

بسم اله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

**Assessment of plasma Calcium and Phosphorus Levels of
Sudanese Pregnant Women in Khartoum state**

تقويم مستويات الكالسيوم والفسفور في بلازما الدم للنساء السودانيات الحوامل
في ولاية الخرطوم

**A dissertation submitted in partial fulfillment for the
requirements of M.Sc degree in Clinical Chemistry**

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2017

الآية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ تَعَالَى: ﴿وَوَصَّيْنَا الْإِنْسَانَ بِوَالِدَيْهِ حَمَلَتْهُ أُمُّهُ وَهْنًا عَلَى وَهْنٍ وَفِصْلَهُ فِي
عَامَيْنِ أَنْ اشْكُرْ لِي وَلِوَالِدَيْكَ إِلَيَّ الْمَصِيرُ ﴿١٤﴾﴾

صدق الله العظيم

سورة لقمان

الآية (14)

Dedication

To the candles which burn to light my life
My mother

To the one who I a lived for making his dreams become
true

My father

To whom I inspired and gave me the meaning of being

My brother

My friends

And special dedication to all who loved me

Acknowledgment

At first I thank my God for giving me health and ability to do this study.

The best thanks for my supervisor Dr: Khalda Mirghani Hamza for helping me.

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And all thanks for my university which gave sciences and knowlegment and gave chance to do this study.

Abbreviations

| | |
|------------------------|---|
| ALP | Alkaline phosphates |
| Ca⁺⁺ | Calcium |
| GFR | Glomerular filtration rate |
| HCG | Human chorionic gonadotropin |
| LNMP | Last normal menstrual period |
| Mg⁺⁺ | Magnesium |
| PH | Potential of hydrogen |
| PTH | Para thyroid hormone |
| PTH-rp | Para thyroid hormone related peptide |
| PUPPP | Pruritic urticarial papules and plaques of pregnancy |
| SD | Stander deviation |

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Abstract

This is a case control study conducted in Soba University Hospital during period from august to Octobers 2014 .

The aim of this study is compare plasma calcium and plasma phosphate between pregnant women in different stages of pregnancy and healthy non pregnant women.

Sixty blood samples were collected from healthy Sudanese pregnant women and divided into first , second , and third trimester as test group and twenty health non pregnant women as control group. Plasma calcium and phosphorus were measured by auto analyzer called cobas its fully automation, then data were analyzed by statistical (SPSS) software .

The study showed insignificant decrease in plasma calcium levels when compared test group (9.02 ± 0.47 mg/dl) with control (9.35 ± 0.75 mg/dl) (p.value 0.137) , and significant reduction when compare first with third trimester (p.value 0.000). No significant change in plasma phosphorus levels in tests group (3.32 ± 0.51 mg/dl) compared with mean of control group (3.59 ± 0.65 mg/dl) (p.value 0.064).

insignificant weak positive correlation between phosphorus and calcium (p.value 0.226) ($r=0.137$).

measurement of serum calcium should be done regularly during pregnancy and pregnant women should take diet rich with calcium or calcium supplement which is needed to compensate increase demand of fetus .

مستخلص الدراسة

هذه دراسته حاله في مستشفى سوبا الجامعي في الفتره من اغسطس حتي اكتوبر 2014.

هدفت هذه الدراسة لقياس مستويات كل من الكالسيوم والفوسفور عند الحوامل في فترات الحمل المختلفه و مقارنتها بغير الحوامل الأصحاء كمجموعة ضبط .
تم اختيار 60 من الحوامل الاصحاء ظاهريا وتم تقسيمهن الي ثلاثه مجموعات (فترة الحمل الاولى , الثانية , الثالثة) كمجموعة اختبار كل مجموعه تحتوي علي 20 أمراه حامل , 20 من الاصحاء غير الحوامل كمجموعة تحكم وقمنا بقياس مستويات الكالسيوم والفوسفور باستخدام جهاز التحليل الذاتي (cobas).
تم تحليل البيانات احصائيا بواسطه برنامج الحزم الاحصائيه للعلوم الاجتماعيه (SPSS) .

نتائج هذه الدراسه اظهرت مستويات الكالسيوم في مصل النساء الحوامل انخفاض ذو دلالة غير إحصائيه عند مقارنه مجموعه التحكم بمجموعة الاختبار (0.75 ± 9.35) (0.47 ± 9.02) على التوالي بإحتمال إحصائي (0.137) كما اظهرت انخفاضا ذو دلالة احصائية معنوية عند مقارنة الفتره الاولى من الحمل بالفتره الثالثه بإحتمال إحصائي (0.000) . اما مستويات الفوسفور لم تظهر اي تغير في كل فترات الحمل المختلفه عند مقارنتها بمجموعات الاختيار.

وقد اثبت التحليل الاحصائي ان الحمل يرتبط بنقصان في مستوي الكالسيوم في فترات الحمل الاولى والثانية والثالثة والذي بدوره يرتبط ارتباط طردي مع الفسوسفور معامل الارتباط (0.137).

خلصت هذه الدراسه الي اهمية قياس مستوي الكالسيوم دوريا عند النساء الحوامل وتناول الغذاء الغني بالكالسيوم او اقراص الكالسيوم وذلك لتعويض الكميته التي ياخذها الجنين

Chapter one

Introduction and Literature review

Chapter one

1. introduction and literature review:

1.1 Introduction

Fetus skeleton is formed during pregnancy period and bone mineralization highly demands minerals, supplied maternally (Prentice, *et al.*,1994).Bone is greatest reservoir for calcium, significant changes may occur in maternal skeletal system during pregnancy that result in osteoporosis and osteomalacia (prentice, *et al.*,1994).Although major part of calcium is absorbed by fetus in third trimester of pregnancy, homeostasis begins from the earliest time of pregnancy (prentice, *et al.*,1994).significant increase is observed in the serum calcium level in the third pregnancy, and phosphorus level is not affected for all pregnancy stages (power, *et al.*,1999) .

