

Article

Assessment of Calcium Levels in Pregnant Versus Non-Pregnant Women in Kirkuk City

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Abstract: The objective of the present study was to determine the levels of serum calcium among pregnant and non-pregnant women in Kirkuk, Iraq, and to study the dynamics of the physiological process responsible for calcium homeostasis among pregnant females. Calcium is an essential element that plays an important role in fetal bone formation and maternal physiological functions. The lack of this element during pregnancy is highly correlated with such serious problems as preeclampsia and intrauterine growth restriction (IUGR). The cross-sectional study was carried out in the Dalia Jumard Specialized Laboratory in Kirkuk. Blood samples from 31 participants (pregnant, $n = 9$ and non-pregnant, $n = 22$) were used to measure their serum calcium concentrations using the Roche Cobas Pure automated analyzer. Differences in serum calcium levels depending on age and physiological state of the participant were analyzed. Unexpectedly, contrary to some traditional tendencies, it was found that the average value of the serum calcium in pregnant women (10.29 ± 0.49 mg/dL) is higher than that in non-pregnant women (8.68 ± 0.72 mg/dL). In addition, both groups have serum calcium concentrations within the physiological limits. Age-related differences demonstrate the temporary reduction in the middle of pregnancy followed by its increase towards the end of the period. Despite the findings, the study was limited by a small sample size and lack of assessment of important factors such as vitamin D status and albumin-corrected calcium, calcium levels in pregnant women in Kirkuk city appear to remain within normal ranges, but continuous monitoring and nutritional support are recommended to prevent potential complications. Further large-scale studies are needed to provide a more comprehensive understanding of calcium metabolism during pregnancy in this population.

Keywords: Calcium, Pregnant, Vitamin D3, Adequate calcium

Introduction

Bone mineral density (BMD) is the single most significant factor when it comes to bone strength. A decrease in BMD by 10% increases the chances of fractures[1]. Calcium (Ca) is the most abundant mineral in the human body, accounting for approximately 2% of the total body weight; furthermore, 99% of calcium is stored in the skeletal system. The remaining 1% is distributed in intra- and extracellular fluids, where it is essential for various regulatory functions, including muscle contraction, enzyme and hormone activity, and the maintenance of bone integrity [2,3,4]. Consequently, ionic

calcium (Ca^{2+}) must be tightly regulated in order to maintain plasma concentrations within normal ranges [5], Calcium levels are influenced by gastrointestinal absorption, renal excretion, and mobilization from the skeleton, which serves as the body's primary calcium reservoir [6]. During pregnancy, the maternal body undergoes significant physiological adaptations in order to maintain homeostasis and support fetal growth and development. These adaptations affect calcium metabolism directly, as calcium plays a critical role in early embryonic stages, particularly in cell signaling and control of cell division, as well as in the third trimester by supporting fetal skeletal mineralization [7,8]. Transfer of calcium through the placenta increases as pregnancy progresses, and this often results in a negative calcium balance for the mother [9,10]. Even women with adequate calcium intake early in pregnancy may experience a neutral or negative balance during the third trimester due to heightened fetal bone accretion demands, The developing fetus requires between 50 and 330 mg of calcium per day for skeletal development, with demands increasing significantly as pregnancy progresses, Insufficient maternal calcium intake may increase the risk of gestational complications, including hypertensive disorders, preterm delivery, and long-term maternal morbidities, such as excessive bone loss [11,12,13,14]. In addition, blood pressure is regulated by intracellular calcium in the vascular smooth muscle cell via vasoconstriction and variations in the vascular volume. Therefore, appropriate calcium intake for pregnant women can prevent future osteoporosis and reduces the risk of PIH [15]. The new fashion of low calcium amongst pregnant ladies is probably due to their incapability to deal with the more call for at some stage in being pregnant and this requires in-intensity studies into the reasons and danger elements so that it will addressing them. The WHO has advocated ordinary calcium supplementation in being pregnant specially in regions of low calcium consumption as one of the measures to lessen the superiority of preeclampsia [16]. Bone metabolism and its minerals are strongly influenced by vitamin D. Increasing the supply and/or supplementing it reduces bone turnover and helps increase bone mineral density. However, it should be noted that excessive vitamin D supplementation and/or supply can negatively affect health, as it belongs to the group of fat-soluble vitamins that have the ability to accumulate in the body [17]. As a biomarker of vitamin D status, 25-hydroxycholecalciferol is used due to its longer half-life [18]. Vitamin D is primarily involved in the metabolism of calcium, phosphorus and regulates bone mineralization. Vitamin D is a fat-soluble vitamin and provides intestinal absorption of calcium by increasing vitamin D- dependent calcium-binding proteins[19]. Pregnant and lactating women require at least 600IU/d of Vitamin D and it had been recognized that at least 1500-2000 IU/d of vitamin D may be need to maintain a blood level of 25-(OH)VitD3 above 30ng/ml [20]. There is accumulating evidence that vitamin D plays a role in the central nervous system (CNS) [21]. Calcium requirement in non-pregnant state is 600 mg/day which increases to 1,200 mg/day during pregnancy. This increased amount of calcium is required for the growth and development of bones and teeth of fetus. This demand can be met by the increased intake of calcium during pregnancy [22]. Serum calcium level decreases during second and third trimester of pregnancy, primarily due to hemodilution [23].

