RELATIONSHIP BETWEEN SERUM 25 OH VITAMIN D AND OTHER SERUM METABOLITE LEVELS OF HYPERTENSIVE AND NON HYPERTENSIVE PREGNANT WOMEN IN NIGERIA

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Abstract: *Hypertension has been estimated to complicate 5 % of all pregnancies, with hypertensive disorders accounting for up to 40 000 maternal deaths annually. This study therefore seeks to investigate the effect hypertension has on some serum metabolites, namely; Serum 25-OH vitamin D (Vit. D), Alkaline phosphates (ALP), Inorganic phosphates (Pi), Calcium and Total protein level in hypertensive and non-hypertensive pregnant women at different gestation period/ trimesters. Vit. D was assayed by Elisa, while the other parameters were all assayed by colorimetric method. Vit. D, Calcium, Pi and total protein had their highest values at non-hypertensive (control) conditions at all trimesters, while other hypertensive conditions from severe, mild to pre-hypertensive cases had steady increase in all the serum metabolites above mentioned except for Pi, which showed a sharp increase in its value between the mild and pre-hypertensive at all three stages studied: 0.09 ± 0.01 and 1.45± 0.12mmol/L at first trimester,0.087±0.01 and 1.23±0.12 mmol/L at second trimester, whileat third trimester 0.07±0.01 and 1.03±0.12 mmol/L. However ALP showed different pattern with its highest mean enzyme activity observed for severe hypertensive cases;192.47±13.11U/L at first trimester, 197.32±18.11U/L at second trimester and 212.47±21.71 U/L at third trimester. Therefore, all the serum metabolites understudied showed significant relationship in the values obtained from the hypertensive and non-hypertensive patients considered and can serve as possible inference for early diagnosis of maternal and foetal complications in hypertension*.

Keywords: Hypertension, pregnancy, serum metabolites

1. INTRODUCTION

Pregnancy, also known as gravidity or gestation, is the time during which one or more offspring develops inside a woman and at this period, maternal physiological changes happen. During pregnancy, there are changes in metabolic, biochemical, physiological, haematological and immunological processes and if there are no complications, all these changes are reversible a few days to a few months after delivery (Sonagra *et al.*, 2012). Globally, an estimated 500,000 women die from complications arising during pregnancy, delivery or puerperium while in Nigeria, a total of 55,000 die which accounts for 10 % of the world's total deaths while Nigeria is 2 % of world's population (Nwosu *et al*., 2009). Hypertensive disorders of pregnancy (HDP) and their complications rank as one of the major cause of maternal mortality and morbidity in the world after obstetric haemorrhage, pre-existing medical disorders, sepsis and abortions (Park, 2011). It is also reported to complicate 1 in 10 pregnancies hence it is the most common medical disorder of pregnancy in both developing and developed countries (Magee and Von Dadelszen, 2004; Podymow and August, 2008). Kamath (2006), reported that HDP occurs in approximately

6-8% of all pregnancies, 10% of first pregnancies, and 20-25% of women with a history of chronic hypertension, while it also accounts for a quarter of all antenatal admissions as it has been associated with fetal growth retardation and leading to largely to perinatal mortality and morbidity (Bansal, 2008; Datta, 2011).

It is also known that maternal nutrition during pregnancy exerts profound effect on the health of both mother and child. Hence a thorough research on all the biochemical changes is thus necessary in order to understand the pathophysiology and identification of biochemical markers that help in early diagnosis of the disorder. This will help in reducing the risk women face in preventing hypertension in subsequent pregnancies and later in their life. This study tried to investigate the effect of hypertension during pregnancy on serum 25-OH vitamin D, Alkaline Phosphates, Inorganic phosphates, calcium and Total Protein and to ascertain any possible correlation.