Most studies of calcium metabolism in pregnancy have examined changes in serum markers of bone formation and urine markers of bone resorption, these studies fraught with a number of confounding variables, including lack of pregnancy base line values, hemodilutional effect on serum markers, increase glomular filtration rate and renal clearance changing in renal excretion. Many studies have reported that urinary markers of bone resorption(24h urine) are increased from early to mid pregnancy conversely serum markers of bone formation are often decrease from early to mid pregnancy and rising to normal value.

It is conceivable that the bone formation marker are artificially lowered by normal hemodilution and increased renal clearance during pregnancy (More, *et al.*,2003). According to the previous effects of pregnancy on health , the study was conducted to evaluation serum calcium and phosphorus. The result of the study may add to the knowledge about health during pregnancy and what about the precaution needed to minimize the bone and dental disease during pregnancy.

1.2 Literature Review

1.2.1 Pregnancy

Pregnancy is the fertilization and development of one or more offspring, known as an embryo or fetus, in a woman's uterus. It is the common name for gestation in humans. A multiple pregnancy involves more than one embryo or fetus in a single pregnancy, such as with twins. Childbirth usually occurs about 38 weeks after conception; in women who have a menstrual cycle length of four weeks, this is approximately 40 weeks from the start of the last normal menstrual period (LNMP). An embryo is the developing offspring during the first 8 weeks following conception, and subsequently the term fetus is used until birth. In many societies' medical or legal definitions, human pregnancy is somewhat arbitrarily divided into three trimester periods, as a means to simplify reference to the different stages of prenatal development. The first trimester carries the highest risk of miscarriage (natural death of embryo or fetus). During the second trimester, the development of the fetus can be more easily monitored and diagnosed. The third trimester is marked by further growth of the fetus and the development of fetal fat stores. The point of fetal viability, or the point in time at which fetal life outside of the uterus is possible, usually coincides with the late second or early third trimesters, and is typically associated with high degrees of morbidity and mortality (Salvolini *et al.*, 1998).

Development of embryo and fetus: The sperm and the egg cell, which has been released from one of the female's two ovaries, unite in one of the two fallopian tubes. The fertilized egg, known as a zygote, then moves towards the uterus, a journey that can take up to a week to complete. Cell division begins approximately 24 to 36 hours after the male and female cells unite. Cell division continues at a rapid rate and the cells then develop into what is known as a blastocyst. The blastocyst arrives at the uterus and attaches to the uterine wall, a process known as implantation. After about 10 weeks of gestational age, the embryo becomes known as a fetus instead. At the beginning of the fetal stage, the risk of miscarriage decreases sharply, when the fetal stage commences, a fetus is typically about 30 mm (1.2 inches) in length, and the heart can be seen beating via ultrasound; the fetus can be seen making various involuntary motions at this stage. During continued fetal development, the early body systems and structures that were established in the embryonic stage continue to

develop. Sex organs begin to appear during the third month of gestation. The fetus continues to grow in both weight and length, although the majority of the physical growth occurs in the last weeks of pregnancy (Kalverboer *et al.*, 2001).

Maternal changes: During pregnancy, the woman undergoes many physiological changes, which are entirely normal, including cardiovascular, hematologic, metabolic, renal and respiratory changes that become very important in the event of complications. The body must change its physiological and homeostatic mechanisms in pregnancy to ensure the fetus is provided for. Increases in blood sugar, breathing and cardiac output are all required. Levels of progesterone and estrogens rise continually throughout pregnancy, suppressing the hypothalamic axis and subsequently the menstrual cycle. Pregnancy is typically broken into three periods, or trimesters, each of about three months. Obstetricians define each trimester as lasting for 14 weeks, resulting in a total duration of 42 weeks, although the average duration of pregnancy is actually about 40 weeks. While there are no hard and fast rules, these distinctions are useful in describing the changes that take place over time (Clark *et al.*, 1986).

First trimester: Minute ventilation is increased by 40% in the first trimester (up to 13 weeks). The womb will grow to the size of a lemon by eight weeks. Many symptoms and discomforts of pregnancy (further described in later sections) appear in the first trimester (www.nhs.uk/condition of pregnancy and baby).

Second trimester: Weeks 13 to 28 of the pregnancy are called the second trimester. Most women feel more energized in this period, and begin to put on weight as the symptoms of morning sickness subside and eventually fade away. The uterus, the muscular organ that holds the developing fetus, can expand up to 20 times its normal size during pregnancy. Although the fetus begins to move and takes a recognizable human shape during the first trimester, it is not until the second trimester that movement of the fetus, often referred to as "quickening", can be felt. This typically happens in the fourth month, more specifically in the 20th to 21st week, or by the 19th week if the woman has been pregnant before. However, it is not uncommon for some women not to feel the fetus move until much later (www.nhs.uk/condition of pregnancy and baby).

Third trimester: Final weight gain takes place, which is the most weight gain throughout the pregnancy. The woman's abdomen will transform in shape as it drops due to the fetus turning in a downward position ready for birth (Stacey *et al.*, 2011).

