بسم الله الرحمن الرحيم

Republic of Sudan
Ministry of Higher Education and scientific Research
Shendi University
Faculty of Graduate Studies and Scientific Research

Title:

Effect 0f Oral Contraceptive on Serum Electrolytes [Ca, Pi, Mg, Iron] Level

A thesis submitted in partial fulfillment for the requirement of MSc degree in Medical Laboratory Science [clinical chemistry]

By:

Alhumira Mohammed Alfaki Haj AL Hassan

BSc in Medical Laboratory Sciences, Faculty of Medical Laboratory Sciences, Clinical Chemistry Department, Shendi University (2013)

Supervisor:

Dr. Haghamad Allzain Mohamed Bulla

Assistant Professor of Biochemistry

Faculty of Medicine, Shendi University

2018

الآية

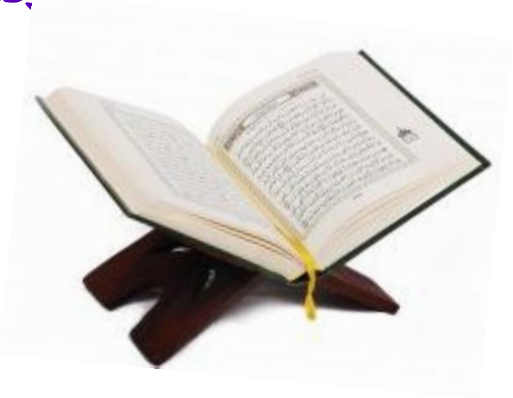
بسم الله الرحمن الرحيم

قال تعالى:

﴿ وَوَصَّيْنَا الْإِنسَانَ بِوَالِدَيْهِ حَمَلَتْهُ أُمُّهُ وَهْنًا عَلَى وَهْنٍ وَفِصَالُهُ فِي عَامَيْنِ أَنِ اشْكُرْ لِي وَلِوَالِدَيْكَ إِلَيَّ الْمَصِيرُ ﴾

صدق الله العظيم

سورة لقمان الآية 14



Dedication

To my mother……

To my father……

To my husband……..

To my brother……

To my sisters ……

To my friends……

I dedicate this work with my best wishes to all

Acknowledgement

All my thanks are in the name of Allah, the most Gracious and the most Merciful.

In this instance, I extended my thanks, deep sincere gratitude and honest appreciation to my supervisor

Dr. Haj hamad bulla

Department of, Biochemistry Chemistry, Shendi University, faculty of medicine, for his kindness, good guidance, valuable direction and generous advice that has kept me on the right track. I am indebted to his kind cooperation.

My thanks are also extended to Faculty of Medical Laboratory Science, Shendi University.

I feel indebted to many people who participated and helped me in this work.

List of Abbreviations

Abbreviations	Term
ATP	Parathyroid gland.
BMP	bone morphogenic protein,
CA	Calcium.
Coc	Combined oral contraceptive.
DMT1	divalent metal transporter 1
ECF	Extracellular fluid.
ER	Endothelial reticulum.
Fe+++	Ferric ion
Fe++	Ferrous ion.
Mg	Magnesium.
Mg	Milligram
OC	Oral contraceptive.
PCT	Proximal convoluted tubules.
Pi	Phosphate.
PTH	Parathyroid hormones.
RBCs	Red blood cells.
Ug	Microgram.

ملخص البحث

أجريت هذه الدراسة بمدينة شندي في الفترة ما بين شهر مارس 2018الى يوليو 2018لقياس تاثير حبوب منع الحمل على مستويات 'كهارل 'الجسم(كالسيوم،فوسفات.مغنسيوم،حديد).

جمعت50 عينة من النساء اللواتى يستخدمن حبوب منع الحمل (عينات دراسة) و50 عينة.من نساء اللواتى لا يستخدمن حبوب منع الحمل(عينات ضابطة)

وقيس مستوى الاكتروليت(كالسيوم،الفوسفات،المغنسيوم،الحديد) تتراوح أعمارهن ما بين 15-50 سنة.

تم تحليل البيانات المجموعة باستخدام برنامج الحزم الإحصائية للعلوم الاجتماعية.

أوضحت النتائج أن هناك انخفاض في مستوى الكالسيوم والمغنسيوم والفوسفات وزيادة فى الحديد فى النساء اللواتى يتناولن حبوب منع الحمل مقارنة مع اللواتى لا يتناولن حبوب منع الحمل .

Abstract

A case control study was conducted in Shendi locality, during the year 2018 to assess effect of oral contraceptive on electrolyte levels (ca, pi, mg, Iron).

The study included [100] woman, [50] of them use oral contraceptive pill and [50] of them as control group. The average ages were ranged from (15-50) years.

The result showed that they lower concentration in calcium ,magnesium level ,and high concentration in iron level in women use oral contraceptive pills was 7.7 ,1.8 ,32.0 mg/dl respectively, while in the control sample was 8.8 ,2.1 ,20.8mg/dl .

The results of the statistical analysis that there is statistically significant difference between the concentration of calcium, magnesium, iron levels ,between woman that use oral contraceptive pills and that non user oral contraceptive pills. The result of study show that age and number of children, educational level, and purpose of use not effect this parameter but period of use have clear effect.

List of contents

List of tables

1.1 Introduction

Oral contraceptive or birth control pills are medication that prevent pregnancy. They are method of birth control. Oral contraceptive are hormonal preparation that may contain single or combination of hormone.(mark h.berr et al,2003)

They are two type of oral contraceptive pill: combination pill which contain progestin and estrogen .they are categorized as monophasic ،biphasic ،or triphasic pills, and progestin-only pills (known as mini pill).(mark h.berr et al,2003).

Oral contraceptive pills primally work by preventing ovulation ,and thin the endometrium ,also cause the cervical mucus to thickening which can stop sperm from getting into the uterus.(mark h.berr et al,2003)

Electrolytes element or compound that, when melted or dissolved in water or another solvent, dissociates into ions and is able to conduct an electric current. Electrolytes differ in their concentrations in blood plasma, interstitial fluid, and cell fluid and affect the movement of substances between those compartments. Proper quantities of principal electrolytes and balance among them are critical to normal metabolism and function. The main electrolytes which lnclude:Ca, Pi,Na,K,Mg,Hco3,Cl-.Calcium and phosphate are essential mineral found in the bone, blood and soft tissue, it can affect by different factor ,so must be maintain within normal range (Ca:2.2-2.6mmol/l) and (Pi:0.8-1.4mmol/l) .

Magnesium is one of the major intracellular cations electrolyte it important for normal neuromuscular activities an important cofactor for various enzymes, transporters, and nucleic acids that are essential for normal cellular function, replication, and energy (Bone and mineral Metabolism in Health and Disease. (Longo DL FA, Kasper DL et al, 2012)

Iron. Iron is a part of heme, which is the active site of peroxidases that protect cells from oxidative injury by reducing peroxides to water. Heme is also the active site

of electron transport in cytochromes. When iron levels are insufficient, proliferation of bacteria or nucleated cells stops. This can cause by some condition and medication such as oral contraceptive. Normal range of serum in men: 55-160ug/dl, women: 50_155ug/dl (Prchal JT KK, Lichtman et al, 2010)

1.2 Objectives

1.2.1 General objective:

To study effect of oral contraceptive on serum electrolytes (ca,pi, mg,iron) levels

1.2.2 Specific objectives:

- To assess the effect of age of women that take oral contraceptive on electrolyte levels (ca,pi,mg , iron).
- To assess effect of type of contraceptive pill on electrolytes levels
- To assess effect of duration uses of oral contraceptive pills on electrolytes levels.

1.3 Rational

Oral contraceptive one of the ways to control pregnancy, which has many side effects and complications.

Contraceptive pill which can effect esstional and important body component like serum electrolytes such as: calcium, phosphate, magnesium, iron .So l was conduct this research.