2. MATERIALS AND METHODS

The study was carried out in State specialist Hospital (SSH) located in Akure, SSH Okitipupa, SSH Ikare and Federal Medical Centre (FMC), Owo. The study spanned between December 2013 and October 2014. Five hundred pregnant women of different trimesters were recruited according to their blood pressure and their samples were collected for the study. They were categorized into four groups of blood pressure. One hundred normotensive (< 120/<80) were used as the control while four hundred that were hypertensive were in three groups of blood pressure. The severe hypertensive blood pressure (≥160/ ≥100), mild hypertensive blood pressure (140-159/90-99) and pre hypertensive blood pressure (120-139/80-89). The pregnant women were within the age range of 26 to 43 years.

Blood sample which was collected from all the subjects three times from the first trimester (1– 3months) to the third trimester (6 – 9 months) of the subjects using method as described by Monica (2000). About 3 ml of blood were collected into sterileplain bottles at each collection period and the serum was pooled 3hours after collection and stored at < –4 ºC before analysis.

Alkaline Phosphatase assay was carried out using colorimetric method, with readings taken at 550 nm wavelength (Babson *et al.*, 1966). Assay of Inorganic Phosphate was done using standard method of Debruyne (1983) and absorbance read on the spectrophotometer was at a wavelength of 340 nm. The assay of Calcium using the method of Fogh-Andersen *et al*., (1978); the preparations in each of the tubes were mixed and read at a wavelength of 600 nm against the reagent blank after 5 minutes of preparation. Vitamin D_3assay was carried out by using Elisa (Enzyme linked immunosorbent Assay) (Randox, UK). The intensity of the yellow colouration obtained is indirectly proportional to the concentration of 25-Hydroxy Vitamin D in the sample. The absorbance was determined with an Elisa reader at 450 nm against 620 nm (Zerwekh, 2008), while the assay of Total protein was carried out according to Biuret method (Layne, 1957). Data collected by questionnaire distributed amongst all considered women to obtain further details and general knowledge pertaining to their blood pressure state and previous medical history, was also analysed.

3. RESULTS AND DISCUSSION

The relationship between the serum metabolite – Alkaline phosphatises (ALP) of pregnant women and their blood pressure at different stages of pregnancy was represented in Figure 1.

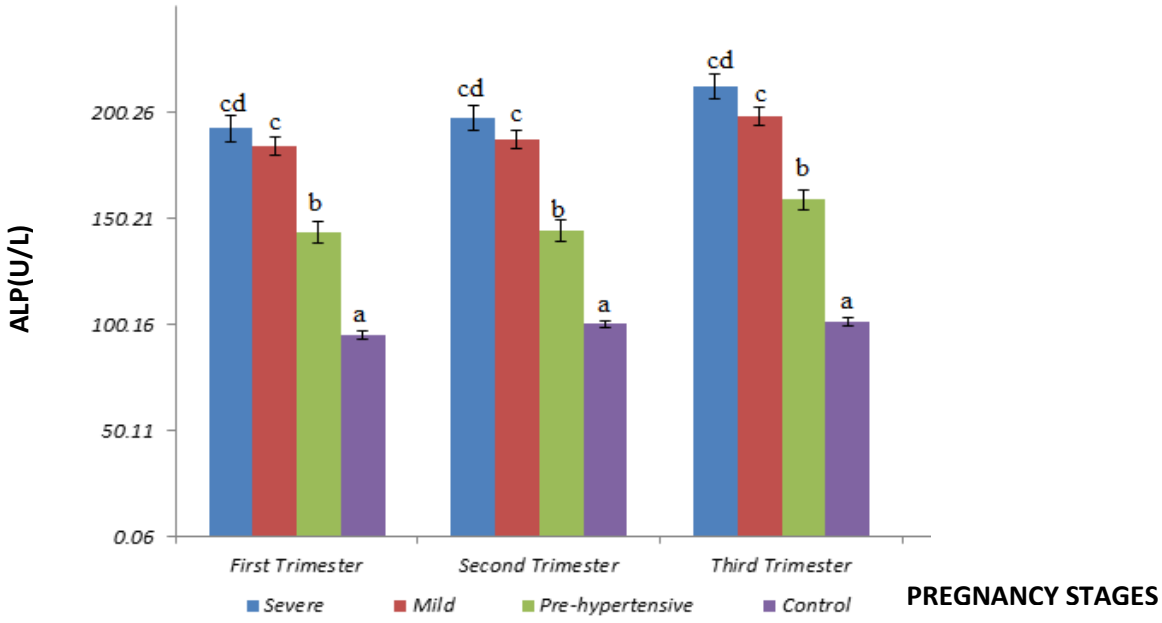

Figure 1: Alkaline Phosphatase (ALP) of pregnant women and their blood pressure at different stages of pregnancy