Materials and Methods

Experimental part

The study was conducted at Dalia Jumard Specialized Laboratory, Kirkuk City, Iraq. A total of 31 samples [22-sample non pregnant and 9 samples pregnant] were collected from the study site and analyzed according to standard laboratory procedures. A case-control study in Dalia Province that evaluated serum electrolytes, including calcium, in women during the third trimester highlighted that disturbances in mineral balance are common and may be associated with pregnancy-related complaints such as muscle spasms, body aches, and cardiovascular symptoms, indicating that electrolyte and calcium homeostasis can be fragile in this population., Blood samples were collected by venipuncture without the use of a tourniquet to minimize hemolysis and physiological alterations in the analysts. The collected blood was transferred directly into gel separator tubes (Gel Tubes). The collected samples were left to clot before being subjected to the process of centrifugation in order to get the serum.

Centrifugation of the samples took place at varying periods during the day depending on the time at which the samples had been collected. After centrifugation, the serum was collected and analyzed.

Instrument Used

Calcium serum concentrations were measured via the use of Roche Cobas Pure integrated solutions system (Roche Diagnostics, Mannheim, Germany) based on the procedures provided by the manufacturer. The use of the specified automated clinical chemistry analyzer guaranteed efficiency, accuracy, and repeatability of results in all groups under investigation (Figure 1).



Figure 1. Shown Cobas Pior Instrument

Results and Discussion

Tables 1-4 highlight the empirical results that have been generated by a study performed on a group of females living in Kirkuk City. Table 1 gives the description of baseline values obtained on a sample size of 22 persons ($n = 22$). In every sample value, there are three distinct variables identified: the unique sample identifier, age (in years), and corresponding biochemistry test values in mg/dL. Age demographic analysis of the age variable indicates a mixed sample of non-pregnant women of various ages from youth up to advanced age. In this sample, the youngest subject is aged 22 years (Sample 13), whereas the oldest is aged 67 years (Sample 8). Looking at the laboratory outcomes, the recorded metrics fluctuate across a relatively narrow baseline, spanning from a minimum value of 7.8 Mg/dl (Sample 21) to a peak value of 10.2 Mg/dl (Sample 13). No immediate, direct correlation appears to exist between advanced age and higher or lower biochemical metrics, as evidenced by Sample 8 (a 67-year-old) and Sample 1 (a 38-year-old) sharing identical results of 9.2 Mg/dl, while younger participants display a wide variety of results themselves. Ultimately, this dataset serves to map out individual health or diagnostic parameters across an adult age spectrum for comparative medical or statistical analysis. According to the National Institutes of Health (NIH), the recommended daily calcium intake for women aged 19–50 years is 1000 mg/day, while it increases to 1300 mg/day for females aged 14–18 years due to the continued process of bone mass development. As for the 15–25 years age group, particularly those under 20 years of age, they are still in the stage of achieving peak bone mass and may therefore be more vulnerable to calcium deficiency if pregnancy occurs during this period, owing to the increased fetal demand and the developmental nutritional needs of the mother.