1.2.1.1 Symptoms and discomforts of pregnancy:

Common symptoms and discomforts of pregnancy include: Tiredness, Constipation, Pelvic girdle pain, Back pain, Braxton Hicks contractions, Edema (swelling), Increased urinary frequency(A common complaint referred by the gravida, caused by increased intravascular volume, elevated GFR (glomerular filtration rate), and compression of the bladder by the expanding uterus), Urinary tract infection, Varicose veins(Common complaint caused by relaxation of the venous smooth muscle and increased intravascular pressure), Haemorrhoids (piles), Regurgitation, heartburn, and nausea, Striae gravidarum, pregnancy-related stretch marks(Vazquez 2010).

1.2.1.2 Diagnosis of pregnancy

Physical signs: Most pregnant women experience a number of symptoms, which can signify pregnancy. The symptoms can include nausea and vomiting, excessive tiredness and fatigue, cravings for certain foods that are not normally sought out, and frequent urination particularly during the night. A number of early medical signs are associated with pregnancy these signs typically appear, if at all, within the first few weeks after conception. Although not all of these signs are universally present, nor are all of them diagnostic by themselves, taken together they make a presumptive diagnosis of pregnancy. These signs include the presence of human chorionic gonadotropin (hCG) in the blood and urine, missed menstrual period, implantation bleeding that occurs at implantation of the embryo in the uterus during the third or fourth week after last menstrual period, increased basal body temperature sustained for over 2 weeks after ovulation, Breast tenderness is common during the first trimester, and is more common in women who are pregnant at a young age. Shortly after conception, the nipples and areolas begin to darken due to a temporary increase in hormones. This process continues throughout the pregnancy (Qasim *et al.*, 1996).

Pregnancy tests: pregnancy detection can be accomplished using one or more various pregnancy tests Which detect hormones generated by the newly formed placenta, serving as biomarkers of pregnancy. Blood and urine tests can detect pregnancy 12 days after implantation. Blood pregnancy tests are more sensitive than urine tests (giving fewer false negatives). Home pregnancy tests are urine tests, and normally detect a pregnancy 12 to 15 days after fertilization. A quantitative blood test can determine approximately the date the embryo was conceived. Testing 48 hours apart

can provide useful information regarding how the pregnancy is doing. A single test of progesterone levels can also help determine how likely a fetus will survive in those with a threatened miscarriage (bleeding in early pregnancy) (Verhaegen *et al.*, 2011).

1.2.1.3 Complications of pregnancy

Pregnancy induced hypertension, Anemia, Postpartum depression, Postpartum psychosis, Thromboembolic disorders(The leading cause of death in pregnant women in the US), skin diseases that develop around the 32nd week Pruritic Urticarial Papules and Plaques of Pregnancy (PUPPP), red plaques, papules, itchiness around the belly button that spread all over the body except for the inside of hands and face, Ectopic pregnancy(implantation of the embryo outside the uterus), Hyperemesis gravidarum(excessive nausea that is more severe than morning sickness), pregnancy can enhance susceptibility to periodontal disease and dental caries(Merck 2010).

1.2.2 Calcium

1.2.2.1 Distribution of calcium

About 99% of calcium in the body is part of bone. The remaining 1% is mostly in the blood and other extra cellular fluid. Little is in the cytosol of most cells. In fact, the concentration of ionized calcium in blood is 5,000 to 10,000 times higher than in the cytosol of cardiac or smooth muscle cells. Maintenance of this large gradient is vital to maintain the essential rapid inward flux of calcium. Calcium in blood is distributed among several forms. About 45% circulates as free calcium ions (referred to as ionized calcium) 40% is bound to protein, mostly albumin, and 15% is bound to anions, such as HCO_3^- , citrate, PO_4^- and lactate (Michael *et al.*, 2010).

1.2.2.2 Calcium level abnormalities

Hypocalcemia

When Para thyroid hormone (PTH) is not present, as with primary hypoparathyroidism, serum calcium levels are not properly regulated. Bone tends to “hang on” to its storage pool and the kidney increases excretion of calcium. Since PTH is also required for normal vitamin D metabolism, the lack of vitamin D’s effects also leads to a decreased level of calcium . Parathyroid gland aplasia, destruction, or removal is obvious reasons for primary hypoparathyroidism. Because hypomagnesemia has become more frequent in hospitalized patients, chronic hypomagnesemia has also become recognized as a frequent cause of hypocalcemia. Hypomagnesemia may cause hypocalcemia by three mechanisms: (1) it inhibits the

glandular secretion of PTH across the parathyroid gland membrane, (2) it impairs PTH action at its receptor site on bone, and (3) it causes vitamin D resistance. Elevated Mg^{++} levels may inhibit PTH release and target tissue response, perhaps leading to hypocalcemia and hypercalciuria (Michael *et al.*, 2010).

When total calcium is the only result reported, hypocalcemia can appear with hypoalbuminemia. Common causes are associated with chronic liver disease, nephrotic syndrome, and malnutrition. In general, for each 1 g/dL decrease in serum albumin, there is a 0.2 mmol/L (0.8 mg/dL) decrease in total calcium levels. About one half of the patients with acute pancreatitis develop hypocalcemia. The most consistent cause appears to be a result of increased intestinal binding of as calcium increased intestinal lipase activity occurs.

Vitamin D deficiency and malabsorption can cause decreased absorption, which leads to increased PTH production or secondary hyperparathyroidism. Patients with renal disease caused by glomerular failure often have altered concentrations of calcium, PO_4^- , albumin, Mg^{++} , and H^+ (PH). In chronic renal disease, secondary hyperparathyroidism frequently develops as the body tries to compensate for hypocalcemia caused either by hyperphosphatemia (PO_4^- binds and lowers ionized Ca^{++}) or altered vitamin D metabolism. Monitoring and controlling ionized calcium concentrations may avoid problems due to hypocalcemia, such as osteodystrophy, unstable cardiac output or blood pressure, or problems arising from hypercalcemia, such as renal stones and other calcifications (Michael *et al.*, 2010).

