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The role of trace and toxic elements in productive of female with polycystic ovary syndrome in AL-Nasiriyah

A thesis

Submitted to The Council of The College of
Science, University of Thi-Qar
in Partial Fulfillment of The Requirement For
The Degree of Master of Science in chemistry

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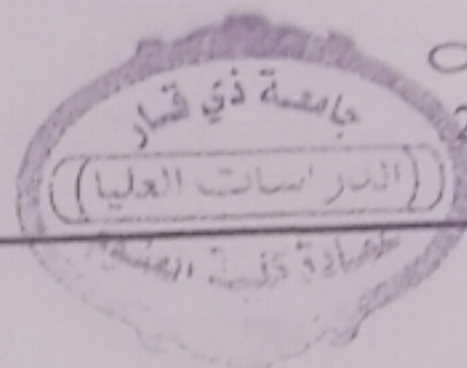
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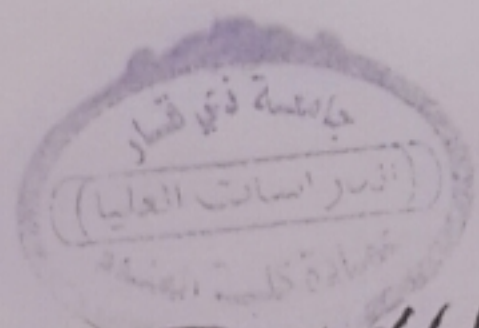
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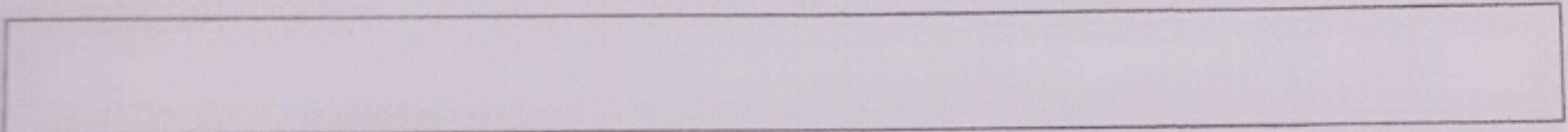
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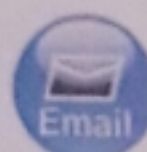
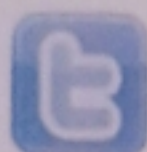
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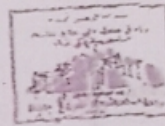
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productive life In polycystic ovary syndrome female
in AL-Nasiriyah





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كلية العلوم

استمارة تعهد الالتزام
بأخلاقيات البحث العلمي

وزارة التعليم العالي والبحث العلمي
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تتمتع بالتفويض التام بما جاء في مضمون كتاب وزارة التعليم العالي والبحث العلمي/جهاز الاشراف والتفويض المرقم ج 425/الصادر بتاريخ 2014/3/24، والمبلغ اليها بكتاب رئاسة جامعة ذي قار/قسم البحث والتطوير المرقم 4704/54/7 والصادر بتاريخ 2014/3/31، عند اعداد الرسالة أو الاطروحة الموسومة:

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- المصطلح العلمي هو ورقة نتائج فكري، وعند الاقتباس ضرورة الإشارة للمصدر، وفي حالات الاقتباس ضرورة إعادة صياغة الفقرة المستنبطة.
- نون الخروج من حدود فكرة الفقرة الأصلية المقبولة والمشار إليها في متن الرسالة أو الأطروحة.
- فصل استمارة التمهيد هذه الرميائل والأطاريح التي لم تتجس حتى تاريخ كلاب جهاز الاشراف والتقويم أدناه.
- أحتت استمارة التمهيد هذه استناداً للفقرة 4- من كلاب وزارة التعليم العالي والبحث العلمي/جهاز الاشراف والتقويم المرقم ج ت/25/ المصدر بتاريخ 2014/3/24 والمبلغ إلينا عن طريق كلاب رئاسة جامعة ذي قار/قسم البحث والتطوير المرقم 4704/54/7 بتاريخ 2014/3/31.

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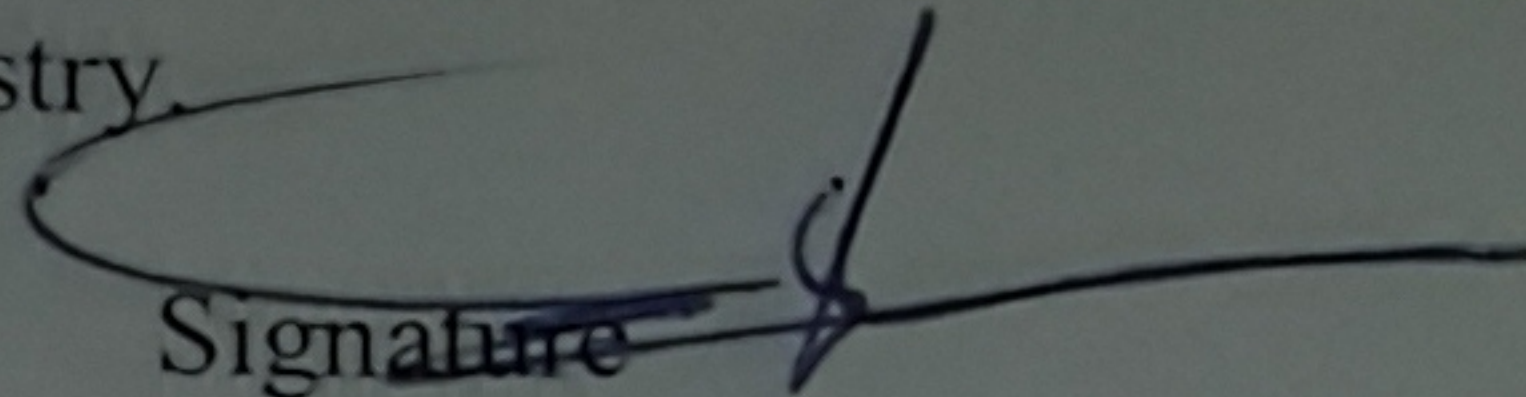
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We the examining committee ,after reading this thesis "**The Role Of Trace And Toxic Elements In Productive Of Female With Polycystic Ovary Syndrome In Al-Nasiriyah**" and examining the student **Nabaa Neama Hameed** in its content, find that it is qualified for pursuing the degree of master of science in chemistry.