Table 1. Sample Distribution of Non-Pregnant Women

No. of Samples	Age (years)	Results Mg/dl
1	38	9.2
2	55	8.7
3	29	9.1
4	24	8.5
5	26	8.8
6	24	8.8
7	28	9.1
8	67	9.2
9	24	8.0
10	39	8.5
11	32	9.7
12	41	8.6
13	22	10.2
14	32	9.6
15	26	8.4
16	26	9.3
17	27	9.0
18	48	8.8
19	58	9.4
20	65	8.1
21	32	7.8
22	56	7.2

Table [2], Summarized biochemical or clinical results (measured in Mg/dl) for a cohort of nine pregnant individuals. The demographic data suggest that the studied group covers the development continuum from early adulthood through advanced maternal age, with the participants being aged between 24 and 39. The mean age of the studied population is 28.4 years, which shows that most people fall into the category of peak reproductive age group. The range of chronological ages extends from the minimum of 24 years (in samples 5 and 9) to the maximum age of 39 years (sample 7). The biochemical measurements show that the serum concentrations (measured in milligrams per deciliter (mg/dL)) range from the minimum of 7.8 mg/dL to the maximum peak of 9.6 mg/dL. It should be emphasized that the lowest and highest values correspond to 32-year-old samples (6 and 2, correspondingly). Thus, the age variance demonstrates that the measured physiological values do not have any linear correlation with age in this particular case. The mean of all the serum concentrations is equal to 8.69 { mg/dL} , which reflects the rather narrow homeostatic range of values. Cross-examination of variables reveals some complicated internal dynamics, rather than linear relationships between the parameters under study. Thus, for example, the two youngest individuals (n=2, aged 24 years) showed different physiological results, namely sample 5 had 8.0 mg/dL while sample 9 had 8.8 mg/dL. At the same time, the oldest subject (aged 39) showed the relatively moderate result of 8.5 mg/dL that corresponds to the overall mean of the studied population. Moreover, the middle concentrations (9.0 mg/dL) are shown by samples 4 (aged 27) and 8 (28). Overall, the lack of clear relationship between increasing maternal age and measured biochemical concentrations implies that there must be some other influencing clinical, gestational or environmental factors.

Table 2. Sample Distribution of Pregnant Women.

No. of Samples	Age (years)	Results Mg/dl
1	29	9.1
2	32	9.6
3	26	8.4

No. of Samples	Age (years)	Results Mg/dl
4	27	9.0
5	24	8.0
6	32	7.8
7	39	8.5
8	28	9.0
9	24	8.8

Table [3], which illustrates the demographic distribution and relative percentages of non-pregnant women across various age cohorts. In the case of the studied population, there is an evident inverse relationship between the age factor and the level of the sample representation, with the most represented individuals being young people. The group that takes the biggest share of the overall studied population consists of individuals aged from 20 to 29 years old and amounts to 10 people (i.e., 45.45% of the total population of $n=22$), so almost half of the investigated non-pregnant females belong to this category of young adults. Subsequently, the level of representation decreases in proportion to aging. The second most represented age category is the one of people aged between 30 and 39 years old, which comprises 5 samples (22.73%). Together, the two smallest groups of young people (aged between 20 and 39 years) account for more than two thirds of the entire dataset (68.18%), which means that the study results reflect mostly the physiological/clinical characteristics of women aged under 40 years old. On the other hand, the representation of older participants is rather low and fragmented. Thus, the group of women aged between 40 and 49 years comprises just 2 samples (9.09%). A slight variation is found in the group of people aged between 50 and 59 years (3 samples, i.e., 13.64%), which then drops down to 2 (9.09%) people in the most senior age category (>60 years old). Statistically speaking, the calculations of the relative frequency show the sum of all individual percentages amounting to precisely 100%, which proves the absence of data loss. Clinically and epidemiologically, it means that the study is based on the data collected.

Table 3. Statistical Distribution and Percentage Calculation of the Non- Pregnant Women

No of Samples	Range of Age	Percentage %
10	20-29	45.45
5	30-39	22.73
2	40-49	9.09
3	50-59	13.64
2	>60	9.09

Figure 2 presents the demographic distribution of a non-pregnant group within four different age groups of five years each within the age ranges of 20 to 40 years. The main vertical axis indicates the percent frequency (from 0% to 50%) while the related data table underneath the graph shows the age groups cross-referenced with their respective number of samples (n) and percentages. An examination of the demographic distribution shows that most of the samples lie within the age group 26 – 30 years, forming the mode and representing 44.4% of the total sample ($n = 4$). On the other hand, the younger and older age groups have smaller percentages of samples. For instance, the younger group (20 – 25 years) and the intermediate group (31 – 35 years) have the same distribution, each representing 22.2% of the total population ($n = 2$). However, the oldest age group (36 – 40 years) is made up of only one sample and represents 11.1% of the sample. In general, the distribution shown in Figure 2 does not have a uniform distribution, with most of the samples falling within the late twenties age range.