2. Literature Review

2.1 Oral contraceptive:

2.1.1 Definition:

Oral contraceptive or birth control pills are medication that prevent pregnancy. They are method of birth control. Oral contraceptive are hormonal preparation that may contain single or combination of hormone. (Mark h.berr et al, 2003)

2.1.2 Type of oral contraceptive pill:

The pill ‘is the common name for oral contraceptive. There are two basic types of birth control pills; progestin-only pill, combination pill. Both are made of hormones like those made by a women ovaries. Combination pill contains estrogen and progestin. Both require a medical evaluation and prescription .Both can prevent pregnancy (.Hatcher RA, Zieman M, et al.2004)

2.1.2.1 The progestin:

Progestin only pills (also called "mini-pills") became available in the 1970. Their use was and has been limited - making up only one to 10% of contraceptive market. This type of pills contains no estrogen. Estrogen-free pills are ideal for breastfeeding women because estrogen reduces milk production. Progestin-only pills are also ideal for women with health conditions that preclude use of combined oral contraceptive pills, such as migraine (vascular) headaches, thromboembolism, and cardiovascular disease. (Hatcher RA, Zieman M et al.2004)

In addition, progestin-only pills do not have most of the estrogen-related side effects of oral contraception: nausea, headaches and other symptoms associated with starting the combined pill are minimal. However, bleeding and spotting days during the intramenstrual period may be higher than with the combined pills and

missed pills may result in a higher chance of pregnancy that with the combined oral contraceptives.(Hatcher RA, Zieman M et al.2004).

2.1.2.2 Combination pills;

The term "birth control pill" it most often refers to oral contraceptives containing estrogen and progestin. Combination pills contain a combination of these two hormones. They are categorized as monophasic, biphasic, or triphasic pills depending on whether the level of hormones stays the same during the first three weeks of the menstrual cycle or changes.(mark h,berr.2003)

2.1.2. 2.1Monophasic pills: have a constant dose of both estrogen and progestin in each of the hormonally active pills throughout the entire cycle (21 days of ingesting active pills). Several of the brands listed above may be available in several strengths of estrogen or progesterone, from which doctors choose according to a woman's individual needs. Because of the uniform hormone level in all the pills, monophasic are least likely to cause side effects, such as mood changes, that can result from fluctuating hormone levels in the body.(mark h ,berr,2003)

2.1.2.2.2Multiphasic oral contraceptives contain varied amounts of hormones and are designed to be taken at specific times throughout the entire pill-taking schedule. They were developed to reduce side effects of oral contraceptives, including breakthrough bleeding, spotting and amenorrhea, associated with higher levels of hormones.(mark h,berr.2003)

*Biphasic pills change the level of progestin hormones once during the menstrual cycle. The progesterone dose is increased about halfway through the cycle.(mark h,berr et al.2003)

*Triphasic pills contain three different doses of progestin hormones in the active pills (changing every seven days during the first three weeks of pills). Triphasic

pills gradually increase the dose of estrogen and some pills also increase the progesterone dose.(mark h ,beer et al.2003).

2.1.3 Mechanism of oral contraceptive pill:

The OC mechanism of contraceptive action on the central nervous system, on the hypothalamus and pituitary, and on peripheral reproductive organs is discussed. Multiple complex reactions occur in the hypothalamus and pituitary as a result of steroid ingestion. The clinical literature now agrees that the inhibition of pituitary gonadotropin secretion is the most important mode of action of OCs. Hormonal contraceptive preparations also affect the ovary, cervical mucus, the oviduct, and the endometrium. Sperm transport into the cervix is impeded by progesterone counteraction of estrogenic stimulation of cervical mucus penetrability. Through continued OC use, the endometrium becomes a hostile environment to implantation and further embryonic growth--due to glandular atrophy and stromal decasualization.(Bronson RA.1981)

2.1.4 Drug interactions of oral contraceptive:

In the very rare cases where a pregnancy occurs during oral contraceptive use, the blame is usually laid against the patient for having forgotten to take the pill. Evidence has started to accumulate to suggest that neither the patient nor the pill is at fault in some contraceptive failures. It may be because the patient is taking other medicines and these may be preventing the pill from suppressing ovulation. Most drug interactions reducing or negating contraceptive activity are due to concomitant use of drugs having microsomal enzyme-inducing activity (e.g., some antibiotics, especially rifampicin, and anticonvulsants, including phenobarbital, phenytoin, and primidone. Other antibiotics (e.g., tetracycline) may also interact by interruption of the enterohepatic circulation of contraceptive steroids. Less well appreciated, oral contraceptive steroids may themselves modify the metabolism

and pharmacological activity of various other drugs (e.g., anticoagulants, benzodiazepines, beta-blockers, caffeine, corticosteroids, and tricyclic antidepressants); in this respect the oral contraceptives are acting as enzyme inhibitors. Contraceptive steroids may also interact with drugs that cause enzyme inhibition and this delays the metabolism of the hormonal agents. Interactions of this type would be expected to potentiate the action of the contraceptive steroids. It is suggested that the effects of such interaction might be presented in terms of increased incidence of side effects, including water retention, diabetogenic effects, hypertension, and an increased risk of thromboembolic disorders. (Darcy P F.1986)

2.1.5Adverse effects of oral contraceptives:

The most important side effects of oral contraceptives (OCs) and their incidence, together with advice and monitoring of the patient at risk, are pointed out. There is a mild increase in blood pressure in long term contraceptive use caused by increased angiotensinogen production by the liver. It is significant only for women with a history of familial hypertension, diabetes mellitus, or pre-eclampsia. Smoking increases this risk. Urinary tract infections are 25-50% more frequent in pill users. Glucose tolerance is slightly decreased. Contraceptives' diabetogenic effect is higher in women with hereditary tendency for diabetes, latent diabetes, and/or obesity. They are contraindicated in latent diabetes. Findings are contradictory in their effects on cholesterol and triglyceride serum level, but the pill is contraindicated in lipid metabolism disorders. There is an increased incidence in cholecystitis and cholelithiasis in pill-users (70-80 additional cases/100,000 user years). Liver diseases, intrahepatic cholestasis, occur rarely and benign liver tumors have not conclusively been proved to be caused by the pill. A variety of laboratory findings have been related to contraceptive use and drug interactions occur with barbiturates, rifampicin, hydantoin, and phenylbutazone.

(Engel HJ. 1979.) Blood coagulation is increased, partially by increased production of various blood coagulation factors; but more importantly, by a decreased synthesis of antithrombin III, a natural protective mechanism against intravascular coagulation. This increases thrombosis risk. Risk doubles with simultaneous cigarette smoking .There is a correlation between contraceptive use and cerebrovascular disorders and myocardial infarction. This risk increases with age and years of pill use. (There is a correlation between contraceptive use and cerebrovascular disorders and myocardial infarction. This risk increases with age and years of pill use. (Engel HJ. 1979.)

2.2 Electrolyte definition:

Chemical substance which, when dissolved in water or melted, dissociates into electrically charged particles (ions), and thus is capable of conducting an electric current. The principal positively charged ions in the body fluids (cations) are sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}). The most important negatively charged ions (anions) are chloride (Cl^-), bicarbonate (HCO_3^-), and phosphorus (PO_4^{3-}). These electrolytes are involved in metabolic activities and are essential to the normal function of all cells. (Anna P, et al, 2000)

2.3. Calcium:

Calcium is the most abundant stored nutrient in the human body. More than 99% (1.2-1.4 kg) is stored in the bones and teeth. Less than 1% is found in extracellular serum calcium. When adults consume calcium as food or supplements, the average absorption rate is approximately 30%. The rate can vary widely due to multiple factors.(Parfitt AM, Kleerekoper M 1980).

2.3.1Calcium distribution:

2.3.1.1Total Body Distribution:

In adults, the body contains -1000 g of Ca, of which 99% is located in the mineral phase of bone as the hydroxyapatite crystal [Ca,,,(PO,),(OH),]. The crystal plays a key role in the mechanical weight-bearing properties of bone and serves as a ready source of Ca to support a number of Ca-dependent biological systems and to maintain blood ion- ized Ca within the normal range. The remaining 1% of total body Ca is located in the blood, extracellular fluid, and soft tissues. In serum, total Ca is lo-’ M and is the most frequent measurement of serum Ca levels. Of the total Ca, the ionized fraction (50%) is the biologically functional portion of total Ca and can be measured clinically; 40% of the total is bound to albumin in a pH-dependent manner; and the remaining 10% exists as a complex of either citrate or PO, ions**.(**Parfitt AM, Kleerekoper M 1980)

2.3.1.2 Cell Levels:

Cytosol Ca is -10-‘M, which creates a 1000-fold ngradient across the plasma membrane (extracellular fluid [ECF] Ca is lo-’ M) that favors Ca entry into the cell. There is also an electrical charge across the plasma membrane of -50 mV with the cell interior negative. Thus, the chemical and electrical gradients across the plasma membrane favor Ca en- try, which the cell must defend against to preserve cell viability. Ca-induced cell death is largely prevented by several mecha- nisms including extrusion of Ca from the cell by ATP- dependent energy driven Ca umps and Ca channels; Na-Ca exchangers; and binding of intracellular Ca by proteins located in the cytosol, endoplasmic reticulum (ER), and mitochondria.