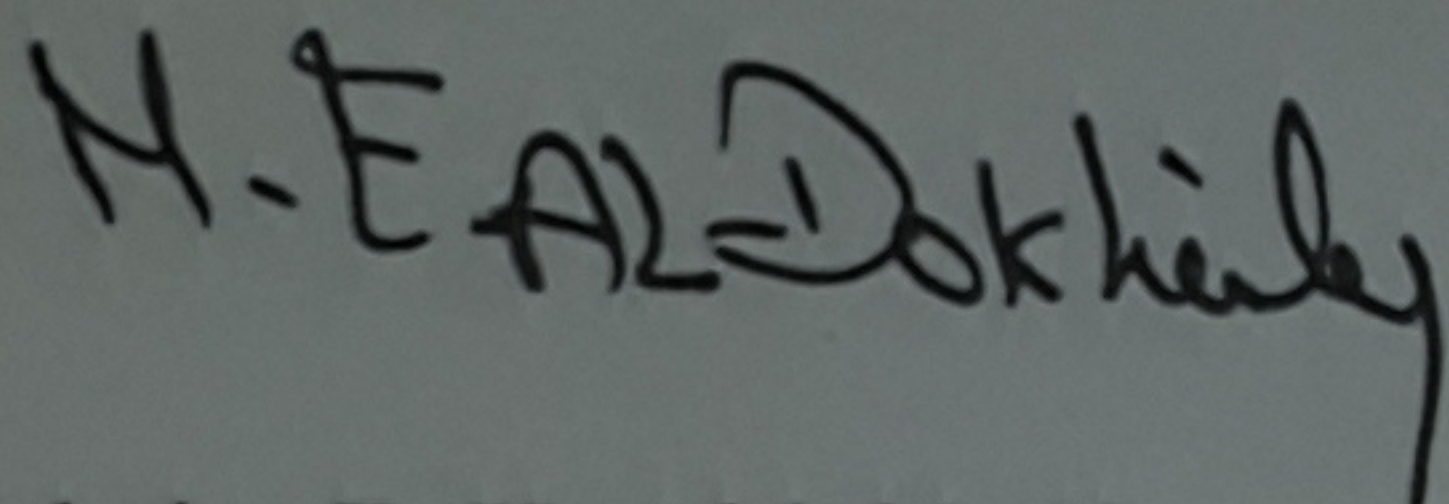

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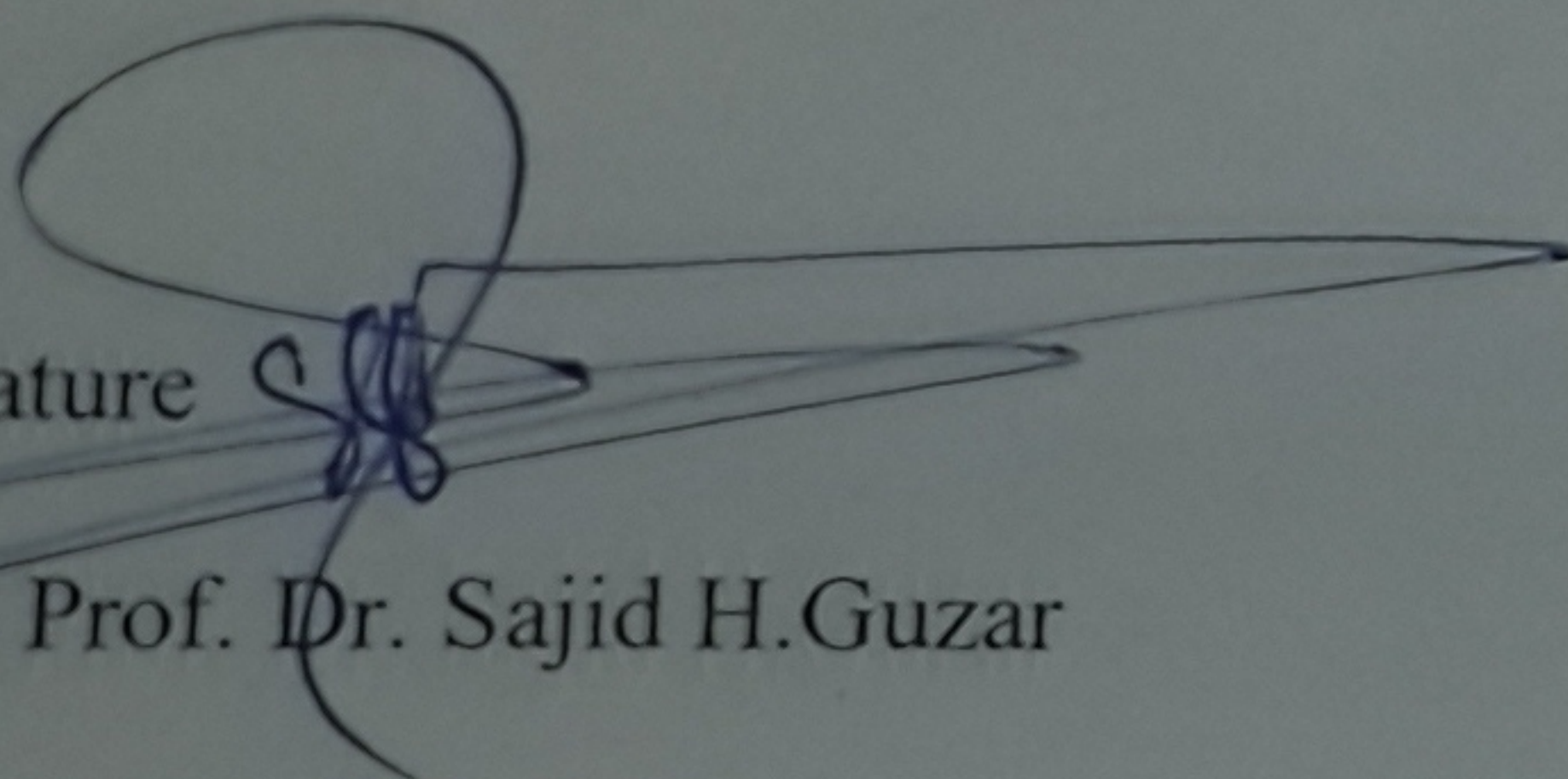


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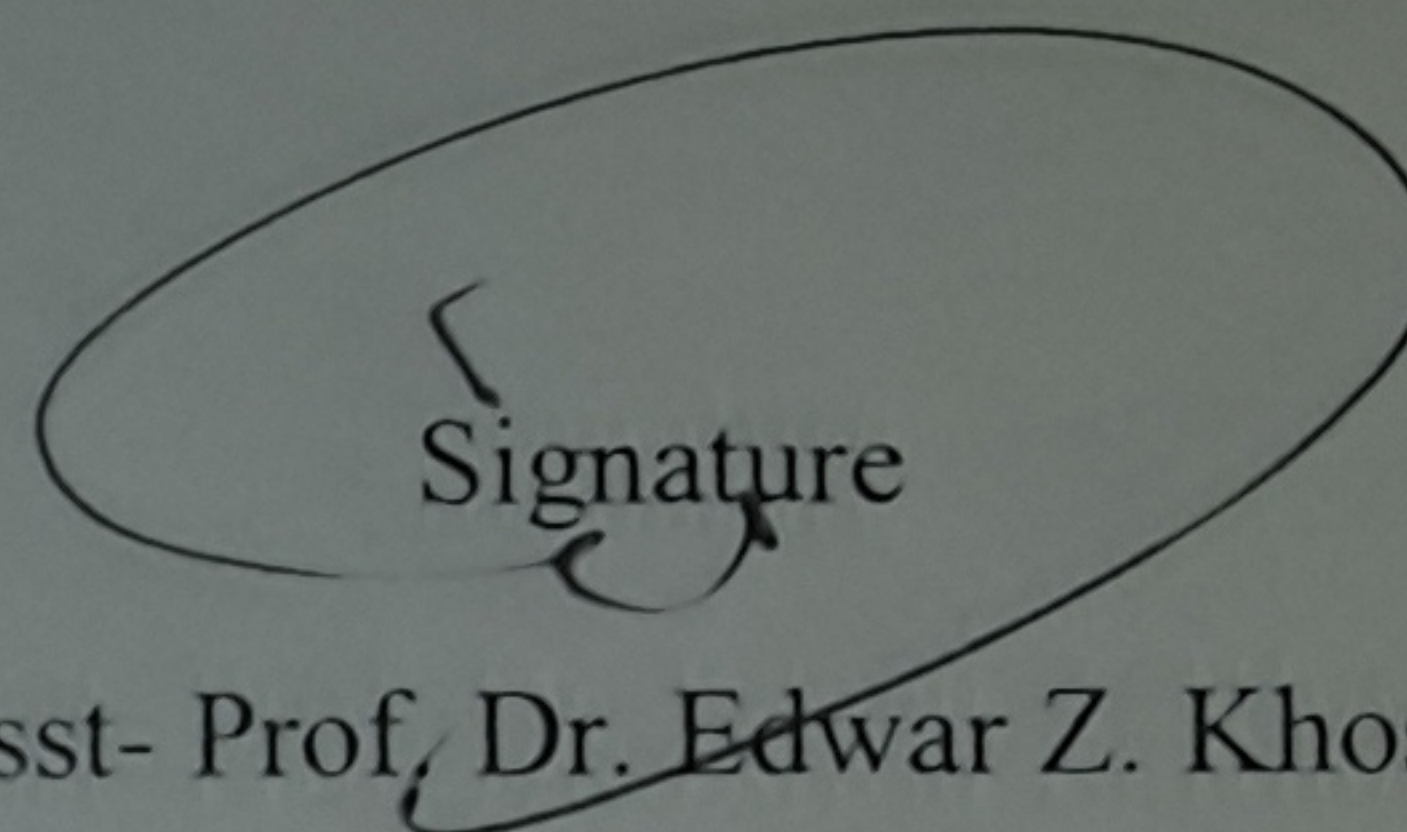


