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EFFECTS SEX HORMONES ON PATIENTS WITH RECURRENT KIDNEY STONE IN ALMUTHANNA GOVERNORATE-IRAQ

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KARRAR KADHIM HUSSEIN ARAJ

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By Karrar Kadhim Hussein ARAJ

July 2021

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

Advisor : Assoc. Prof. Dr. Hakan ÇOLAK
Co-Advisor : Prof. Dr. Husam Muhammad KREID

Examining Committee Members:

Chairman : Assoc. Prof. Dr. Şevki ADEM
Chemistry Science -Biochemistry
Çankırı Karatekin University

Member : Asst. Prof. Dr. Hakan ÇOLAK
Chemistry Science -Inorganic chemistry
Çankırı Karatekin University

Member : Asst. Prof. Dr. Ümit YIRTICI
Medical Laboratory
Kırıkkale University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. İbrahim ÇİFTÇİ
Director of Graduate School

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Karrar Kadhim Hussein ARAJ

ABSTRACT

EFFECTS SEX HORMONES ON PATIENTS WITH RECURRENT KIDNEY STONE IN AL-MUTHANNA GOVERNORATE-IRAQ

Karrar Kadhim Hussein ARAJ

Master of Science in Biochemistry

Advisor: Assoc. Prof. Dr. Hakan ÇOLAK

Co-Advisor: Prof. Dr. Husam Mohammed KREIDI

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Kidney stones or Urolithiasis is an illness that affects a large number of people around the entire globe. The present research aims to know the impact of sex hormones in the frequency of stone inpatient forming residing in Al-Muthanna Governorate-Iraq. The study included 70 patients of males diagnosed with kidney stones more than once and 70 healthy controls. The patients were divided into 35 cigarette smokers and 35 non-smokers (the control class was divided in the same way). In the blood of patients and the control group, Testosterone, Estrogen, Progesterone, Vitamin D3, Calcium, Magnesium, Phosphorous, and Uric Acid concentrations were examined. It was seen that there was a significant reduction in the concentrations of Testosterone, Estradiol, Magnesium, and Phosphorus, this reduction was statistically important ($P \leq 0.05$). Concentration Calcium of the patients was similar to the Calcium focus of the control group, and it was found that there was a rise in the concentration of Uric Acid in the patients compared with a control group, and it was found that all patients had a decrease in the concentration of Vitamin D3 comparison with the control group, No clear behavior of the results of the hormone Progesterone in the patients in this study. Finally, we need to expand the number of examined samples and the research time so that we can understand the influence of sex hormones on patients with kidney stones.

2021, 71 pages

Keywords: Recurrent kidney stone, Sex hormones, Vitamin D3 concentrations

ÖZET

ALMUTHANNA VALİLİK - IRAK'TA SEKS HORMONLARININ YENİDEN BÖBREK TAŞI OLAN HASTALAR ÜZERİNE ETKİSİ

Karrar Kadhim Hüseyin ARAJ

Biyokimya, Yüksek Lisans

Tez Danışmanı: Doç. Dr. Hakan ÇOLAK

Eş Danışman: Prof. Dr. Hüsam Muhammed KREID

Temmuz 2021

Böbrek taşları veya Ürolitiyazis, tüm dünyada çok sayıda insanı etkileyen bir hastalıktır. Bu araştırma, Al-Muthanna Governorate-Irak'ta yaşayan taş yataklı hasta oluşum sıklığında seks hormonlarının etkisini bilmeyi amaçlamaktadır. Çalışmaya birden fazla böbrek taşı tanısı almış 70 erkek hasta ve 70 sağlıklı kontrol dahil edildi. Hastalar 35 sigara içen ve 35 sigara içmeyen olarak ayrıldı (kontrol sınıfı da aynı şekilde ayrıldı). Hasta ve kontrol grubunun kanlarında Testosteron, Östrojen, Progesteron, Vitamin D3, Kalsiyum, Magnezyum, Fosfor ve Ürik Asit konsantrasyonlarına bakıldı. Testosteron, Estradiol, Magnezyum ve Fosfor konsantrasyonlarında anlamlı azalma olduğu görüldü, Bu azalma istatistiksel olarak önemliydi ($P \leq 0,05$). Hastaların Kalsiyum Konsantrasyonunun Kontrol grubunun Kalsiyum odağına benzer olduğu ve kontrol grubuna göre hastalarda Ürik Asit konsantrasyonunda artış olduğu ve tüm hastalarda azalma olduğu tespit edildi. Vitamin D3 konsantrasyonunda kontrol grubu ile karşılaştırıldığında, bu çalışmada hastalarda Progesteron hormonunun sonuçlarında net bir davranış yok. Son olarak, seks hormonlarının böbrek taşı olan hastalar üzerindeki etkisini anlayabilmemiz için incelenen örneklerin sayısını ve araştırma süresini genişletmemiz gerekiyor.

2021, 71 sayfa

Anahtar Kelimeler: Tekrarlayan böbrek taşı, Cinsiyet hormonları, Vitamin D3 konsantrasyonları

PREFACE AND ACKNOWLEDGEMENTS

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Karrar Kadhim Hussein ARAJ

Çankırı-2021

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LIST OF ABBREVIATIONS

KSD	Kidney stone disease
CT	Computerized tomography
AHA	Acetohydroxamic acid
vitamin D3	Cholecalciferol-D3
CKD	Chronic Kidney disease
CAOX	Calcium Oxalate
CaP	Calcium phosphate
HIV	Human immunodeficiency virus
Ksp	Thermodynamic solubility product
COM	Calcium oxalate monohydride
VDR	Vitamin D receptor
BMI	Body mass index
RPM	Revolutions per minute
Testo	Testosterone
Prog	Progesterone
E2	Estradiol
Ca	Calcium
Mg	Magnesium
PO_4	Phosphate
UA	Uric acid
DT	Disc tube Technology
MTS	Metabolic syndrome

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1. INTRODUCTION

Urolithiasis or Nephrolithiasis is currently considered a relatively popular clinical condition, and the estimates indicate that the disease prevalence around the world is estimated between 1 to 20%(Grabe *et al.* 2020), Nevertheless, the number of injuries around the world varies, with the number of injuries in Europe reaching 5-9% (it is frequent in the British Scandinavian islands), in Asia 1-5% (mostly in India, Pakistan, the Philippines, Thailand, and Indonesia), the United States of America 13% and Canada 12%. In the Middle East, the largest number of people with kidney stones was reported estimated at 20% (Sudan, Saudi Arabia, Egypt, and Iran) (Jayaram and Gurusamy 2018) ,The cause of this number of injuries in the Middle East may be due to the hot climate, which can cause drought, which in turn is that important environmental agent in the build of kidney stones. All ages and genders suffer from kidney stones (the infection rate in elderly men is higher than the number of injuries in women by 2/1, and the rate of infection in children is estimated to be 1-2% only).(Romulo and Vitale 2000). This disease chronic and has a great frequency creation of It may lead to kidney failure, as the rate from recurrence of kidney stones growth is estimated at 10% during the first year and from 30 to 40% within five years (Jayaram and Gurusamy 2018).

1.1 Sign and Symptoms

When the stones move and travel from the kidneys to the bladder through the ureter, some stones remain in the ureter and thus will prevent the flow of urine and cause the kidneys to enlarge or swell, and this condition is known as hydronephrosis. Because of hydronephrosis, the patient suffers great pain in the kidneys and has many symptoms, including sudden and severe pain in the back and along its side (Singh *et al.* 2019), and the pain may move to the genital area or the lower abdomen, involuntary and sudden urination occurs for the patient, the presence of burning when urinating, the color of urine becomes dark or red and the reason is the presence of blood particles in it resulting from the injury of the ureter due to the movement of the stones, the patient suffers from nausea and vomiting, and if the patient is male, he feels pain at the tip of the penis (Tewari and Shukla 2005).

1.2 Diagnosis and Tests

Examinations and analyzes are necessary to make a correct diagnosis and to determine the location for the stone, size, type (Jayaram and Gurusamy 2018).

1.2.1Blood tests

The findings of the blood analysis (which measure calcium and uric acid in the serum of blood) aid in kidney monitoring and maybe urge the specialist to examine the patient's another medical problems (Pak *et al.* 1986).

1.2.2 Urine testing

In a twenty-four hours hour urine summation analysis, the urine might display many minerals Which are involved in the formation of stones, and these minerals are excreted by the kidneys, or they excrete a little of inhibitors of stone formation (Coe *et al.* 2001).

1.2.3 Imaging

The imaging test tells us to know the amount or number of stones in the urinary system. The types of imaging tests start from the simple, which are the x-ray of the abdomen, which may not reveal to us small stones in size, CT which is a high-speed or dual-energy computed tomography, which shows the Small stone(Daniele J *et al.* 2005), other imaging options are non-invasive testing, ultrasound, urography, in which the patient is injected with a colored dye into a vein (in the arm) and either a Computerized tomography of the urinary system while the tincture moves through the kidney and the urinary bladder, or an image is taken X-ray (intravenous pyelogram) (Griffith *et al.* 1988).

1.3 Treatment

If the stones are small in size, they do not need more than one treatment to get rid of them, as they leave the body when drinking 4-5 liters of water daily. This amount is

sufficient to get rid of the stone through the urine, and when the rocks move, they cause pain and can be treated by taking some painkillers (Kurita *et al.* 1994). As a treatment for renal calculi, doctors refer to the use of alpha-blockers, this helps to expel stones from the kidneys faster by relaxing the walls of the ureter. Also, diuretics are important because they increase the diuresis, which leads to a tightening of the gravel (Birkenhager *et al.* 1981). The stone Large remain attached to tubule renal, and for this reason, it isn't easy to get rid of them. This type can be harmful to the body. It causes damage to the kidneys, loss of the nephron, internal bleeding, and urinary tract infection (Michell 1989), Large stones are broken up using the Extracorporeal shock wave lithotripsy technique outside the body and turn into small pieces that come out with the urine; this technique uses shock waves or sound waves that cause strong vibrations to cause the stones to break down (Laerum and Larsen 1984). If physicians discover big stones in or around the kidneys that are more than 2 cm in diameter, then the stone excision process is an available option, as in this process, the patient is given general anesthesia, and the excision device is used that contains thin telescopic tools that work to remove kidney stones, it is preferable to use this tool in If there are stones in the pelvic area (Escribano *et al.* 2010) . If stones come out of the kidneys and are stuck either in the ureter or in the bladder, doctors have to perform an ureteroscopy examining rocks in the upper urinary tract. Ureteroscopy contains a wire at its end there is a camera. Wire inserted into the urinary tract up to the bladder and then into the ureter if the stones are stuck in the ureter. The process of removing the stones is done by a cage connected to the wire (Huen and Goldfarb 2007). Drinking fluids and adjusting the diet is not sufficient to prevent or eliminate kidney stones. Hence, doctors have to prescribe treatments to eliminate rocks throughout the human body, and the sort of therapy defined lean on knowing the volume for stones, their type, and urine abnormalities(Moe *et al.* 2011). Thiazide diuretics are one of these treatments as they work to reduce the amount of calcium in the urine, meaning that it helps calcium stones in the kidneys as it works to excrete the excess calcium out of the body with the diuretics. Also, thiazides can be used in a low-sodium diet where it is placed with the amount of salt added to the food (Hess *et al.* 1998). Potassium citrate can be used in individuals that have uric acid, Cystine, and Calcium stones with decrease citrate in the urine, as it works to make diuresis less acidic. Citrate is caused does urine extra alkaline, and aforementioned

feature aids deny uric acid stones and cysteine from forming; The higher the concentration of citrates excreted with urine, it works to stop the formation form stones Calcium(Duncan *et al.* 2002). Struvite stones are removed by surgery, so Acetohydroxamic acid (AHA) should be used as a treatment for patients who suffer from this type of gravel or have urinary tract infections caused by some types of bacteria, where repeated infections guide to the forming of this type of stones, (AHA) dilutes the wee (lowers the level from urea) and makes it unsuitable for the construction of struvite stones and also works to prevent urinary tract infections and thus inhibits the formation of the struvite stones (Intake 2002). One of the diseases that cause high uric acid into the serum of blood and the reason uric acid deposition at a joint is gout. Allopurinol is used to treat gout by lowering uric acid levels in the urine and blood, as well as preventing the development of calcium rocks into the kidneys (Forest and Carolina 2001).

1.4 The Study's Goal

The purpose of this study is to look into the influence of sex hormones and other variables on the incidence of stone production in People with this disease living in the Almuthanna Governorate-Iraq. The study was recommended since the governorate has a high number of these pathological cases.

2. LITERATURE REVIEW

2.1 Kidney

The kidneys (Figure 2.1), which are a pair of bean-like organs, are located above the waist between the abdominal serous membrane (peritoneum) and the posterior part of the abdomen; both the eleventh and twelfth ribs help to protect them. The Kidneys are a complex organ of the urinary system, where the kidneys perform many functions such as getting rid of foreign substances and also waste products by excreting them with the urine, and it works to regulate many properties for the blood such as the regulation of a number of blood ion concentrations and this process is called ionic synthesis, and the production of some hormones such as the active form Of the vitamin D called citiriol and erythropoietin, blood pH, blood volume, blood pressure, glucose level, and osmolarity (Killeen 2017).

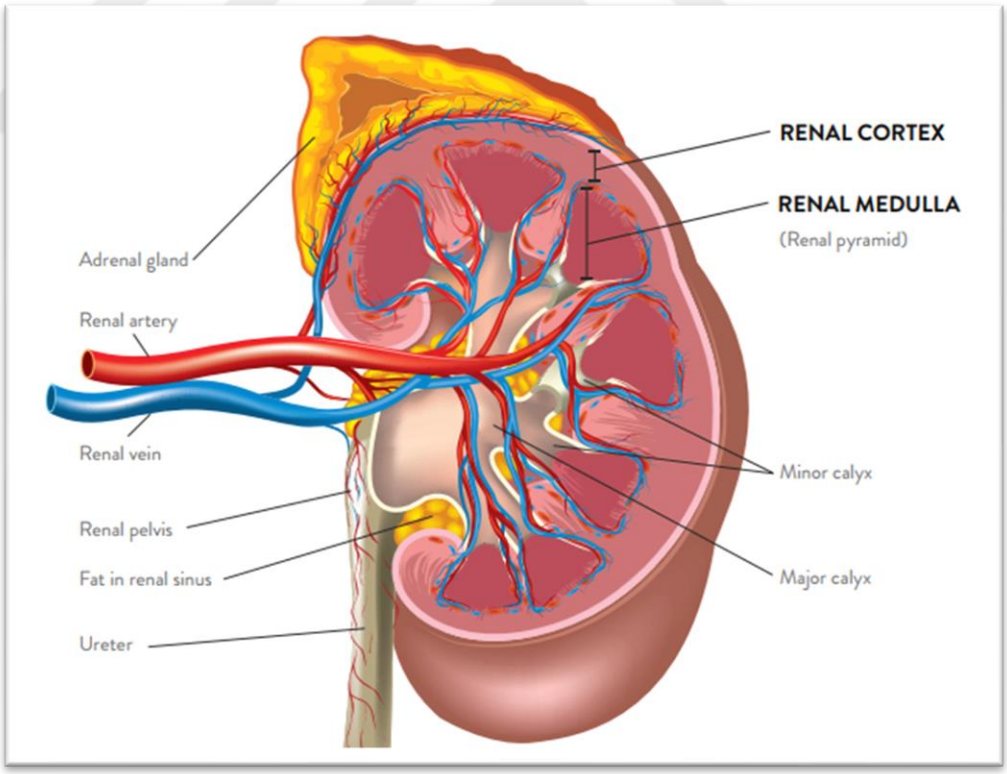


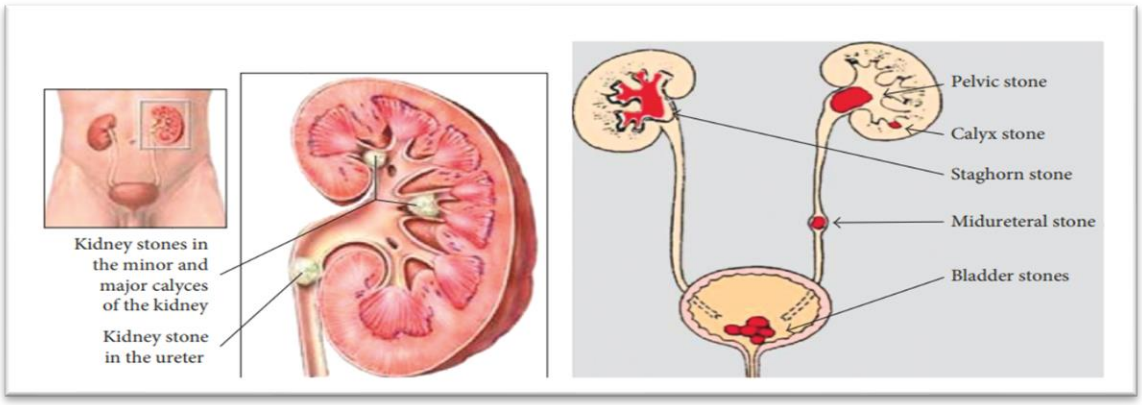
Figure 2.1 The kidneys Internal anatomy (Killeen 2017)

2.1.1 Chronic kidney disease

Chronic, in a long-term meaning, is defined as damage to the kidney and its structure, retardation in its functions, damage to its functions, and deterioration of its functions for a period of 3 months or more. This condition is considered a major health issue that is spread at a rate of 13.4 percent globally and the mortality rate due to it is 1.2 million people within one year (Kakitapalli *et al.* 2020). Kidney stone disease (KSD) and chronic kidney disease are among the main general health troubles. Several previous studies in epidemiology have demonstrated a link between the date of KSD and the danger of developing the chronic disease of the kidney. Recurrent KSD predisposes to CKD, and although CKD reduces the risk of KSD, it seems necessary to stay away from recurrent KSD not only because of the clinical symptom for KSD that appears quickly but because the KSD is estimated to increase the incidence of CKD (Uribarri 2020).