Ca binding to ER and mitochondria1 sites buffer intracellular Ca and can be mobilized to maintain cytosol Ca levels and to create pulsatile peaks of Ca to mediate membrane receptor signaling that regulate a variety of biological systems. (Parfitt AM, Kleerekoper M 1980).

2.3.1.3 Blood Levels;

Ca in the blood is normally transported partly bound to plasma proteins (-45%), notably albumin, partly bound to small anions such as phosphate and citrate and partly in the free or ionized state (-45%). Although only the ionized Ca is available to move into cells and activate cellular processes, most clinical laboratories report total serum Ca concentrations. Concentrations of total Ca in normal serum generally range between 8.5 and 10.5 mg/dl (2.12-2.62 mm),

And levels above this are considered to be hypercalcemic.

The normal range of ionized Ca is 4.65-5.25 mg/dl (1.16-1.31 mm). When protein concentrations, and especially albumin concen- trations, fluctuate, total Ca levels may vary, whereas the ion- ized Ca may remain relatively stable. Dehydration or hemo- concentration during venipuncture may elevate serum albumin and falsely elevate total serum Ca. Such elevations in total Ca, when albumin levels are increased, can be "corrected" by sub- tracing 0.8 mg/dl from the total Ca for every 1.0 g/dl by which the serum albumin concentration is >4 g/dl. Conversely, when albumin levels are low, total Ca can be corrected by adding 0.8 mg/dl for every 1.0 g/dl by which the albumin is <4 g/dl. Even in the presence of a normal serum albumin.(Parfitt AM, Kleerekoper M 1980).

2.3.2Calcium regulation:

Three hormones, PTH, vitamin D, and calcitonin, are known to regulate serum ca by altering their secretion rate in response to changes in ionized calcium. PTH secretion in blood is stimulated by decrease in ionized ca2+, and conversely PTH secretion.is stopped by an increase in ionized ca2+.PTH exerts three major effect on both bone and kidney .in the bone ,PTH activated process known as bone resorption ,in which activated osteoclasts break down bone and subsequently release ca2+ into the ECF. In kidneys PTH conserves ca2+ by increasing tubular reabsorption of calcium ions PTH also stimulates renal production of active vit D.

Vitamin D3 acholecalciferol is obtained from the diet or exposure of skin to sunlight. Vitamin D3 is then converted in liver to25_ hydroxycholecalciferol(25_OH_D3) still an inactive from of vitamin D3. In the kidney, 25_OH_D3 is specifically hydroxylated to form 1, 25_hydroxycholecalciferol, The biologically active form .This active form of vitamin D3 increase ca2+ absorption in intestine and enhance the effect of PTH on bone resorption.(George A,2006,).

Calcitonin, which originates in the medullary cells of the thyroid gland, is secreted when the concentration of ca2+ in blood increase. Calcitonin exerts it's ca2+ lowering effect by inhibiting the actions of both PTH and vitamin Although calcitonin is apparently not secreted during normal regulation of the ionized ca concentration in blood, it’s secreted in ray to a hypercalcemic stimulus.(George A,2006)

2.3.3 Calcium function:

Calcium plays a range of roles in the body, these include:

2.3.3.1Bone health:

Around 99 percent of the calcium in the human body is found in the bones and teeth; it is essential for the development, growth, and maintenance of bone. Calcium continues strengthening the bones of humans until they reach the age of 20-25 when bone density is highest. After that age, bone density declines, but calcium continues to help maintain bones and slow down bone density loss, which is a natural part of the aging process (.Frances et al, 1997)

People who do not consume enough calcium before the age of 20-25 have a considerably higher risk of developing brittle bone disease or osteoporosis later in life; this is because calcium is drawn from the bones as a reserve.(Frances et al,1997).

2.3.3.2Muscle contraction:

Calcium regulates muscle contraction, including the beating of the heart muscle. When a nerve stimulates a muscle, calcium is released; it helps the proteins in muscle carry out the work of contraction. The muscle only relaxes again once the calcium is pumped back out of the muscle.(Frances et al,1997)

2.3.3.3Blood clotting:

Calcium plays a key role in normal blood coagulation (clotting). The process of clotting is complex with a number of steps; a host of chemicals are involved. Calcium plays a part in a number of these steps.(Frances et al,1997).

2.3.3.4Calcium also have other roles which include:

_Calcium is a co-factor for many enzymes; this means that without the presence of calcium, these important enzymes cannot work as efficiently.(Frances et al,1997)

_Calcium affects the smooth muscle that surrounds blood vessels,

Causing it to relax. It is important to note that calcium is not easily absorbed without the presence of vitamin D.(Frances et al,1997)

2.3.4 Calcium disorder:

Disorders of calcium metabolism occur when the body has too little or too much calcium.

Hypocalcaemia is common and can occur unnoticed with no symptoms or, in severe cases, can have dramatic symptoms and be life-threatening.Hypocalcemia can be parathyroid related or vitamin D related. Parathyroid related hypocalcaemia includes post-surgical hyperparathyroidism, inherited hyperparathyroidism, pseudo hypo parathyroidism, and pseudo-pseudohypoparathyroidismPost-surgical hypoparathyroidism is the most common form, and can be temporary (due to suppression of tissue after removal of a malfunctioning gland) or permanent, if all parathyroid tissue has been removed. Inherited hypoparathyroidism is rare and is

due to a mutation in the calcium sensing receptor (. Murphy, E; Williams 2009) Pseudohypoparathyroidism is maternally inherited and is categorized by hypocalcaemia and hypophosphatemia. Finally, pseudo-pseudohypoparathyroidism is paternally inherited. Patients display normal parathyroid hormone action in the kidney, but exhibit altered parathyroid hormone action in the bone Vitamin D related hypocalcaemia may be associated with a lack of vitamin D in the diet, a lack of sufficient UV exposure, or disturbances in renal function. Low vitamin D in the body can lead to a lack of calcium absorption and secondary hyperparathyroidism (hypocalcaemia and raised parathyroid hormone).Symptoms of hypocalcaemia include numbness in fingers and toes, muscle cramps, irritability, impaired mental capacity and muscle twitching(Murphy, E; Williams 2009)

Hypercalcaemia is suspected to occur in approximately 1 in 500 adults in the general adult population. Like hypocalcaemia, hypercalemia can be non-severe and present with no symptoms, or it may be severe, with life-threatening symptoms. Hypercalcemia is most commonly caused by hyperparathyroidism and by malignancy, and less commonly by vitamin D intoxication, familial hypocalciuric hypercalcemia and by sarcoidosis. [Hyperparathyroidism occurs most commonly in postmenopausal women. Hyperparathyroidism can be caused by a tumor, or adenoma, in the parathyroid gland or by increased levels of parathyroid hormone due to hypocalcaemia. Approximately 10% of cancer sufferers experience hypercalcemia due to malignancy. Hypercalcemia occurs most commonly in breast cancer, lymphoma, prostate cancer, thyroid cancer, lung cancer, myeloma, and colon cancer It may be caused by secretion of parathyroid hormone-related peptide by the tumor (which has the same action as parathyroid hormone), or may be a result of direct invasive on of the bone, causing calcium release Symptoms of hypercalcemia include anorexia, nausea, vomiting, constipation, abdominal pain,

lethargy, depression, confusion, polyuria, polydipsia and generalized aches and pains(Waters, M,2009).

2.3.5 Reference Range of calcium:

Calcium is maintained within a fairly narrow range from 8.5 to 10.5 mg/dl (4.3 to 5.3 mEq/L or 2.2 to 2.7 mmol/L). (Goldstein DA.1990).

2.4 Phosphate:

Phosphate is a mineral that makes up 1% of a person's total body weight. It is the second most abundant mineral in the body. It is present in every cell of the body. Most of the phosphate in the body is found in the bones and teeth.

