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# Serum Calcium Levels among Pregnant Women Attending Jos University Teaching Hospital (JUTH)

**A. I. Ekwempu [a*], Roseline Emeji [b], D. B. Yakubu [a], N. Vwamdem [a] and A. Walong [a]**

*[a] Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, University of Jos, Nigeria.*
*[b] School of Medical Laboratory Science, Rivers State College of Health Science and Management, Port Harcourt, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Pregnancy is a period characterized by heightened calcium demand which is necessary for the optimum growth and development of the fetus and placenta. The physiological changes that occur in pregnancy may as well affect calcium level amongst other biochemical parameters among these subjects. The aim of this study was to evaluate calcium levels in pregnant women attending antenatal clinic in Jos University Teaching Hospital (JUTH), Plateau State Nigeria. A total of 90 subjects were randomly selected for the study, they were between 15 to 45 years. Of the 90 subjects, 30 were non-pregnant women who served as control while 60 of these subjects were pregnant women and they were the test group. The subjects were selected using a simple random technique. Blood (5mls) was collected using venipuncture technique and the sample was assayed for calcium using o-cresolphthalein complexone method. The result revealed that there was no significant difference in calcium level between pregnant and non-pregnant women, p-value>0.05. Also, demographic characteristics had no effect on calcium level among pregnant women, p-value>0.05. In addition, trimester had no significant impact on calcium level among pregnant women, p-value>0.05. This study has shown that pregnancy does not have any impact on serum calcium level among pregnant women at any stage in pregnancy and their demographic identification is no factor to consider in monitoring their calcium level.

**Corresponding author: Email: ekwempua@unijos.edu.ng;*

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## 1. INTRODUCTION

Calcium is the major mineral of the skeletal system and the most abundant cation in the human body. Calcium as a nutrient is most commonly associated with the formation and metabolism of bone. Over 99 percent of total body calcium is found as calcium hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$) in bones and teeth, where it provides them with their strength [1]. The skeleton is a major reservoir for providing calcium for both the extracellular and intracellular pools. In serum, about 50% of calcium is free or ionized, 40% is bound to serum proteins, and 10% is complexed with anions such as bicarbonate, phosphate, lactate, and citrate. Albumin is the predominant calcium-binding protein with 1g/dL of albumin binding to approximately 0.8mg/dL of calcium. Serum level of calcium influences many extracellular and intracellular processes such as neural transmission, membrane stability, bone structure, blood coagulation, muscle movement, and intracellular signaling. It is also an important cofactor for hormonal secretion in endocrine organs [2] for optimal and normal functioning of these processes, the total serum calcium concentrations need to be normally maintained within the very narrow range of 8.5 and 10.5 mg/dL (2.12 to 2.62 mmol/L) [3,4].

Pregnancy is a period characterized by heightened calcium demand which is necessary for the optimum growth and development of the fetus and placenta. Women with low calcium intake may manifest with hypocalcaemia in pregnancy. The fetal calcium accretion often occurs at the expense of the mother who compensates by doubling the intestinal calcium absorption aided by increased vitamin D levels and slight decrease in parathyroid hormones [5].

Many physiologic changes such as falling albumin level, expansion of extracellular fluid volume, increase in renal function and placental calcium transfer [6] occur during pregnancy resulting in an increase of red blood cells and plasma volume and a reduction of micronutrients and circulating nutrient-binding proteins. In most developing countries, poor nutrition combined with the usual physiological pregnancy changes can lead to micronutrient deficiency states [7]. This implies that pregnant women may be at a greater risk of hypocalcaemia. Therefore, this study focuses on the evaluating serum level in pregnant women attending Jos Teaching Hospital.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was carried out in Jos University Teaching Hospital (JUTH) which is located in Lamingo, Jos North Local Government Area of Plateau State, Nigeria. JUTH is a tertiary health institution in North Central Zone of Nigeria and also serves as a referral Centre for neighbouring States such as Bauchi, Gombe, Nasarawa, Taraba, Adamawa and parts of Kaduna State.