Symptoms of hypocalcemia

Neuromuscular irritability and cardiac irregularities are the primary groups of symptoms that occur with hypocalcemia. Neuromuscular symptoms include paresthesia, muscle cramps, tetany, and seizures. Cardiac symptoms may include arrhythmia or heart block. Symptoms usually occur with severe hypocalcemia, in which total calcium levels are below 1.88 mmol/L (7.5 mg/dL) (Michael *et al.*, 2010).

Hypercalcemia

Primary hyperparathyroidism is the main cause of hypercalcemia. Hyperparathyroidism, or excess secretion of para thyroid hormon, may show obvious clinical signs or may be a symptomatic. The patient population seen most frequently with primary hyperparathyroidism is older women. Although beither total or ionized calcium measurements are elevated in serious cases, ionized calcium is more frequently elevated in bsubtle or asymptomatic hyperparathyroidism. In general, ionized calcium measurements are elevated in 90% to 95% of cases of hyperparathyroidism, whereas total calcium is elevated in 80% to 85% of cases. The second leading cause of hypercalcemia is associated with various types of malignancy, with hypercalcemia sometimes being the sole biochemical marker for disease. Many tumors produce PTH-related peptide (PTH-rP), which binds to normal PTH receptors and causes increased calcium levels (Michael *et al.*, 2010).

Symptoms of hypercalcemia: A mild hypercalcemia (2.62–3.00 mmol/L [10.5–12 mg/dL]) is often asymptomatic. Moderate or severe Ca^{++} elevations include neurologic, GI, and renal symptoms. Neurologic symptoms may include mild drowsiness or weakness, depression, lethargy, and coma. GI symptoms may include constipation, nausea, vomiting, anorexia, and peptic ulcer disease. Hypercalcemia may cause renal symptoms of nephrolithiasis and nephrocalcinosis. Hypercalciuria can result in nephrogenic diabetes insipidus, which causes polyuria that result in hypovolemia, which further aggravates the hypercalcemia. Hypercalcemia can also cause symptoms of digitalis toxicity (Michael *et al.*, 2010).

1.2.2.3 Calcium abnormalities during pregnancy:

Calcium metabolism is dramatically altered by pregnancy and lactation. The normal fetal skeleton accumulates approximately 30g of calcium by term, proportional to the fetal weight. **The largest proportion (80%) of that accretion occurs in the third trimester, at a rate of about 250-300 mg/day.** Total serum calcium levels fall early in pregnancy, due to hemodilution and the consequent decline in serum albumin. Ionized calcium levels and phosphate levels remain normal throughout pregnancy. Urinary calcium excretion increases early in gestation secondary to an increased calcium load filtered by the kidneys and the increased glomerular filtration rate of pregnancy (Kovacs *et al.*, 1997)

1.2.3 Phosphorus:

Phosphorus is predominant intracellular anion about 80% of total phosphorus contained in bone and 20% in soft tissue and less than 1% is active in serum (Bishop *et al.*,2010)

Dietary sources of phosphorus:

- Bran of wheat.
- pumpkin, squash, and water melon seeds.
- Sun flower seeds.
- Toasted wheat germ.
- Cheese.
- Sesames seeds.
- Flax seeds(National research council.,1989).

Regulation of plasma phosphorus:

Phosphorus absorbed in intestine from dietary source, released from all in to blood and lost from bone, in healthy individuals all these processes are relatively constant and easily regulated by renal excretion or reabsorbed ion of phosphorus.

Disturbance to any processes can alter phosphate concentration in the blood (Bishop *et al.*,2005).

As with calcium the majority of body phosphorus (approximately $\approx 85\%$) is present in the mineral phase of bone. The remainder of body phosphorus is a variety of inorganic compounds distributed within both intracellular and extracellular compartments. (Bihlg *et al.*, 2001).

1.2.3.1 phosphorus abnormalities :

1-Hyperphosphatemia:

Is the state of blood level higher than expected normal range in health population (Bishop *et al.*,2005).

Normal range of phosphorus is (2.7-4.5mg/dl or .87-1.45mmol/l). often calcium level is lowered (hypocalcemia) due to precipitation of phosphate with calcium in tissue(Bishop *et al.*,2005).

Causes of hyperphosphatemia:

Causes include ectopic calcification, secondary hyperparathyroidism and renal osteodystrophy (Bowen, et al., 2008).

-Hypoparathyroidism: in this situation there are low level of parathyroid hormone (PTH). PTH normally inhibits renal reabsorption of phosphorus, and so without enough PTH there is more reabsorption of the phosphorus.

- Chronic renal failure: when the kidneys are not working well, there will be increased phosphorus retention.

Drugs for hyperphosphataemia can also be caused by taking oral sodium phosphorus solutions prescribed for bowel preparation for colonoscopy (Fine et al., 1997)

2-Hypophosphataemia:

Hypophosphataemia is state of blood phosphorus level below than expected normal range in health population. (Bishop et al., 2010)

A decreased phosphorus in the blood is sometimes associated with increase phosphorus in the urine, this is hypophosphatemia and phosphaturia. (Thomas *et al.*, 2003)

Signs and Symptoms:

- Muscle dysfunction and weakness
- Mental status change this may from irritability to gross confusion.
- White cell dysfunction causing worsening of infection.
- Instability of cell membranes due to low ATP levels this may cause rhabdomyolysis and also hemolytic anemia. (Toy *et al.*, 2007)

Causes:

Hypophosphatemia is caused by the following 3 mechanisms:

- Inadequate intake.
- Increased excretion in hyperparathyroidism, hypophosphatemic rickets.
- Alcohol abuse and alcohol impairs phosphate absorption. (Toy *et al.*, 2007).