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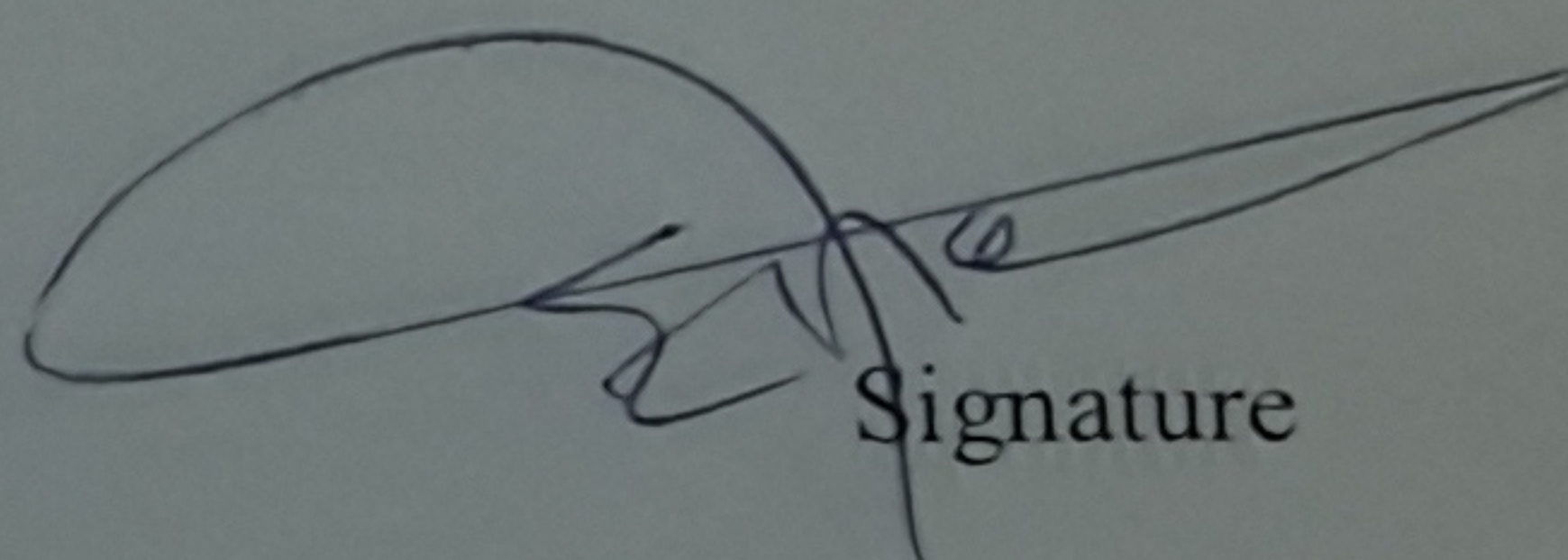


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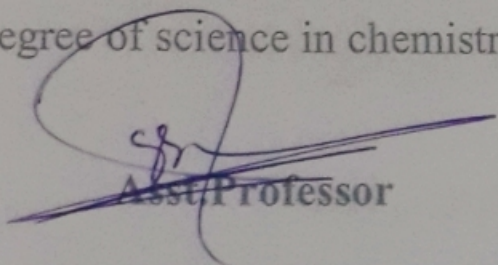
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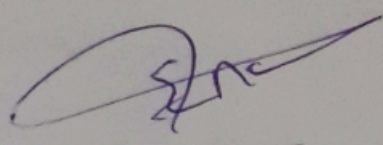
CERTIFICAT

We certify that this thesis, the role of trace and toxic element in productive of female with polycystic ovary syndrom in AL-Nasiriyah by Nabaa Neema Hameed is under our supervision at the Department of Chemistry, College of Science , University of Thi-Qar (Iraq) in partial fulfilment of the requirements for the Master degree of science in chemistry.



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Dr. Sajid Hassan Guzar



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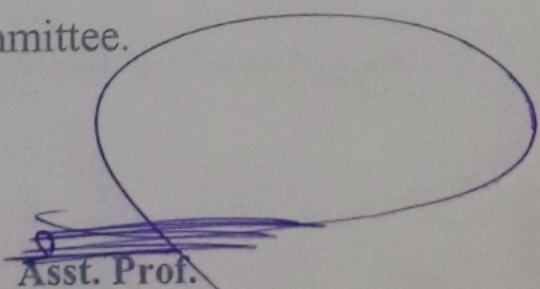
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In view of the available recommendation, I forward this thesis for debate by the examining committee.



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الاهداء

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الى كل من ساعدني ووقف الى جانبي

اضع بين ايديهم هذا الجهد المتواضع

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Abbreviations

AAS	Atomic Absorption Spectroscopy
AMH	Anti-Mullerian hormone
BMI	Body mass index
CV	Cardiovascular
E2	Follicular phase estradiol
FSH	Follicle-stimulating hormone
PID	pelvic inflammatory disease
GH	Growth hormone
HIV	Human Immunodeficiency Virus
HDLs	High-density lipoproteins
IR	Insulin resistance
LH	Luteinizing hormone
LSD	Low density lipoproteins
NIDDM	Noninsulin- dependent diabetes mellitus
No	Number
PCOS	Polycystic ovary syndrome
SHBG	Sex-hormone-binding-globulin
STD	Sexually transmitted disease
SD	Standard Deviation

TT	Testosterone
T2DM	Type2 diabetes mellitus
TSH	Thyroid stimulating hormone
TG	Triglycerides
ROS	Reactive oxygen species

Summary

The present study is designed to role of trace elements (Zn, Co, Cd, Fe, Cr, pb, Ni, Cu) in PCO patient. Measuring of trace elements in patients and control serum .Samples were collected from a total of 120 (total 70 patients and 50 control). After statistical analysis was found that both the concentration of copper, nickel, lead, cadmium and iron in patients levels were significant increase in (Ni, Pb, Fe, Cu and Cd) than control group ,also significant decrease in concentration of serum (Zn and Cr)in patient and control group while in no significance in the concentration of serum Co between patient and control groups , levels of trace element in case classification based on body mass index into three group(es)(non obese , obese and over wight) found the same of result trace element concentration according to age (Ni, Fe,Cd ,Cu, Pb) higher significant while (Zn ,Cr) lower significant and Co non significant between patient and control group(es) . In partition according to waist is found increase in elements (Ni ,Cd,Pb, Fe,Cu) while decrease in elements (Zn,Cr) and Co is no different in patient and control group(es) . These results were based on mean , standard deviation and LSD below the level of probability 0.05 These result can change with environmental changes and dietary habits .

CHAPTER

ONE

INTRODUCION

1- INTRODUCTION

1.1: Polycystic ovary syndrome (PCOS)

Is the most frequent endocrine disorder in women of reproductive age with heterogeneous clinical and biochemical features ^(1,2). The diverse and complex female endocrine disorder PCOS affects 1 in 15 women worldwide ⁽³⁾. PCOS produces symptoms in approximately 5% to 10% of women of reproductive age 12–45 years old ⁽⁴⁾. The major characteristics of the syndrome are chronic anovulation, menstrual abnormalities (oligomenorrhea or amenorrhea), hyper androgen, and PCO appearance using ultrasound ⁽⁵⁾.

PCOS is associated with obesity, (IR), and increased risk of (T2DM), dyslipidemia, cardiovascular disease, and endometrial carcinoma and elevated (TT) levels⁽⁶⁾. Research suggests that anxiety, depression, and various other psychological problems are greater in PCOS than in healthy women ⁽⁷⁾.