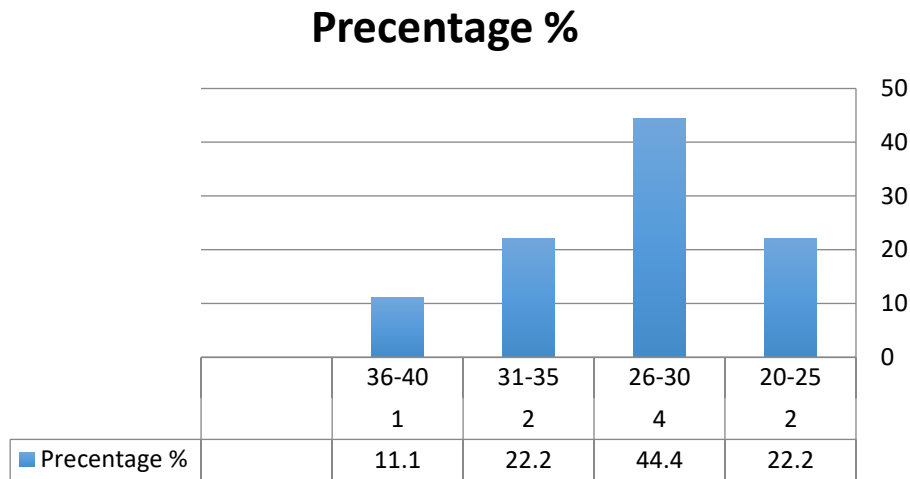


Figure 2. Percentage Distribution of Participants According to Age Groups[Non- Pregnant]

Table[4] is an example of a quantitative demographic representation of a study sample population of nine pregnant females allocated into certain age groups. This academic assessment seeks to establish a demographic profile for the maternal age that is considered an important independent variable in obstetric and epidemiological studies because of the direct relationship between maternal age and pregnancy outcome. The size of the sample population (N=9) is divided into four different five year-age group ranging from twenty years to forty years old. The equation utilized for determining the percent frequency is:

$$\text{Percentage}\% = \left(\frac{n}{N}\right) \times 100$$

When *n* be the number of specimen in a certain age interval, while *N* will be equal to the total sample size (N=9). Analysis of stratified data demonstrates that there is a clear presence of a bell-shaped curve of distribution, characteristic of a normal distribution in a relatively small sample, with a clear peak in the young adult mothers’ demographic segment. The youngest age group, represented by pregnant females between 20-25 years old, constitutes 22.2% of the total sample (n=2). Similarly, the next age group between 31-35 years old is also represented by 2 subjects and constitutes 22.2% of the total sample size. Overall, these two groups form almost half of the whole population in question. There is an obvious peak of distribution in the 26-30 year age group with a frequency of 4 and accounting for 44.4% of the entire population. Such distribution is typical and can be explained using biological and physiological grounds as it is often defined as the period of peak fertility and associated with fewer pregnancy complications compared to older age mothers. The oldest group (36-40 years old) is characterized by the lowest frequency of only one person (11.1% of the total sample). It is worth noting that people from this age group or approaching it have often been classified as Advanced Maternal Age (AMA) in various clinical and scientific literature. This particular age group of females is noted to have an elevated risk coefficient when it comes to complications such as gestational diabetes, chromosomal abnormalities, and hypertension problems. This explains why they form a lesser percentage among other females when studying general maternal health issues. To summarize, the above figures in Table 4 reveal that 66.6% of pregnant females sampled were aged between 20 to 30 years, and most of them belonged to late twenties. The representation falls off as we approach late thirties, and this gives us a stratified demographic base which is important in explaining any clinical findings in the study.

