Evaluation of Serum Level of Minerals, Alkaline phosphatase and Vitamin D in Healthy Pregnant Women of Jodhpur.

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ABSTRACT

There is believe that vitamin D deficiency is rare in sunny, semi hot arid parts of tropical country like India. Calcium and vitamin D play major roles in calcium homeostasis and skeletal development especially during pregnancy. This study is aimed to determine and evaluate the serum vitamin D, calcium, magnesium, phosphorus and alkaline phosphatase levels in healthy pregnant subjects around Jodhpur, "The Sun City" district of Rajasthan. The study involves determining alterations or otherwise of these parameters during each trimester of normal pregnancy when compared with healthy non-pregnant controls. Total of 120 subjects were recruited for this study. Of this total, 90 were healthy pregnant women (30 subjects for each trimester) and 30 were healthy non-pregnant controls. First trimester subjects were designated as group I, second trimester subjects as II, third trimester subjects as III and control subjects as group IV. Venous blood was drawn to test calcium, magnesium, phosphorus, vitamin D, and alkaline phosphatase levels in the serum. Of the total 90 pregnant subject, 37% had vitamin D deficiency, 33% had insufficiency and only 30% had normal or optimal vitamin D level. This is in contrast to control group where 40% of the 30 subjects therein had deficiency, 20% had insufficiency and 40% had optimal level. Serum calcium in third trimester (group III) was significantly lower ($p<0.05$) as compared to controls. Difference in serum calcium was not significant when comparing group, I with IV, I with II or II with IV. Mean serum magnesium was higher but not significant in the first trimester as compared to controls. However, it decreased in the second and third trimester and value was significantly lower in third trimester as compared to controls. Vitamin D deficiency or insufficiency parallels low level of calcium and magnesium. Significant increase in serum alkaline phosphatase was observed throughout pregnancy. Subtle but insignificant changes in serum phosphorus was observed during pregnancy. Supplementation of these minerals, fortification of food and adequate sun exposure are the possible ways of combatting vitamin D and minerals deficiency.

Keywords; Vitamin D, Calcium, Pregnancy, Hypocalcemia, Alkaline Phosphatase, trimester, Serum, Women.

1.0. INTRODUCTION.

Pregnancy is a delicate stage where maternal adaptation happens early to provide a good upshot for both the mother and the foetus [1]. These physiological changes occur at varying rates throughout the whole body which allow the pregnant woman to accumulate additional energy in preparation for labour and gestation [2]. Often times than not, pregnancy is characterized by profound changes in almost every organ and system to accommodate the demands of fetoplacental unit [3]. In pregnancy, Mineral metabolism in the mother must adapt to the demand created by the foetus and placenta, which together draw calcium and other minerals from the maternal circulation to mineralize the growing foetal skeleton. In the same vein, mineral metabolism must adapt in the lactating woman to supply sufficient calcium to milk and the suckling neonate. Potential adaptations include increased intake of mineral, increased efficiency of intestinal absorption of mineral, mobilization of mineral from the skeleton, and increased renal conservation of mineral [4]. Despite a similar magnitude of calcium demand by pregnant and lactating women, the adjustments made in each of these reproductive periods differ significantly [5]. A pregnancy is influenced by many factors, some of which include culture, environment, socioeconomic status, and access to medical care. The haematological indices also have an impact on pregnancy and its outcome [6].

Calcium, phosphorus, magnesium and zinc are the primary bone-forming minerals. At birth an infant contains approximately 20-30g calcium, 16g phosphorus, 750mg magnesium and 50mg zinc, of which approximately 98%, 80%, 60% and 30%, respectively, are in the skeleton [7]. Quantitatively, the greatest period of fetal mineral accretion takes place from mid-gestation and is maximal during the third trimester. For instance, fetal accretion of calcium increases from around 50mg/d at 20 weeks' gestation to 330mg/d at 35weeks, averaging around 200mg/d for the third trimester. After delivery, the mineral accretion accompanying skeletal and somatic growth continues, the rate being higher in the first months and slowing progressively with age [7]. Typical whole-body mineral accretion rates for infancy are 140 mg/d calcium, 70 mg/d phosphorus, 3mg/d magnesium and 0.4 mg/d zinc (Prentice and Bates, 1994). There are several biological strategies for meeting the extra demands that pregnancy and lactation put on the mineral economy of the mother. An increase in mineral intake is one; physiological adaptations through elevated gastrointestinal absorption, decreased mineral excretion or mobilization of tissue stores is another