2.4.1Distribution of phosphate:

Although the concentration of all phosphate compounds in blood in blood is about 12mg/dl (3.9 mmol/l), most of that is organic phosphate and only about 3to 4mg/dl is inorganic phosphate. Phosphate is the predominant intracellular anion, with intracellular concentration varying, depending on the types of cell. About 80% of the total body pool of phosphate is contained in bone, 25% in soft tissue, and less than 1% is active in serum, plasma.(George A,2006)

2.4.2 Regulation of phosphate:

Phosphate in blood may be absorbed in the intestine from dietary sources, released from cells into blood, and lost from bone. In healthy individuals, all these processes are relatively constant and easily regulated by renal excretion or reabsorption of phosphate.

Distribution to any of these processes can alter phosphate concentration in the blood, however the loss of regulation by kidneys will have the most profound effect. Although other factors such as vitamin D, calcitonin, growth hormone, and acid base status, can affect renal regulation of phosphate, the most important factor

is PTH, which overall lowers blood concentrations by increasing renal excretion.(George A,2006).

Vitamin D acts to increase phosphate in the blood .Vitamin D increases phosphate absorption in the intestine and phosphate reabsorption in the kidney.(George A,2006).

Growth hormone, which helps regulate skeletal growth, can affect circulating concentrations of phosphate. In case of excessive secretion or administration of growth hormone, phosphate concentration in the blood may increase because of decreased renal excretion of phosphate. (George A, 2006).

2.4.3 Function of phosphate:

The roles of phosphate in the human body: Building bones and teeth, Use of carbohydrates (sugar) and fat.

Protein synthesis Growth, maintenance and healing of cells and tissues serves as a source of energy (in the molecule adenosine triphosphate - ATP and creatine phosphate).Muscle contractions.

- Regulation of heart rate Transmission of nerve impulses.
- Production of genetic material (DNA, RNA)
- Part of the cell membrane (within the phospholipid).Necessary in all the metabolic pathways in the body (energy production, cell division, etc...).A part of many enzymes and hormones. Maintain normal acid-base balance in the body (pH)(Ehrid.fannin,marilyns ,2014)

2.4.4 Imbalance of phosphate:

Phosphate, or Pi, is an electrolyte used in several functions throughout the body. Although a phosphate imbalance isn’t as well-known as some of the other imbalances, it can still cause problems with your patient’s condition.(Burtis CAet al, 2005).

Hypo phosphotemia occur when levels of phosphate in the blood are below the normal range, the symptoms generally include muscle weakness, heart failure, seizure, and coma. It may be caused by vitamin D deficiency, hyperparathyroidism, or alcoholism. Hypophosphatemia may also be present, in addition to other electrolyte disturbances, in re-feeding syndrome, which is associated with the commencement of total parental nutrition .Hyperphosphataemia, when levels of phosphate in the blood are above the normal range, can be caused by kidney disease, parathyroid issues, and metabolic or respiratory acidosis. Symptoms are usually not present, and they are related to hypocalcaemia. Renal patients can experience hardened calcium deposits when this condition goes untreated.(Burtis CAet al, 2005)

2.4.5 Reference Range:

Normal serum phosphate concentration is:

Adults: 2.5-4.5 mg/dL = 0.81-1.45 mmol/L(Burtis CA et al,2005)

2.5 Magnesium:

Magnesium (Mg) is the fourth most abundant essential element in the body and the second most abundant intracellular cation.(J. Griffin,2003)

2.5.1 Magnesium Distribution :

Magnesium is the fourth most abundant cation in the body and second most abundant intracellular ion. The average human body (70kg) contains 1 mol (24g) of mg2+.approximately 53% of magnesium in the body is found in bone, 46% in muscle and other organs and soft tissue ,and less than 1% is present in serum and RBcs(Elin Rj .1994) .Of the magnesium present in serum ,about one-third is bound to protein, primarily albumin .Of the remaining two-third,61%exist in the free or ionized State and about 5% is complexes with other ions, such as po4- and citrate

.similar to calcium it's the free ion that is physiologically active in the body(polancic JE,1991).

2.5.2. Regulation of magnesium:

The regulation of body Mg2+ is controlled largely by the kidney, which can reabsorb Mg2+ in deficiency States or readily excrete excess Mg2+ in overload States. Of the nonprotein _bound Mg2+ that get filtered by the glomerulus,25% to 30% is reabsorbed by the proximal convoluted tubule(PCT) ,unlike Na ,in which 60% to 75% is absorbed in the PCT .Henle,s loop is the major renal regulatory site, where 50%to 60% of filtered Mg2 is reabsorbed in the distal convoluted tubule (Whang R,1997). The renal threshold for Mg2+ is approximately 0.60 to 0.85mmol/l(~1.46_2.07mg/dl).Because this is close to normal serum concentration ,slight excess of Mg2 in serum are rapidly excreted by the kidneys. Normally only about 6% of filtered Mg2 is excreted in urine per day (Elin Rj,1994).

Mg2 regulation appears to be related to that of calcium and sodium. Parathyroid hormone increase the renal reabsorption of Mg2 and enhance the absorption of Mg2 in the intestine. However changes in ionized ca2+ have greater effect on PTH secretion .aldosterone and thyroxin apparently have the opposite effect of PTH in the kidney, increasing the renal excretion of magnesium (Polancic JE, 1991).

2.5.3Role of magnesium in body:

The role of magnesium in the body is widespread, it’s an essential cofactor of more than 300 enzymes, including those important in glycolysis; transcellular ion transport; neuromuscular transmission; synthesis of carbohydrates, protein, lipid and nucleic acids; and the release of and response to certain hormones.(George A,2006).

The most significant findings are the relationship between abnormal serum Mg2 Levels and cardiovascular, metabolic, and neuromuscular disorders. Although serum levels may not reflect total body stores of Mg2, They are useful in determining acute change in ion. (George A, 2006).

2.5.4 Magnesium disorders:

*Magnesium deficiency or hypo magnesia (not to be confused with hypomagnesaemia) refers to inadequate intake of dietary magnesium or impaired absorption of magnesium, which can result in numerous symptoms and diseases.] It is generally corrected by an increase of magnesium in diet, oral supplements, and in severe cases, intravenous supplementation (Rude RK, Shill's ME.2006).

*Symptoms of magnesium deficiency include hyper excitability, muscular symptoms (cramps, tremor, fasciculations, spasms, tetany, and weakness), fatigue, loss of appetite, apathy, confusion, sound and light sensitivity, anxiety, insomnia, irritability, poor memory, and reduced ability to learn. Moderate to severe magnesium deficiency can cause tingling or numbness, heart changes, rapid heartbeat, continued muscle contractions, nausea, vomiting, migraines, personality changes, delirium, hallucinations, low calcium levels, low serum potassium levels, retention of sodium, low circulating levels of parathyroid hormone (PTH)(Rude RK, shill's ME,2006).

Causes of magnesium deficiency include diet, alcohol abuse, chronic stress, poorly controlled diabetes, excessive or chronic vomiting and/or diarrhea. Certain drugs can deplete magnesium levels such as osmotic diuretics, cisplatin, ciclosporin, amphetamines, and possibly proton pump inhibitors (Rude RK, Shils ME.2006)

Hyper magnesium is an electrolyte disturbance in which there is a high level of magnesium in the blood. Most cases of hypermagnesemia occur in people who have kidney failure. Hypermagnesemia occurs because the process that keeps the

levels of magnesium in the body at normal levels does not work properly in people with kidney dysfunction and end-stage liver disease. (Kasper DL, et al, 2005).

Some treatments for chronic kidney disease, including proton pump inhibitors, can increase the risk of hypermagnesemia. Malnourishment and alcoholism are additional risk factors in people with chronic kidney disease. (Kasper DL, et al, 2005).

It is rare for someone who has normal kidney function to develop hypermagnesemia. They are other causes of hypermagnesemia include: lithium therapy, hypothyroidism, Addison's disease milk-alkali syndrome drugs containing magnesium, such as some laxatives and antacids, lithium therapy, hypothyroidism, Addison's diseases. Milk-alkali syndrome, rugs containing magnesium, such as some laxatives and antacids. Familial hypocalciuric hyperglycemia.(Kasper DL,et al,2005).