### 2.2 Study Design

The research was a cross sectional study conducted in the antenatal clinic of Jos University Teaching Hospital (JUTH), Plateau State. A total of 90 subjects were randomly selected for the study, they were between 15 to 45 years of age. Of the 90 subjects, 30 were non-pregnant women who served as control while 60 of these subjects were pregnant women and they were the test group. Consent form and questionnaire were given to each subject before commencement of the study.

### 2.3 Sample Size

The sample size was determined using the formula as described by Frankline [8] as follows.

The calculated sample size was 86.68 which was rounded up to 90

### 2.4 Eligibility Criteria

All apparently healthy pregnant and non-pregnant women of between 15 to 45 years attending Jos University Teaching Hospital who gave their consent for the study were included. Their health status was confirmed from the clinic folder.

All subjects (pregnant and non-pregnant women) with some conditions such as chronic hypertension, chronic renal disease/renal osteodystrophy, retroviral infection/acquired immunodeficiency syndrome, sickle cell anaemia, metabolic syndrome and thyroid disease were excluded from the study because such conditions have been shown to influence calcium levels in the study population [9]. All subjects who did not give their consent for the study were also excluded.

## 2.5 Sampling Method

Subjects were recruited into the study through simple random sampling technique using a numbering system [10-12].

## 2.6 Sample Collection and Preparation

From each subject, 5mls of whole Blood was collected through venipuncture from antecubital fossa using aseptic precautions into plain tubes and labeled appropriately. The blood samples were allowed to clot and later retracted and centrifuged at 3000rpm for 5 minutes to obtain serum. The serum sample was then transferred into another labeled plain bottle using disposable Pasteur pipette.

## 2.7 Laboratory Analysis

### 2.7.1 Procedure

Three test tubes were labeled as blank, standard and test. To each of them, 500µL of solution R1 (Calcium Base) and R2 (Calcium Dye) were added. Another 10µL of standard was added to the standard test tube. Then 10µL of serum sample was added in the tube labeled for test. All tubes were mixed gently for few seconds and incubated at room temperature for 5 minutes. Absorbance was read at 578nm and optical density (OD) was recorded.

### 2.7.2 Calculation of results

The concentration of serum calcium was determined using the formula below:

$$Serum\ calcium\ (mmol/l) = \frac{OD\ of\ Test - Blank}{OD\ of\ Std - Blank} \times Concentration\ of\ Std\ (mmol/L)$$

Where:

OD = Optical Density
Std = Standard

Normal values for serum calcium: 2.12 - 2.62mmol/L [3,4].

## 2.8 Statistical Analysis

Data obtained was analyzed using the statistical package for social sciences (SPSS) version 26. Results were expressed as mean, standard deviation and frequency tables were also used. Bar charts were used to illustrate data generated. Mean values were compared using T-test and ANOVA. P≤ 0.05 was considered statistically significant.

## 3. RESULTS

Table 2 shows the comparison of serum levels in each demographic parameter. The demographic parameters include, age, level of education, occupation, regular diet, calcium supplement and calcium supplement intake per day. The result showed that there was no significant difference in calcium levels in each of the demographic parameter.

**Table 1. Demographic characteristics of the pregnant women**

| Characteristics | Number (%) of participants |
|---|---|
| **Age (years)** | |
| 15-25 | 24 (40) |
| 26-35 | 20 (33) |
| 36-45 | 16 (27) |
| Total | 60 (100) |
| **Level of education** | |
| Primary | 01(02) |
| Secondary | 25(42) |
| Tertiary | 34 (57) |
| Total | 60 (100) |
| **Occupation** | |
| Teacher | 08 (13) |
| Student | 10 (17) |
| House wife | 08 (13) |
| Artisan | 07 (12) |
| Business | 15 (25) |
| Civil Servant | 12 (20) |
| Total | 60 (100) |
| **Regular diet** | |
| Carbohydrate | 40 (67) |
| Protein | 12 (20) |
| Fruit | 05 (08) |
| Vegetables | 03 (05) |
| Total | 60 (100) |
| **Ca supplement** | |
| Yes | 06 (10) |
| No | 54 (90) |
| Total | 60 (100) |
| **Ca supplement intake per day** | |
| Nil | 54 (90) |
| Once | 04 (07) |
| Twice | 02 (03) |
| Total | 60 (100) |