[8]. This research looks into the extent to which physiological adaptations occur during human reproduction and the importance of maternal diet and vitamin D status on the growth and bone development of the baby and the bone health of the mother

2.0. Experimental design.

Healthy adult pregnant and Non-pregnant women within the age range of 20-45 years living in Jodhpur attending Vasundhara Hospital and Fertility Research Centre Jodhpur, Rajasthan India were included in this study. Total of 120 subjects, out of which 90 were healthy pregnant (different gestational age) and 30 were healthy non-pregnant control, were recruited. Institutional ethical committee clearance was obtained prior to the study. Written informed consent was got from every subject before collecting blood. The 90 pregnant women were grouped into three based on gestational age while the 30 non-pregnant were used as control. The subjects were grouped as follows;

Group I: Healthy pregnant women in first trimester.

Group II: Healthy pregnant women in second trimester.

Group III: Healthy pregnant women in third trimester.

Group IV: Healthy non-pregnant control.

2.1. Inclusion Criteria:

1. Healthy pregnant and non-pregnant subjects age range of 20-45 years.
2. Subjects attending Vasundhara Hospital and Fertility Research Centre Jodhpur.

2.2.Exclusion Criteria:

1. Subjects on medication.
2. Subjects with special illness.
3. Lactating women.

2.3. Sample collection.

By venopuncture technique, 10ml of blood sample was collected from each pregnant woman from each group above. Sample was collected from control subjects by similar means. In both cases, the antecubital fossa was cleaned with methylated spirit and allowed to dry. A tourniquet was then

applied a few centimetres above the antecubital fossa to detect the veins. Blood was taken with 10ml sterile syringe. This was transferred into a plain bottle. Serum samples were separated from the collected blood samples by centrifugation for 5minutes at 3000 revolutions per minute. The serum was separated from the cells and were used for calcium, inorganic phosphate, magnesium, 25(OH) vitamin D and alkaline phosphate analysis within 24 hours of storage at -20^{0}C.

2.4. Methods of Estimation.

Various methods of estimation for various kits were available. The biochemistry parameters assessed in this study were determined using the following methods; principles and procedures of the methods explained below:

2.4.1. Serum Calcium (Barnett *et al*., 1973, Moorehead and Briggs, 1974).

This was done by using modified O-cresolphthalein Complexone method using Erba Mannheim Calcium estimation kit. Normal values of calcium using this kit will be 8.4-10.4mg/dl.

Principle:

O-cresolphthalein complexone react with calcium in alkaline solution to form a purple coloured complex. The intensity of the colour formed is proportional to the calcium concentration and is measured photometrically between 540nm and 600nm with maximum absorbance at 575nm.

Procedure:

Into a test tube, 1000μl working reagent was pipetted followed by 10 μl of serum. The tube was shaken (to mix the content uniformly) and incubated for 5 minutes. Absorbance as well as result of the test was measured using semi auto analyser by aspirating the solution.

2.4.2. Serum phosphorus (Molybdate UV Method).

This was done by molybdate UV method using Liquimax Phosphorus–SLR kit. In this method, normal values of serum Phosphorus is in a range of 2.5-5.0mg/dl.

Principle:

Phosphate ions in acidic medium react with ammonium molybdate to form a phosphomolybdate complex. This complex has an absorbance in the ultraviolet range and is measured at 340nm. Intensity of the complex formed is directly proportional to the amount of phosphorus present in the serum.

Procedure:

Into a test tube, 1000μl working reagent was pipetted followed by 10 μl of serum. The tube was shaken (to mix the content uniformly) and incubated for 5 minutes. Absorbance as well as result of the test was measured using semi auto analyser by aspirating the solution.

2.4.3. Serum Magnesium.

This was done using Erba kit. In this method, normal value of serum magnesium is in the range of 1.6-2.6mg/dl.

Principle:

Magnesium reacts with Xylidyl Blue to form a coloured compound in alkaline solution. The intensity of the colour formed is proportional to the magnesium concentration in the sample. Interference with calcium is prevented by the use of GEDTA.

Procedure:

As per directions provided with kit, 1ml of working solution was poured in tube. Then 10µl sample was added to the tube, mixed and incubated at 37^0C for 3 minutes. The concentration of magnesium was estimated by aspirating the sample through semi-auto analyser.

2.4.4. Serum Alkaline phosphatase (IFCC Method).

This was done quantitatively by using International Federation of Clinical Chemist method using Liquimax alkaline phosphatase-Erba kit. Normal range of alkaline phosphatase ranges as follows; 78-306IU/L.