2.5.5Reference Range:

Normal serum concentration is:1.26_2.10mg/dl (0.63_1.0mmol/l).(George A,2006).

2.6 Iron:

Iron is a mineral that is naturally present in many foods, added to some food products, and available as a dietary supplement. Iron is an essential component of hemoglobin, an erythrocyte protein that transfers oxygen from the lungs to the tissues. As a component of myoglobin, a protein that provides oxygen to muscles, iron supports metabolism. Iron is also necessary for growth, development, normal cellular functioning, and synthesis of some

Hormones and connective tissue.((Anderson,G.J,1991).

2.6.1 Distribution of iron:

The adult human body contains approximately 3–5 g of iron (45–55 mg/kg of body weight in adult women and men, respectively), with more than two-thirds (~2 g) incorporated in the hemoglobin of developing erythroid precursors and mature red blood cells. The remaining body iron is mostly found in a transit pool in reticuloendothelial macrophages (~600 mg) or stored in hepatocytes (~1000 mg) within ferritin, an iron storage protein. A smaller fraction is found in muscles within myoglobin (~300 mg), while only a minuscule amount (~8 mg) is constituent of other cellular iron containing proteins and enzymes. A healthy individual absorbs daily 1–2 mg of iron from the diet, which compensates nonspecific iron losses by cell .desquamation in the skin and the intestinal, Iron bound to plasma transferrin corresponds to less than 0.1% of total body iron, but represents, in kinetic terms, the most active pool.(Anderson,G.J,1991).

2.6.2 Iron metabolism:

A well-balanced diet contains sufficient iron to meet body requirements. About 10% of the normal 10 to 20 mg of dietary iron is absorbed each day, and this is sufficient to balance the 1 to 2 mg daily losses from desquamation of epithelia. Greater iron utilization via growth in childhood, greater iron loss with minor hemorrhages, menstruation in women, and greater need for iron in pregnancy will increase the efficiency of dietary iron absorption to 20%.(Fuqua et al, 2012).

Iron is mainly absorbed in the duodenum and upper jejunum. A transporter protein called divalent metal transporter 1 (DMT1) facilitates transfer of iron across the intestinal epithelial cells. DMT1 also facilitates uptake of other trace metals, both good (manganese, copper, cobalt, zinc) and bad (cadmium, lead). Iron within the enterocyte is released via ferroportin into the bloodstream. Iron is then bound in the bloodstream by the transport glycoprotein named transferrin. Both DMT-1 and

ferroportin are found in a wide variety of cells involved in iron transport, such as macrophages. (Fuqua et al, 2012).

Normally, about 20 to 45% of transferrin binding sites are filled (the percent saturation). About 0.1% of total body iron is circulating in bound form to transferrin. Most absorbed iron is utilized in bone marrow for erythropoiesis. Membrane receptors on erythroid precursors in the bone marrow avidly bind transferrin. About 10 to 20% of absorbed iron goes into a storage pool in cells of the mononuclear phagocyte system, particularly fixed macrophages, which is also being recycled into erythropoiesis, so there is a balance of storage and use. The trace elements cobalt and manganese are also absorbed and transported via the same mechanisms as iron. (Nemeth, 2008).

2.6.3Iron Regulation:

Iron one of the most dietary mineral that can be regulate by**:**

*Dietary regulator: a short-term increase in dietary iron is not avidly absorbed, as the mucosal cells have accumulated iron and "block" additional uptake.

*Stores regulator: as iron stores increase in the liver, the hepatic peptide hepcidin is released that diminishes intestinal mucosal iron ferroportin release and the enterocytes retain any absorbed iron and are sloughed off in a few days; as body iron stores fall, hepcidin diminishes and the intestinal mucosa is signaled to release their absorbed iron into circulation.(Fleming RE, Bacon BR.2005)

The composition of the diet may also influence iron absorption. Citrate and ascorbate (in citrus fruits, for example) can form complexes with iron that increase absorption, while tannates in tea can decrease absorption. The iron in heme found in meat is more readily absorbed than inorganic iron by an unknown mechanism. Non-heme dietary iron can be found in two forms: most is in the ferric form (Fe+++) that must be reduced to the ferrous form (Fe++) before it is absorbed.

Duodenal microvilli contain ferric reductase to promote absorption of ferrous iron.(Fleming RE, Bacon BR.2005).

Only a small fraction of the body's iron is gained or lost each day. Most of the iron in the body is recycled when old red blood cells are taken out of circulation and destroyed, with their iron scavenged by macrophages in the mononuclear phagocyte system, mainly spleen, and returned to the storage pool for re-use. Iron homeostasis is closely regulated via intestinal absorption. Increased absorption is signaled via decreased hepcidin by decreasing iron stores, hypoxia, inflammation, and erythropoietic activity. The 'set point' for hepcidin synthesis may also be influenced by the bone morphogenetic protein (BMP) pathway (Fleming RE, Bacon BR.2005).

Storage iron occurs in two forms:

*Ferritin.

*Hemosiderin.

Iron is initially stored as a protein-iron complex ferritin, but ferritin can be incorporated by phagolysosomes to form hemosiderin granules. There are about 2 mg. of iron in the adult female, and up to 6 mg iron in the adult male. About 1.5 to 2 mg. of this total is found in red blood cells as heme in hemoglobin, and 0.5 to 1 gm. occur as storage iron, mainly in bone marrow, spleen, and liver, with the remainder in myoglobin and in enzymes that require iron.(Fleming RE, Bacon BR.2005)

2.6.4 Iron function in body:

Iron has several vital functions in the body. It serves as a carrier of oxygen to the tissues from the lungs by red blood cell hemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues. (Brock, J.H., Halliday, 1994).

Most of the iron in the body is present in the erythrocytes as haemoglobin, a molecule composed of four units, each containing one heme group and one protein chain. The structure of haemoglobin allows it to be fully loaded with oxygen in the lungs and partially unloaded in the tissues (e.g., in the muscles). The iron-containing oxygen storage protein in the muscles, myoglobin, is similar in structure to haemoglobin but has only one heme unit and one globin chain. Several iron-containing enzymes, the cytochromes, also have one heme group and one globin protein chain. These enzymes act as electron carriers within the cell and their structures do not permit reversible loading and unloading of oxygen. Their role in the oxidative metabolism is to transfer energy within the cell and specifically in the mitochondria. Other key functions for the iron-containing enzymes (e.g., cytochrome P450) include the synthesis of steroid hormones and bile acids; detoxification of foreign substances in the liver; and signal controlling in some neurotransmitters, such as the dopamine and serotonin systems in the brain. Iron is reversibly stored within the liver as ferritin and hemosiderin whereas it is transported between different compartments in the body by the protein transferrin.(Brock, J.H., Halliday, 1994)

2.6.5. Imbalance of iron:

2.6.5.1 Iron deficiency anemia:

Is a condition where there are too few red blood cells in the body due to a shortage of iron

The body uses iron to produce red blood cells, which transport oxygen around the body.

Without enough iron, there may be too few healthy red blood cells to carry sufficient oxygen to satisfy the body's needs.

The result of this situation is called iron deficiency anemia, which can leave a person feeling extremely tired and out of breath. (Bermejo, F., & García-l, 2009)

Iron deficiency anemia relates directly to a lack of iron in the body. The cause of the iron deficiency varies, however, some common causes include:

***Poor diet:**

Diets that lack iron are a leading cause of iron deficiency.

Foods rich in iron, such as eggs and meat, supply the body with much of the iron it needs to produce hemoglobin. If a person does not eat enough to maintain their iron supply, an iron deficiency can develop. (Bermejo, F., & García-l, 2009).

***Blood loss:**

Iron is found primarily in the blood, as it is stored in red blood cells. An iron deficiency may result when a person loses a lot of blood from an injury, giving birth, or heavy menstruation.

In some cases, slow loss of blood from chronic diseases or some cancers can lead to an iron deficiency. (Bermejo, F., & García-l, 2009).

*Decreased ability to absorb iron:

Some people are not able to absorb enough iron from the food they eat. This may be due to a problem with the small intestine, such as celiac disease or Crohn's disease, or if a portion of the small intestine has been removed.(Bermejo, F., & García-l,2009)

*Pregnancy:

Low iron levels are a common problem for pregnant women. The growing fetus needs a lot of iron, which can lead to an iron deficiency. Also, a pregnant woman has an increased amount of blood in her body. This larger volume of blood demands more iron to meet its needs. (Bermejo, F., & García-l, 2009).