Table 4. Statistical Distribution and Percentage Calculation of the Pregnant Women

Samples	Range of Age	Percentage %
2	20-25	22.2
4	26-30	44.4

Samples	Range of Age	Percentage %
2	31-35	22.2
1	36-40	11.1

Figure [2] shows the distribution of non-pregnant female participants belonging to four different age groups ranging from 20 to 40 years. In this case, the sample is characterized by non-linear, negatively skewed distribution, with 26-30 year age group accounting for the largest part 44.4% (n = 4). Two other age groups, 20-25 and 31-35 years old, are characterized by 22.2% (n = 2) distribution rate, whereas the smallest age group (36-40) accounts for 11.1% (n = 1). From the biochemical and physiological point of view, the isolation and stratification of non-pregnant individuals within this age period is very important because it enables to create proper baseline biological parameters. In particular, the period between 20 and 40 years corresponds to the time of maximum reproductive and metabolic potential in females when the homeostatic systems are significantly influenced by the fluctuations of steroid hormones, namely, estrogens and progesterone. They act as powerful biochemical regulators affecting the cellular metabolism, lipids, enzyme production, and blood sugar levels. The stratification into specific age intervals and the isolation of non-pregnant individuals allow minimizing the significant metabolic and hormonal changes connected with pregnancy and gestational biochemistry (hemodilution, insulin resistance, increase of hormonal secretion of placenta). That is why it is extremely important for creating proper baseline data.

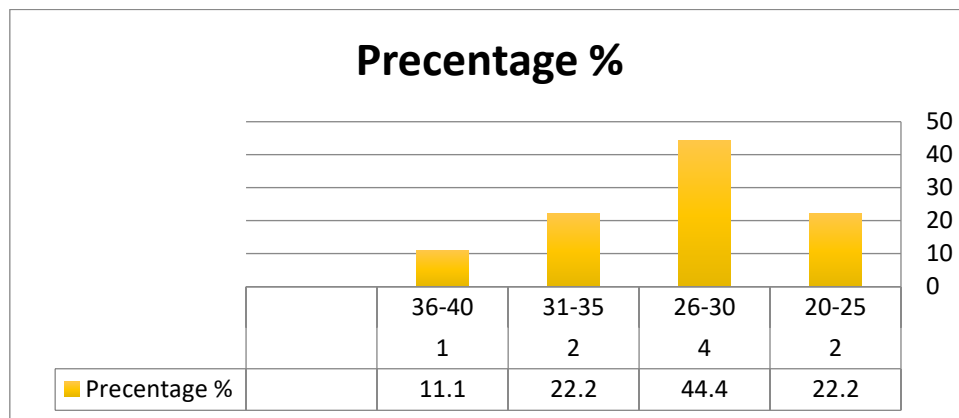


Figure 3. Percentage Distribution of Participants According to Age Groups [Pregnant]

Conclusion

The study done in Kirkuk city clearly showed that the homeostasis of serum calcium is well-regulated physiologically in pregnant women as well as in non-pregnant women. The marginal increase in mean serum calcium found in pregnant women group (10.29 ± 0.49 mg/dL) compared to that of non-pregnant reference group (8.29 ± 0.72 mg/dL) biochemically indicates a number of physiological adaptations by mother for meeting the increased needs due to pregnancy, especially the cellular requirements for metabolism of signaling as well as mineralization in the skeleton during the third trimester. The significant transplacental transport of calcium because of fetal growth would result in maternal negative calcium balance under physiological conditions; this is compensated through the up-regulation of calcium absorption and retention hormones. The changes in structure at different stages of gestation in terms of decreased levels in the mid-term of pregnancy followed by an increase in the third trimester show the interdependence of dietary, supplementation, and systemic regulatory influences. Though there is no clear linear relation between the clinical parameters and chronological age of the participants in this study, the biochemical adequacy of these profiles is largely influenced by the influence of confounders such as vitamin D, which regulates bone turnover and synthesis of intestinal calcium binding proteins. However, the limitations of these results are seen in the form of low number of pregnant women, a wide age group that includes the period of acquisition of peak bone

mass till postmenopause, and lack of stratification in terms of albumin corrected calcium and serum levels of 25-hydroxycholecalciferol. Overall, the homeostasis of calcium appears to be adequate in the Kirkuk population, and it is important to conduct more studies to establish calcium-phosphate-vitamin D metabolism and prevent any complications like preeclampsia or intrauterine growth restriction.

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