2.2 Urinary System and Stone

The urinary system is a system made up of several organs, namely the kidneys, ureters, bladder, and urethra. The main function of this device is to purify the bloodstream and eject excess fluids and other substances through the urine. Urine is a yellowish liquid that contains various substances, such as vitamins and minerals, which are redundant with the remnants of the metabolism process. The urinary system works in cooperation with the rest of the other organs of the body to balance water, salts, and acid basic in the blood (Sohgaura and Bigoniya 2017) .The stones may be present in different locations in the urinary system as shown in the (Figure 2.2) (Alelign and Petros 2018).



a1` a2

Figure 2.2 The locations of Kidney stones in the urinary system. (a1) taken from (Zahid *et al.* 2013), (a2) taken from (Evan 2010).

2.3 Kidney Stones

The primary site for kidney stones is the kidney. Humanity has been affected by kidney stones for many centuries, dating appears to 4000 years BC. Kidney stones are the most common urinary tract illness. There is a relationship between kidney stones on the one hand and an increased risk of end-stage renal failure, Diabetes mellitus, chronic kidney illness, vessels blood, and raise blood pressure. Kidney stone disease has been linked to MTS illness and is a systemic problem .If nephrolithiasis is present, nephrolithiasis is responsible for 2% to 3% of end-phase renal disease. Whether the stones are in the kidneys, ureters, or bladder, the marks of kidney stones are the same. At the beginning of the formation of stones, there are no symptoms, and the symptoms appear later, where the symptoms of kidney stones are severe pain (renal colic) and dilation of the kidney, inflammation of the urinary tract, pain in the back, and urinary tract morbidity, and these cases lead to nausea and vomiting (Alelign and Petros 2018).

2.3.1 Kidney stone compositions

The stone of the kidney is a solid piece that forms like a result of several crystals gathering together, either in one kidney or in the two kidneys. The size of the stones varies; they vary in size from small millimeters to many centimeters. The majority of pebble passes through the urine without interference and some need intrusion in order to be removed. Diet, in general, it affects the concentricity traction of certain chemicals in

urine as well as urine acidity. By collecting and analyzing urine samples for 24 hours, he found some characteristics that augmentation the hazard of stone production, namely:

- There is an increase in the concentration of oxalate excreted during urine
- Increase the calcium levels
- Low level of citrate
- The too much uric acid level

Urinary tract stones are formed by binding to crystals of uric acid, magnesium ammonium phosphate, struvite phosphate, or apatite phosphate Oxalates, calcium, citrate, and uric acid are natural elements located within the blood. The tartness of any liquid is known as the pH, and it is divided into two parts, where when the pH is greater than 7, it is referred to as alkalinity, and when the pH is less than 7, it is referred to as acidic. During one day, the pH of the urine can vary, usually from 5 to 8 (Singh *et al.* 2019).

2.3.2 Kinds of kidney stones

The installation of the chemicals that make up kidney stones is determined by chemical abnormalities in urine production. Because of differences in mineral content and Pathogenesis, kidney stones vary in size, chemical composition, and shape. *(*Chhiber *et al*. 2014).

2.3.2.1 Calcium stones

Generally, Calcium stones made up of two types calcium oxalate and calcium phosphate. The most frequent form of kidney stone is calcium stone and is found in 80% of total sorts of urinary stones. Calcium stones can be distributed as follows: Unmixed calcium oxalate (CAOX) accounts for 50% of the total, calcium phosphate (CaP designated with apatite) accounts for 5%, and a combination of the two accounts for 45 percent. Hydroxyapatite or brushite (calcium hydrogen phosphate) is the primary item of calcium stones. Many factors intervention to the configuration of CaOX stones like an increase in the calcium excretion in the blood (which is due to renal seepage or

absorption diseases or metabolic diseases), hyperoxaluria, Increased excretion of uric acid in the urine, decreased esters excreted in the urine, increased urination (Hyperuria), lack of magnesium in diuresis. Generally, the pH of the urine affects stone formation, where when it is from 5.0 to 6.5 it leads to the formation of CaOX stones, and when it is higher than seven and a half it leads to the generation of the stones of CaP type (Alelign and Petros 2018 , Sah and Jauhari 2017) .

2.3.2.2 Stones of struvite

They may be called infectious stones and triphosphate stones. The most common types of bacteria present in the urine that cause this type of stones are Klebsiella and Proteus. When ammonia production increases and the acidity of the urine increases, and consequently the solubility of phosphates decreases, the formation of struvite stones occurs. Urea is broken down into carbon dioxide and ammonia by the urea enzyme. Men are less than likely to develop struvite stones than women, and this type of stones is large and likely to be large, filling the nephritic tub and figuration coral stones named (Staghorn calculus), their evolution is fast and they will likely grow again after removal by surgery (Flannigan *et al.* 2014).

2.3.2.3 Uric acid stones

It accounts for roughly 3% to10% of the overall number of stone kinds (Giannossi and Summa 2012). Diets that contain a high amount of purines, particularly those containing animal proteins like seafood and meat, cause high uric acid in the urine, The likelihood of uric acid stone formation is aggravated by a reduced volume of urine and a low urinary pH (pH<5.05) (Ngo and Assimos 2007). People who suffer from gouty arthritis may develop kidney stones of this type. Men are more probable to get uric acid stones than women (Dursun *et al.* 2015).

2.3.2.4 Cystine -type kidney stones

Nephrolithiasis is the essential clinical appearance of cystine stone and an outcome of irregular renal tubule transmission which in turn leads to the secretion of large quantities of cysteine during the urine. Males and females are equally affected by Cystinuria; Even so, males are extra vulnerable to infection. Stones begin to crystallize in the first to fourth decades and become diverse, big, and binary. In the analysis of cysteine stone, yonder are some exist of model hexagonal crystals in the urine (Fattah *et al.* 2014).

2.3.2.5 Drug-induced stones

The percentage of stones that are formed due to the drug is 1% of all varieties of kidney stones, and they are two main mechanisms to them, first includes stones that form due to the drug and/or treatment metabolites that are identified or known by two methods infrared spectroscopy or X-ray diffraction according to the specified graphs of them. Protease antagonists (one of which is indinavir) is the most commonly utilized ones currently by HIV patients, and sulfadiazine overdose, which is used to treat cerebral toxoplasmosis. And taking preparations containing guaifenesin/ephedrine, earlier, triamterene was considered one of the less frequent drugs of concern. The second type contains stones known or commonly formed that result from the metabolic influence of the medication on the secretion of calcium, urinary pH, purines, oxalates, citrates, phosphates, or acid Uric. The most common causes are vitamin D and calcium supplements taken without control, and Topiramate is a type of carbonic anhydrase inhibitor, which is an anticonvulsant. Stones consist of curative origin due to high doses of treatment or long-term use of treatment, low solubility, a boost in renal secretion, a lowering in the amount of urine, or insufficient pH of urine, and that the common in patients with previous stones (Daudon *et al.* 2018).

2.3.3 Epidemiology of kidney stones

There are a limited number of viable therapies for pissed tract stone illness at a time when the disease is spreading and recurring across the world (Knoll 2010). This disease affects the world's population12% at an age of their lives (Chauhan *et al.* 2009), as it affects all age groups as well as all sexes and races (Moe 2006), but at the age of 20-49, it affects more men than women (Edvardsson *et al.* 2013). If the patient does not apply the metaphylaxis system, then the risk of forming a second stone is estimated to be around 10-23% per year, 50% in 5-10 years, and 75% within the patient's 20 years (Moe 2006), and despite that, the recurrence increases for males more than females (Afsar *et al.* 2016). In recent studies, in recent decades, there has been an upsurge in the prevalence of urinary stones illness in both developed and developing nations. This increase in growth is attributed to physical activity, dietary habits, lifestyle (Sofia *et al.* 2016), and global warming (Romero *et al.* 2010). Kidney stones affect 1-11 persons in the USA (Manuscript 2012), where it is predestined that American people suffering from kidney stones number 600,000 per year. In India, around12% of them are predicted to develop urinary tract stones, with 50% of them losing kidney function (Joseph *et al.* 2005).

2.3.4 Kidney stones stimulants and inhibitors

Even if supersaturation bases are present, inhibitors operate to minimize stone formation. Citrate is a strong inhibitor as it has two characteristics as it works to form complexes that have the ability to dissolve with calcium (as it reduces urine saturation with calcium) and also it has the capacity to prevent the growth and aggregation of crystals. Magnesium may act as an inhibitor, but its mechanism of action is not yet clear, as laboratory experiments have suggested the magnesium possesses the capacity to disturb the stability of the electron pair of calcium oxalate. However, clinical trials have not proven the effectiveness of magnesium in reducing kidney stones. Alkaline pH alone is not considered a catalyst, the pH of 6.7 alters the amount of supersaturation leading to crystallization of calcium phosphate observed in sick people with milk-alkali syndrome or Remote nephritic tubular acidosis or an increase in the primary

hyperparathyroidism or in patients treated with carbonic anhydrase inhibitors. (Ex, topiramate, used for migraine, and acetazolamide, used to treat glaucoma), On the other hand, when the pH reaches 5.5 this leads to the precipitation of uric acid. By converting urine to an alkalinization pH, it can prevent the ability to crystallize when cystine or uric acid stones are present (Pfau and Knauf 2016).

2.4 Stages of Stone Formation

The process of stone formation consists of several steps like Nucleation, aggregation, and Crystal Growth (Gupta and Shamsher 2018) .

2.4.1 Crystal nucleation

The process by which free ions bind to small molecules within a solution is called the nucleation process. Crystallization can occur on surfaces such as those inside cells, on the surface of cells (Lieske *et al.* 1996), or inside a nephron, and this represents environments that are sensitive to the solution (Olszta *et al.* 2003). There is disagreement about the importance of crystallization in a free solution compared to crystallization on the walls of the bladder or in the renal tubules or in areas that have been exposed to infection and cells have been stripped of them because of this injury or in the event. Those cells are damaged or normal or in the same cell sites (Evan *et al.* 2003).

2.4.2 Aggregation

When crystals that grow in a free solution coalesce in the form of big molecules consisting of many components, this process is called aggregation. It might also refer to the process of nucleation, which happens as a result of the growth of modern crystals on the surfaces of previously existing crystals. One or more of these steps must happen, depending on the composition of the stones, for the stone to grow Size that is clinically significant (Sandersius and Rez 2007). Stone can resemble concrete, as it is made up of materials including a bonding agent and particles such as gravel, glass, or sand. Stones

occur when crystals combine with an organic matrix that is made up of fats, sugars, cell-derived materials, and proteins (a binding agent)(Ratkalkar and Kleinman 2011).

2.4.3 Crystal growth

the growth of a microscopic crystal occurs when ions move from the solution into the developing crystal. In general, the large single crystals that form the stone are not observed, meaning that the growth process of some nuclear crystals is limited. Mostly, stone growth is achieved by a grouping of previously formed crystals or the nucleation that occurs secondary to the crystal on another surface covered by the matrix. As it has been submitted, the tiny crystals cannot grow to the extent that the crystal remains in the inside of the kidneys depending on the size alone Rather, the crystals must aggregate or bind to specific parts within the kidney (Kok and Khan 1994).

2.5 Pathophysiology

Many different theories explain the process of forming kidney stones, one of these theories assumes that kidney stones are formed when a salt dissolves in the urine to the point of saturation for example calcium oxalate salt (which is considered one of the salts that dissolve in the urine naturally) and then a crystal is formed where if it is large enough, and then a crystal is formed where if it is large enough, It can anchor in the terminal part of the aggregation channels(urothelium) and then growing of it be slowly. Another type of theory suggested that the interstitial tissue is the place where the stone is first formed and then we go to the Randall plaque in the papilla where crystals such as (phosphate or calcium oxalate) are deposited in it (Sanchez and Sarano 2011, Dasgupta *et al.* 2009). At the stage when the urine passes through the kidneys, these large particles are formed that can settle inside the kidneys and be the nucleus of future stone formation (Sanchez and Sarano 2011). The solution that provides the conditions for the urolithiasis is a saturated solution, meaning that it contains the highest concentration of a particular substance that can dissolve in it, that is if any excess amount is added to this solution from the same substance, this addition will lead to precipitation of this substance in the solution and the formation of crystals, Ksp or thermodynamic solubility product is the concentration at which a solution reaches a state of saturation and at

which crystallization begins (Aggarwal *et al.* 2013 , Dardamanis 2013).During clinical examinations, it was found that saturation may occur for two reasons, either because of a decrease in the amount of urine (the reasons are as for the loss of fluid outside the kidneys or a decrease in fluid intake) or that saturation occurs because of a rise in urine excretion of solvents for examples oxalate, calcium, or cysteine (Lin *et al.* 2014). Another mechanism, calcium oxalate monohydride (COM) crystals adhere rapidly to the outer roof of the renal epithelial cells, and the cause of occurring this condition is the outer cover of the COM crystals work like if it is a positive charge carrier, and the outer shell of the tubular epithelial cells work like if it carries a negative charge, so the This sticking is caused by electric charges, causing the crystals to work as if they were positively charged and bound to negatively charged particles emerging of the outer roof of the tubular cell, Through the research done by Lieske et al, which decided that the crystal that adhered to the outer surface of the tube cell could act as a new preferred site for the adhesion of additional crystals, after which the crystal was inserted into the tube cell through its plasma membrane, which after this assimilation appears to have the ability to increase binding with the crystals because during the 24 hours of the first crystal adhesion there was an increase in the number of crystals adherent to it, and for this reason, if there are any crystal linked with plasma membrane will guide to an improvement in the figure of adhesion sites of the crystals or bring these sites closer to each other on the surface of the tube cell (Tostes and Cardoso 2001 , Lieske *et al.* 1997). It stumbled upon anionic molecules present outside of epithelial cells, which work like receptors for calcium oxalate monohydride crystals. Anions were also present dissolved and attached to the roof of the crystals in the tubes; they were unable to adhere to epithelial cells as a result of this. The modification that occurs affects the composition and amount of specific particles (which found on the outside of the epithelial cells of the tubes or present in dissolved form into the urine) and other anions (which have the ability to dissolve in the urine) on an attachment of crystals to the cells and thus contribute to urolithiasis (Dardamanis 2013) .