Iron deficiency anemia often takes a long time to develop. People may not know they have it until the symptoms are severe.(Bermejo, F., & García-l,2009).

In some cases, an iron deficiency may improve with no intervention, as a person's situation changes, such as after a woman has given birth.(Bermejo, F., & García-l, 2009).

Iron deficiency can have some of the following symptoms:

general weakness, dizziness or lightheadedness, extreme fatigue fast heartbeat, easily broken and brittle nails, paler than normal skin, chest pain, shortness of breath.headaches,cold hands and feet cravings for non-nutritive things, such as dirt, starch, or ice poor appetite, especially in children.(Bermejo, F., & García-l,2009).

2.6.5.2 Iron overload:

Iron overload could be defined as an excess of iron in the body, regardless of the presence or absence of tissue damage. However, progressive accumulation of iron in vital organs increases the risk of hepatic, cardiovascular, and pancreatic dysfunctions. Moreover, and in line with previously discussed, individuals with iron overload diseases have an increased susceptibility to infectious diseases. Iron overload disorder can be either primary or secondary. (Muñoz M, 2011).

2.6.5.2.1 Primary iron overload:

The classical example of primary iron overload is HH. HH is a common genetic disorder among Caucasian population, with an autosomal recessive inheritance, and is characterized by an excessive absorption of dietary iron. Excess iron progressively accumulates in different organs, particularly in liver, heart, and pancreas. The common complications are cirrhosis and carcinoma of the liver, cardiomyopathy, diabetes mellitus, and if not treated, death.((Muñoz M, 2011)

HH occurs in patients with mutations in specific genes involved in iron metabolism (e.g., HFE gene), and has been classified into four subtypes (types 1, 2, 3, and 4).

The majority of patients (approximately 85–90%) have type 1, which is associated with mutations in HFE gene (OMIM 235200). This gene is located on the short arm of chromosome 6 (6p21.3) and encodes for a Trans membrane protein. This protein associates with β-2-microglobulin and regulates the interaction between transferrin and its receptor. Two missense mutations in this gene were identified: C282Y (C, cysteine replaced by Y, tyrosine) and H63D (H, histamine replaced by D, aspartic acid). Although the whole mechanism leading to iron overload remains to be elucidated, there are evidences that it could also arise from hepcidin dysregulation.(Muñoz M, 2011) .

2.6.5.2.2 Secondary iron overload:

Secondary iron overload typically occurs as a result of a wide range of conditions, namely ineffective erythropoiesis, chronic liver diseases, parenteral administration, or ingestion of excessive amounts of iron. One of the best studied examples of iron overload secondary to ineffective erythropoiesis and blood, transfusions is thalassemia.(Muñoz M, 2011)

*****The symptoms of hemochromatosis:

People with early hemochromatosis have no symptoms and are unaware of their condition. The disease may then be suspected when elevated iron blood levels are noted by routine blood testing. Symptoms may not appear until 30-50 years of age. Iron deposits in the skin cause darkening of the skin. Since females lose iron through menstrual blood loss, no menstruating women develop symptoms 15 to 20 years later. Iron deposits in the pituitary gland and testicles cause shrinkage of the testicles and impotence. Iron deposits in the pancreas cause a decrease in insulin production resulting in diabetes. Iron deposits in the heart muscle can cause cardiomyopathy and lead to heart failure as well as abnormal heart rhythms. Iron

accumulation in the liver causes scarring of the liver (cirrhosis) and an increased risk of developing liver cancer. (www.medicine net.com).

2.6.6 Reference Range:

Normal range of serum iron is:

Men: 65 - 175 µg/dL = 11.6 – 31.3 µmol/L

Women: 50 - 170 µg/dL = 9.0 – 30.4 µmol/.(Burtis CA et al,2005)

Material and Methodology

3.1: Study design:

Case control study.

3:2: Study area:

The study was conducted in shendi locality which is located 180km north to capital Khartoum, southern part of river Nile state, and covering area about 30km2 there are several medical centers for different service and purposes, also there is shendi university with various medical faculties like of medicine and health science [Medical laboratory, Nursing, Public health].Shendi has three big hospitals all of them have different departments which provide good health's service for the population.

3:3: Study Period:

The study conducted in period between March2018-August2018**.**

3:4: Study population sampling:

Fifty of venous blood sample were obtained from women use contraceptive pills as case group (15-50) year, and [50] venous blood samples were obtained from women without use contraceptive pills as control group.

3:5: material and instrument:

- Cotton
- Lithium heparinize container.
- test tube
- Automatic pipettes.
- arenezo lll Reagent
- Methylthymol reagent
- Analyzer [spectrophotometer].

*spectrophotometry is method use to estimate the level of analyte in solution .it relies on the principle that materials absorb light of a certain wavelength as it passes through the solution

3.6 Exclusion criteria:

People with any disease that have effect electrolytes level such as [renal disease, thyroid disease, malabsorption……..etc.].

3.7 Ethical consideration:

Each woman was told before taken sample and they all agree to participate in this study.

3.8 Tools of data collection:

Information from participate women was collected in preformed questionnaire [Age, Type of OC, period of use OC].

3.9 Data analysis:

Data was analyzed by using online t test calculator is computer program.

3.10 Specimen:

Serum sample obtain by take venous blood sample from patient in plan container.

3.11 methodology:

3.11.1principle of methyltymol method for calcium:

Calcium in the sample reacts with methylthymol blue in alkaline medium forming a colored complex that can be measured by spectrophotometry. Hydroxyquinoline is included in the Reagent to avoid magnesium interference.

3.11.1.2 Samples:

Serum, heparinized plasma collected by standard procedures.

3.11.1.3 Procedure:

1. Pipette into labelled test tubes: (Notes 1, 2)

Tubes	BLANK	SAMPLE	STANDE
Calcium Reagent	-	-	10ul
Sample	-	10ul	-
Working Reagent	1.0ml	1.0ml	1.0ml

2. Mix thoroughly and let stand the tubes for 2 minutes at room temperature.

3. Read the absorbance (A) of the Standard and the Sample at 610 nm against the Blank. The colour is stable for at least 1hour.

3.11.1.4 Calculation:

The calcium concentration in the sample is calculated using the following general formula:

A sample\A stander ×C stander × Sample dilution factor = C Sample

3.11.2 Principle of phosphomolybdate method for phosphate:

Inorganic phosphorus in the sample reacts with molybdate in acid medium forming phosphomolybdate complex that can be measured spectrophotometer.

3.11.2.2 Sample.

Serum, heparinized plasma collected by standard procedures.

3.11.2.3 Procedure:

1. Pipette into labelled test tubes: (Note 1):

	Reag.Blank	Sample Blank	Sample	Stander
Distilled water	10ul	-	-	-
Sample	-	10ul	10ul	-
Phospho stander(S)	-	-	-	10ul
Reagent	-	1ml	-	-
Working Reagent	1ml	-	1ml	1ml

2. Mix thoroughly and let stand the tubes for 5 minutes at room temperature.

3. Read the absorbance (A) of the Sample Blanks at 340 nm against distilled water.

4. Read the absorbance (A) of the Samples and of the Standard at 340 nm against the Reagent Blank.

3.11.2.4 Calculation:

The phosphorus concentration in the sample is calculated using the following general formula:

A sample - A sample Blank ÷ A stander ×C stander × Sample dilution factor = C Sample.

3.11.3principle of the method for magnesium:

Magnesium in the sample reacts with xylidyl blue in alkaline medium forming a colored complex that can be measured by spectrophotometry. EGTA is included in the reagent to remove calcium interference.

3.11.3.2 Sample:

Serum, plasma collected by standard procedure.

3.11.3 Procedure:

1. Bring the Working reagent to room temperature.

2. Pipette into labelled test tubes: (Notes 1, 2)

Tubes	BLANK	SAMPLE	STANDE
Magnesium Stander	-	-	10ul
Sample	-	10ul	-
Working Reagent	1ml	1ml	1ml

3. Mix thoroughly and let stand the tubes for 2 minutes at room temperature.

4. Read the absorbance (A) of the Standard and the Sample at 520 nm against the Blank. The colour is stable for at least 1 hour.