1.3 Rationale

Pregnancy is often associated with physiological abnormalities lead to various complications in serum calcium and phosphorus lead to problem in bone and dental diseases for pregnant women and also cause defect in structure of bone and teeth of baby after delivery.

In Sudan, few studies about the effects of pregnancy on serum calcium and phosphorus, and alkaline phosphates was conducted, so the study was carried out to measure these parameters in serum during pregnancy.

1.4 Objective:

1.4.1 General objective:

Assessment of calcium and phosphorus plasma levels serum levels in Sudanese pregnant women.

1.4.2 Specific objective:

1-To determine calcium and phosphorus plasma levels and in Sudanese pregnant women compared to non-pregnant women.

2-To correlate calcium and phosphorous levels according to trimester of pregnancy.

Chapter two

Materials and Methods

Chapter Two

2. Material and method

2.1 Material:

2.1.1 Study design :

This is case control study .

2.1.2 Study area :

This study was done in Soba University Hospital.

2.1.3 Study population :

Eighty Sudanese women were participated in this study.

Sixty healthy pregnant women in different trimesters they were selected as case of this study and divided in three groups ,each group contain twenty women according to trimester . twenty healthy non pregnant women as control group .

2.1.4 inclusion criteria :

Healthy Sudanese pregnant women were included in this study as case group and healthy non pregnant women were included as control group who they age ranged from (17-40) years old. Attended in Soba University Hospital during period from Augustus to October 2014

2.1.5 Exclusion criteria :

Women with cholestasis , bone disease , renal impairment or chronic disease and any disease that may affect the parameters under study .

2.1.6 Ethical consideration:

Subjects who voluntarily accepted to participate in the study were included.

2.1.7 Data collection:

A questionnaire was specifically designed to collect demographic data such as age, trimesters and any calcium supplement.

2.1.8 blood sample collection:

after informed consent was used alcohol antiseptic (70 ethanol) to collect 3ml of venous blood in lithium heparin container and centrifuged for 3 minutes at 30000/rpm.

2.1.9 Quality control:

Each laboratory should establish its own internal Quality control scheme and procedure for corrective action if controls do not recover within the acceptable tolerance .

2.1.10 method

COBAS

Principle of phosphorus estimation:

Inorganic phosphate forms an ammonium phosphomolybdate complex with ammonium molybdate in the presence of sulfuric acid.

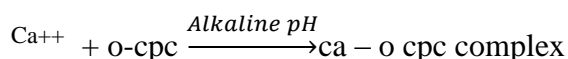
Phosphate molybdate + ammonium → ammonium-phosphomolybdate

The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration . it is determined by measuring the increase in absorbance at 340nm .

Normal range: 2.7 – 4.5 mg/dl

Principle of calcium estimation:

Ions react with o-cresolphthalein under alkaline condions to form a violet colored complex. The addition of 8-hydroxy quinoline prevents interference by magnesium and ferric ions



The color intensity of complex formed is directly proportionalto calcium concentration . it is determined by measuring the increase in absorbance at 552nm.

Normal range 8.2 – 10.5 mg /dl

2.1.11 Data analysis :

The Data collected in this study were analyzed using statistical package for social science (spss) computer program: ANOVA test and correlations were calculated. P significant level was set at $p \leq 0.05$

Chapter three

Results

CHAPTER THREE

RESULTS

3. Results:

Sixty pregnant women age range from (17_40 years old) and 20 non pregnant women were included in this study. Samples was collected from Soba University Hospital. The samples (plasma) were collected from pregnant women during period from Augustus 2014 to October 2014.

insignificant difference in calcium plasma level of pregnant women in the second and third trimesters compared to control but significantly different in third trimester compared to first.

insignificant difference in phosphorus when compared study group with control and no difference in all trimesters.

insignificant weak positive correlation between calcium serum level and phosphorus plasma level ($r=0.137$, $P= - 0.226$)

Table (3.1): plasma calcium, phosphorus of prgenent women compared to control group.

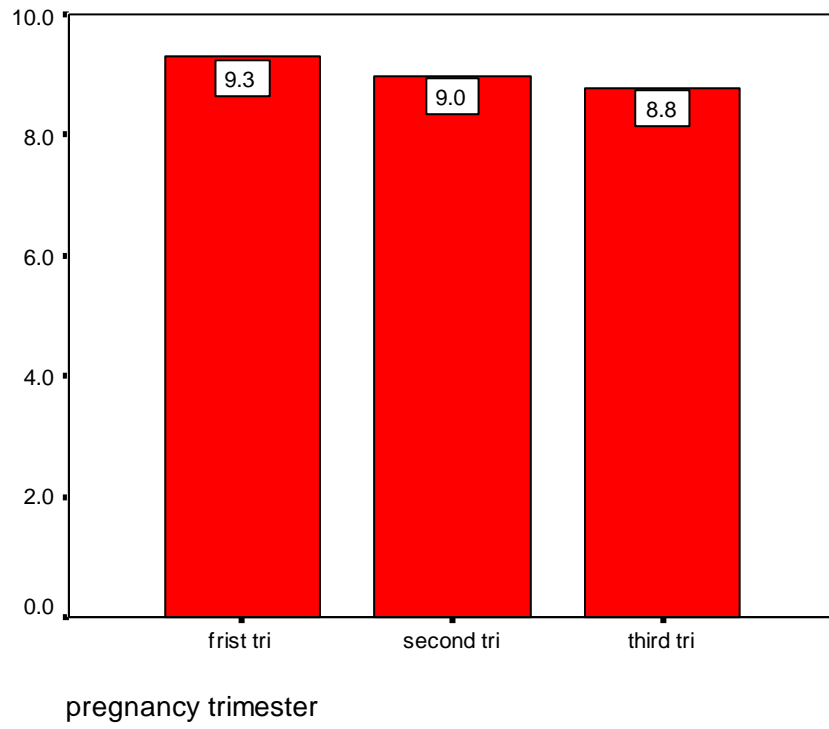
| Variable | Pregnant women mean±SD N=60 | Control mean±SD N=20 | P. value |
|------------------------------|-----------------------------------|-------------------------|----------|
| plasma calcium (mg/dl) | 9.02±0.47 | 9.35±0.75 | 0.137 |
| plasma phosphorus (mg/dl) | 3.32±0.51 | 3.59±0.65 | 0.064 |