2.5.1 Testosterone

Studies indicate that testosterone promotes stone forming and this is done by inhibiting Osteopontin in the kidneys and also leads to raised oxalate secretion in the urine (Yagisawa *et al.* 2001). Osteopontin is a protein that prevents the crystallization of calcium phosphate and calcium oxalate. These crystals remain the usual popular kind of crystal found in urine stones (Mohamaden *et al.* 2014).

2.5.2 Estradiol (E2)

Estrogen may contribute, even partially, to stop the development of stones, because estrogen affects the tube cells by causing changes in the cellular protein of these cells, and thus this will cause a loss in the surface area of the receptors from crystals calcium oxalate, promote cell proliferation, tissue healing and reduce the metabolism process Inside cells (Peerapen and Thongboonkerd 2019).

2.5.3 Progesterone.

In the kidneys, progesterone is metabolized into testosterone and dihydrotestosterone. For this reason, some of the effects of progesterone on the kidneys may be attributed to his androgenic impacts (Organ *et al.* 2003). The study on progesterone was not good, but it may act as an antidiuretic and antioxidant and may cause chronic vasoconstriction (Yanes *et al.* 2008).

2.5.4 Calcium

Most patients with kidney stones have increased calcium excretion in urine (a great level of calcium in the urine leads to the production of crystals and these crystals collect on Randall plaques and form stones) and a small number of patients have the systemic disease (the most common disease condition that causes a high level of calcium in the blood It is hyperparathyroidism, Cancer, Sar deactivating vitamin D receptor (VDR) polymorphism and sarcoidosis) Which causes a chronic increase in the calcium level of blood and thus promotes the creation of stones (Dawson and Tomson 2012).

2.5.5 Magnesium

Previous studies have shown that magnesium acts as an inhibitor of kidney stones, especially the type of calcium oxalate, and this evidence is controversial due to the conflicting results in recent studies. Magnesium affects the formation of calcium oxalate crystals as it works to make the interaction between Ca^{2+} ions and Ox^{2-} This reduces the link and The action of magnesium as an inhibitor is affected by the density of magnesium and its location in relation to Ox^{2-}, where magnesium binds with citrate, and this link remains effective in acidic media and this is its inhibitory effect (Riley *et al.* 2013). The depletion of magnesium (which occurred gradually due to giving the rat a low-magnesium diet for 7 days) points to a change in the structure of the nearby tubular cells, which prepares to the forming of calcium crystals, and these calcium stones are formed. Being a consequence, it was considered that magnesium maybe have a function in the production of kidney stones in humans (Bunce *et al.* 1978).

2.5.6 Vitamin D3

Vitamin D3 has a significant part in balancing phosphorus, calcium, and bone strength. The high level of vitamin D3 is deemed a danger factor for the synthesis of calcium stones, and its decrease leads to the forming of the kidney stone, and the proposed mechanism for this relationship is secondary hyperparathyroidism and other effects similar to the decrease in vitamin D3 and consequently the formation of calcium stones. It has been proven that a decrease in the level of vitamin D3 causes oxidative stress and inflammation, as the author suggested that there is a relationship between calcium stones in the urine and a decrease in vitamin D3 that may occur due to the role of deficiency vitamin D3 which causes inflammation and oxidative stress in the kidney tissues (Umekawa *et al.* 2006, Tavasoli and Taheri 2019) .

2.5.7 Phosphorus

The decrease in blood phosphate caused by renal phosphate leaks may predispose to the development of calcium stones via increasing the level of calcitriol in the blood and the

elimination of calcium through urine and urinary saturation (Prié *et al.* 2001) As it was found that there is a decrease in the level of phosphorus in infected cases with kidney stones, and as well changes in phosphorus metabolism may share in the formation of stone calcium in the kidneys (Taylor *et al.* 2015).

2.5.8 Uric acid

Urine pH is the factor that affects crystallization and sedimentation of uric acid stones, not urine volume, nor urine uric acid level (Charles *et al*. 2005), A decrease in the acidity of the urine may lead to an increased danger of receiving kidney stones kind of uric acid stones. when the acidity is at the number 5.3 the risk of crystallization of the uric acid stones increases (50% of uric acid at this number is poorly soluble). Most cases of uric acid crystallization are deposition of uric acid hyper-saturated diuresis, and this is likely to occur after eating a meal rich in protein with this to form uric acid stones (It goes through the stages of crystallization, nucleation, aggregation, and stone formation). All people have differences in the pH of urine during the day (Wiederkehr and Moe 2011) .

2.6 Risk Factor of Kidney Stone

Kidney stones are a complex process and are affected by many factors, including internal factors (age, gender, and genetics), including external factors (climate, geography, nutrition, water consumption and mineral composition) (Moe 2006). Through studies, it has been shown that many hazard factors can lead to kidney stones, which we will explain briefly. The first being Diet is any eating foods high in proteins and salts such as sodium can lead to kidney stones(Sohgaura and Bigoniya 2017), gender and age (Moudi *et al.* 2017, Edvardsson *et al.* 2013), Metabolic disorders Such as decreased urine, increased calcium excretion in the diuresis, excess uric acid in the urine, and gout (Joshi *et al.*2012), Hypercalcemia, the disorders that lead to hypercalcemia are incipient hyperparathyroidism and another disorder of calcium metabolism (Heilberg and Schor 2006).the composition of Urine, In other words, an increase in the crystallization stimuli in the urine and a decrease in the inhibitors (Giannossi and Summa 2012), cigarette smoking (Liu *et al.* 2009), the decrease in the

volume of diuresis, which is caused by not drinking enough water (hyper-saturated urine and dehydration) (Straub and Hautmann 2005), Recurrent infections of the urinary tract that occur due to the abnormalities in the urine pH and the conversion of the urine to be alkaline by urease like bacteria Proteus mirabilis (Dursun *et al.* 2015 , Heilberg and Schor 2006) , Heredity, that is, genetic susceptibility(the family history of stones), renal tubular acidosis, and diseases that are inherited and are caused by one gene (monogenic)(Joshi *et al.* 2012), Anatomical anomalies, including many conditions, such as ureteropelvic junction stenosis, horseshoe kidney, and defects in medullary sponge kidney, polycystic renal disease, and pyeloureteral duplication(Gambaro *et al.* 2006), Hypertension(Obligado and Goldfarb 2008), Obesity (Poore *et al.* 2020), Climatic conditions, seasonal changes (higher in summer than winter), and global warming(Lieske *et al.* 2014), Lithogenic medicines: like protease inhibitor, sulfonamides (sulfadiazine), indinavir (Crixivan), ceftriaxone (aloft potion in long terms), and uricosuric factors who have weak soluble and promotes the forming of stone (Barbas *et al.* 2002).

2.7 Complication of Kidney Stone

Many complications may arise because of the growth of kidney stones, according to studies; it is possible that the infection of kidney stones even if the first injury has negative consequences on the kidneys, including a risen danger of forming kidney disease in the final stage (Means the kidneys are no longer able to work as they should. To meet the needs of the body) (Todd Alexander *et al.* 2012), the severe obstruction may occur due to stones and needs urgent surgical intervention, and This blockage occurs when the stones pass from the kidneys to the ureter, and thus the blockage leads to a reduction in the blood specific to the kidneys and the renal liquidation average (Miller and Lingeman 2007), as this Clogging and Bilateral hindrance in the affected kidney is deemed a contingency case and requires urgent surgery (Bultitude and Rees 2012). The stagnation of urine resulting from infection in the urinary tract is deemed a contingency case that needs requires necessary surgery to prevent the lack of urine that threatens the life of the patient (Cunningham *et al.* 2016).

3. MATERIALS AND METHOD

3.1 Study Design

Our study was obtained 70 blood samples from sick people with recurrent kidney stones who had been diagnosed by a urologist. The samples were collected from AL Hussein Teaching Hospital and a special central laboratory, and all samples were exclusively from ALMuthanna Governorate. Samples were collected from October / 2020 to February 2021. All samples are male, and they are divided into two parts, 35 samples of a non-smoker and 35 samples of a smoker (also the healthy male 70 samples divided into two parts, 35 non-smokers and 35 smokers). All patient data (age, weight, and height) were recorded. The patients (and healthy people) were divided into two main parts, the first based on age and the second on body mass index. Age contains two groups, the first group of 30-45 years old was called age group1, and the second age group from 46-65 it was called age group 2. Body mass index was also divided into two categories, the first category was patients who had a normal BMI average of 18.5 to 24.9 (kg / m^2), and the second category had overweight and its value (BMI $\geq$ 25) (kg / m^2).

3.2 Collection of Blood Samples

5 ml was drawn from the patient's blood, taking into account not to use Tourniquet during blood draw, it is then placed in a tube containing the separation gel and waited for it to coagulate at room temperature for 15 minutes and then separated using a centrifuge at 4000 rpm shown (Table 3.2) for 5 minutes. The serum was taken and placed in the tube of the laboratory devices, and 8 types of analyzes were done for all samples directly (on the same day as the sample was drawn) and the analyses were (testosterone, estradiol, progesterone, vitamin D3, calcium, magnesium, phosphorous, uric acid).

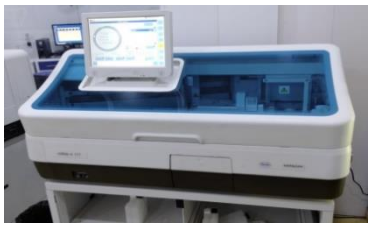
3.3 Chemicals

Table 3.1 Table of chemicals

no	Chemicals	The manufacture company	Ref
1	Testosterone II	Roche Diagnostics GmbH	05200067 190
2	Estradiol III	Roche Diagnostics GmbH	06656021 190
3	Progesterone III	Roche Diagnostics GmbH	07092539 190
4	Vitamin D total	Roche Diagnostics GmbH	05894913 190
5	Calcium gen .2	Roche Diagnostics GmbH	05061504 190
6	Magnesium	Roche Diagnostics GmbH	05401712 190
7	Phosphate ver.2	Roche Diagnostics GmbH	05401780 190
8	Uric acid ver.2	Roche Diagnostics GmbH	04657608 190

3.4 Instrument

Table 3.2 Types of laboratory Instrument used and their names

no	Instrument name and Model	The manufacture company
1	The cobas e 411 analyzer Is a fully automated analyzer that uses a patented ElectroChemiLuminescence (ECL) technology for immunoassay analysis, it is designed for both quantitative and qualitative in vitro assay determinations for a broad range of applications (including anemia; bone, cardiac and tumor markers; critical care; fertility/hormones; maternal care; and infectious diseases). The analyzer is available as a rack or disk sample handling system.	Roche – Hitachi 
2	The cobas c 111 analyzer Is the smallest member of the cobas® serum work area platform family and the ideal solution for clinical chemistry testing in laboratories running 10 to 50 samples per day.	Roche 
3	Centrifuge EBA 200 The EBA 200 and EBA 200 S are practical, compact centrifuges for small sample sizes. The high-speed S model can deliver reliable results in 3 minutes or less. An 8-place fixed angle rotor is included to hold standard blood and urine tubes up to 15 ml in capacity.	Hitachi 

3.5 Biochemical Parameters

3.5.1 Testosterone II

Serum testosterone concentration was measured by a Cobas e 411 shown in (table 3.2) which is a fully automatic machine.

3.5.1.1 The working principle of the analysis

The full duration of the analysis is only 18 minutes.

- The first incubation involves incubating 20 milliliters of the material with a biotinylated monoclonal testosterone-specific antibody. The sample analyte binds to the labeled antibody's binding sites (depending on its concentration)
- 2nd incubation: the complex gets attached to the solid phase via biotion and streptavidin interaction with the addition of streptavidin-coated microparticles and a testosterone derivate tagged with a ruthenium complex.
- The reactants are drawn into the cell in which the measurement is made, where the tiny particles are trapped on the electrode roof by magnetism. A ProCell/ProCell M is then used to extract the unbound material. When a voltage is applied to the electrode, a chemical luminous emission is produced, which is detected by a photomultiplier.
- The results are obtained using a calibration curve, which is devise specifically generated by 2-point calibration, and a master curve is given by the reagent barcode or barcode.

3.5.1.2 Specialized detection and working solutions

The mark on the detector shelf is TESTO II.

M 1 vial, 6.5 mL, streptavidin-coated microparticles (clear cap): Streptavidin-coated microparticles 0.72 mg/mL, preservative.

R1 1 glass, 10 mL Anti-testosterone-Abbiotin (gray cover): 40 ng/mL biotinylated monoclonal anti-testosterone antibody (sheep); 2-bromoestradiol as a releasing reagent; MES buffer 50 mmol/L, pH 6.0; A substance for preserving the solution.

R2 1 vial, 9 mL testosterone-peptideRu(bpy) (black cover): ruthenium compound 1.5 ng/mL testosterone derivative; MES buffer 50 mmol/L, pH 6.0; A substance for preserving the solution.

3.5.1.3 The calculate of results

Each sample analyte concentration (in ng/mL, ng/dL, or nmol/L) is automatically calculated by the analyzer

Transformation constants: ng/mL x 3.47 = nmol/L

ng/mL x 100 = ng/dL

nmol/L x 0.288 = ng/mL

3.5.2 Estradiol III

Estradiol concentration was measured by Cobas e 411 shown in (Table 3.2) which is a fully automatic machine.

3.5.2.1 The principle of working of the test

The full duration of the analysis is only 18 minutes.

- First incubation: Immunocomplexes are produced by incubating the sample (25 µL) with two estradiol-specific biotinylated antibodies, the amount of which is determined by the analyte concentration in the model.
- 2nd incubation: The biotinylated antibodies' still-vacant sites become occupied after streptavidin-coated microparticles and an estradiol derivative tagged with a ruthenium complex is added, resulting in the development of an antibody hapten complex. The interaction of biotin with streptavidin binds the whole complex to the solid phase.
- The reactants are drawn into the cell in which the measurement is made, where the tiny particles are trapped on the electrode roof by magnetism. A ProCell/ProCell M is then used to extract the unbound material. When a voltage is applied to the electrode, a chemical luminous emission is produced, which is detected by a photomultiplier.
- A calibration curve, an instrument created expressly by 2point calibration, and a master curve given by the reagent barcode or barcode are used to determine the results.

3.5.2.2 Used reagents and solutions for analysis

The mark placed on the detector shelf is E2 III.

M1 container, 6.5 mL, streptavidin-coated microparticles (clear cap): 0.72 mg/mL streptavidin-coated microparticles; preservative.

R1 vial, 9mL AntiestradiolAb biotin (gray cap): 2.5 ng/mL and 4.5 ng/mL biotinylated monoclonal anti estradiol antibodies (rabbit); mesterolone 130 ng/mL; MESb) buffer 50 mmol/L, pH 6.0; preservative

R2 bottle, 9 mL estradiol peptide Ru(bpy) (black cap): 4.5 ng/mL ruthenium complex estradiol derivative; MES buffer 50 mmol/L, pH 6.0; preservative (b) MES = 2-morpholino-ethane sulfonic acid.