3.11.3.4 Calculation:

The magnesium concentration in the sample is calculated using the following general formula:

A sample ÷ A Stander × C stander = C sample

3.11.4 Principle of the method for iron:

Transferrin-bound ferric ions in the sample are released by guanidinium and reduced to ferrous by means of ascorbic acid. Ferrous ions react with ferrozine forming a colored complex that can be measured by spectrophotometry.

3.11.4.2 Sample:

Serum or heparinized plasma collected by standard procedures.

3.11.4.3 Procedure:

1. Bring the Reagent to room temperature.

2. Pipette into labelled test tubes: (Notes 1, 2)

	Reag.Blank	**Sample Blank**	**Sample**	**Stander**
Distilled water	200ul	-	-	-
Sample	-	200ul	200ul	-
Iron stander(S)	-	-	-	200ul
Reagent	-	1ml	-	-
Working Reagent	1ml	-	1ml	1ml

3. Mix thoroughly and let stand the tubes for 5 minutes at room temperature.

4. Read the absorbance (A) of the Sample Blanks at 560 nm against distilled water.

5. Read the absorbance (A) of the Samples and of the Standard at 560 nm against the Reagent Blank.

3.11.4.4 Calculation:

The iron concentration in the sample is calculated using the following general formula:

A Sample - A sample Blank ÷ C stander =C Sample.

4. Results

Table (4-1): show the mean of calcium in case and control group:

Group		N	Mean	Std. Deviation
Calcium	Test	50	7.706	.7493
	control	50	8.808	.8750

P.value = 0.000

P.value less than or equal 0.05 is considered to be significant.

Table (4-2): show the mean of phosphate in case and control group

Group		N	Mean	Std. Deviation
Phosphate	test	50	3.312	1.2165
	control	50	3.352	.8671

P.value = 0.850

Table (4-3): show the mean of magnesium in case and control group

Group		N	Mean	Std. Deviation
Magnesium	test	50	1.8320	.41066
	control	50	2.1240	.31270

P.value = 0.000

Table (4-4): show the mean of iron in case and control group:

Group		N	Mean	Std. Deviation
Iron	test	50	32.022	6.6965
	control	50	20.888	6.5536

P.value = 0.000

Table (4-5): show frequency, mean, percentage of ca, pi,mg,iron according to duration of use among case:

Using duration		calcium	phosphate	Magnesium	iron
Less than 1yrs	Mean	8.509	4.036	2.0482	31.018
	N	11	11	11	11
	Total of N%	22.0%	22.0%	22.0%	22.0%
1- 3yrs	Mean	7.577	3.240	1.8149	31.369
	N	35	35	35	35
	Total of N%	70.0%	70.0%	70.0%	70.0%
More than 3yrs	Mean	6.625	1.950	1.3875	40.500
	N	4	4	4	4
	Total of N%	8.0%	8.0%	8.0%	8.0%
Total	Mean	7.706	3.312	1.8320	32.022
	N	50	50	50	50
	Total of N%	100.0%	100.0%	100.0%	100.0%
	P.value	0.000	0.008	0.017	0.027

Table (4-6): show frequency, mean, percentage of ca, pi, mg,iron according to age among case:

Age		Calcium	phosphate	Magnesium	Iron
21-26 yrs	Mean	7.743	3.743	1.8236	31.921
	N	14	14	14	14
	Total of N%	28.0%	28.0%	28.0%	28.0%
27-32 yrs	Mean	7.778	3.100	1.8528	30.289
	N	18	18	18	18
	Total of %N	36.0%	36.0%	36.0%	36.0%
More than 32 yrs	Mean	7.606	3.189	1.8178	33.833
	N	18	18	18	18
	Total of N%	36.0%	36.0%	36.0%	36.0%
Total	Mean	7.706	3.312	1.8320	32.022
	N	50	50	50	50
	Total of N%	100.0%	100.0%	100.0%	100.0 %
	P.value	0.777	0.294	0.965	0.288

Table (4-7): show frequency, mean, percentage of ca, pi, mg, iron according to educational level among case:

Educational level		calcium	phosphate	magnesium	Iron
Primary	Mean	7.800	2.150	1.5500	34.250
	N	2	2	2	2
	Total of N%	4.0%	4.0%	4.0%	4.0%
Secondary	Mean	7.330	3.090	1.7900	33.635
	N	20	20	20	20
	Total of N%	40.0%	40.0%	40.0%	40.0%
University	Mean	7.968	3.554	1.8821	30.711
	N	28	28	28	28
	Total of N%	56.0%	56.0%	56.0%	56.0%
Total	Mean	7.706	3.312	1.8320	32.022
	N	50	50	50	50
	Total of N%	100.0%	100.0%	100.0%	100.0%
	P.value	0.071	0.167	0.465	0.299

Table (4-8): show frequency, mean, percentage of ca,pi,mg,iron according to Purpose of use among case:

Purpose of use		calcium	Phosphate	magnesium	Iron
Planning	Mean	7.692	3.288	1.8402	32.061
	N	49	49	49	49
	Total of N%	98.0%	98.0%	98.0%	98.0%
Medical	Mean	8.400	4.500	1.4300	30.100
	N	1	1	1	1
	Total of N%	2.0%	2.0%	2.0%	2.0%
Total	Mean	7.706	3.312	1.8320	32.022
	N	50	50	50	50
	Total of N%	100.0%	100.0%	100.0%	100.0%
	P.value	0.355	0.329	0.328	0.775

Table (4-9): show frequency,mean,percentage of ca,pi,mg,iron according to number of children among case:

No.of children		Calcium	Phosphate	Magnesium	Iron
1-3 ch	Mean	7.728	3.306	1.8766	31.244
	N	32	32	32	32
	Total of N%	64.0%	64.0%	64.0%	64.0%
4-6 ch	Mean	7.665	3.406	1.7265	33.582
	N	17	17	17	17
	Total of N%	34.0%	34.0%	34.0%	34.0%
More than 6 ch	Mean	7.700	1.900	2.2000	30.400
	N	1	1	1	1
	Total of N%	2.0%	2.0%	2.0%	2.0%
Total	Mean	7.706	3.312	1.8320	32.022
	N	50	50	50	50
	Total of N%	100.0%	100.0%	100.0%	100.0%
	P.value	0.963	0.494	0.323	0.50

Table (4-10): show the relation between educational level and purpose of use

Educational level	Purpose of use		Total
	Planning	Medical	
Primary	2	0	2
Secondary	20	0	20
University	27	1	28
Total	49	1	50

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.802	2	.670

p.value .067.

Table (4-11): the relation between educational level and number of children

Educational level	No.of children			Total
	1-3 ch	4-6 ch	More than 6 ch	
Primary	0	2	0	2
Secondary	9	10	1	20
University	23	5	0	28
Total	32	17	1	50

Chi-Square Tests:

Value		df	Asymp. Sig. (2-sided)
Pearson Chi-Square	**11.563**	**4**	**.021**

p.value; 021

5.1 Discussion

The study conducted in shendi locality during the period between March 2018_to August 2018,this study aimed to determine the levels of calcium, phosphate ,magnesium ,iron ,in woman that use oral contraceptive pill and compare it with control group, the study include [100] lady's,[50] of them were case of study (use oral contraceptive pill) and[50] of them were healthy woman as control group. Their ages ranged from [15_50] years old.

The study showed that there was significant difference of calcium, magnesium, iron between case and control group.

Table [4.1] showed that the calcium level in case lower than control, the mean of Calcium among case was (7.7mg/dl), and the mean of calcium among control group was (8.8mg/dl) that level of P.value is (0.00),This is disagreement with study of [Harted et al] who reported significantly increased of serum calcium,

Table [4.2] showed that the phosphate level in case lower than control group, the mean of phosphate among case was (3.31mg/dl), and the mean among control group was (3.35mg/dl), that at level. Of P.value is (0.8). This is agreement with study of [Hameed et al] who reported significantly decreased of serum phosphate.

Table [4.3] showed that magnesium in case group lower than control group, the mean of magnesium among case group was (1.8mg/dl), and the mean among control group was (2.1mg/dl) that at P.value is (0.00), This is agreement with study of [Hameed et al] who reported significantly decreased of serum magnesium.

Table [4.4] showed that the iron level in case group higher control group, the mean of iron among case group was (32.0mg/dl), and the mean among control group was (20.8mg/dl), that at P.value is (0.00).