The table (3-1) showed the mean ±SD, and the probability (P). No significant difference of plasma calcium, phosphorus levels of test compared to control.

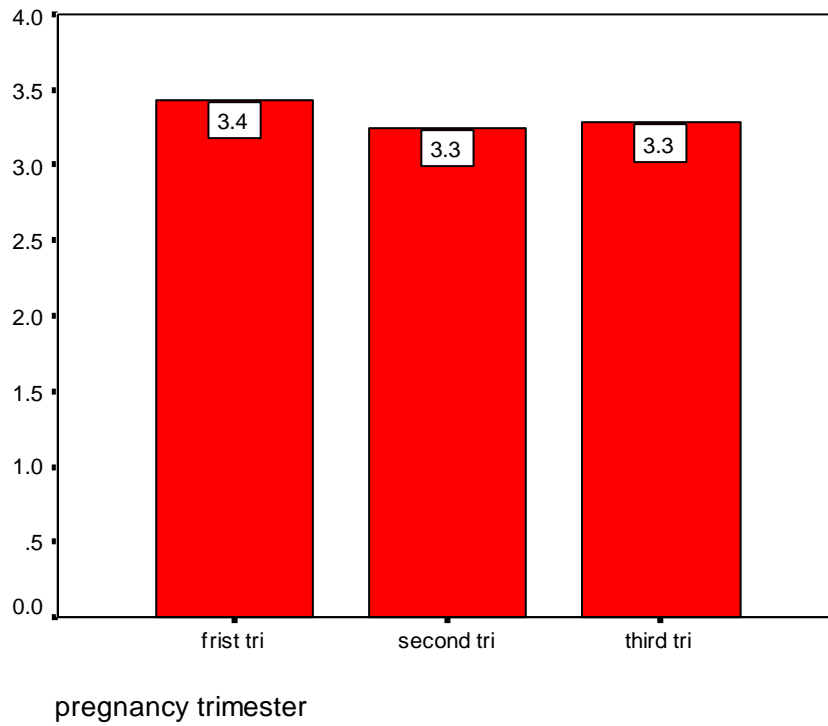
Table (3.2): Comparison between plasma calcium, phosphorus of test group in different three trimesters of pregnancy.

| Variables | Trimesters of pregnancy | | P value |
|----------------------------------|-------------------------|------------------|---------|
| Plasma calcium level mg/dl. | First trimester | Second trimester | 0.027 |
| | | Third trimester | 0.000 |
| | Second trimester | Third trimester | 0.143 |
| Plasma phosphorus level mg/dl | First trimester | Second trimester | 0.258 |
| | | Third trimester | 0.358 |
| | Second trimester | Third trimester | 0.830 |

(*The mean difference is significant at the .05 level.



(Figure 3.1): plasma Calcium levels in pregnant women in different trimesters of pregnancy.



(Figure 3.2): phosphorus plasma level in pregnant women with different trimesters of pregnancy.

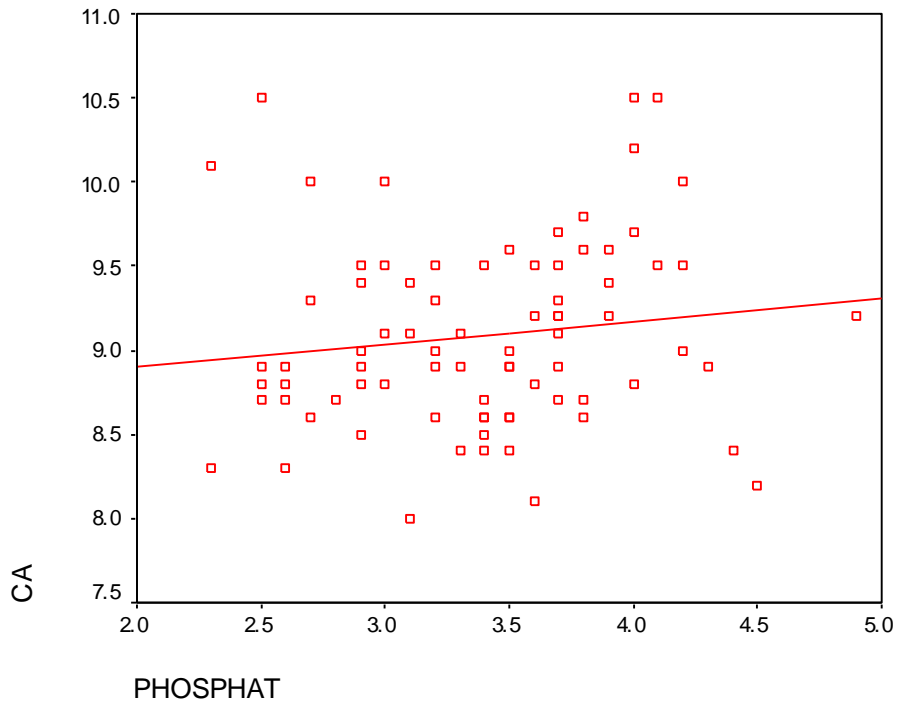


Figure (3.3) show correlation between calcium and phosphorus plasma levels of pregnant women.