Steady increase in placental alkaline phosphatase (PALP) was observed at all hypertensive stages of pregnancy, control (normotensive) conditions inclusive. Highest mean unit of enzyme activity observed was 212.47±21.71 U/L, recorded for severe hypertensive pregnant women in their third trimester. A statistical increase at different probability level of ALP was shown in this research work. The test groups for each trimester increased with increase in the gestational age of the subjects as seen from the results, in reference to the control. This was in agreement with Jeacock *et al*., (1963) which showed that phosphatase activity in the placenta usually increases during pregnancy and Sussman and Bowman (1968) validated that claim by showing that placental phosphatase first appears in the first trimester of pregnancy and increases during gestation to comprise 40 – 67% of the total serum phosphatase during the third trimester. Also the increase in ALP can be attributed to the increase in blood pressure of the subjects because there is an increase of ALP activity in pre-eclampsia.

At all hypertensive conditions tested, the mean values of Pi reduced with severity in blood pressure considered (figure 2). The highest concentration of Pi been recorded was for control at all trimesters;1.83±0.19, 1.85±0.19 and 1.78±0.19mmol/L for first, second and third trimesters respectively. Also, mean concentration of Pi reduced at all hypertensive conditions as gestation age increases, except in control (normotensive), which had a slight increase at second trimester before decline in mean concentration at the last trimester.

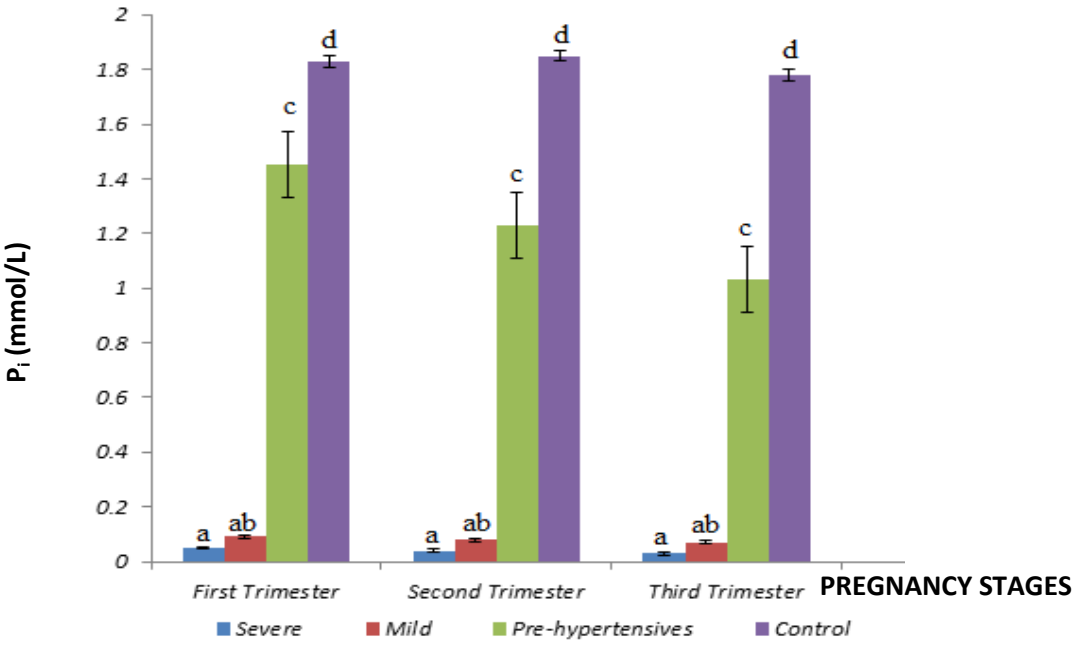

Figure 2: Inorganic Phosphate (P_i) of pregnant women and their blood pressure at different stages of pregnancy

Calcium (Ca^{2+}) of pregnant women and their blood pressure at different stages of pregnancy as observed in figure 3 shows thatat all hypertensive conditions tested, the mean values of Ca^{2+} reduced with severity in blood pressure considered. The highest concentration of Ca^{2+} been recorded was for control at all trimesters;2.67±0.28, 2.42±0.16 and 2.34±0.08 mmol/L for first, second and third trimesters respectively.