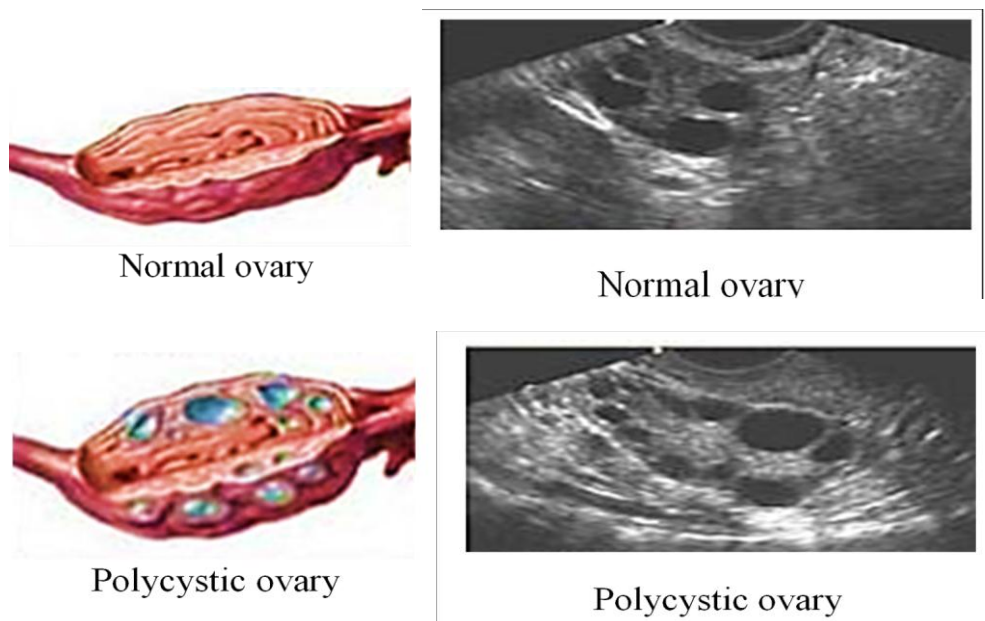


Figure 1-1 polycystic ovary and normal ovary

1.1.1: Symptom of PCOS

The symptoms of PCOS usually become apparent in teens and early 20s. It varies from woman to woman. Symptoms can vary from mild to severe conditions. The common symptoms of PCOS (occur after adolescence and does not respond to usual treatment), rapid weight gain, difficulty in losing weight, hirsutism (excess hair growth on face, chest, abdomen, upper thighs, back or buttocks, affect 70% of women with PCOS), mental health problems, metabolic dysfunction, difficulty getting pregnant, thinning hair and hair loss from the head, oily skin, depression and mood changes, patches of thickened velvety, darkened skin on the neck, arms, breast or thighs which is known as **Acanthus's nigricans**, pelvic pain and obesity (80% of women with PCOS are obese)^(8, 9).

1.1.2 : Causes of PCOS

PCOS effected women may have abnormalities in the metabolism of androgens and estrogen, while other effected women are genetic pre disposition for the syndrome. It is questionable why the clinical appearances of this syndrome are so different. Being a complex syndrome of uncertain cause. There are many different mechanisms leading to PCOS, First of all there are irregularities in the metabolism of androgens and in the control of androgen production. These patients may be encountered with high serum concentrations of androgenic hormones, such as testosterone and androstenedione. Nevertheless, a patient may have normal androgen levels, though there is considerable individual variation⁽¹⁰⁾. Hyperinsulinemia and peripheral insulin resistance are also associated with PCOS.

High levels of insulin may augment the gonadotropin levels, thereby affecting the function of the ovaries. Insulin resistance can lead to metabolic and reproductive disorders. There might also be a correlation between hyperinsulinemia and suppression of hepatic generation of (SHBG), which may increase androgenicity⁽¹¹⁾ .

A suggested mechanism for high androgen levels and anovulation is that under the increased effect of (LH), the ovarian theca cell stimulation is also increased ⁽¹²⁾ .

These cells then increase the production of androgens. Because of a decreased FSH ratio of LH. the granulosa cells are not able to aromatase the androgens to estrogens, which can lead to reduced levels of estrogen and anovulation ⁽¹³⁾ .

1.1.3 : Diagnosis of PCOS

1.1.3.1: Menstrual disorders: abnormal menstrual cycle might be the ⁽¹⁴⁾. Most common symptom history of menstrual disturbance dating back to menarche has been often typical amongst patients with PCOS. Oligomenorrhea occurs in some women, also menstrual bleeding that occurs with intervals of 35 days to 6 months or amenorrhea. Infertility is another consequence of anovulatory menstrual cycles. The higher rate of abortions amongst PCOS women has been observed, but the causes are not quite known ⁽¹⁵⁾ .

1.1.3.2: Hyperandrogenis: manifests clinically as excess body hair in a male distribution pattern. Hair is seen on the upper lip, on the chin, around the nipples and a long line Alba of lower abdomen⁽¹⁶⁾. In some patients acne occurs as well. Having seborrheic skin as a PCOS patient,

the chances of getting acne as a consequence of oil, bacteria, and dead skin cells getting trapped inside the pores of the skin are higher compared to normal or dry skin. In women with PCOS, androgen levels are often elevated, including dihydrotestosterone.

This metabolite promotes oil production, which can lead to the pores getting clogged. Rise in oil production also manifests as greasy hair in patients with PCOS ⁽¹⁷⁾. Increased muscle mass, voice deepening and hyperandrogenism are related to more extreme forms of PCOS. As a differential diagnosis, symptoms of hyperandrogenism could also be consistent with androgen producing tumors ⁽¹⁵⁾.

1.1.3.3: Hormone analyses:

Follicle stimulating hormone (FSH) level should be checked in patients with PCOS. In most cases, FSH levels are within the reference or even low. There are elevated levels of (LH), and the LH:FSH ratio is higher than 3 ⁽¹³⁾. Measuring total and free (TT) levels are sensitive indicators of androgen excess. Levels of (SHBG) are usually low in women with PCOS.

Elevated levels of androstenedione are also common. TSH levels should be evaluated, because thyroid dysfunction can cause amenorrhea and hirsutism. Other laboratory analytical such as Cholesterol levels should be measured because in some women with PCOS an abnormal lipid profile is often shown ⁽¹⁸⁾.

IR and inflammatory markers have been shown to be useful in identifying adolescent girls with PCOS who have a higher risk of developing metabolic syndrome ⁽¹⁹⁾.

1.1.3.4: Ultrasonographic Imaging of Ovaries

Gynecologic ultrasonography, especially using a transvaginal probe, is a good screening tool for PCOS patients. Ultrasonography can be used to evaluate the ovarian morphology, specifically looking for small ovarian follicles. Bilaterally enlarged ovaries with multiple small follicles are seen on ultrasound. The ovarian size is increased, with 12 or more follicles measuring 2-9 mm and most follicles of similar size. The endometrium may appear thickened, resembling that of a proliferative endometrium. A transrectal approach with ultrasound is an alternative in virginal patients ⁽²⁰⁾.

Serum concentrations of AMH hormone have been shown as a useful indicator in the diagnosis of PCOS. AMH is usually at least two to three times higher in women with PCOS compared to normal women ⁽¹⁵⁾.

1.1.4:Treatment of PCOS

1.1.4.1:Oral contraceptive.To induce regular menses and to increase (SHBG) production are recommended for long term management of menstrual dysfunction ⁽²⁰⁾.

If hirsutism is not reduced enough, adding an androgen-blocking agent has been shown effective. For patients with PCOS, the oral contraceptive of choice is called Diane. It contains estrogen (ethinylloestradiol) which is the most common estrogen used in nearly all the oral contraceptive pills. Cyproterone acetate is an anti-androgen hormone, and it therefore reduces the conversion of testosterone to its more active forms ⁽²¹⁾.