Chapter four
**Discussion, conclusion and
recommendation**

CHAPTER FOUR

4. Discussion, conclusion and recommendation :

4.1 Discussion:

This is case control study aimed to determine plasma level of calcium and phosphorus , age ranged between 17-40 years females in all trimesters were included compared with control and then with trimesters.

Several studies reported association between pregnancy and changes in plasma calcium and phosphorus levels (Christopher *et al.* , 1995)

The fetus needs nearly 30 gram calcium ,80% of which is achieved in the third trimester of pregnancy, and this amount is about 3% of the total maternal calcium.

Inspite of this achievement by the fetus in absorbing the major port of it is calcium in third trimester of pregnancy (200mg/day) ;maternal calcium homeostasis begins from the earliest time of this period (8,9) (Gharedaghi M,*et al.*,1986) .

In the present study , plasma calcium of pregnant women insignificantly decreased when compared to the control (p.value = 0.137) , but calcium significantly decreased in the third trimester compared with other trimester (p.value = 0.000).

Theses findings agreed with various studies done by Christopher who reported a reduction of calcium during pregnancy.

In the first trimester there is fall in total plasma calcium due to the decrease in serum albumin that accompanies the normal hemodilutional during pregnancy, low parathyroid hormone during first trimester but increase steadily to the mid pregnancy and reached normal range, increased serum calcitonin, intestinal absorption of calcium is increased during pregnancy and phosphorus levels is normal (Kovac, *et al.*, 1997).

The extra demand for calcium from the growing fetus, espically during the third trimester is compensated through changes in hormonal levels lead to increase intestinal absorption, decrease renal excretion and intestinal calcium absorption becoming the primary source of calcium.(kovac,*et al.*, 2001)

plasma phosphorus level showed insignificant decrease in pregnant women, when compared to the controls table (3-1) (p.value = 0.064), and no difference in all trimesters. that means the level of phosphorus concentration is not affected by pregnancy. Similar previous studies reported that there is no change in plasma phosphorous concentration during pregnancy and plasma calcium and phosphorus

mainly regulated by parathyroid hormone and the active form of vitamin D and interference with the action of vitamin D and parathyroid hormone must be reflected on the plasma concentration of calcium and phosphorous (Varley, 2000).

4.2 Conclusion:

1-serum calcium of pregnant women insignificantly decreased compared with control and significant difference when compare first with third trimesters.

2-serum alkaline phosphates significantly increase when compared first with third and second with third trimesters.

3-significant negative correlation between serum calcium and serum alkaline phosphates.

4- no relationship between serum phosphate and alkaline phosphates.

4.3 Recommendation:

1-measurment of serum calcium should be done regularly during pregnancy .

2-pregnant women should take diet rich with calcium supplement if needed to compensate increase demand of fetus.

References

References:

Bihlg G, Meyers A. (2001) Recurrent Renal Stone Disease-Advances in pathogenesis and clinical management-358(9282):615-656.

Christophers, Kovacs CS. (2001) Calcium and Bone Metabolism in Pregnancy and Lactation of Clinical Endocrinology and Metabolism, 86 (6) 2244 .

Clark DA, Chaput A and Tutton D (March 1986). Active suppression of host vs graft reaction in pregnant mice VII 136(5):1668_75.

Kovacs CS and Kronenberg HM (1997), maternal fetal calcium and bone metabolism during pregnancy 18:832.

March of Dimes, PMNCH2012 . Save the Children, WHO. Born too soon: the Global Action Report on Preterm Birth. Howson CP, Kinney MV, Lawn JE, eds. Geneva, World Health Organization.

Michael J Bishop , Edward P .Foudy Larry E .Schoess . (2005) . Clinical Chemistry Principle , Procedure and Correlation . Phosphate Regulation 5th ed : 332-337 .

Merck (2010). pregnancy complicated by disease. Merck Manual, Home health hand book. Michael L, Bishop and Edward P 2010 . Clinical chemistry: techniques, principles, correlation, sixth Edition.

National Research council (1989) , food and nutrition board , Recommended Dietary allowance 10th ed .

Power M I . Heary RP, Kalwarf H Y . Amjobstet (1999) . The role of calcium in health and disease in gynecology 181 (3) : 9-13.

Prentice A. Amjelin (1994), Maternal Requirement of During Pregnancy and Lactation the American Journal of Clinical Nutrition 90(3) : 4775-48835 .

Qasim S, Callanc C, Choe JK (1996). The predictive value of an initial serum beta human chorionic gonadotropin level of pregnancy outcome following in vitro fertilization. Journal of assisted reproduction and genetics 13(9):705_8.

Salvolini E, Digorgio R, Curatola A (1998). Biochemical modification of human whole saliva induced by pregnancy 105:656-660.

Stacey T, Thompson JM and Cowan LM (2011). Association between maternal sleep practice and risk of late still birth.

Toy L , Giradet H, Horman P (2007) Case Files Pediatric 2ed , 103 .