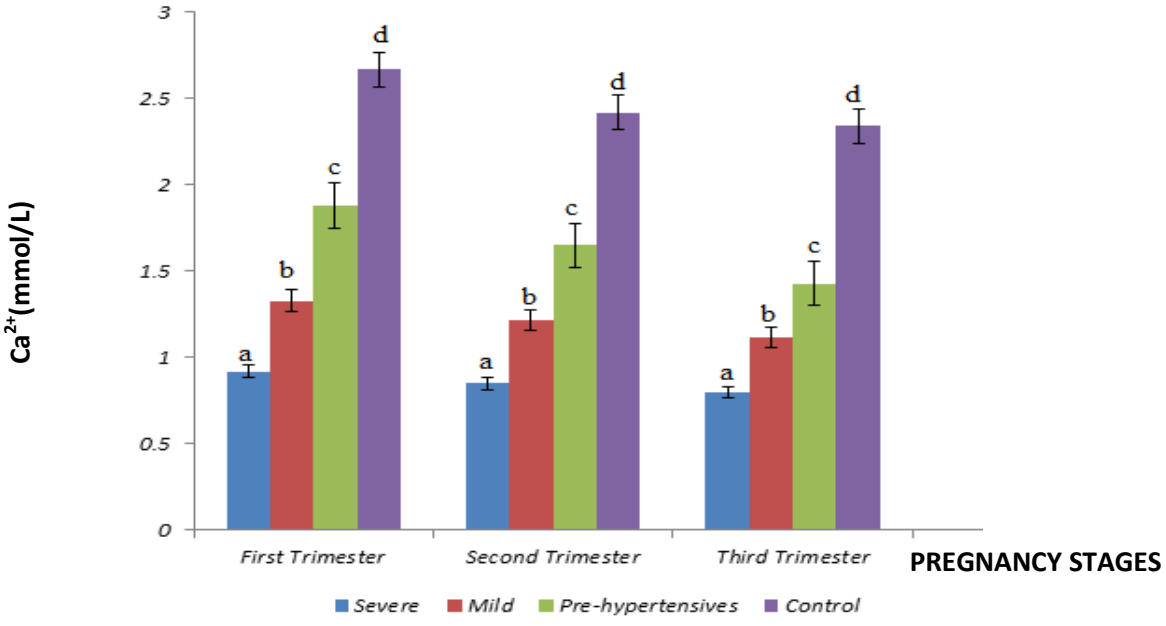

Figure 3: Calcium (Ca^{2+}) of pregnant women and their blood pressure at different stages of pregnancy.

It was observed, that there was a steady and marked decrease in the serum levels of both factors as gestation age increases that is for inorganic phosphate and serum calcium; while the concentration of both parameters, was much lower in severe hypertensive patients with a corresponding increase in Mild and pre-hypertensive patients. Following the questionnaire the tested patients filled, the calcium intake from different supplements by the subjects appears to be less than the amount recommended for pregnant women due to socioeconomic status, this may also account for the low concentration as gestation period nears. It is therefore suspected that the inadequate dietary intake of calcium possibly contributed to the marked decrease in calcium and inorganic phosphate of the subjects. Putting into consideration the work of Seelig, (1980), the importance of low serum calcium levels in the pathogenesis of such conditions as in-coordinate uterine action, pre-eclampsia and intra-uterine growth retardation should also be considered as a possible explanation for the decrease in the serum calcium in for the hypertensive pregnant women (test group) when compared to non-hypertensive pregnant women (control). Also Blum *et al*., (2008) confirmed that the possible decrease in serum calcium and inorganic phosphate as the gestational age increases might be as a result of their utilization for foetal growth.