There is ample evidence that hyperinsulinemia results in increased ovarian androgen biosynthesis in vivo and in vitro and decreased (SHBG) synthesis from the liver leading to increased bioavailability of free androgens⁽²²⁻²⁴⁾. This excess in local ovarian androgen production augmented by hyperinsulinemia causes premature follicular atresia and anovulation⁽²⁵⁾.

Therapeutic measures directed at lowering insulin secretion in women with PCOS should theoretically ameliorate their hyperandrogenism and restore normal follicular growth, thus facilitating ovulation⁽²⁶⁾. The most extensively studied insulin-sensitizing drug in the treatment of PCOS is metformin^(27,28).

1.1.4.2: Metformin (dimethylbiguanide) is an orally administered drug used to lower blood glucose concentrations in patients with noninsulin- dependent diabetes mellitus (NIDDM)⁽²⁹⁾. It is anti-hyperglycemic in action and does not cause hypoglycemia Metformin enhances insulin sensitivity in both the liver, where it inhibits hepatic glucose production, and the peripheral tissue, where it increases glucose uptake and utilization in muscle tissue. By increasing insulin sensitivity, metformin reduces IR, insulin secretion and hyperinsulinemia⁽³⁰⁾.

The pathophysiology of PCOS may have a genetic component, although it can be suggested that the main factors responsible for the increasing prevalence of the PCOS are related to the influence of the environment, including dietary habits, behaviour and other still undefined factors⁽³¹⁾. The clinical features of PCOS are heterogeneous and may change throughout the lifespan, starting from adolescence to postmenopausal age⁽³²⁾.

1.1.5 :Obesity and metabolic syndrome:

Obesity is strongly associated with the PCOS although the cause of this association remains unknown but obesity is present in at least 30% of case, in some series the percentage may be as high as 75%⁽³¹⁾.

Although it is not possible to evaluate whether the present prevalence rates of PCOS and that of associated obesity is higher than in the past due to the lack of cross-sectional and longitudinal studies, nonetheless it can be hypothesised that the increasing worldwide prevalence of obesity may play a key role in favouring the development of PCOS in susceptible individuals. Therefore we should expect an increasing rate of problems affecting women with PCOS with particular reference to infertility, since PCOS is one of the most common causes of infertility in women. Obesity in fact is associated with hyperinsulinaemia that compensates for the presence of insulin resistance, This is what occurs particularly in the presence of the abdominal phenotype of obesity which develops as a consequence of a prevalent enlargement of visceral fat depots^(33,34).

Although PCOS per se may be associated with alterations of both lipid and lipoprotein metabolism the coexistence of obesity usually leads to a more atherogenic lipoprotein pattern, a greater reduction of (HDLs) together with a higher increase of both TG and total cholesterol levels has in fact been observed in obese women with PCOS, with respect to normal weight women with PCOS. Obesity per se represents a condition of sex hormone imbalance in women. Levels of the (SHBG) tend to linearly decrease with increasing body fat and this may lead to

an increased fraction of free androgens delivered to target sensitive tissues ⁽³⁵⁾.

1.1.6: Treatment of obesity in PCOS Significance of weight loss in obese women with PCOS

Weight loss is a first line management in PCOS women who are obese ⁽³⁶⁾. Further because almost 80% of overweight adolescents remain overweight as adults it is warranted to have weight reduction interventions as soon as possible ⁽³⁷⁾.

1.2: Trace elements

Trace elements are defined as those elements occurring in human body but constituting $< 0.01\%$ of the body weight include essential elements (Zn,Fe,Co(B12),Cr(required),andCu(cofactor) and non-essential (toxic) (Cd,Pb,and Ni)⁽³⁸⁾, generated from mining operations, metal-plating facilities, power-generating facilities, electronic device manufacturing units, and tanneries, and commonly exist in process waste streams ⁽³⁹⁾. Cadmium (Cd), lead (Pb), and arsenic (As) are ubiquitous in the environment following many years of industrial use, with most adults having measurable levels of these nonessential elements in their blood.

Human exposure to the elements may occur through occupational contact, environmental pollution, and accumulation in the food. The serum levels trace elements can change in patients with PCOS ⁽⁴⁰⁾.

Environmentally relevant levels of heavy metals are heavy metals are group of metallic elements that have certain chemical and electrical characters and its density is greater than 5g/cm^3 ⁽⁴¹⁾. Also associated

with modest changes in reproductive hormone levels ⁽⁴²⁾, However, few studies have directly examined the serum trace element concentrations in relation to PCOS ^(40, 43)

These elements are considered as natural constituents of the earth's crust and are non-biodegradable and so effect on the living things in the environment. So it can enter the body through the respiratory system through the air and digestive system through ingestion and bio accumulate for a long period of time ^(44, 45)

The pollution by heavy metals can occur by many different ways, directly or indirectly, may contaminate soils, water and plants by material from the air or by direct deposition of pollutants "generally humans are exposed to these metals by ingestion (drinking or eating) or inhalation (breathing)" ⁽⁴⁶⁾. Because of their contact with human life and other living organisms, these dangerous and important pollutants are interesting to many researchers and have been a high number of mortality because of their toxicity ⁽⁴⁵⁾.

Toxic elements pollution are widespread due to many sources such as traffic and fuel stations⁽⁴⁷⁾. Some of trace elements emission are factors for cars, vans, trucks, buses and motorcycles for each of the emission sources, fuel consumption, engine oil, tire wear, brake wear and road abrasion, the emission components contain (Cd,Cr,Cu,Ni,Pb, Co,and Zn)⁽⁴⁸⁾.Some of these elements are accumulating pollutants cause environmental hazards and are reported to be exceptionally toxic⁽⁴⁹⁾.

1.2.1: Classifications of trace elements exposure:

Exposure to toxic elements is generally classified as acute 14 days or less, intermediate 15-365 days, and chronic are more than 365 days because elements are not easily biodegradable, they can accumulate in vital human organs, which cause varying degrees of sickness based on the period and type of exposures ⁽⁵⁰⁾. Chronic exposure to low levels of trace elements have negative effects on human beings and other animals due to the fact that mechanism for their elimination from the body is not easy ⁽⁵¹⁾.

Some of a trace elements are cumulative poisons and these elements are considered as an environmental hazards and to be exceptionally toxic like (Pb,Cd)⁽⁵²⁾. An accumulation of a trace element in the human body can cause disruption of numerous biochemical processes such as cardiovascular, nervous, kidney and bone diseases. Furthermore the consumption of a trace elements-contaminated food can seriously deplete some essential nutrients in the body causing a decrease in immunological defenses, impaired psycho-social behavior, intrauterine growth retardation, disabilities associated with malnutrition and a high prevalence of upper Gastrointestinal cancer ^(53, 54).

Long term storage of some toxic metals takes place in hard tissues such as teeth and bones. In addition to samples of keratinous tissue components such as nails and hair are commonly used for routine clinical screening and diagnosis of longer-term exposure to heavy metals. For example levels of lead in hair, bones and teeth increase With age, due to a gradual accumulation of lead in the body. Therefore pollution of food with lead and may be lead intoxication through the diet is a chronic this need for constant monitoring⁽⁵⁵⁾.

1.2.2: Zinc:

Zn is a chemical element with symbol Zn and atomic number 30. It is the first element of group 12 of the periodic table. This element was discovered by German chemist Andreas Sigismund Marggraf in 1746 at Germany⁽⁵⁶⁾.

It has an atomic weight of 65.4. It is found throughout the entire body system with half in the muscle tissue. Is an essential trace element that functions as a cofactor for certain enzymes involved in metabolism and cell growth, it is found in nearly 300 specific enzymes ⁽⁵⁷⁻⁵⁹⁾.

In women zinc is involved in the growth process of the oocyte or egg and deficiency causes immaturity of the egg and impeding of ovulation as result causes infertility. Adequate zinc allows women to use estrogen and progesterone efficiently supporting reproductivity and ensuring that estrogen does what it's supposed to do in the body. When estrogen levels become too high or are inefficiently metabolized they can cause poor reproductive health and breast cancer ⁽⁶⁰⁾.