3.5.2.3 The computation of the results

With the MODULAR ANALYTICS E170, Cobas e 601, and Cobas e 602 analyzers, the analyzer automatically determines the analyte concentration of each sample (either in pmol/L, pg/mL, ng/L, or furthermore in nmol/L)

Transformation constants: pmol/L x 0.272 = pg/mL (ng/L)
pg/mL x 3.67 = pmol/L
pg/mL x 0.00367 = nmol/L

3.5.3 Progesterone III

The blood Progesterone concentration was measured by the Cobas e411 shown in (Table 3.2) which is a fully automatic machine.

3.5.3.1 The principle of working of the test

Rivalry standard is the total term of the assay: 18 minutes.

1st incubation period Immune complexes are produced by incubating the sample (20 µL) with a progesterone-specific biotinylated antibody, the amount of which is depending on the analyte concentration in the sample. The still empty sites of the biotinylated antibodies get occupied with the creation of an antibody hapten complex

with the addition of streptavidin-coated microparticles and a progesterone derivative tagged with a ruthenium complex. The interaction of biotin with streptavidin binds the whole complex to the solid phase. The reaction mixture is sucked into the measurement cell, where the microparticles are trapped magnetically on the electrode's surface. ProCell/ProCell M is then used to remove any unbound compounds. A photomultiplier detects chemiluminescent light when a voltage is applied to the electrode. A calibration curve, an instrument particularly created by 2-point calibration, is used to determine results, and a master curve is given by the reagent barcode or barcode.
(a) Tris (2, 2'-bipyridyl) Ruthenium (II)-complex (Ru) (bpy).

3.5.3.2 Used reagents and solutions for analysis

The mark placed on the detector shelf is PROG III.
M 1 Container, 6.5 mL, Streptavidin-coated microparticles (transparent cap): Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1 1 Bottle, 10 mL Anti-progesterone-Abbiotin (gray cap): Anti-progesterone monoclonal antibody biotinylated (Recombinant, Sheep) 30 ng/mL, 25 mmol/L phosphate buffer, and pH 7.0; preservative.
R2 1 vial, 9 mL progesterone-peptideRu(bpy) (black cap): 2 ng/mL progesterone (from plants) combined with a synthetic peptide tagged with ruthenium complex; phosphate buffer 25 mmol/L, pH 7.0; preservative.

3.5.3.3 The computation of the results

Each sample's analyte concentration is calculated automatically by the analyzer. (Either in nmol/L, ng/mL, or in µg/L).
Conversion constants: nmol/L x 0.314 = ng/mL (µg/L)

Ng/mL x 3.18 = nmol/L

3.5.4 Vitamin D3

The serum of vitamin D3 concentration was measured by Cobas e411 shown in (Table 3.2) which is a fully automatic machine.

3.5.4.1 Analysis principle

The full time to complete the analysis is only 27 minutes

▪ 1st incubation: The sample (15µL) is incubated with pretreatment reagents 1 and 2 to release bound vitamin D (25OH) from the vitamin D binding protein.

▪ 2nd incubation: A complex between vitamin D (25OH) and the runny late vitamin D binding protein is produced by incubating the pretreatment sample with the ruthenium labeled vitamin D binding protein.

▪ 3rd incubation: Unbound ruthenium labeled vitamin D binding proteins becomes occupied with the addition of streptavidin-coated microparticles and vitamin D (25OH) tagged with biotin. The runny late vitamin D binding protein and biotinylated vitamin D (25OH) create a complex that interacts with biotin and streptavidin to become bound to the solid phase.

▪The reaction mixture is Enter into the measurement cell, where the microparticles are caught magnetically on the electrode's surface. ProCell/ProCell M is then used to remove any unbound compounds. When a voltage is applied to the electrode, chemiluminescent emission is induced, which is detected by a photomultiplier.

▪The results are determined by using a calibration curve, which is equipment created particularly for 2-point calibration, and a master curve is given by the reagent barcode or barcode.

3.5.4.2 Used reagents and solutions for analysis

VITDT is the label for the reagent rack pack (M, R1, and R2) and the pretreatment reagents (PT1, PT2).

PT1 1 vial, 4 mL pretreatment reagent 1 (white cover): 1 g/L dithiothreitol, pH 5.5

PT2 1 bottle, 4 mL pretreatment reagent 2 (gray cover): Sodium hydroxide 55 g/L.

M 1 container, 6.5 mL, Streptavidin-coated microparticles (transparent cover): Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 1 vial, 9 mL vitamin D binding protein BPRu (gray cap): 150 g/L ruthenium labeled vitamin D binding protein; 200 mmol/L bis-tris propane buffer; 25 g/L human albumins; pH 7.5; preservative

R2 1 vial, 8.5 mL, 25 hydroxyvitamin Dbiotin (black cover): 14 g/L biotinylated vitamin D (25OH); 200 mmol/L bistris propane buffer; pH 8.6; preservative.

3.5.4.3 The computation of the results

Each sample's analyte concentration (in ng/mL or nmol/L) is automatically calculated by the analyzer

Conversion constant: nmol/L x 0.40 = ng/mL

ng/mL x 2.50 = nmol/L

3.5.5 Calcium gen.2

The blood Calcium concentration was measured by the Cobas c 111 shown in (Table 3.2), a fully automatic machine.

3.5.5.1 Analysis principle

Under alkaline circumstances, calcium ions form a compound with 5-nitro-5'-methyl-BAPTA (NM-BAPTA). In the second stage, this complex interacts with EDTA.

$$Ca^{+2} + \text{NM-BAPTA} \xrightarrow{\text{alkalin}} Ca^{+2}\text{-NM-BAPTA complex} \quad (3.1)$$

$$Ca^{+2}\text{-NM-BAPTA complex} + \text{EDT} \longrightarrow \text{NM-BAPTA} + Ca^{+2}\text{-EDTA complex} \quad (3.2)$$

The change in absorbance is photometrically recorded and is directly proportional to the calcium content.

3.5.5.2 Used reagents and solutions for analysis

R1 CAPSO:[a]) 557 mmol/L; NM-BAPTA: 2 mmol/L, pH 10.0; non-reactive surfactant; preservative

SR EDTA: 7.5 mmol/L, pH 7.3; non-reactive surfactant; preservative

(a) 3-[cyclohexylamino]-2-hydroxy-1-propanesulfonic acid.

3.5.5.3 The computation of the results

The analyte concentration of each sample is automatically calculated by the Cobas c 111 analyzers.

Conversion factor: mmol/L x 4.01 = mg/dL

In studies with 24-hour urine, multiply the value obtained by the 24-hour volume in order to obtain a measurement in mg/24 h or mmol/24 h.

3.5.6 Magnesium

The blood magnesium concentration was measured by the Cobas c 111 shown in (Table 3.2), a fully automatic machine.

3.5.6.1 Test principle

Chlorophosphonazo III (CPZ III) binds to magnesium and produces a rise in absorbance in the colorimetric technique. EGTA (ethylenebis(oxyethylenenitrilo)tetra-acetic acid) is used to inhibit calcium binding to Chlorophosphonazo III.

$$Mg^{+2} + \text{CPZ III} \xrightarrow{\text{pH 75}} \text{Mg-CPZ III complex} \qquad (3.3)$$

The addition of EDTA (ethylenediaminetetraacetic acid), which eliminates magnesium from the magnesium-CPZ III complex and enables correct sample blank measurement, reduces nonspecific absorbance interferences.

$$\text{Mg-CPZ III complex} + \text{EDTA} \xrightarrow{\text{pH 7.5}} \text{CPZ III} + \text{Mg-EDTA} \qquad (3.4)$$

The absorbance difference between the magnesium-CPZ III combination and the EDTA-treated complex is attributable to the magnesium alone.

3.5.6.2 Used reagents and solutions for analysis

R1 TES[a]: pH 7.5, 145 mmol/L, 0.2 mmol/L chlorophosphonazo III, 10 mmol/L EGTA, nonreactive surfactant, preservatives.

SR 100 mmol/L TES; pH 7.5; 16 mmol/L EDTA; nonreactive surfactant; preservatives (a) N-[Tris(hydroxymethyl)-methyl]-2-aminoethanesulfonic acid.

3.5.6.3 The computation of the results

The analyte concentration of each sample is automatically calculated by the Cobas c 111 analyzers. Conversion factors: mmol/L x 2.43 = mg/dL

mval/L x 0.5 = mmol/L

mval/L x 1.22 = mg/Dl

mval/L = mEq/L

3.5.7 Phosphate ver.2

The blood Phosphate concentration was measured by the Cobas c 111 shown in (Table 3.2), a fully automatic machine.

3.5.7.1 Test principles

Endpoint method with sample blanking, In the presence of sulfuric acid, inorganic phosphate forms an ammonium phosphomolybdate complex with ammonium molybdate with the formula $(NH_4)_3[PO_4(MoO_3)_{12}]$.

$$\text{Phosphate + ammonium molybdate} \xrightarrow{H_2SO_4} \text{ammonium phosphomolybdate complex} \qquad (3.5)$$

The amount of phosphomolybdate produced is proportional to the amount of inorganic phosphate present and maybe quantified photometrically.

3.5.7.2 Used reagents and solutions for analysis

R1 0.36 mol/L sulfuric acid; detergent

SR 3.5 mmol/L ammonium molybdate; 0.36 mol/L sulfuric acid; 150 mmol/L sodium chloride.

3.5.7.3 The computation of the results

The analyte concentration of each sample is automatically calculated by the Cobas c 111 analyzers.
Conversion factor: mmol/L x 3.10 = mg/d

3.5.8 Uric acid ver.2

The blood Uric acid concentration was measured by the Cobas c 111 shown in (Table 3.2), a fully automatic machine.

3.5.8.1. Analysis principle

Colorimetric enzymatic test Uricase degrades uric acid, releasing allantoin and hydrogen peroxide in the process.

$$\text{Uric acid} + 2\,H_2O + O_2 \xrightarrow{\text{uricase}} \text{allantoin} + CO_2 + H_2O_2 \quad (3.6)$$

$$2\,H_2O_2 + H^+ + \text{TOOS}^a + \text{4-aminophenazone} \xrightarrow{\text{peroxidase}} \text{Quinone-diimine dye} + 4H_2O \quad (3.7)$$

(a) N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methyl aniline, the color intensity of the quinone-diimine produced is related to the amount of uric acid present and may be measured by measuring the rise in absorbance.

3.5.8.2 Reagents - working solutions

R1 TOOS: 7 mmol/L; fatty alcohol polyglycol ether: 4.8 percent; ascorbate oxidase (EC 1.10.3.3: zucchini): 2 83.5 ukat/L (25 °C); stabilizers, preservative
SR 4 aminophenazone: 22.5 mmol/L; uricase (EC 1.7.3.3; Arthrobacter protophormiae): 283.4 ukat/L (25 °C); peroxidase (POD) (EC 1.11.1.7; horseradish): 2 50.0 ukat/L (25 °C); stabilizers; preservative.

3.5.8.3 Calculation

The Cobas c111 analyzer determines the analyte concentration of each sample automatically.

Conversion factors: umol/L < 0.0168 = mg/dL

mg/dL x 59.5 = umol/L

mg/dL < 0.059 = mmol/L

3.6 Statistical Analysis

The concentrations of testosterone, progesterone, estradiol, vitamin D3, calcium, magnesium, phosphorous and uric acid in the patient's blood serum (and also the control group) were measured and reported as Mean ±S.E. (Standard error) using [SPSS 26.0] program, where this program was used in all comparisons made between samples by independent T-test. In the study, a P-value of less than 0.05 was considered statistically significant.

4. RESULTS

In the present study, a total of 140 individuals was enrolled viz. patient (n=70) and control (n=70). Both the groups were further distributed into two groups, i.e., smoking group and the non-smoking group.

4.1 Calculation of Serum Parameters in Smokers and Non-Smokers

Table 4.1 depicts the comparison between various serum parameters in smokers and non-smokers individuals. Serum testosterone ($p \leq 0.05$), estradiol, magnesium, vitamin D3, and uric acid were found to be decreased in the smokers as compared to non-smokers. The progesterone and phosphate were increased in the smokers as compared to non-smokers. Serum calcium was found to be comparable in the smoking and non-smoking individuals.

Table 4.1 Variation of serum parameters between smoking and non-smoking individuals

Variables	Non-smoking(n=35) Mean±S.E	Smoking (n=35) Mean±S.E	P - value
Testosterone (ng/ml)	3.15±0.07	3.44±0.4**[1]	0.002
Progesterone (ng/ml)	0.46±0.03	0.61±0.04**	0.003
Estradiol (pg/ml)	17.89±0.86	16.00±0.58	0.075
Calcium (mg/dl)	9.24±0.12	9.25±0.07	0.974
Magnesium (mg/dl)	2.6±0.08	2.25±0.07	0.008
Phosphate (mg/dl)	2.75±0.05	3.01±0.10	0.034
Vitamin D3 (mg/dl)	16.45±.54	15.10±0.50	0.073
Uric Acid (mg/dl)	5.53±0.28	4.68±0.14	0.009

[1] **P≤0.01

4.2 Estimate of Serum Parameters in the Smokers' Collection

4.2.1 Analysis based on the control and patient of smoking individuals' group

Table 4.2 represents the serum parameters of the enrolled individuals (n=70). The patients showed decreased in the serum testosterone ($p \leq 0.001$), estradiol ($p \leq 0.05$), calcium, magnesium content ($p \leq 0.001$), phosphate ions ($p \leq 0.05$), and vitamin D3 content as compared to control individuals. While the concentration of progesterone ($p \leq 0.05$) increased uric acid in the patient's serum compared to the control serum.

Table 4.2 The enrolled participants' serum parameters

Variables	Control (n=35) Mean±S.E	Patient (n=35) Mean±S.E	P - value
Testosterone (ng/ml)	5.39±0.19	3.44±0.04***[1]	<0.001
Progesterone (ng/ml)	0.49±0.03	0.61±0.04*[2]	0.016
Estradiol (pg/ml)	32.68±0.69	16.00±0.58*	<0.001
Calcium (mg/dl)	9.29±0.08	9.25±0.07	0.656
Magnesium (mg/dl)	4.85±0.12	2.25±0.07***	<0.001
Phosphate (mg/dl)	4.16±0.11	2.75±0.05*	<0.011
Vitamin D3 (mg/dl)	17.20±0.62	16.90±0.50	0.402
Uric Acid (mg/dl)	4.52±0.13	4.68±0.14	0.446

4.2.2 Comparative analysis based on individuals' age

The enrolled individuals were further grouped on the basis of their age, i.e., age below 45 years (Group 1) and individuals above 45 years till 65 years (Group 2).

4.2.2.1 Comparative analysis between two groups

Serum parameters of group 1 and group 2 are represented in the (Table 4.3). All the serum parameters of control individuals in both the groups showed non-significant variations. In the Group 1, serum of patient individuals showed decrease in testosterone ($p \leq 0.001$), estradiol ($p \leq 0.001$), magnesium ($p \leq 0.001$), phosphate ($p \leq 0.001$), vitamin

[1] ***P≤0.001
[2] *P≤0.05

D3, calcium and uric acid values as compared to the control individuals. Serum progesterone level was increased in the patients as compared to control group.

In the group 2, similar results were observed in all serum parameters except calcium, and uric acid. Serum calcium is high in patients when compared with the control group, but there is no significant in this increase (p-value = 0.417). Serum uric acid was found to be significantly increased in the patients as compared to control.