Principle:

Alkaline Phosphatase at an alkaline pH hydrolyses para-nirophenylphosphate (p-NPP) to form yellow coloured para-nitrophenol (p-NP). The rate of para-nitrophenol formation is directly proportional to the ALP activity. Absorbance was measured at 405nm.

Procedure:

Into a test tube, 1000µl of the working reagent was pipetted followed by 20µl of the serum. The resulting solution was mixed well and aspirated into semi-auto analyser for measurement.

2.4.5. Estimation of Serum Vitamin D.

Method used was chemiluminescence micro particle assay. Architect 25-OH Vitamin D kit is loaded in Architect I system to estimate serum Vitamin D levels. In this method, levels below 20 ng/ml is considered as deficiency, 20-30 ng/ml is insufficiency and levels between 30-80ng/ml is optimum.

2.5. Statistical Analysis.

Analysis was performed using SPSS version 16. Continuous variables were expressed as mean±SD. Discrete variables were expressed as frequency (percentage). A p value of <0.05 was considered as significant. Significant difference between groups was analysed using independent t test and results were considered statistically significant if the p value is <0.05.

3. RESULT AND DISCUSSION.

3.0. Result:

Table 4.1 shows a comparison of Mean±SD of the parameters in different trimesters of pregnancy and in controls. Differences in serum calcium among first trimester, second trimester and controls were not significant. Serum calcium in third trimester was significantly lower ($p<0.05$) as compared to controls. Mean serum magnesium was higher but not significant in the first trimester as compared to controls. However, it decreased in the second and third trimester and value was significantly lower in third trimester as compared to controls. No significant differences in serum phosphorus among different trimesters. Serum concentration of 25(OH) vitamin D_3 did not vary significantly between pregnant and control subjects. Mean serum alkaline phosphatase concentration was significantly different when compared with controls. It increases in first, second and was significantly higher in the third trimester as compared to controls. Table 4.2 shows cumulative mean serum concentrations of all the parameters of pregnant subjects and controls. Significant difference in serum calcium, magnesium, phosphorus and vitamin D was not observed when mean serum of these parameters in pregnant subjects (first, second and third trimester treated as one) was compared with that in controls. However, such difference was found to be significant in serum alkaline phosphatase. Distribution of serum 25(OH) vitamin D_3 in the various trimesters of pregnancy and of the control subjects is shown in Table 4.3. Deficiency of 25(OH) vitamin D_3 were seen in 40, 30 and 40% of pregnant women in the first, second and third trimesters respectively. The corresponding figures for insufficiency of vitamin D were 40, 30 and 30% respectively. A deficiency of 25(OH) vitamin D_3 was found in 40% of controls and insufficiency was seen in 20% of this subject. Table 4.4 shows the distribution of serum calcium concentration in the three trimesters and in controls. Low level of calcium is most frequent in third trimester with 50% of the subjects having calcium level below 8.4mg/dl. Low level of calcium reads 10% in first trimester subjects, 20% in second trimester subjects and 10% in controls as shown in the table.

Table 1.1: Mean ± Standard deviation of the biochemical parameters in various trimesters of pregnant subjects and among controls.

Biochemical Parameters	Trimester of Pregnancy			Control (n=30)
	First (n=30)	Second (n=30)	Third (n=30)	
Calcium (mg/dl)	9.18±0.76	9.05±0.79	8.59±0.81*	9.14±1.04
Magnesium(mg/dl)	2.17±0.69	1.93±0.75	1.72±0.68*	2.08±0.68
Phosphorus(mg/dl)	4.10±0.98	4.19±1.05	4.17±1.00	4.16±1.07
25(OH) Vitamin D_3(ng/ml)	22.95±7.10	21.06±6.49	20.25±7.21	23.74±6.27
Alkaline Phosphatase (IU/L)	130.41±37.17	148.89±45.17*	199.81±48.83*	121.84±30.23

*significant difference at $p<0.05$ when compared with controls.

Table 1.2: Mean ± Standard deviation of the parameters in pregnant subjects and Non pregnant controls.

Biochemical parameters.	Pregnant subjects (Test) (n=90)	Controls (n=30)
Calcium (mg/dl)	8.94±0.82	9.14±1.04
Magnesium (mg/dl)	1.94±0.72	2.08±0.68
Phosphorus (mg/dl)	4.15±1.00	4.16±1.07
25(OH) vitamin D_3 (ng/ml)	21.42±7.00	23.74±6.27
Alkaline Phosphatase (IU/L)	159.70±46.24*	121.84±30.23

* significant difference at $p<0.05$ when compared with controls.