Zn is found in wheat, brown rice, oats, lentils soybeans, dried peas, black-eyed peas, lima beans, walnuts, peanuts, cashews, brazil nuts, many cheeses any kind of liver, and animal flesh such as beef, lamb chicken, turkey, and various fish and seafood. It is also found in most vitamin mineral supplements as sulfate, citrate, or oxide and these are inexpensive and bioavailable sources ⁽⁶¹⁻⁶³⁾.

In more severe cases, Zn deficiency causes hair loss, delayed sexual maturation, impotence, hypogonadism in males, and eye and skin

lesions, weight loss, delayed healing of wounds, taste abnormalities, and mental lethargy can also occur ⁽⁶⁴⁻⁶⁶⁾.

1.2.3: Chromium:

Cr is a chemical element with symbol Cr and atomic number 24. It is a steely-gray, lustrous, hard, and brittle metal ⁽⁶⁷⁾. It has an atomic weight of 52.0. In 1797, chromium oxide was discovered by the French pharmacist and chemist Louis Nicolas Vauquelin. In the following year 1798, Vauquelin discovered that he could isolate metallic chromium by heating the oxide in a charcoal oven, which making him the discoverer of the element ⁽⁶⁸⁾.

Cr is a trace element that humans require in trace amounts. It is found primarily in two forms: Trivalent Cr^{+3} which is biologically active and found in food and hexavalent Cr^{+6} a toxic form that results from industrial pollution ⁽⁶⁹⁾.

Is a trace element that is not yet a well studied medication in PCOS and not all its effects on the body have been demonstrated. There are several reports on its effect on glucose and lipid metabolism in humans and animals ⁽⁷⁰⁾. Cr is widely distributed in the food supply, but most foods provide only small amounts of it. It is found in egg yolks, whole-grain products high-bran breakfast cereals, coffee, nuts, green beans, broccoli, meat, and brewer's yeast ^(71,72).

1.2.4: Iron:

Fe is a chemical element with symbol Fe and atomic number 26 and has been known since the beginning of time. It is by mass the

most common element on earth, forming much of Earth's outer and inner core. It is the fourth most abundant elements after oxygen, silicon, and aluminum, respectively. It has an atomic weight of 55.8. Fe is the most abundant metal in the human body⁽⁷³⁾.

Is an essential nutrient in humans and states of both iron deficiency and iron excess result in deviation from optimal health⁽⁷⁴⁾.

Iron serves as a cofactor for several enzymes involved in oxidation-reduction reactions due to its ability to exist in two ionic forms , ferrous (Fe^{+2}) and ferric (Fe^{+3}). Most of the body Fe is contained within two proteins: (1) hemoglobin(an oxygen transport protein in erythrocytes, (2) myoglobin(an oxygen storage protein present in the muscle cells)⁽⁷⁵⁾.

1.2.5: Copper:

Cu is a chemical element with symbol Cu and atomic number 29. It is in the top of group 11, of the periodic table, above silver and gold. It has an atomic weight of 63.5. Cu is a reddish metal with a face-centered cubic crystalline structure, it reflects red and orange light and absorbs other frequencies in the visible spectrum. It is malleable, ductile, and an extremely good conductor of both heat and electricity second only to silver in electrical conductivity^(76,77).

Cu is also an important trace element involved as a cofactor of highly diverse enzymes in a broad range of processes some critical to life in general specific to mammals important for the production of hemoglobin and red blood cells ⁽⁷⁸⁾. The best dietary sources of Cu to human body include wheat, barley, sunflower seeds ,almonds, pecans,

walnuts, peanuts, cashews, prunes, raisins apricots, various dried beans, mushrooms, chicken, and most fish⁽⁷⁹⁾. Cu is an essential micronutrient necessary for the hematologic and neurologic systems. It is necessary for the growth and formation of bone, formation of myelin sheaths in the nervous systems, helps in the incorporation of Fe in hemoglobin, assists in the absorption of Fe from the gastrointestinal tract, and in the transfer of Fe from tissues to the plasma⁽⁸⁰⁾.

1.2.6: Cobalt:

Cobalt is a chemical element with symbol Co and atomic number 27. It has an atomic weight of 58.9. Co was first discovered in 1739 by the chemist Georg Brandt of Swedish. Co is hard, shiny, crisp, bluish-gray metal. Is one of the three magnetic minerals (in addition to Fe and nickel) that is often used in magnet alloys. It has considerable industrial applications. It is used in paints and dyes, where it has been used since the middle ages in the production of a blue colored glass smalt⁽⁸¹⁾. Is one part of components vitamin B12 which is essential for all animals including humans⁽⁸²⁾.

At present the use of Co increased significantly because of its resistance to corrosion and its magnetic qualities and durability of alloys. Co is also used in paints, varnishes, inks, foam stabilizers in beverages, Agrochemicals and many other applications⁽⁸³⁾. Co is an essential trace element for the human body where it is a key constituent of cobalamin (the scientific name of vitamin B12). It also has a substantial role in the formation of amino acids and neurotransmitters Human body can get Co ions through several pathways: With food, by the respiratory system, by the skin, and as a component of biomaterials.

The cobalt ions enter the body through any of the above routes and bind with proteins within the bloodstream and get transported with blood to be deposited in tissues and cells ^(84,85).

1.2.7: Cadmium:

Cd atomic number 48 relative atomic mass 112.41 is a metal , which belongs to group IIB of the periodic table, half life 38 years ⁽⁸⁶⁾. Is a non-essential heavy metal it can be toxic to humans and some biological systems even at very low levels of concentration and is considered a risk factor as associated with premature arteriosclerosis and high blood pressure which can lead to cardiovascular disease and bound to metallothionein ⁽⁸⁷⁾.

1.2.8: Lead:

Pb is a chemical element with atomic number 82 and symbol Pb and has an atomic weight of 207.2. Pb is a soft, malleable, and heavy metal. It is a bluish-white metal but on exposure to air it tarnishes to a dull gray metal ⁽⁸⁸⁾. Is a non-essential and toxic metal for human even at low concentrations and can cause several biological effects ⁽⁸⁹⁾. Because of Pb is a serious metal for human and the fuel consumption is the main source of Pb emission and release to the environment ⁽⁹⁰⁾

There are several ways to be exposed to lead, usually by inhalation, digestion of food and water and skin contact and due to it is used as gasoline additives ⁽⁸⁹⁾. Pb is an extremely common metal in our life. Water, paint, electric storage batteries, insecticides, and gasoline

are considering some common sources of lead. It is rapidly absorbed into the bloodstream of human body through inhalation, ingestion, or by skin contact. Through the bloodstream, lead is distributed among three main compartments: Blood, soft tissue that includes kidney, bone marrow, liver, brain, and mineralized tissue that includes bones and teeth⁽⁹¹⁾. In fact there are no any known health benefits or biological role of lead for the human body. On the contrary, lead has adverse effects that deleterious the human body. It can affect almost every organ and system in the human body. To a great extent, it can cause damage to the brain, kidneys, nervous system, reproductive system, and can cause high blood pressure. From these dysfunctions^(92,93). Although there is no safe level of exposure to lead has been found, chronic toxicity of it is much more common and occurs at blood levels of about 40-60 µg/dL⁽⁹¹⁾. Pb exposure has also been linked to miscarriage, hormonal changes, reduced fertility in men and women, menstrual irregularities, delays in puberty onset in girls⁽⁹⁴⁾. It also has an impact on the composition of hemoglobin which is associated with anemia⁽⁹⁵⁾.

1.2.9: Nickel:

Ni is a chemical element with symbol Ni and atomic number 28. Ni is a silvery-white metal, hard, malleable, and ductile metal. It is of the Fe group and it is a fairly good conductor of heat and electricity. It has an atomic weight of 58.7. Ni is a naturally occurring element that is present in soil, water, air, and biological materials. It is a natural component of earth's crust and is present in igneous rocks⁽⁹⁶⁾.