Table 4.3 Group 1 and Group 2 smoking participants' serum parameters

	Group 1			**Group 2**		
Variables	**Control (n=12) Mean±S.E**	**Patient (n=12) Mean±S.E**	**P value**	**Control (n=23) Mean±S.E**	**Patient (n=23) Mean±S.E**	**P value**
Testosterone (ng/ml)	5.57±0.35	3.61±0.05***	0.001	5.31±0.21	3.35±0.04***	0.001
Progesterone (ng/ml)	0.46±0.03	0.57±0.05	0.125	0.50±0.04	0.64±0.05	0.055
Estradiol (ng/ml)	32.45±1.22	13.95±1.15***	<0.001	32.79±0.85	17.07±0.55***	0.001
Calcium (mg/dl)	9.34±0.14	9.00±0.11	0.068	9.27±0.09	9.38±0.08	0.417
Magnesium (mg/dl)	4.75±0.22	2.27±0.13***	0.001	4.90±0.15	2.25±0.08***	<0.001
Phosphate (mg/dl)	4.66±0.19	2.67±0.06***	0.001	3.91±0.09	2.80±0.71***	<0.001
Vitamin D3 (mg/dl)	17.36±1.15	16.14±1.17	0.458	17.11±0.74	16.86±0.45	0.396
Uric Acid (mg/dl)	4.15±0.23	4.27±0.25	0.814	4.61±0.16	4.89±0.16	0.240

4.2.2.2 Comparison of various serum parameters of patients between group 1 and 2

The serum of patients enrolled in group 2 showed significantly increased estradiol ($p \leq 0.01$), calcium ($p \leq 0.05$), and uric acid ($p \leq 0.05$) levels in a contrast to the patients enrolled in group 1. However, testosterone ($p \leq 0.05$) level was found to be decreased. The levels of the rest of the other parameters were similar between the group 1and the group 2 (Table 4.4).

Table 4.4 Serum parameters of patients between group 1 and 2 of smoking individuals

	Group 1	Group 2	
Variables	**(n=12) Mean±S.E**	**(n=23) Mean±S.E**	**P – value**
Testosterone (ng/ml)	3.61±0.05	3.35±0.04**	0.002
Progesterone (ng/ml)	0.57±0.05	0.64±0.05	0.410
Estradiol (pg/ml)	13.95±1.15	17.07±0.55**	0.009
Calcium (mg/dl)	9.00±0.11	9.38±0.08*	0.013
Magnesium (mg/dl)	2.27±0.13	2.25±0.08	0.898
Phosphate (mg/dl)	2.67±0.06	2.80±0.71	0.238
Vitamin D3 (mg/dl)	16.14±1.15	16.86±0.45	0.140
Uric Acid (mg/dl)	4.27±0.25	4.89±0.16*	0.040

4.2.3 Comparative analysis based on body mass index (BMI) of enrolled individuals

4.2.3.1 Comparative analysis between normal and overweight

Serum parameters of normal and overweight individuals are represented in (Table 4.5). In the normal-weight patient group, serum testosterone ($p \leq 0.001$), estradiol ($p \leq 0.001$), calcium, magnesium ($p \leq 0.001$), phosphate ($p \leq 0.001$), uric acid, and vitamin D3 were decreased as compared to the respective group in charge. Serum progesterone was non-considerable increased in the patients of normal weight as compared to its control.

The overweight patient showed a significantly ($p \leq 0.001$) decreased serum testosterone, estradiol, magnesium, and phosphate as compared to the respective control. Serum progesterone ($p \leq 0.01$) and uric acid were increased in the patient group of normal weight as compared to its control group. Serum calcium level was comparable with each other in the control and patient group of overweight individuals. Serum vitamin D3 level decreased in comparison to the respective healthy men.

Table 4.5 Serum parameters of normal and overweight individuals

Variables	Normal Weight			Overweight		
	Control (n=08) Mean±S.E	Patient (n=08) Mean±S.E	P value	Control (n=27) Mean±S.E	Patient (n=27) Mean±S.E	P value
Testosterone (ng/ml)	5.42±0.33	3.67±0.05***	0.001	5.39±0.22	3.37±0.04***	<0.001
Progesterone (ng/ml)	0.47±0.09	0.49±0.03	0.904	0.49±0.30	0.65±0.05**	0.008
Estradiol(pg/ml)	32.12±1.59	16.87±1.15***	0.001	32.83±0.77	15.74±0.67***	0.001
Calcium (mg/dl)	9.43±0.13	9.13±0.16	0.174	9.25±0.08	9.28±0.08	0.814
Magnesium (mg/dl)	4.51±0.15	2.70±0.10***	<0.001	4.94±0.14	2.12±0.07***	0.001
Phosphate (mg/dl)	4.31±0.27	2.89±0.09***	0.001	4.12±0.11	2.71±0.05***	0.001
Vitamin D3 (mg/dl)	18.98±1.33	18.12±0.47	0.622	18.67±0.69	17.87±0.44	0.610
Uric Acid (mg/dl)	4.04±0.23	4.15±0.22	0.296	4.56±0.16	4.86±0.15	0.193

4.2.3.2 Comparison of body mass index (BMI) in the patient group

Serum parameter comparison between normal weight and overweight patients is depicted in table (4.6). The patients enrolled in the overweight group showed a significant increase in serum uric acid ($p \leq 0.05$) level as a comparison to the patients with normal weight. Overweight patients showed a significant decrease in serum testosterone ($p \leq 0.01$), and magnesium ($p \leq 0.001$), as a comparison to the normal-weight patients. Other serum parameters were non-significantly different from each other.

Table 4.6 Serum parameter comparison between normal weight and overweight patients

Variables	Normal weight (n=8)	Overweight (n=27)	p value
Testosterone (ng/ml)	3.67±0.05	3.37±0.04**	0.002
Progesterone (ng/ml)	0.49±0.03	0.65±0.05	0.091
Estradiol (pg/ml)	16.87±1.15	15.74±0.67	0.424
Calcium (mg/dl)	9.13±0.16	9.28±0.08	0.397
Magnesium (mg/dl)	2.70±0.10	2.12±0.07***	<0.001
Phosphate (mg/dl)	2.89±0.09	2.71±0.05	0.152
Vitamin D3 (mg/dl)	18.12±0.47	17.87±0.44	0.360
Uric Acid (mg/dl)	4.15±0.22	4.86±0.15*	0.015

4.2.4 Correlations among the studied parameters

Table 4.7 depicts the correlation of testosterone, BMI, progesterone, estradiol, calcium, magnesium, phosphate, vitamin D3, and uric acid in the smoking individuals. Vitamin D3 showed positive correlation with BMI (P≤0.01) and testosterone (P≤0.05). While, it showed negative correlation with uric acid (P≤0.01). The BMI recorded negative correlation with testosterone (P≤0.05), phosphate (P≤0.01), and magnesium (P≤0.01). The testosterone showed adverse relationship with progesterone (P≤0.01). The calcium showed positive correlation with magnesium (P≤0.05) and phosphate (P≤0.01). The magnesium displayed a plus relationship with phosphate (P≤0.01).

Table 4.7 Correlations among the studied parameters in smoking individual

Variables	**Vit D3[1]**	**BMI**	**Testo[2]**	**Prog[3]**	**E2[4]**	**Ca[5]**	**Mg[6]**	**PO_4[7]**	**UA[8]**
Vit D3	1								
BMI	-0.587**	1							
	0.001								
Testo	0.404*	-0.367*	1						
	0.016	0.030							
Prog	-0.011-	0.271	-0.548**	1					
	0.952	0.115	0.001						
E2	0.279	-0.154	-0.302	0.074	1				
	0.105	0.378	0.078	0.674					
Ca	-0.316-	-0.127	-0.186	-0.061	-0.049	1			
	0.065	0.467	0.286	0.729	0.778				
Mg	0.320	-0.790**	0.268	-0.330	-0.036	0.389*	1		
	0.061	0.000	0.120	0.053	0.837	0.021			
PO_4	0.229	-0.551**	0.118	-0.175	-0.069	0.523**	.488**	1	
	0.185	0.001	0.501	0.315	0.694	0.001	0.003		

[1] Vitamin D3
[2] Testosterone
[3] Progesterone
[4] Estradiol
[5] Calcium
[6] Magnesium
[7] Phosphate
[8] Uric acid

Table **4.7**(continue)

UA	-0.448**	0.167	-0.278	0.248	-0.083	0.140	-0.208	-0.061	1
	0.007	0.337	0.106	.150	0.634	0.421	0.230	0.730	

4.2.4.1 Correlation between testosterone and other parameters

By studying the correlation between testosterone and other variables in smoking patients, it was found that testosterone showed a negative correlation with calcium, as shown in (Figure 4.1), testosterone showed a negative correlation with uric acid, as shown in (Figure 4.2), while testosterone showed a positive correlation With magnesium as shown in (Figure 3.4), it also showed a positive correlation with phosphorous as in (Figure 4.4), and testosterone showed a positive correlation with Vitamin D3 as shown in (Figure 4.5).

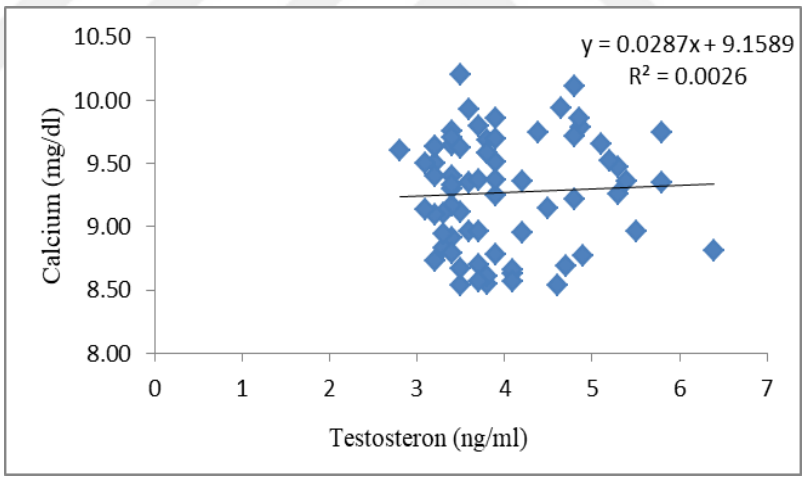


Figure 4.1 Correlation between testosterone and calcium in smoking individual

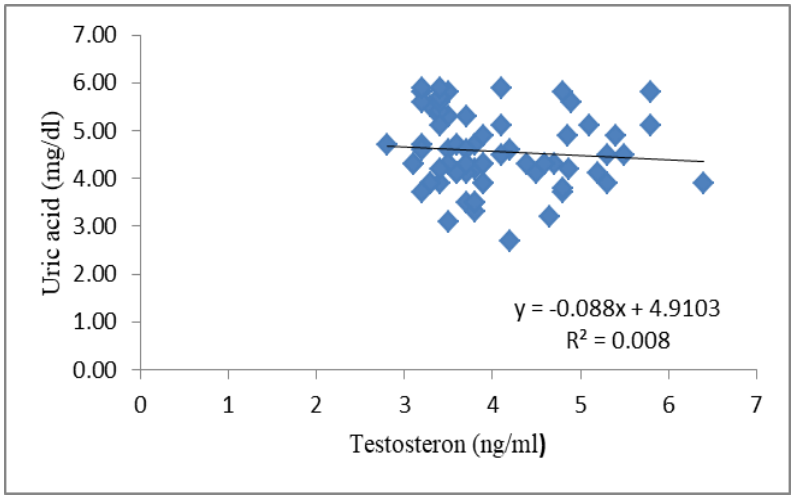


Figure 4.2 Correlation between testosterone and uric acid in smoking individuals

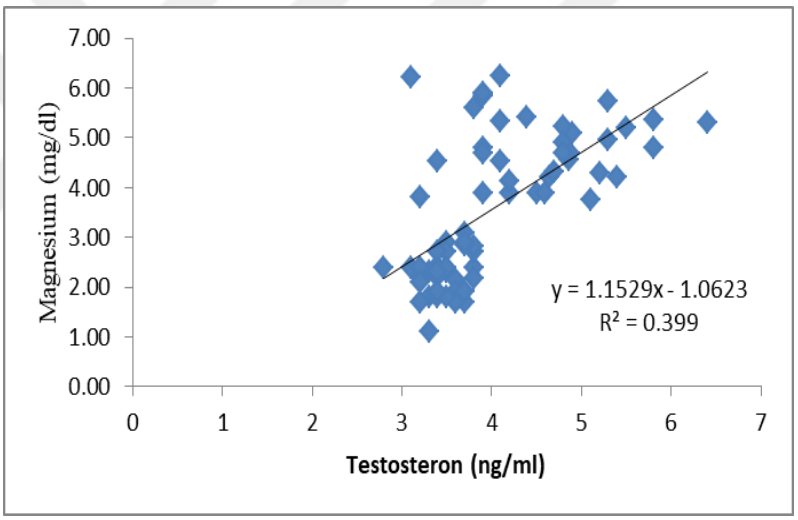


Figure 4.3 Correlation between testosterone and magnesium in smoking individuals

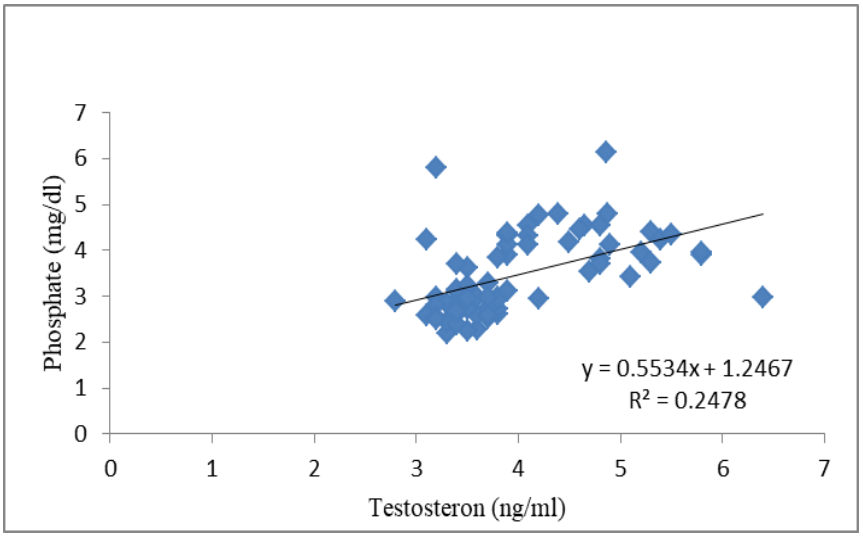


Figure 4.4 Correlation between testosterone and phosphate in smoking individuals

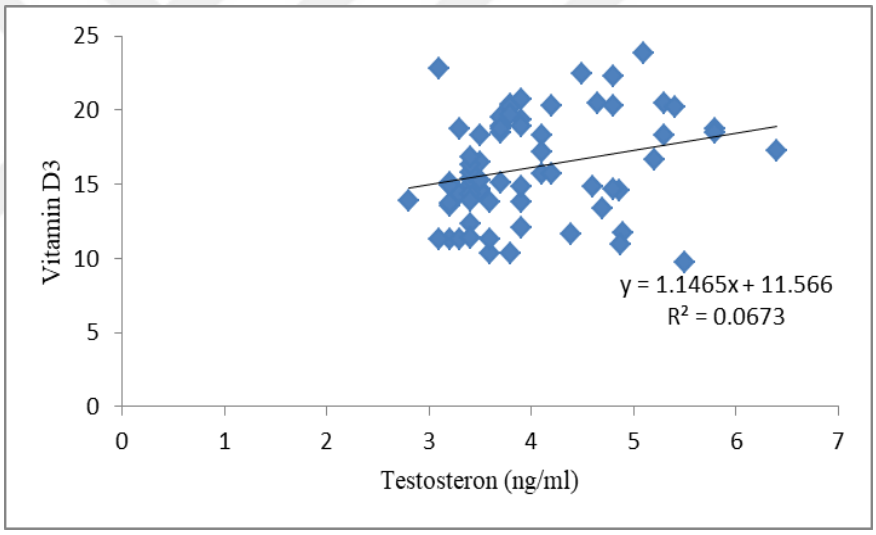


Figure 4.5 Correlation between testosterone and vitamin D3 in smoking individuals

4.2.4.2 Correlation between Estradiol and other parameters

By studying the association between estradiol hormone and other variables in smoking patients, it was found that estradiol showed a negative correlation with calcium, as shown in (Figure 4.6), estradiol showed a negative correlation with uric acid, as shown in (Figure 4.7), it showed Estradiol showed a negative correlation with magnesium as shown in (Figure 4.8), and estradiol also showed a negative correlation with

phosphorous as in (Figure 4.9), and estradiol showed a positive correlation with vitamin D3 as shown in (Figure 4.10).