**Table 2. Serum calcium levels based on demographic characteristics of the pregnant women**

| Characteristics | Number (%) of participants | Mean serum Ca level (mmol/L) | SD | *P*-value |
|---|---|---|---|---|
| **Age (years)** | | | | |
| 15-25 | 24 (40) | 2.50 | 0.21 | 0.90 |
| 26-35 | 20 (33) | 2.47 | 0.14 | |
| 36-45 | 16 (27) | 2.47 | 0.23 | |
| Total | 60 (100) | 2.48 | 0.19 | |
| **Level of education** | | | | |
| Primary | 01(02) | 2.60 | 0.00 | 0.83 |
| Secondary | 25(42) | 2.48 | 0.16 | |
| Tertiary | 34 (57) | 2.48 | 0.21 | |
| Total | 60 (100) | 2.48 | 0.19 | |
| **Occupation** | | | | |
| Teacher | 08 (13) | 2.50 | 0.23 | 0.57 |
| Student | 10 (17) | 2.45 | 0.18 | |
| House wife | 08 (13) | 2.45 | 0.13 | |
| Artisan | 07 (12) | 2.55 | 0.25 | |
| Business | 15 (25) | 2.54 | | 0.22 |
| Civil Servant | 12 (20) | 2.42 | 0.11 | |
| Total | 60 (100) | 2.48 | 0.19 | |
| **Regular diet** | | | | |
| Carbohydrate | 40 (67) | 2.49 | 0.17 | 0.97 |
| Protein | 12 (20) | 2.49 | 0.25 | |
| Fruit | 05 (08) | 2.48 | 0.21 | |
| Vegetables | 03 (05) | 2.43 | 0.20 | |
| Total | 60 (100) | 2.48 | 0.19 | |
| **Ca supplement** | | | | |
| Yes | 06 (10) | 2.58 | 0.25 | 0.20 |
| No | 54 (90) | 2.47 | 0.18 | |
| Total | 60 (100) | 2.48 | 0.19 | |
| **Ca supplement intake per day** | | | | |
| Nil | 54 (90) | 2.47 | 0.18 | 0.25 |
| Once | 04 (07) | 2.52 | 0.18 | |
| Twice | 02 (03) | 2.70 | 0.42 | |
| Total | 60 (100) | 2.48 | 0.19 | |

** SD= Standard Deviation*

**Table 3. Mean serum calcium levels in pregnant and non-pregnant women**

| Variable | Number of participants | Serum Ca level (mmol/L) | SD | *P*-value |
|---|---|---|---|---|
| **Pregnant women:** | 60 | 2.48 | 0.22 | 0.31 |
| **Non-pregnant Women:** | 30 | 2.50 | 0.12 | |

** SD= Standard Deviation*

Table 3 shows the mean serum calcium levels of pregnant and non-pregnant women, a total of sixty (60) participants were pregnant while thirty (30) participants were non-pregnant. The mean serum±SD calcium level for the pregnant women was 2.48±0.22mmol/L while that of the non-pregnant women was 2.50±0.12mmol/L. There was no statistically significant difference between both groups (*P-value*=0.31).

The mean serum calcium level in relation to trimesters of pregnancy is shown in Table 4, a total of 60 pregnant women participated in the study with 60 being pregnant women, while 20 pregnant women were in their first trimester(1-12 weeks), 20 in their second trimester (13-27 weeks), and 20 in their third trimester (28-40 weeks) of pregnancy. First trimester had mean±SD serum calcium of 2.48±0.17mmol/L,

**Table 4. Mean serum calcium levels in relation to trimesters of pregnancy**

| Trimester (Weeks) | Number of participants | Serum Ca level (mmol/L) | SD | *P*-value |
|---|---|---|---|---|
| FIRST (1-12 weeks) | 20 | 2.48 | 0.17 | 0.34 |
| SECOND (13-27 weeks) | 20 | 2.52 | 0.24 | |
| THIRD (28-40 weeks) | 20 | 2.45 | 0.16 | |
| Total | 60 | 2.48 | 0.19 | |

