of Lopenz-Jaramillo P (2000)[19].

The present finding is similar to the previous studies performed by Malas et al (2001).[20] Derangement in calcium homeostasis may contribute to the increased vascular sensitivity which is documented in pregnancy induced hypertension. Calcium metabolism is under strain during pregnancy. Expectant mothers need to store about 30-50 gm of calcium during the course of pregnancy, of which 25gms are needed by the fetus. Eighty percent of the total fetal calcium is deposited during the third trimester. The transport of ionized calcium from the mother to the fetus increases from about 50 mg/day at 20 weeks of gestation to a maximum of about 350 mg/day at 35 weeks of gestation. Decreased serum calcium levels lead to an increase in the parathyroid hormone levels, thereby

increasing the intracellular calcium levels, which leads to an increase in the vascular smooth muscle contraction and thus, an increase in the blood pressure. Despite the low circulating calcium levels, the intracellular level of calcium ions is high, which leads to hypertension. Varner et al. were the first to report that calcium metabolism in term pregnancy associated with essential hypertension without other complications was characterized by significant reduced levels of ionized calcium[21]. It is possible that the blood pressure lowering effect of calcium supplementation could be even greater in women with PIH. In support of this hypothesis, Knight et al in a randomized controlled clinical trial involving both normotensive and hypertensive pregnant women reported that calcium supplementation (1000 mg. /d) significantly lowered the diastolic blood pressure in the hypertensive group only[2].

CONCLUSION

In conclusion, the evaluation of oxidant/antioxidant imbalance in pregnant women could be valuable in the early identification of PIH, and calcium supplementation in the early weeks of gestation might improve maternal and perinatal outcome in pregnant women. Therefore, the treatment with calcium in the initial stages of the disease may be useful as secondary therapy to avert the oxidative damage. The present study showed that Calcium supplements are excessively used to attenuate the cellular changes which occur due to lipid peroxidation. However more studies are needed to confirm the relationship between calcium levels and pregnancy induced hypertension.

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