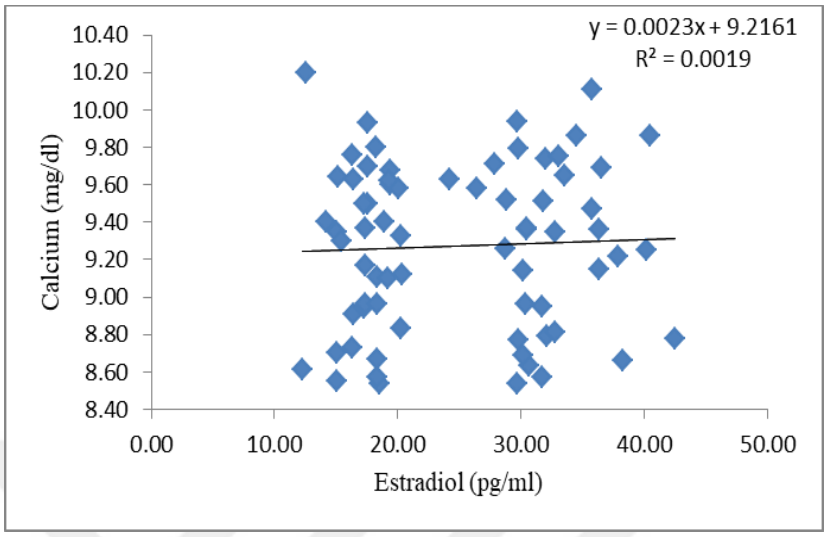


Figure 4.6 Correlation between estradiol and calcium in smoking individuals

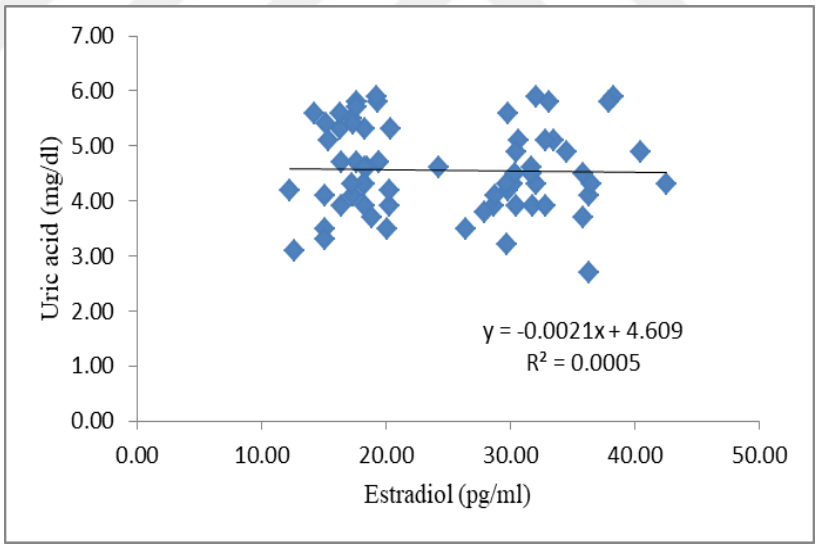


Figure 4.7 Correlation between estradiol and uric acid in smoking individuals

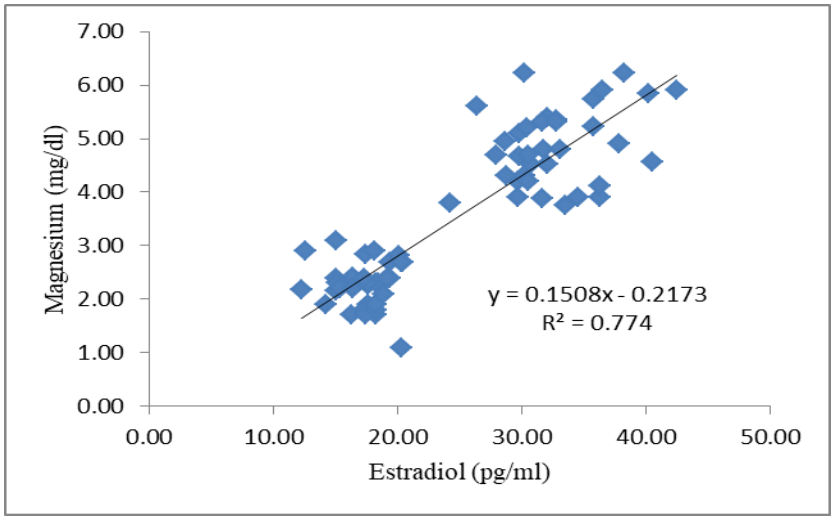


Figure 4.8 Correlation between estradiol and magnesium in smoking individuals

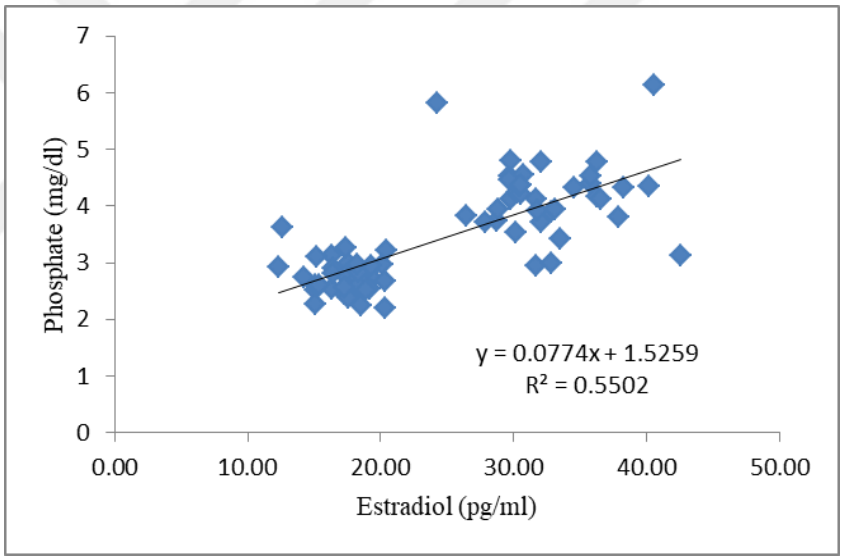


Figure 4.9 Correlation between estradiol and phosphate in smoking individuals

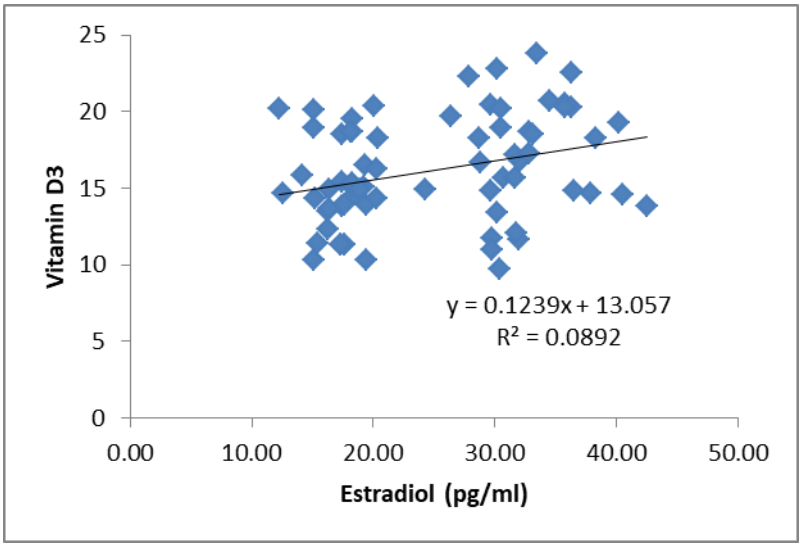


Figure 4.10 Correlation between estradiol and vitamin D3 in smoking individuals

4.3 Estimation of Serum Parameters in Non-Smoking Individuals

4.3.1 Analysis based on the control and patient of non-smoking individuals' group

Table (4.8) represents the serum parameters of the enrolled individuals (n=70). The patients showed decreased in the serum testosterone ($p \leq 0.001$), progesterone, estradiol ($p \leq 0.001$), calcium, magnesium content ($p \leq 0.001$), phosphate ions ($p \leq 0.001$), and vitamin D3 content as compared to control individuals. While, uric acid ($p \leq 0.05$) was increased in the patient serum as compared to the control serum.

Table 4.8 Serum parameters control and patients enrolled in the non-smoking group

Variables	Control (n=35)	Patient (n=35)	p-value
Testosterone(ng/ml)	5.24±0.14	3.15±0.07***	<0.001
Progesterone(ng/ml)	0.53±0.03	0.46±0.03	0.084
Estradiol (ng/ml)	30.58±1.05	17.89±0.86***	<0.001
Calcium (mg/dl)	9.31±0.10	9.24±0.12	0.679
Magnesium (mg/dl)	5.18±0.11	2.56±0.08***	<0.001
Phosphate (mg/dl)	3.96±0.05	3.01±0.10***	<0.001
Vitamin D3 (mg/dl)	18.70±0.82	17.45±0.54	0.325
Uric acid (mg/dl)	4.75±0.11	5.53±0.28*	0.013

4.3.2 Comparative analysis based on individuals' age

4.3.2.1 Comparative analysis between two groups

Serum parameters of the two groups are represented in the (Table 4.9). All the serum parameters of control individuals in both the groups showed non-significant variations. In the Group 1, serum of patient individuals showed a decrease in testosterone ($p \leq 0.001$), progesterone, estradiol ($p \leq 0.001$), calcium content, phosphate content ($p \leq 0.001$), magnesium content ($p \leq 0.001$), and vitamin D3, values as opposed to the control individuals. Serum uric acid ($p \leq 0.05$) level was increased in the patients as opposed to the healthy group. In the group 2, similar results were observed in all serum parameters except calcium. There was no elevation in serum calcium levels in the patient as a comparison to the control.

Table 4.9 Serum parameters of the two groups of non-smoking individuals

	Group 1			**Group 2**		
	Control (n=18)	**Patient (n=19)**	**P value**	**Control (n=17)**	**Patient (n=16)**	**P value**
Testosterone (ng/ml)	5.60±0.20	3.25±0.07***	<0.001	4.85±0.15	3.08±0.12***	<0.001
Progesterone (ng/ml)	0.52±0.04	0.46±0.05	0.420	0.55±0.04	0.45±0.02	0.067
Estradiol (pg/ml)	30.35±1.75	16.15±1.44***	<0.001	30.83±0.50	19.94±0.50***	<0.001
Calcium (mg/dl)	9.47±0.16	9.22±0.20	0.344	9.14±0.11	9.27±0.11	0.401
Magnesium (mg/dl)	5.01±0.16	2.53±0.12***	<0.001	5.37±0.14	2.59±0.11***	<0.001
Phosphate (mg/dl)	3.87±0.06	3.06±0.19***	<0.001	4.07±0.08	2.95±0.08***	<0.001
Vitamin D3 (mg/dl)	17.49±1.12	16.47±0.86	0.474	17.17±1.18	16.44±0.92	0.402
Uric acid (mg/dl)	4.50±0.17	5.70±0.49*	0.032	5.02±0.11	5.33±0.18	0.179

4.3.2.2 Comparison of various serum parameters of patients between group 1 and 2

Table 4.10 represents the serum parameters of group 1 and group 2 of non-smoking individuals. The patients enrolled in the group 2 showed increased serum testosterone, uric acid and estradiol ($p \leq 0.05$) levels as compared to the patients enrolled in the group 1. However, serum phosphate content was found to be decreased in the group 2 as compared to group 1. Serum progesterone, calcium content, magnesium content, and vitamin D3 showed comparable levels with each other in group 1 and 2.

Table 4.10 Serum parameters of patients between group 1 and 2 of non-smoking individuals

Variables	Group 1 (n=19)	Group 2 (n=16)	P value
Testosterone (ng/ml)	3.25±0.08	3.08±0.12	0.287
Progesterone (ng/ml)	0.46±0.05	0.45±0.26	0.923
Estradiol (pg/ml)	16.15±1.44	19.95±0.50*	0.027
Calcium (mg/dl)	9.22±0.20	9.27±0.11	0.820
Magnesium (mg/dl)	2.53±0.12	2.59±0.11	0.740
Phosphate (mg/dl)	3.06±0.17	2.95±0.08	0.605
Vitamin D3 (mg/dl)	16.47±0.86	16.44±0.92	0.979
Uric Acid (mg/dl)	5.71±0.49	5.33±0.19	0.518

4.3.3 Comparative analysis based on BMI of enrolled individuals.

4.3.3.1 Comparative analysis between normal and overweight.

Serum parameters of group 1 and 2 individuals are represented in the (Table 4.11). In the normal weight patient group, serum testosterone ($p \leq 0.001$), progesterone, phosphate ($p \leq 0.01$), estradiol ($p \leq 0.001$), magnesium ($p \leq 0.001$), and uric acid were decreased as compared to the respective control group. Serum calcium content and vitamin D3 level were non-significantly increased in the patient of normal weight as compared to its control.

The overweight patient showed decrease in serum testosterone ($p \leq 0.001$), progesterone, estradiol ($p \leq 0.001$), magnesium ($p \leq 0.001$), and phosphate ($p \leq 0.001$) as compared to the

respective control. Serum uric acid ($p \leq 0.01$) was increased in the patient group of normal weight as compared to its control. Serum calcium level and vitamin D3 were comparable with each other in the control and patient group of overweight individuals.

Table 4.11 Comparison of serum parameters in normal-weight and overweight individuals' patients

Variables	Normal Weight			Overweight		
	Control (n=07)	**Patient (n=07)**	**P value**	**Control (n=28)**	**Patient (n=28)**	**P value**
Testosterone (ng/ml)	5.62±0.32	3.29±0.19***	0.001	5.14±0.16	3.12±0.08***	0.001
Progesterone (ng/ml)	0.50±0.07	0.47±0.06	0.758	0.55±0.04	0.45±0.03	0.084
Estradiol (ng/ml)	31.30±1.28	18.16±2.26***	0.001	30.40±1.29	17.82±0.95***	0.001
Calcium (mg/dl)	9.08±0.22	9.18±0.13	0.697	9.36±0.11	9.26±0.15	0.567
Magnesium (mg/dl)	5.37±0.22	3.13±0.12***	0.001	5.14±0.13	2.42±0.08***	<0.001
Phosphate (mg/dl)	4.03±0.19	3.19±0.14**	0.004	3.95±0.05	2.96±0.12***	<0.001
Vitamin D3 (mg/dl)	18.97±1.81	18.83±1.36	0.461	19.02±0.91	18.67±0.56	0.410
Uric Acid (mg/dl)	4.54±0.22	4.77±0.32	0.672	4.71±0.12	5.72±0.33**	0.007

4.3.3.2 Comparison of BMI in the patient group

Serum parameter comparison between normal weight and overweight patients is depicted in the (Table 4.12). The patients enrolled in the overweight group showed significant increase in serum uric acid and calcium level as a comparison to the patients with normal weight. Overweight patients showed significant decreased in serum testosterone, estradiol, magnesium ($p \leq 0.001$), and phosphate as compared to the normal weight patients. Serum progesterone and vitamin D3 were non-significantly differs with each other in both the groups.