Lower serum calcium and phosphate levels were reported in pregnancy-induced hypertension (PIH) compared to normotensive pregnant women by Almasganj *et al*., (2004). A significant correlation between serum calcium and hypertension has been shown in PIH (Nasser and Ziad, 2011). Gary *et al.*, 2005 and others confirmed that high serum calcium level in pregnancy can lead to decreased incidence of preeclampsia and vice versa (Daniel and David, 1994; Gary *et al.,* 2005). The result obtained in this study was in line with the above claim, as there was a statistical decrease in the serum calcium level of different blood pressure category in each trimester when compared to the control. Likewise, inorganic phosphate showed same correlation.

1,25-Dihydroxyvitamin D of pregnant women and their blood pressure at different stages of pregnancy is presented in figure 4. The concentration of metabolite steadily reduced as gestation period progresses in all hypertensive except in normotensive control which had a slight increase in concentration at second trimester (i.e. from 89.21±5.17 to 91.03±7.36 mmol/L), before a final drop at the third trimester (87.03±7.36 mmol/L).

Serum calcium also has an integral pathogenesis with 1,25-dihydroxyvitamin D. Physiologically, 1,25-dihydroxyvitamin D stimulates calcium influx in a variety of cells, including vascular smooth muscle cells. This effect is rapid, as it is mediated by vitamin D receptor rather than via a classical nuclear-receptor-mediated mechanism. As a consequence, 1,25-dihydroxyvitamin D exerts a repressor effect, serving to promote contraction and increase peripheral vascular resistance. Consequently, low calcium diets, which elicit a 1, 25-dihydroxyvitamin D response, would be expected to increase blood pressure, whereas high calcium diets, by virtue of suppressing 1,25- dihydroxyvitamin D levels, would be expected to reduce vascular smooth muscle cell intracellular calcium, peripheral vascular resistance and then blood pressure (Zemel, 2001). This fact was validated again by this study showing the decrease of 1,25-dihydroxyvitamin D as the blood pressure of the subjects increases in each trimester.

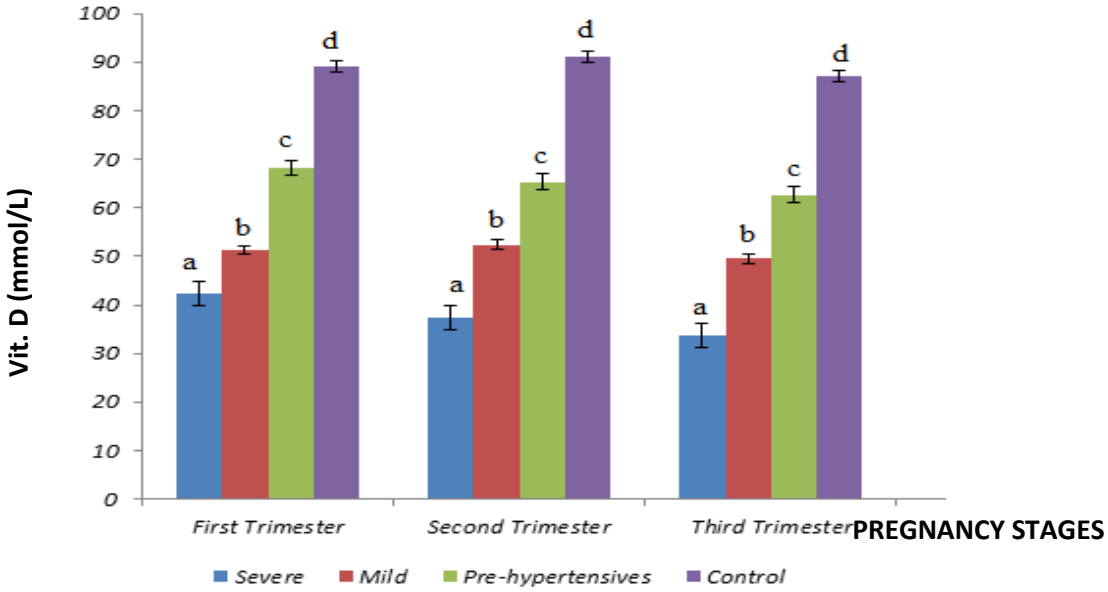

Figure 4: 1,25- Dihydroxyvitamin D of pregnant women and their blood pressure at different stages of pregnancy

Total protein of pregnant women and their blood pressure at different stages of pregnancy was shown in figure 5. A continuous decrease in total protein concentration was observed with severity in blood pressure of pregnant cases considered. Likewise concentration also decreased as gestation period progresses for all categories of hypertensive cases and its control.