Table 1.3: Distribution of Serum 25(OH) Vitamin D_3 concentration in various trimesters of pregnant subjects and in controls.

Groups	Serum 25(OH) Vitamin D_3 (ng/ml)		
	Deficiency <20ng/ml	Insufficiency 20-30ng/ml	Normal >30ng/ml
First trimester (n=30)	12(40)[α]	12(40)	6(20)
Second trimester (n=30)	9(30)	9(30)	12(40)
Third trimester (n=30)	12(40)	9(30)	9(30)
Control (n=30)	12(40)	6(20)	12(40)

[α]Numbers in parenthesis represent percent.

Table 1.4: Distribution of Serum Calcium concentrations in various Trimesters of pregnant subjects and in controls.

Calcium	First Trimester (n=30)	Second Trimester (n=30)	Third trimester (n=30)	Control (n=30)
Low <8.4 mg/dl	3(10)[α]	6(20)	15(50)	3(10)
Normal Range 8.4-10.4 mg/dl	24(80)	21(70)	12(40)	18(60)
High >10.4 mg/dl	3(10)	3(10)	3(10)	9(30)

[α]Numbers in parenthesis represent percent.

3.2. Discussion:

This research determines the status of 25(OH) vitamin D, calcium and other parameters related to calcium metabolism during different stages of pregnancy in pregnant women in Jodhpur. 25(OH) vitamin D deficiency was present in 40% of pregnant women in first trimester. However, the value has decreased in the second trimester as well as third trimester. Insufficiency of 25(OH) vitamin D_3 was seen in 40% of those pregnant women in their first trimester, so that overall, 80% of the first trimester pregnant women had either deficiency or insufficiency of 25(OH) vitamin D_3 as compared to 60% of the control women. In the present study, we compared the trends of biochemical changes of different subjects during different trimesters of pregnancy. In a study in Nigeria among young pregnant women, it was found that 25(OH) vitamin D_3 concentration in the first trimester decreased as compared to non-pregnant women [9]. In a comprehensive study conducted in Iran, Mirsaeid Ghazi *et al*. [10] demonstrated high prevalence WAZ of 25(OH) vitamin D_3 deficiency in the population of Tehran; this was more pronounced in women. In the present study, 25(OH) vitamin D_3 deficiency is more common in the third trimester of pregnancy, the decrease being attributable to less sun exposure. A gradual decrease in concentration of 25(OH) vitamin D_3 seen in this study from control down to third trimester is attributable to less exposure to sun as pregnancy grows. The decrease in 25(OH) vitamin D concentration in first, second and third trimester is however not significant when compared with controls due to supplementation intake during pregnancy. In a study by Bruinse [11], there was no difference in the levels of vitamin D during pregnancy in comparison to non-pregnant women. A vitamin D deficiency was also observed in a study in rural pregnant women of Barabanki [12]. Since there is positive correlation between sun light and vitamin D status [13], the observed decreased vitamin D concentration and deficiency in pregnant women can be attributed to sedentary indoor life style in which there is less exposure to sun. Another possible reason of vitamin D deficiency in pregnant women as observed in this study is attributable to diets that are not rich in vitamin D. Dietary sources are very low in Vitamin D content. Indians are not usually eating salmon, sardines, tuna, mackerel which are rich in D content. Egg yolk is also having only 20IU of Vitamin D. Dietary source of Vitamin D for vegetarians is unfortified milk which contain only 2IU of Vitamin D /100ml. Dilution and adulteration of milk will not be able to provide sufficient D vitamin [14]. In India, Vitamin D fortified food products are not commonly sold as in case of Western countries which made study subjects vulnerable to Vitamin D deficiency. The finding in this research was supported by other

researches which reported insufficient calcium and vitamin D intake increased phytate intake as inhibitor of vitamin D absorption and also lack of sun exposure were the causes of vitamin D deficiency [15], [16].