Table 4.12 Comparison of serum parameters of normal-weight patients and are obese patients

Variables	Normal weight (n=7)	Overweight (n=28)	p value
Testosterone (ng/ml)	3.29±0.19	3.12±0.08	0.381
Progesterone (ng/ml)	0.47±0.06	0.46±0.04	0.863
Estradiol (pg/ml)	18.15±2.26	17.82±0.94	0.880
Calcium (mg/dl)	9.18±0.13	9.26±0.14	0.817
Magnesium (mg/dl)	3.13±0.11	2.42±0.08***	<0.001
Phosphate (mg/dl)	3.19±0.14	2.96±0.12	0.418
Vitamin D3 (mg/dl)	18.83±1.36	18.67±0.56	0.834
Uric Acid (mg/dl)	4.77±0.32	5.72±0.34	0.181

4.3.4 Correlations among the studied parameters in non-smoking individuals

Table 4.13 depicts the correlation of testosterone, BMI, progesterone, estradiol, calcium, magnesium, phosphate, vitamin D3, and uric acid in the non-smoking individuals. Vitamin D3 showed negative correlation with uric acid ($p \leq 0.05$) and estradiol ($p \leq 0.05$). BMI showed a negative association with magnesium ($p \leq 0.01$). The testosterone showed positive correlation with estradiol ($p \leq 0.01$) and a negative relationship with phosphate ($p \leq 0.01$). The estradiol showed a negative association with calcium ($p \leq 0.05$) and phosphate ($p \leq 0.05$). The phosphate content showed negative correlation with uric acid ($p \leq 0.05$).

Table 4.13 Correlations among the studied parameters in non-smoking individuals

Variables	D 3	BMI	Testo	Prog	E2	Ca	Mg	PO_4	UA
Vit D3	1	-0.267	-0.162	0.064	-0.366*	-0.019	.270	.177	-.388*
		0.121	0.353	0.716	0.030	0.914	.117	.308	.021
BMI		1	-0.009	.039	0.269	-0.075	-0.590**	-.295	.063
			0.960	.822	.119	.667	.000	.085	.719
Testo			1	.059	.555**	-.025	.126	-.477**	.091
				.736	.001	.888	.472	.004	.604
Prog				1	.035	-.105	-.002	-.029	-.010
					.842	.549	.993	.870	.954
E2					1	-.342*	.084	-.420*	.182
						.044	.632	.012	.297

Table 4.13 (continue)

Ca						1	-.262	-.044	.311
							.129	.800	.069
Mg							1	.191	-.289
								.272	.092
PO_4								1	-.369*
									.029
UA									1

4.3.4.1 Correlation between testosterone and other parameters in non-smoking individuals.

By studying the correlation between testosterone hormone and other variables in non-smoking patients, it was found that testosterone showed a negative correlation with calcium, as shown in (Figure 4.11), and testosterone showed a positive correlation with uric acid, as shown in (Figure 4.12), it showed Testosterone showed a positive correlation with magnesium as shown in (Figure 4.13), and testosterone showed a negative correlation with phosphorous as in (Figure 4.14), and testosterone showed a negative correlation with Vitamin D3 as shown in (Figure 4.15).

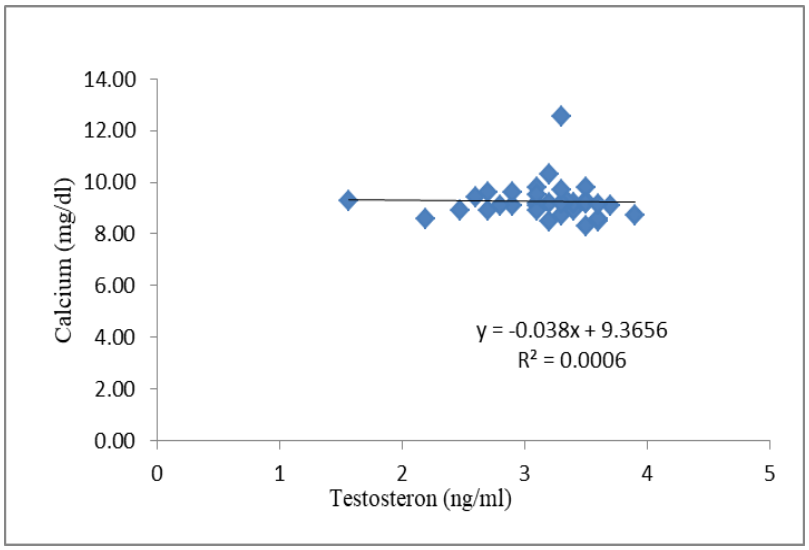


Figure 4.11 Correlation between testosterone and calcium in non-smoking individuals

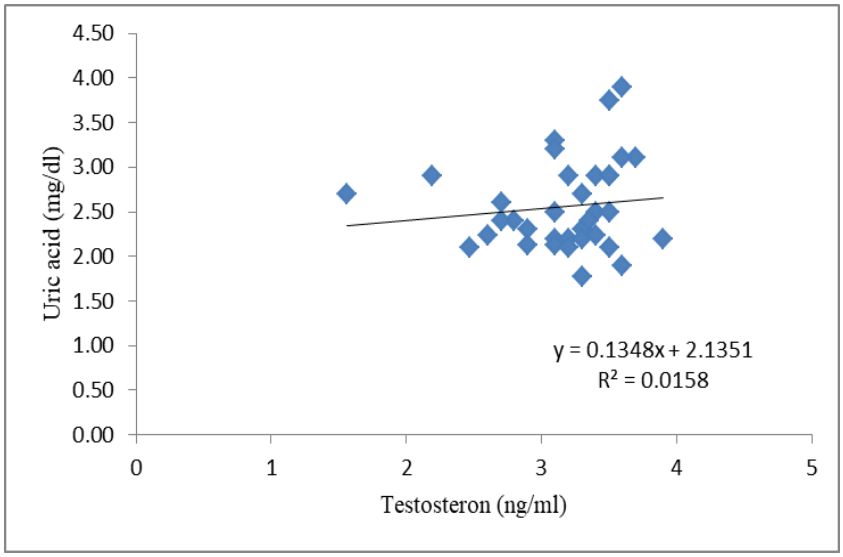


Figure 4.12 Correlation between testosterone and uric acid in non-smoking individuals

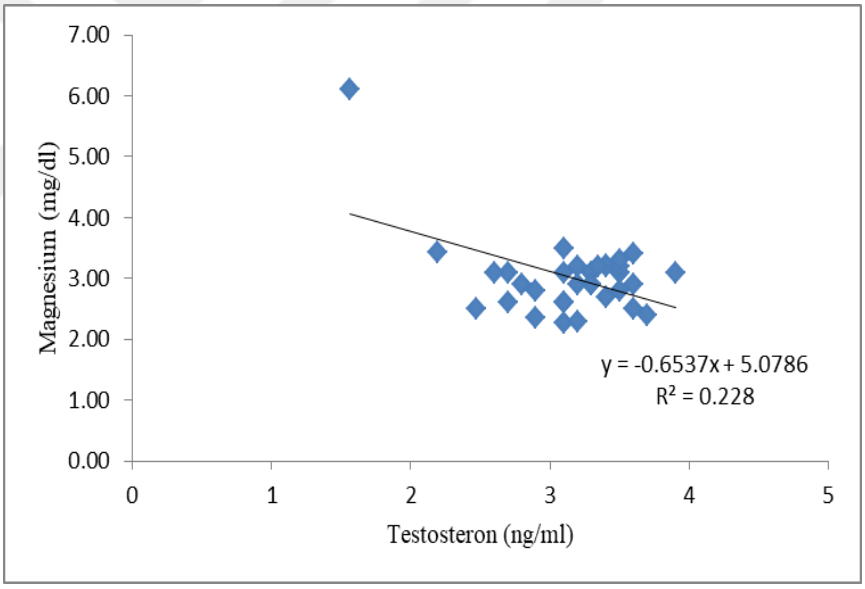


Figure 4.13 Correlation between testosterone and magnesium in non-smoking individuals

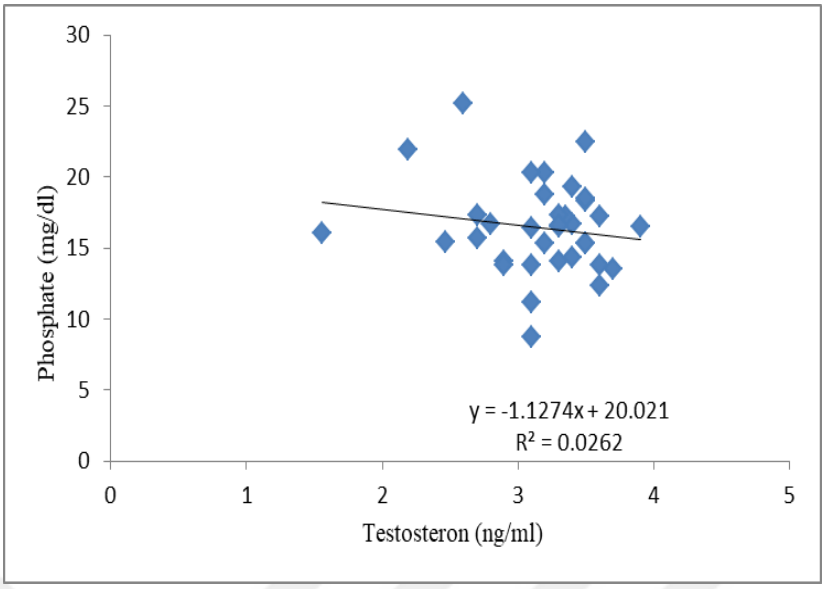


Figure 4.14 Correlation between testosterone and phosphate in non-smoking individuals

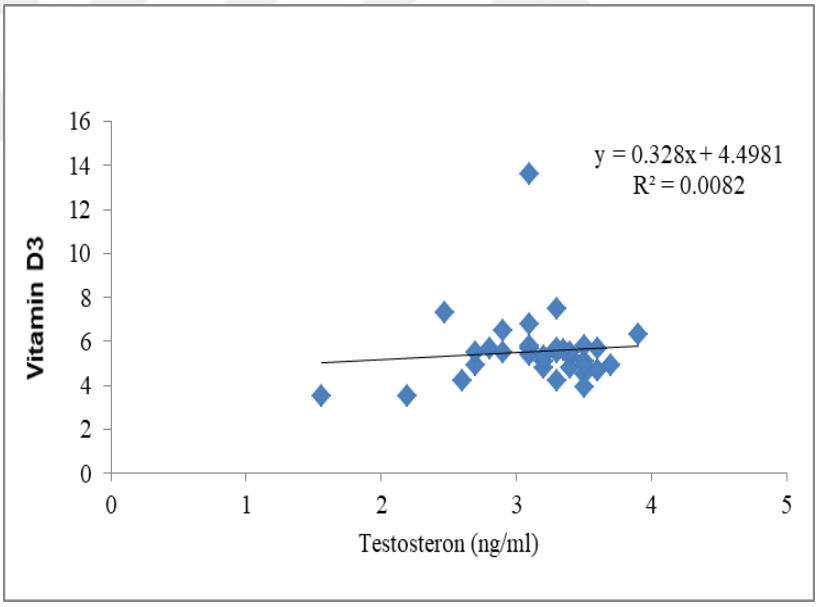


Figure 4.15 Correlation between testosterone and vitamin D3 in non-smoking individuals

4.3.4.2 Correlation between Estradiol and other parameters in Non-smoking individuals

By studying the correlation between estradiol hormone and other variables in non-smoking patients, it was found that estradiol showed a negative correlation with

calcium, as shown in (Figure 4.16), and estradiol showed a negative correlation with uric acid, as shown in (Figure 4.17), it showed estradiol showed a positive correlation with magnesium as shown in (Figure 4.18), and estradiol showed a negative correlation with phosphorous as in (Figure 4.19), and estradiol showed a negative correlation with Vitamin D3 as shown in (Figure 4.20).

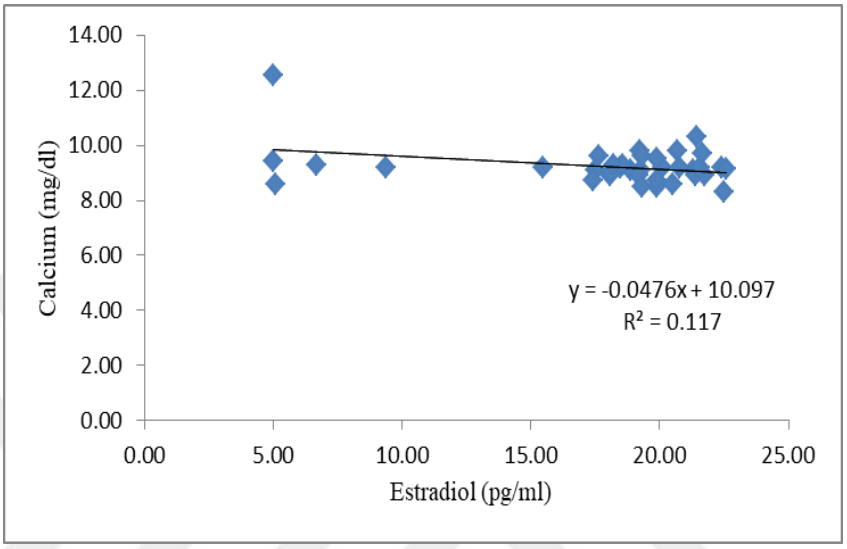


Figure 4.16 Correlation between estradiol and calcium in non-smoking individuals

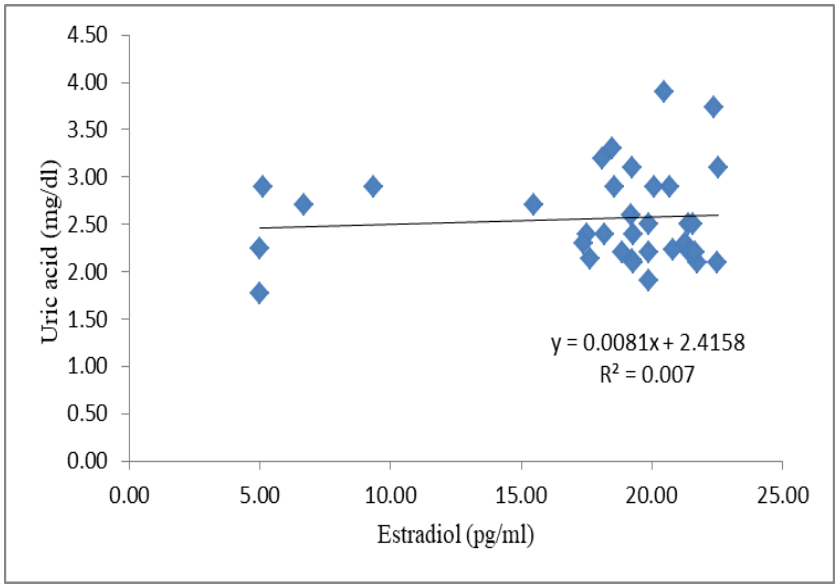


Figure 4.17 Correlation between estradiol and uric acid in non-smoking individuals