It has been found that humans may be exposed to nickel during breathing air, eating food, or smoking cigarettes. Skin contact with

nickel- contaminated soil or water may also result in nickel exposure. In fact, small quantities of nickel are essential for the body, but when the uptake is too high it can be a danger to human health. Studies have shown that acute exposure of human body to nickel may cause several health problems such as liver, kidney, spleen, brain and tissue damage, vesicular eczema, lung, and nasal ^(97,98). Ni is contained in many foods such as, beans, chocolate, soybeans, lentils, split, green peas, oats, buckwheat, barley, and corn. Nuts, such as walnuts and hazelnuts, are the best sources of nickel. Many vegetables and some fruits, such as bananas and pears, have moderate amounts of nickel ⁽⁹⁹⁾.

It has been observed that the exposure to nickel (especially, nickel in jewelry) may result in the development of a dermatitis known as “nickel allergy” in sensitized individuals. The first symptom is usually itching, before skin eruption occurs. Ni is an important cause of contact allergy^(100,101).

1.3: Atomic Absorption Spectroscopy AAS

Atomic absorption spectroscopy (AAS) is based on the principle that when a beam of light is passed through a sample, the radiation may either be transmitted or absorbed according to the radiation wavelength. The absorption causes an increase in the energy of the atom. The energy absorbed by the atom is directly proportional to the radiation wavelength. Electronic excitation due to increase in the energy of the free atom, when electrons jump to higher energy levels. The atom can absorb particular wavelength depends upon the charges in vibrational, or rotational or electronic state. The determination of the elements can

be obtained by different methods according to the element itself of concentration^(102,103).

1.3.1: Principles

There are many ways in which element atoms can be stirred and moved from the ground state to the excited state. The simplest way to stir the material is to heat the sample to a high temperature causing excitation due to collisions between sample atoms. Used in Flame Atomic Absorption Spectrometer, it works to examine the wavelengths of photons absorbed while stirring atoms. It is known that each element absorbs featured groups of discrete wavelengths according to its electronic composition. Atomic absorption spectrometry determines the elements in their solutions. The idea of determination depends on turning the sample solution into nebulization then mixing the Nebulizer products with a mixture of gases such as acetylene, air or acetylene and nitrous oxide.

The element is converted into the atomic image by the flame resulting from the burning of these gases, which is exposed to the Hollow cathode lamp for each element measured. Required. These methods are⁽¹⁰⁴⁾.

1.3.1.1-Flame Atomic Absorption (FAAS)

In this technique the atomization occurs by using a flame and a hollow cathode lamp. Hollow cathode mainly contains a tungsten anode and hollow cylindrical cathode made of the element to be measured^(105,106).

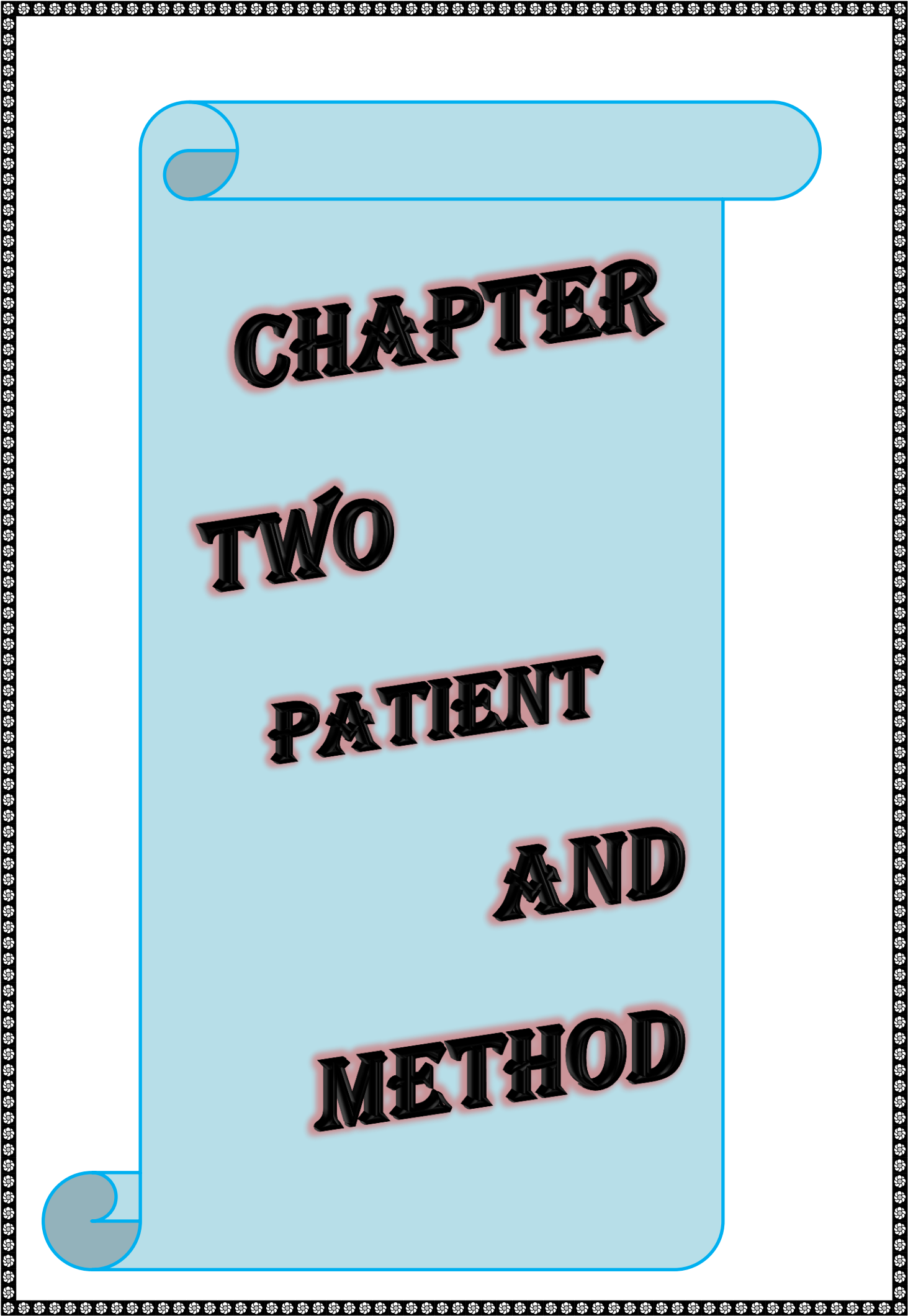
1.3.1.2:Flameless Atomic Absorption (FLAAS)

In this technique the atomization occurs by using other methods rather than flame, those methods include graphite tube which use a graphite coated furnace to vaporize the sample⁽¹⁰⁵⁾.

Aim of study

1-determination the serum concentrations trace elements (Cd, Ni,Co,Fe,Cr,Cu,Zn, and Pb) in PCOS patients and controls.

2- evaluation effective levels of determined elements of PCOS

The image features a light blue scroll with a decorative border. The scroll has a rolled-up top edge and a rolled-up bottom edge. The text "CHAPTER TWO PATIENT AND METHOD" is written in a bold, black, serif font with a red glow effect. The scroll is set against a white background with a black border featuring a repeating floral pattern.

CHAPTER

TWO

PATIENT

AND

METHOD

2-Patient and Methods

2.1: Chemicals:

The chemicals that were used in this study with their companies Which are shown with suppliers, and listed in the table 2.1: below.