Table (4.6) show that calcium, phosphate, magnesium, iron levels in case group with age from 21_26year was (7.7،3.7،1.8،31.9mg/dl) respectively. And within age from 27_32year was(7.7,3.1,1.8,30.2mg/dl),and within age more than 32yearwas(7.6,3.1,1.8,33.8mg/dl)and this difference is insignificant (p.value:0.77,0.29,0.96,0.28).

Table(4.5)show that calcium ,phosphate,magnesium,iron levels in case group with period of use less than year was(8.5,4.0,2.0,31.0mg/dl) and within period of use from 1_3year was(7.5,3.2,0,1.8 ,31.3 mg/dl) and within period more than 3year was(6.6 ,1.9 ,1.3 ,40.5 mg/dl).and this difference is significant (p.value:0.00 ,0.008 ,0.01 ,0.02).

Table (4.7) showed that calcium,phosphate,magnesium ,iron in case group with primary level was(7.8 ,2.1 , 1.5 ,31.2 mg /dl),and within secondary level was(7.3 ,3.0 ,1.7 ,33.6 mg/dl) ,and within university level was (7.8 ,3.5 ,1.8 ,30.7 mg/dl) and this difference is insignificant(p.value:0.07 ,0.16 ,0.46 ,0.29).

Table (4.8)showed that calcium, phosphate ,magnesium ,iron in case group with planning was (7.6 ,3.2 ,1.8 ,32 mg/dl) and within medical use was (8.4 ,4.5 , 1.4 ,30.1 mg/dl) and this difference is insignificant (p.value:0.35 ,0.32 ,0.32 ,0.77).

Table(4.9) showed that calcium ,phosphate,magnesium,iron in case group with number of children from 1_3 was(7.7 ,3.3 ,1.8 ,31.2mg/dl) and with number of children from 4_6 was(7.6 ,3.4 ,1.8 ,31.2mg/dl) and within number of children more than 6was(7.7 ,1.9 ,2.2 ,30.4 mg/dl) and this difference is insignificant (p.value :0.9 ,0.4 ,0.3 ,0.5).

Table (4.10) showed that have insignificant difference between educational level and purpose of use (p.value 0.6).

Table (4.11) showed that have significant difference between educational level and number of children (p.value 0.02

5.2 Conclusion

By the end of this was conducted that the Calcium, phosphate. Magnesium lower in woman that use oral contraceptive pill rather than non-user OCPs.and iron level increase in woman that non user oral contraceptive pill. Also show that age and number of children, educational level, and purpose of use not effect this parameter but period of use have clear effect.

5.3Recommendations

- It's is suggested that these contraceptive pill should be used with care and with proper investigation of woman before and during use of these pills.
- Health education programs would apply for use of oral contraceptive pills and its disorder and complications.
- Further studies in different area were needed to give accurate results.
- Dietary supplements may be necessary, especially where levels are significantly reduced in some individuals on contraceptives. This should be borne in mind by the prescribing physician.

6.1 Reference

- Anderson GJ,Walsh's MD,Powell Lw,hallidy J British journal of haematology,1991,pg 229_236.
- Anna p,Barbara H, Norma J,Walters ,Basic medical laboratory techniques ,2000,pg 440.
- Bermego F and Garcia Lopez, world journal of Gastroenteroloy, 2009, pg 4638_4643.
- Brock JH,hallidy,m.Jp and Powell ,Iron in health and disease,1994, pg 114_122.
- Broson RA,clinical obstelognecol,1981,pg 138_139.
- Brurtis CA,Ashwood ER,Bruns D,B.Saunders, text book of clinical chemistry and molecular diagnosis,2005.
- Daery PF,Drug intellclin pharm ,1986,pg 102_103.
- Erid, fannin,Marilyns,Sommer,disease and disorder:Anursing Therapeutic.manual,2014,pg 555.
- Elin RJ,The fifth but forgotten electrolyte, 1991,pg 616_622.
- Engel HS,med moutatssdr ,1979,pg 98_102
- Flemina RE,Bacon BR ,orchestratio of iron homeostasis,2005,pg 1741_1749.
- Frances and Whithney, E Leanon, nutrition Conceptation and Cortra varsies 1997 , 7th edition
- Fuqua BK, Vwpo Co, anderson GJ, Jtrace elemmed Biol, 2012, 26(2_5) 115_148.
- George A, HarwELL,clinical, correlation and analytic procedures,7th edition, pg 359_368.
- Hameed A,Majeed T,Rauf S, Ashraf M, Jalil A, Effect of oral and injectable contraceptive on serum calcium ,phosphours , magneasum in women,J Ayub Med Coll Abbottabad 2001;13;24-25.
- Hartard M,Bottermnn P, Bartenstein P,Jesehke D,Sehwaiger M, Effect on bone mineral deposit of low-dosed oral contraceptive compared to acombined with physical activity ,contraception,1997

- Hatcher RA, Trussel JE, Stewart FD, contraception technology, 1994, 16th edition.
- Kasper DL.et al, Harrison’s principle of internal medicine, 2005, 16edition, pg 320_324.
- Longo DL, Kasper DL, et al, Harrison’s principle of internal medicine ، 18 ،2012th edition.
- Mark H, Beers et al, The merck manual of medicine information, 2003, 2nd edition.
- Munozm, Garri Ercd JA, Remach Molecular basis of iron, 2011, pg 281_286.
- Murphy E,William,medicine ,2009,
- Perfit AM, Kleere koperM, clinical disorder of fluid and electrolyte, 1980 ، 3edition.
- PrchalJT, lichtmanMA, KippsTJ, Seligsohn, Williams’s hematology2010 ،, 8edition.
- Polanic JE, clinical importantance and analysis, 1991, pg. 105_109.
- Rude RK, shils ME, modern nutrition in health and disease2006 ،, 10edition ، pg48.
- Water's M, calcium metabolism and disorder, 2009, pg. 465_468.
- Whang Clinical disorder of magnesium, 1997, pg. 168_173.
- www.medicinenet.com.

استمارة الموافقة الأخلاقية

استمارة موافقة المريض على المشاركة بالبحث

يجب أن تكتب الاستمارة بلغة عربية واضحة ومفهمة وتحتوي على الفقرات الآتية

اسم الباحث : .....

عنوان الباحث : .....

مكان اجراء البحث : .....

أنت مدعو للمشاركة ببحث علمي سريري في (.....)

ترجى أن تأخذ الوقت المناسب لقرأة المعلومات الآتية بتأني قبل أن تقرر إذا ما كنت راغبا بالمشاركة أم لا وبإمكانك طلب مزيدا من الايضاحات أو المعلومات الاضافية عن أي أمر مذكور باستمارة أو عن الدراسة من طبيبك .

1. وصف مشروع البحث وأهدافه ومساره
2. الفوائد الايجابية المحتملة للمشارك التي قد تنتج من هذا البحث
3. التأثيرات السلبية أو الاعراض الجانبية المحتملة التي يتعرض لها المشارك

وفي حالة موافقتك على مشاركتك في هذه الدراسة سيبقى اسمك قيد الكتمان ولا يسمح لأي شخص حق الاطلاع على الملف الطبي الخاص بك .

وثيقة الموافقة التحريرية

لقد اوضحت للمشارك بالتفصيل البحث وطبيعته ومجرياته فوائده المحتملة وسلبياته المحتملة أيضا

وأجبت عن كل استفساراته واسئلته بوضوح وسأعلم المشارك بأي تغيرات في مجريات البحث أو فوائده أو سلبياته حال حصولها أثناء البحث .

اسم الباحث : ..... التوقيع التاريخ / /

موافقة المشارك

لقد اطلعت على استمارة الموافقة وأدركت مضمونها وبناء عليه فإني حرا مختارا أوافق على المشاركة بالبحث

وفهمت أن الدكتور / ( ) وزملاءه ومساعديه سيكونون مستعدين للاجابة عن

أسئلتي المستقبلية وبإستطاعتي الاتصال بهم على رقم الهاتف ( ) كما أعلم تماما بأنني حر في الانسحاب من هذا البحث متى شئت ولو بعد الموافقة التحليلية ومصادقتها من أن يؤثر ذلك على العناية الطبية المقدمة لي

اسم المشارك ..... التوقيع التاريخ / /