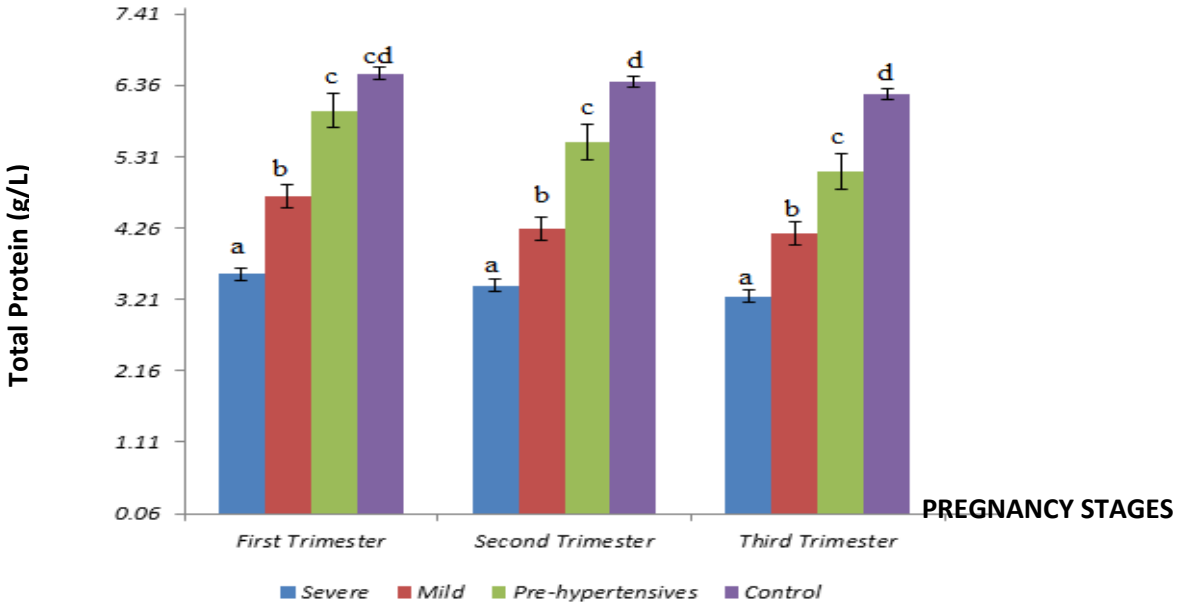

Figure 5: Total serum protein of pregnant women and their blood pressure at different stages of pregnancy

Total serum protein represents the sum of albumin and globulin in broad terms. It was significantly reduced in the hypertensive test groups compared to the control. This was in line

with the work of Hofmeyr *et al*., (1991) who showed that total proteins were significantly less than those of their internal controls in hypertensive women. In the case of trimesters, the decrease in the second trimester is most likely due to decrease in albumin fractions and probably hypervolemia which is consistent with pregnancy and consequentially causes dilution effect (Mustafa *et al*., 2012). Moreover due to its molecular weight, albumin is the fraction that is normally lost during proteinuria in pregnancy. Thus hypoalbuminaemia in pregnancy may not necessarily be due to decrease in production but also dilution (Greenwood *et al*., 1970).

4. CONCLUSION

Since the relationship between the serum metabolites of hypertensive and non-hypertensive pregnant women was noteworthy, inference can be drawn from them by medical experts in better understanding the pathological process of pregnancy induced hypertension and to develop strategies for prevention and early diagnosis of any maternal and foetal complications in hypertension.

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