Varley L. Practical (2000) Clinical Biochemistry 5th ed , 850-882.

www.nhs.uk/condition of pregnancy and baby.

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College of Graduate Studies

Questionnaire

Patient number...

Name ...

Age (year)

Gestational age

Supplements

History of other diseases

-bone diseases

-liver diseases

Results

-calcium (mg/dl)

-phosphate (mg/dl)

Appendixes

| No | trimester | age | calcium | phosphate |
|-----------|------------------|------------|----------------|------------------|
| 1 | 3 | 39 | 10.2 | 4.0 |
| 2 | 2 | 23 | 9.7 | 3.7 |
| 3 | 2 | 22 | 8.9 | 3.7 |
| 4 | 2 | 24 | 9.7 | 4 |
| 5 | 2 | 30 | 9.5 | 3.2 |
| 6 | 3 | 34 | 9.5 | 3.6 |
| 7 | 3 | 35 | 8.6 | 3.8 |
| 8 | 2 | 20 | 8.9 | 2.6 |
| 9 | 3 | 28 | 9.2 | 3.6 |
| 10 | 2 | 27 | 8.9 | 2.5 |
| 11 | 2 | 26 | 9.1 | 3.1 |
| 12 | 2 | 27 | 9.4 | 3.9 |
| 13 | 3 | 24 | 9.5 | 4.3 |
| 14 | 3 | 27 | 9.0 | 2.9 |
| 15 | 3 | 20 | 10.0 | 3.0 |
| 16 | 3 | 30 | 9.2 | 3.7 |
| 17 | 3 | 26 | 9.6 | 3.8 |
| 18 | 3 | 24 | 9.5 | 4.2 |
| 19 | 2 | 25 | 8.0 | 3.1 |
| 20 | 2 | 33 | 9.4 | 2.9 |
| 21 | 6 | 24 | 8.7 | 3.7 |
| 22 | 5 | 32 | 8.6 | 3.4 |
| 23 | 4 | 30 | 9.3 | 2.7 |
| 24 | 6 | 26 | 8.9 | 2.9 |
| 25 | 5 | 23 | 8.6 | 3.5 |
| 26 | 5 | 24 | 9.0 | 3.2 |
| 27 | 6 | 29 | 8.5 | 2.9 |
| 28 | 6 | 32 | 8.7 | 2.6 |
| 29 | 6 | 33 | 8.7 | 2.5 |

| | | | | |
|----|---|----|-----|-----|
| 30 | 5 | 25 | 8.8 | 2.6 |
| 31 | 5 | 35 | 9.8 | 3.8 |
| 32 | 5 | 20 | 8.8 | 2.5 |
| 33 | 6 | 25 | 9.1 | 3.3 |
| 34 | 5 | 24 | 8.9 | 3.5 |
| 35 | 5 | 30 | 9.1 | 3.7 |
| 36 | 6 | 20 | 8.6 | 3.2 |
| 37 | 4 | 17 | 9.6 | 3.5 |
| 38 | 4 | 20 | 9.6 | 3.9 |
| 39 | 4 | 32 | 9.4 | 3.1 |
| 40 | 5 | 30 | 9.0 | 3.5 |
| 41 | 7 | 18 | 8.6 | 3.4 |
| 42 | 7 | 27 | 8.7 | 2.8 |
| 43 | 7 | 24 | 8.6 | 2.7 |
| 44 | 7 | 23 | 8.8 | 3.0 |
| 45 | 7 | 30 | 8.3 | 2.6 |
| 46 | 7 | 36 | 8.8 | 2.6 |
| 47 | 8 | 26 | 9.1 | 3.0 |
| 48 | 9 | 20 | 8.4 | 4.4 |
| 49 | 8 | 29 | 9.2 | 3.9 |
| 50 | 9 | 23 | 8.9 | 3.2 |
| 51 | 7 | 18 | 8.1 | 3.6 |
| 52 | 9 | 28 | 8.8 | 3.6 |
| 53 | 9 | 34 | 8.8 | 2.9 |
| 54 | 9 | 29 | 8.7 | 4.0 |
| 55 | 9 | 30 | 9.5 | 3.8 |
| 56 | 9 | 36 | 9.3 | 2.9 |
| 57 | 9 | 36 | 8.4 | 3.2 |
| 58 | 9 | 34 | 8.9 | 3.4 |
| 59 | 9 | 30 | 8.3 | 4.3 |
| 60 | 8 | 35 | 9.5 | 2.3 |
| 61 | | 24 | 9.2 | 3.7 |

| | | | | |
|----|--|----|------|-----|
| 62 | | 26 | 9.3 | 4.7 |
| 63 | | 26 | 8.7 | 3.4 |
| 64 | | 33 | 8.9 | 3.5 |
| 65 | | 30 | 8.4 | 3.5 |
| 66 | | 27 | 9.2 | 4.9 |
| 67 | | 35 | 8.5 | 3.4 |
| 68 | | 26 | 9.5 | 4.1 |
| 69 | | 26 | 8.9 | 3.3 |
| 70 | | 26 | 10.5 | 2.5 |
| 71 | | 26 | 9.5 | 3.0 |
| 72 | | 20 | 10 | 2.7 |
| 73 | | 24 | 10.5 | 4.1 |
| 74 | | 30 | 8.2 | 4.5 |
| 75 | | 22 | 8.4 | 3.3 |
| 76 | | 26 | 10.1 | 2.3 |
| 77 | | 34 | 9.0 | 4.2 |
| 78 | | 35 | 8.6 | 3.5 |
| 79 | | 26 | 10.5 | 4.0 |
| 80 | | 33 | 10.0 | 4.2 |