Vitamin D deficiency state leads to secondary hyperparathyroidism which results in loss of phosphorus in the urine and decreases intestinal absorption of phosphorus. This causes low calcium or low normal phosphorus concentration. Low normal calcium and low normal phosphorus both will cause insufficient calcium phosphorus product which is important for bone mineralization process. Defective mineralization causes rickets in children and osteomalacia in adults [17], [18], [19]. During deficiency of Vitamin D, there should be low levels of calcium and phosphorus but alkaline phosphatase levels should raise. But in this study, calcium was reduced but phosphorus and alkaline phosphatase levels were within normal limits indicating that bone mineralization was not yet affected. While in this study, the status of vitamin D is not significantly different during pregnancy, the rise in the rate of low serum calcium from 10% in first trimester to 20% in the second trimester and even 50% in third trimester as shown in table 4.4 may be due to deficiency and insufficiency of vitamin D caused by both lack of sun exposure, lack of access to vitamin D fortified food and intake of dietary foods that are poorly rich in vitamin D as vitamin D source. The fact that vitamin D increases the efficiency of intestinal absorption of calcium to 30-40% [20] explains why a subtle change in vitamin D concentration can alter serum calcium level. The significant decrease in serum calcium (when compared with controls and first trimester subjects) during third trimester can be explained by increase fetal calcium deposition which is shown to be 350mg/day in third trimester [21] and increased calcium transfer from mother to meet fetal calcium demand. Therefore, the significant reduction we observed in serum calcium and in the present study could be as a result of mineral transfer from maternal circulation to the developing fetus. About 80% of the transfer occurs during the third trimester [22]. Muller *et al*. [23] also noted that calcium level increases in the fetus with increase in gestational age. However, the finding on serum calcium in this study contradict that of [24] which reports maternal serum calcium does not vary with increase in gestational age, and that there is increase in serum calcium in pregnant women compared to non-pregnant controls.

In this study, the concentration of phosphorus shows a subtle increase during pregnant women but this increase is not significant. In other studies, the same findings have been reported in the trimesters in comparison to non-pregnant women [25]. Vitamin D increases the efficiency of

intestinal absorption of phosphorus by approximately 80% [26] and this high percentage may be the reason why a subtle change in vitamin D level hardly affect serum phosphorus. Hence serum phosphorus shows no significant variation among all the groups in this study. It has been reported by a similar study that supported that no major changes in either bone mass or blood inorganic phosphorus have been reported during pregnancy [27], [28], thus, phosphorus supplementation is not recommended during pregnancy. Major increment in alkaline phosphatase as observed in this study which parallels gestational age was due to placental production of the enzyme. Therefore, serum alkaline phosphatase activity increased progressively throughout gestation to a peak level at the end of gestation. Similar observation was reported on serum alkaline phosphatase by Jong and Soo [30].

This study revealed that magnesium level during third trimester was significantly lower in the pregnant population when compared with non-pregnant subjects. In fact, serum magnesium decreases as pregnancy advanced, the fall in serum magnesium value being observed most between the first and second trimesters. Young age, previous delivery and low social class were associated with magnesium deficiency. In this study, a reduction in serum magnesium was observed with advancing gestational age. The reduction in serum magnesium with advancing gestation reported in this study is similar to the findings of previous workers [31], [32]. Some suggested reasons for the low levels of magnesium in pregnancy include inadequate intake, increased metabolic demand of pregnancy especially as gestation advanced, physiological haemodilution in pregnancy, increasing parity and low socio-economic status [33]. Pathak *et al* [34] reported 43.6% amongst rural Indian women in a community-based cross-sectional study and found a higher prevalence in higher parity women, while another hospital based pilot study involving urban Indian dwellers reported magnesium deficiency in 4.6% of all pregnant women included in the study reported a prevalence of 25.6% while Kumar and co-worker [35] in Mauritius reported magnesium deficiency of 48% but found no difference in prevalence between their urban and rural participants.

There are conflicting reports indicating possible effect of maternal vitamin D status on fetal growth and bone development [35]. Insufficient intake of dairy products and vitamin D makes individuals including pregnant women susceptible to inadequate vitamin D status and vitalizes the need for other nutritional supplements. Other very important sources of vitamin D are fish oils, which these pregnant subjects rarely consumed. Exposure to the sun also plays a very important in providing sufficient vitamin D. The fact that these pregnant and non-pregnant subjects are mostly vegetarians

also contributes to the observed common vitamin D deficiency and insufficiency in the study subjects. This inadequacy of vitamin D in the study subject has contributed immensely to the observed abnormal serum mineral concentration in the study subjects since homeostasis of these minerals in the body is mediated by vitamin D in one way or the other. Therefore, a defect in vitamin D level is likely to be reflected in serum minerals concentration.

3.3. ACKNOWLEDGMENT

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