** SD= Standard Deviation*

**Table 5. Serum calcium levels at age interval in the various trimesters of pregnancy**

| Age (Years) | Number (%) of participants | Serum calcium (mmol/L) | Standard deviation | *P*-value |
|---|---|---|---|---|
| **First trimester** | | | | |
| 15-25 | 10 (50) | 2.52 | 0.18 | 0.55 |
| 26-35 | 06 (30) | 2.47 | 0.22 | |
| 36-45 | 04 (20) | 2.44 | 0.10 | |
| Total | 20 (100) | 2.48 | 0.17 | |
| **Second trimester:** | | | | |
| 15-25 | 08 (40) | 2.45 | 0.27 | 0.16 |
| 26-35 | 07 (35) | 2.54 | 0.18 | |
| 36-45 | 05 (25) | 2.63 | 0.25 | |
| Total | 20 (100) | 2.52 | 0.24 | |
| **Third trimester:** | | | | |
| 15-25 | 05 (25) | 2.50 | 0.16 | 0.10 |
| 26-35 | 08 (40) | 2.45 | 0.07 | |
| 36-45 | 07 (35) | 2.40 | 0.22 | |
| Total | 20 (100) | 2.45 | 0.16 | |

second trimester had 2.52±0.24mmol/L, while those in third trimester had 2.45±0.16mmol/L respectively. There was no statistically significant difference in calcium level among the trimester groups (*P-value*=0.34).

Table 5 show the comparison of calcium level among various age groups in each trimester. The age groups were 15-25, 26-35, and 36-45. In the first trimester, there was no significant difference in calcium level. Similar finding was observed in second and third trimesters, *P*-value>0.05.

## 4. DISCUSSION

Findings from the study done to determine the serum calcium levels in pregnant women attending antenatal clinic in Jos University Teaching Hospital (JUTH) showed that the serum calcium of pregnant women based on age distribution indicates that there was no significant difference in serum calcium level among the age groups. This is in agreement with the work of Ben-chioma et al. [13] who worked among pregnant women attending tertiary hospital in Port Harcourt Metropolis, Nigeria and reported that the non-significant differences could be as a result of habitual prenatal routine drugs and calcium-rich diet as advised in antenatal clinics especially for the adolescent women. Despite the fact that the pregnant women had different levels of education, their serum calcium levels showed a non-significant difference when compared to the control group which disagrees with the work of Bako et al. [14] who carried out a study in Maiduguri, Nigeria among women attending antenatal clinic in University of Maiduguri Teaching Hospital (UMTH); they also reported that this differences may be as a result of the precarious nutritional status in the state occasioned by the long-standing Boko haram conflict. The result of this study also shows that participants' occupations as Teachers, Students, House wives, Artisans, Business women and Civil Servant had no impact on their serum calcium levels as there was no statistically significant difference in their mean values.

Regular diet showed that most of the pregnant women consumed carbohydrate followed by protein, fruits and vegetables; however, their mean serum calcium level was not statistically significant, this agrees with the findings of [15] who worked among pregnant and non-pregnant women attending clinic at Korle-Bu Teaching Hospital, Ghana and found out that there was a non-significant difference in total serum calcium and magnesium levels among pregnant and non-pregnant women; reasons for this findings were because the participants were apparently healthy women who had proper staple diet and were also exposed to sunlight for vitamin D synthesis which in turn has a vital role in maintaining calcium balance [15].

The findings of this research shows that women who took calcium supplement had a higher level of serum calcium compared to those who were not on calcium supplement however, the difference was not statistically significant. The findings also suggested that over 90% of these pregnant women went through their pregnancy without proper calcium supplementation; this observation is in agreement with that of Agueh et al. [16] who carried out a cross sectional survey in Benin on calcium uptake among pregnant women, their study indicated that over 90% of pregnant women in Southern Benin had low calcium supplementation during pregnancy due to low socio economic status and lack of education as their diets were discovered to be mainly grains and legumes, only 10% who had secondary and tertiary education and were from the upper tercile of economic status had proper calcium supplementation during pregnancy [16]. Frequency of calcium intake per day suggested that there was an association between frequency of calcium supplement intake and serum calcium level; however their difference was not statistically significant; this is because the participants were young adults and adolescents between 15-45 years of age in which bone formation and resorption are almost balanced in the adolescents and balanced in the young adults and therefore, calcium supplementation during this period did not differ between controls and calcium supplemented individuals as reported by Matkovic et al. who conducted a randomised clinical trial among American females from childhood to young adulthood [17].