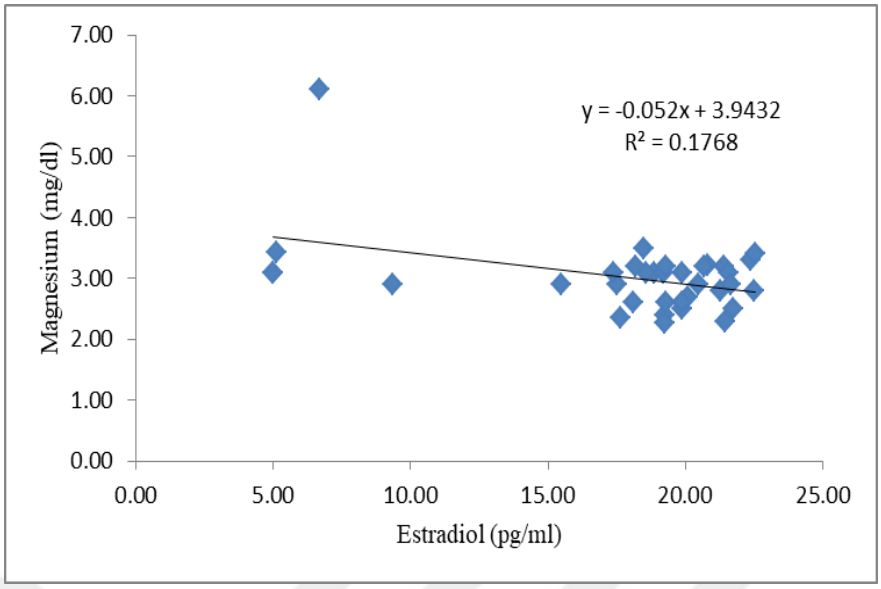


Figure 4.18 Correlation between estradiol and magnesium in non-smoking individuals

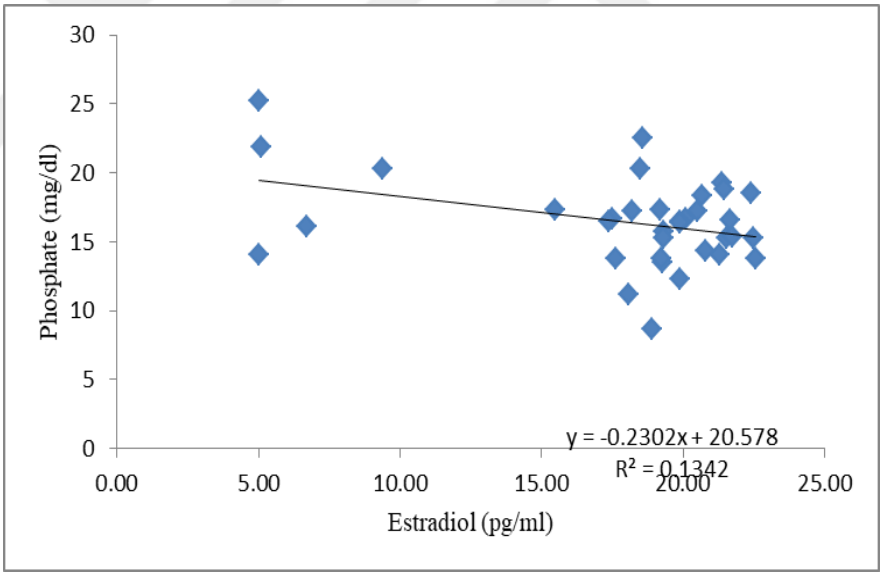


Figure 4.19 Correlation between estradiol and phosphate in non-smoking individuals

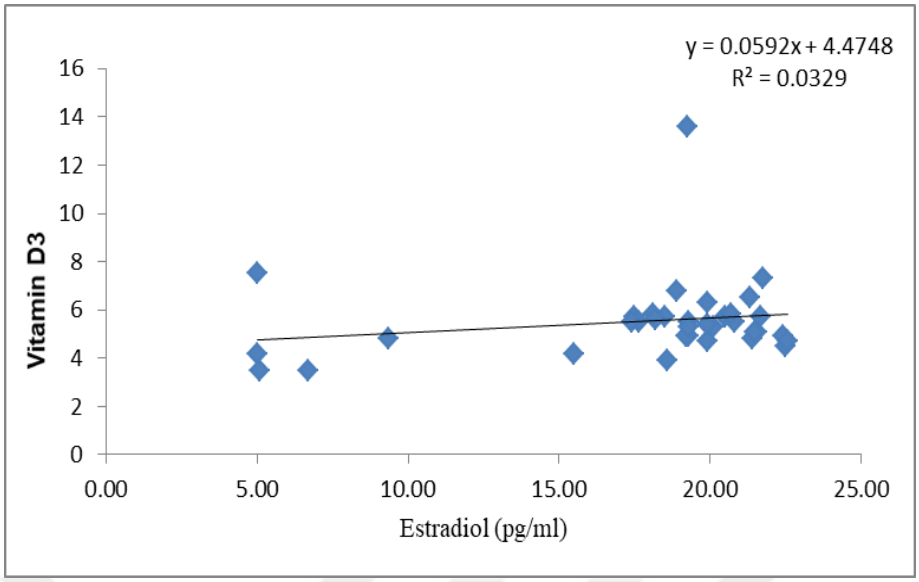


Figure 4.20 Correlation between estradiol and vitamin D3 in non-smoking individuals

5. DISCUSSION

From the last few years, kidney stone has been steadily increasing global prevalence. It is assumed to increase the risk of chronic kidney diseases. Generally, a kidney stone happens when urine has insufficient fluid. Due to less fluid in urine, high concentration of minerals and distinct substances like calcium, uric acid and oxalate accumulate and come together to form small tiny crystals. These tiny crystals are difficult to detect at an early stage, hence it results in stones with severe throbbing pain. The kidney stones can very tiny as grain size that can pass unconsciously through urine without ever knowing. Furthermore, if the size is increased, it can block the normal urine flow causing severe pain. Few patients say the pain can be as agonizing as childbirth. Different parameters are responsible for kidney stones like ones eating habits, consumption of less liquid; prolong medications, or genetic disorder in rare cases. Different types of stones are caused due to different substances. Hence, it is foremost important to know the type of stone one has; therefore, one can identify the root cause and utmost efforts to prevent it. Different minerals and hormones that are responsible for kidney stones are listed below:

5.1 Hormones and Kidney Stone

Serum testosterone levels were significantly associated with higher uric acid urinary excretion in kidney stone patients (Shakhssalim *et al.* 2011). Furthermore, the occurrence of kidney stones was unswervingly more in men than women. Since biologically the men have higher testosterone levels than women (Manuscript 2013). In men, the relationship between testosterone and kidney stones could be elucidated by the calcium oxalate biosynthesis upregulation by an androgen receptor (Li *et al.* 2010, Liang *et al.* 2014), and testosterone might have a biological role in kidney stone formation (Naghii *et al.* 2014), In the study of Majuran Perinpam et al, reported that age affects urinary excretion of factors that are major aspects in the kidney stones development risk (Perinpam 2016), and another study of Jianfeng Zenga et al, disclosed that the majority of patients with kidney stones more than once had an increase in weight (Zeng *et al.* 2019), the study of Roger D's Stanworth and others showed an inverse relationship between age and serum testosterone level (Stanworth and Jones

2008), and the study of M Diaz-Arjonilla et al showed that the increase in BMI has an inverse relationship with serum testosterone (Diaz-Arjonilla *et al.* 2009). The results of the current study follow these studies, where our study showed a decrease in testosterone in general when compared with the control, when comparing the two age groups between them, it was found that there is a decrease in the testosterone hormone in older men and also when comparing patients with a normal body mass index (BMI) with patients who have a high BMI we found that there is a decrease in the testosterone hormone in obese patients, and on although testosterone is generally decreased, which can reduce the incidence of kidney stones, according to a study conducted by Yucel *et al,* decreased testosterone levels in overweight patients may decrease the kidney stones development risk (Yucel *et al.* 2018). However, despite this decrease in the level of testosterone hormone in the study patients, it did not lead to a decrease in the kidney stones incidence, perhaps due to the combined effect of age and BMI, where the proportion of the elderly in this study was 55% and the proportion of patients who had an increase in BMI was 78 %.

Progesterone is involved in the Ca^{2+} uptake by the distal tubule membranes of kidney nephron through a direct non-genomic mechanism (Brunette and Leclerc 2002). In the present study, serum progesterone of smoking patients was found to be increased as compared to non-smoking patients. In non-smoking individuals, BMI is not affecting the progesterone level.

A study conducted by Sirpi Nakkeeran *et al*. stated that the estradiol level is low in kidney stones history patients (Nackeeran *et al.* 2020), and two current studies follow the results of this study, where we found that estradiol was low compared to the control group in all study patients. If estrogen therapy is applied to women in menopause, there are more chances of developing kidney stones. Maalouf et al conducted a comparative study between the two groups, i.e., one group was treated with estrogen therapy and another treated with estrogen plus progestin. The results reveal that estrogen therapy had more chances of kidney stone formation. Hence, it was proved that an excess of estrogen may lead to the formation of kidney stone (Naim *et al.* 2010), Jasuja *et al,* In their research, they reported that estradiol was higher in the elderly than in younger men

(Jasuja *et al.* 2013). Our results are following these two studies and showed that patients with more than 45 years of age had an increase in the level of estradiol compared to the younger age group.

5.2 High Serum Calcium May Lead to Recurrence of Kidney Stone

The kidney stones are of various types such as calcium phosphate, calcium oxalate, uric acid stones, struvite stones, cystine stones, etc. However, calcium oxalate stones are very usual. A higher concentration of calcium in the serum may cause the formation or recurrence of kidney stones (Pabico *et al.* 1983, Sorensen 2014, Han *et al.* 2015). The hormones such as parathyroid hormone are playing a key role in the balancing of ion concentration in the body (Williams *et al.* 1996). Studies reveal that the higher amount of calcium is consumed from dietary products there will be fewer the chances of calcium stones occurrence in the kidney (Worcester *et al.* 2007, Rendina *et al.* 2012). Nevertheless, along with calcium dietary products, if calcium supplements are prolong taken, it is also responsible for kidney stones. Thus, it can be concluded that increasing the kidney stone development risk is majorly due to calcium excess or calcium traces in the urine. This phenomenon is commonly known as hypercalciuria (Worcester *et al.* 2007). The active metabolite such as calcitriol of Vitamin D3 increases calcium absorption. This is directly related to digestive calcium absorption (Rendina *et al.* 2012). Snjezana Milicevic and others reported in their study that most kidney stone patients who do not have hyperthyroidism or malignant disease, do not suffer from an increase in calcium secretion in the blood (Milicevic *et al.* 2014). Eric N. Taylor and others reported in their study that the serum calcium levels were similar to the serum calcium levels of the control group (Taylor *et al.* 2015). The results of our current study are similar with Snjezana Milicevic and Eric N. Taylor, where there was no change in the calcium level of the current study patients with their two categories (smokers and non-smokers) and the calcium level in the control group**.**

5.3 Vitamin D3 is Directly Associated with Stone Formation

In combination with calcium, vitamin D3 also play a vital role in kidney stone development (Ticinesi *et al.* 2016, Letavernier and Michel 2018). Consumption of a high amount of vitamin D3 as a supplement is linked with a higher risk of kidney stone formation (Lung 2006, Malihi *et al.* 2016, Wang *et al.* 2016, Lappe *et al.* 2017). Hu *et al* (Hu *et al.* 2017) reported that the patients with a higher serum 25-hydroxyvitamin D3 level ware also reported to have hypercalciuria. In this study, all patients showed low vitamin D3 levels in the blood compared to the control and these results are similar to the two studies Chrysoula Pipili et al and Ticinesi *et al.* In both studies, there is a prevalence of vitamin D3 deficiency in kidney stone patients (Pipili and Oreopoulos 2012, Ticinesi *et al.* 2016).

5.4 The Uric acid, Magnesium, and Phosphorus Role in Kidney Stone Development.

Increase uric acid in the serum is associated with the formation of kidney stones (Kim *et al.* 2017). Patients suffering from obesity, diabetes, or any other kidney disorder accounting for high urine pH can produce acidic urine (Manish and Stephen 2020). Thus, when uric acid dissolves in urine tiny crystals are formed which leads to the formation of stones. Similarly, in the acidic pH, phosphate is less soluble, so it precipitates in the formation of insoluble ammonium products, yielding a large staghorn stone development (Dursun *et al.* 2015).

Phosphorous is involved in maintaining the balance of calcium along with vitamin D in the blood. It does not directly take part in kidney stones formation. However, phosphorous imbalance with other minerals may contribute to kidney stone formation (Khan *et al.* 2016, Alelign 2018). Lower concentration of serum phosphate in patients may result in the formation of the kidney stone (Williams *et al.* 1996, Worcester *et al.* 2007), Dominique pire *et al.* stated that the decrease in blood phosphate, which is caused by the leakage of phosphate during urine, could cause an increase the serum cholesterol concentration, and that it would lead to an upsurge in calcium secretion in

the urine to the point of saturation, which leads to the formation of calcium stones (Prié *et al.* 2001) . Our results are in accordance with these reports. In the present study, patients showed a lower concentration of serum phosphate.

(Kim et al. 2017) Studied Korean adults cohort and published the association between serum uric acid levels and kidney stones incident risk. In this study, the ultrasonographic examination was performed for the identification of kidney stones. The authors found that in the men, new kidney stone formation was directly associated with the increased serum uric acid concentration with the 70% higher risk in the highest category. However, no such association was found in the women. Our results are following these reports. In the present study, serum uric acid concentration was higher in the patient as compared to their control. The serum uric acid was more in the patient with age above 45 years. However, a similar trend was observed in the smoking and non-smoking individuals.

The lower magnesium content in urine may lead to lithogenic conditions, whereas a higher concentration is linked with a lower kidney stone risk (Chaudhary *et al.* 2010). Magnesium regulates the dissolution of calcium in the blood resulting in a lower kidney stone recurrence risk. Besides, magnesium citrate is also paramount important in reducing the risk of kidney stone formation. A study conducted in rural northeastern Thailand reports that magnesium deficiency is common among kidney stone patients. After magnesium supplementation, urinary citrate excretion was increased which indicates that magnesium affects citrate metabolism and thus may cause kidney stones formation (Reungjui *et al.* 2002). Our results are in the same line with these reports. In the present study, all patients showed lower levels of serum magnesium as compared to the control individuals. This may be one of the causes of the formation of kidney stones.

6. CONCLUSIONS

1- There is a clear decrease in the level of both testosterone and estradiol, and this decrease had a significant statistical effect.

2- There was a significant positive correlation between age, BMI, and risk of developing kidney stones

3- Calcium has no clear statistical relationship with the development of kidney stones

4- Progesterone did not show any statistical effect on the forming of kidney stones

5- that is a significant decrease in the level of vitamin D3.

6- It was found that there was a rise in the level of uric acid in the serum of blood of patients compared to the level of uric acid in the control group.

7- The low level of phosphorus may be connected with the forming of kidney stones

8- Lower serum magnesium concentration in patients may be linked with the danger of developing kidney stones.

7. RECOMMENDATIONS

1- There is a need to raise the number of studied samples and increase the period of study since we do not have a database on this disease so that we can understand the impact of sex hormones on patients with kidney stones.

2- Patients who suffer from malignant diseases or hyperthyroidism should be studied in conjunction with kidney stones so that we can know the impact of these diseases on patients with kidney stones in our society.

3- A 24 urine test should be done on each patient with kidney stones to find out the concentrations of phosphorous, magnesium, uric acid, and calcium, besides measuring the pH of urine to see the extent of their positive or negative impact on patients with this disease.

4- The prevailing diet in our society must be studied and the extent of its impact on the kidneys, in general, must be studied.

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CURRICULUM VITAE

Personal Information

Name and Surname : Karrar Kadhim Hussein ARAJ

Education

Undergraduate	Al Muthanna university College of science Department of Chemistry	(2010 -2011)