The mean serum calcium levels among pregnant and non-pregnant women showed that pregnant women had reduced serum calcium compared to the non-pregnant women. This may be due to the physiological demands and calcium transfer from the mother to the developing foetus during pregnancy as reported in the work of Kovacs et al. among pregnant women [18]. However this was not statistically significant; this finding agrees with the work of Djagbletey et al. [15] who compared serum calcium of pregnant and non-pregnant women in Korle Bu, Ghana and found out that there was non-significant difference in serum calcium between pregnant and non-pregnant women. This may be so because Nigeria has geographical and cultural similarities with Ghana. In addition, this finding contrasts with that of Egesie and Dike in Yanagoa, Nigeria [19] and Sultana et al. [20] in Bangladesh who reported a significant decrease in serum calcium of pregnant women when compared to their non-pregnant counterparts.

Serum calcium levels in relation to trimesters of pregnancy indicated that there was reduced serum calcium in the first trimester when compared to the second trimester; this may be as a result of late visit to the antenatal clinic especially in the later stages of the first trimester when calcium demand begins to rise. The increase in serum calcium in the second trimester suggests that proper adherence to antenatal drugs has taken effect. The reduced level of serum calcium in the third trimester may be as a result of increase in calcium demand for maternal and foetal needs which was the conclusion of Almaghamsi and his colleagues in their study among pregnant women [21]. However, there was no statistically significant difference in the mean serum calcium across the trimesters of pregnancy, which agrees with the work of Bako et al. [14] who carried out a similar study in Maiduguri-Nigeria among women attending antenatal clinic in University of Maiduguri Teaching Hospital (UMTH).

Serum calcium levels in relation to various trimesters of pregnancy showed a significant increase when the age interval of 15-25 years were considered in the first trimester compared to other age intervals; the increase seen among this group could be tied to the fact that the degree of foetal demand of calcium at this stage of pregnancy is reduced as compared to the second and third trimesters; this increase in serum calcium at this age interval of 15-25 years could also be as a result of maximal absorption of calcium or increased calcium accretion during this developmental period as the participants in this age interval are still teenagers. There was reduction of serum calcium level in the second

trimester as the demand increased, this may be due to expansion of extracellular fluid volume and fall of total serum concentration due to haemodilution as reported by Cooper et al who worked on developmental origins of osteoporotic fracture in pregnant women attending University of Southampton general hospital, United Kingdom [22]. The increase in serum calcium during the third trimester may be as a result of adaptation illustrated by doubling of intestinal calcium absorption driven by calcitriol in order to store more calcium in advance of the peak fetal demand that occurs in the third trimester as reported by Cross et al. [23] who worked among pregnant women in University of Missouri, Columbia [23].

The results of this study illustrate an increase in serum calcium level from age group 26-35 years with subsequent drop during the third trimester. This undulating rise and fall may be a reflection of the foetal calcium requirement. The results also showed a decrease in serum calcium in the first trimester which then increased in the second trimester when the age group of 36-45 years were considered, this increase may be associated with habitual prenatal routine drugs and calcium rich diet as advised in the antenatal clinic. However, the decrease observed in the third trimester could be due to behavioral pattern among these pregnant women not adhering to the routine antenatal drugs including calcium and calcium rich diets as reported by Ben-chioma et al. [13].

## 5. CONCLUSION

This study has shown that calcium level is not affected throughout pregnancy. In addition, this study has equally showed that certain demographic variables such as age, education, diet, occupation and calcium supplementation do not have effect on calcium level among pregnant women.

## CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

Ethical clearance was sought and obtained from the ethics committee of Jos University Teaching Hospital before study was commenced. The reference number of the ethical clearance was JUTH/DCS/IREC/127/XXX1/2446.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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