Table 2.1:chemicals with their supplies company , concentration and molecular weight

No.	Chemical	Conc.	Supplies company	Purity
1	Nitric acid	70%	Scharlau	Analytical grade
2	Perchloric acid	70%	Scharlau	Analytical grade
3	Hydrochloric acid	30-35%	Scharlau	Analytical grade
4	Deionized water			

2.2: instrument

The instruments are available in the laboratories of chemistry A department in the college of science (university of Thi-Qar) which are Shown in table 2.2 :

Table 2.2: the instruments and their models and supplied company an original

No.	Instruments	Model	Supplied company	Original
1	Drying Oven	LIB-060M	Lab tech	Korea
2	Atomic Absorption Spectroscopy	AL 1200	Aurora	Canada
3	Centrifuge	Rotofix 32 A	Hettich	Germany
4	Hot Plate		Lab tech	Korea

2.3: Design of study

This study was conducted at an Alhussien teaching hospital in infertility unit Thi - Qar governorate. Measurements were carried out chem during the period from (De/ 2016 to April/2017). Informed consent was obtained Verbally from all participants.

The present study includes (120) women, which were divided into two groups

1- Control group: including (50)

2- patient group (PCOS women): including (70) women with polycystic ovarian syndrome .

Details of the number and age of the two groups are explained in the following table.

Table 2.3: details of Numbers and age of the Subject

Groups	No.	Range of age (years)	Waist	Rang of body mass kg
Control group	50	16-42	28-40	40-100
PCOS group	70	10-42	25-45	40-115
Total	120	10-42	25-45	40-115

Table 2.4 : clinical chart

Patient profile	
Name	
Age	
Weight	
Waist	
Height	

2.4: Body Mass Index BMI

BMI is determined from division of weight (measured in kg / square hight)
 Normal BMI 18.5-24.9. Overweight BMI 25-29.9. Obesity BMI ≥ 30 ⁽¹⁰⁷⁾.

2.5: Collection of blood sample

Blood samples were from 120 collected women. Based on these blood samples, 70 female PCOS patients. PCOS were defined when at least two of the following three features were present: oligo- /amenorrhea, Hyperandrogenism (and/ or hirsutism), and polycystic ovaries.

In order to carry out a control, we randomly selected 50 samples from the remaining 120 samples. None of the women had symptoms of Hyperandrogenism, a history of menstrual dysfunction, infertility. Women were excluded if they had a personal history of hypertension, diabetes, or cardiovascular events, or had received treatment with oral contraceptives, antiandrogens, insulin sensitizers, or drugs that might interfere with clinical and or bio-chemical variables, for at least 6 months prior to analysis.

All women were non-pregnant, non-smokers. All women were studied within the first (1- 5) days after onset of menstruation in the case of mild oligomenorrhea or at random in those suffering from severe oligo-/amenorrhea.

2.6 : Preparation of serum

A 5 ml of blood was centrifuged (2500 rpm) for 10 minutes, then serum, kept at -20 °C until the digestion. The concentration of the elements was determined by the flame atomic absorption spectroscopy. This Measurement is an optimal choice to measure the amount of trace elements in serum owing to its high sensitivity and specificity^(108,109,110).

2.7: Digestion of serum

The digestion of serum was achieved by adding Nitric acid (2ml, 70%) and Perochloric acid (1ml, 70%) to serum (0.5ml). The mixture was heated for one hour at 160°C by using oil bath in a Pyrex tube. After cooling the solution was completed to 10ml with Hydrochloric acid (0.3N)⁽¹¹¹⁾.

2.5: Table show the condition in the analysis by the flame atomic absorption of each element

Element	$\lambda_{\max}$	Flame type	Detection limit (ppm)
Cr	357.9	Air/ Acetylene	0.005
Fe	248.3	Air/ Acetylene	0.0046
Cd	228.8	Air/ Acetylene	0.0028
Zn	213.9	Air/ Acetylene	0.003
Ni	240.7	Air/ Acetylene	0.01
Cu	324.7	Air/ Acetylene	0.004
Co	240.7	Air/ Acetylene	0.01
Pb	217	Air/ Acetylene	0.012

2.8: Statistical Analysis:

The statistical analysis was achieved by using Microsoft Excel 2010 the results were expressed as mean $\pm$ standard deviation (mean $\pm$ SD). One way ANOVA was used to compare parameters in different studied groups. Pearson's correlation was applied to determine the relationship between the present study parameters. P-values ($P \leq 0.05$) were considered statistically significant.

2.9: Study design

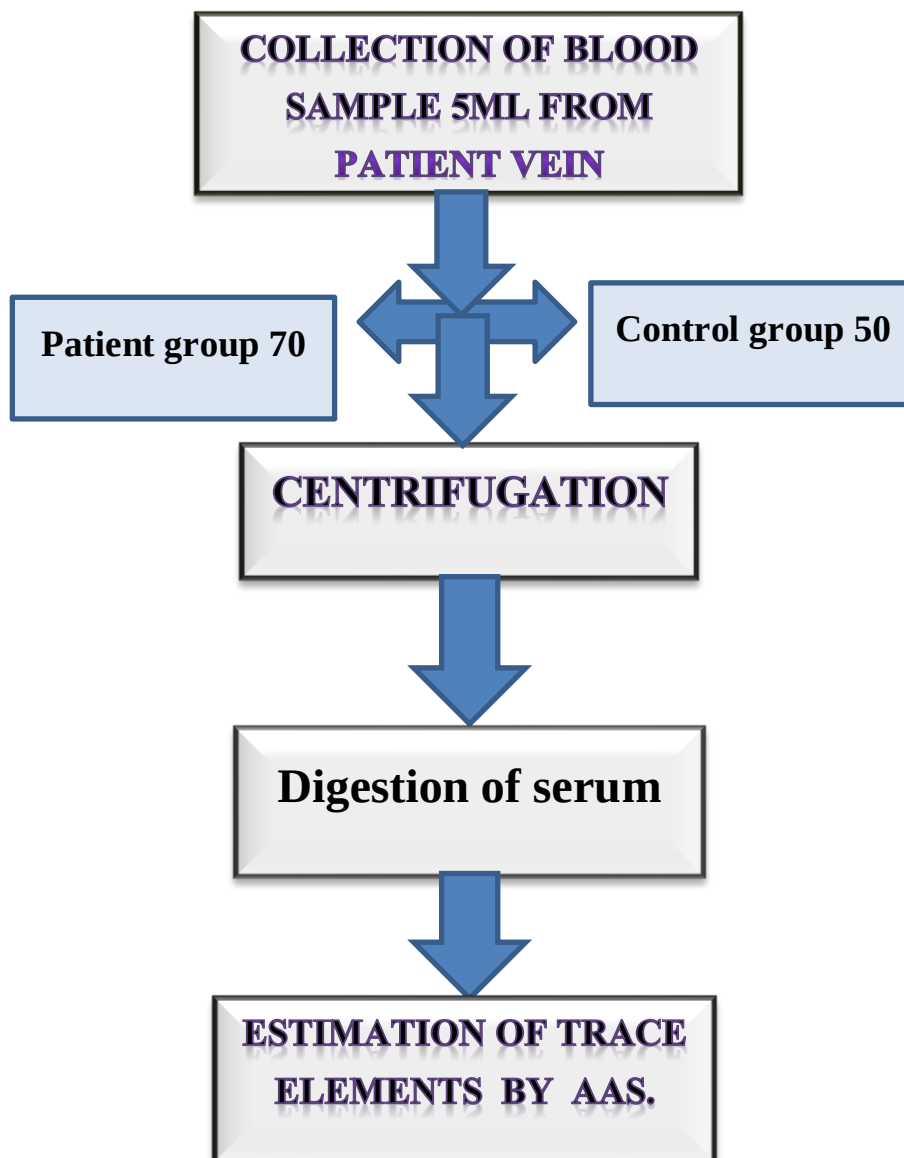


Figure (2-1): Study design

CHAPTER
THREE
RESULT
AND
DISCUSSION

Results & Discussion

3.1: General Comparison elements concentration in patient and control groups.

3.1.1: Determination zinc concentration in Serum patient and control groups

The results showed a significant decrease ($p \leq 0.05$) in the concentration of Zn in patient serum in comparison with control groups

Table 3.1.1 Zinc concentrations (ppm) in patient and control groups

Groups	*No.	Zn (Mean $\pm$ SD**)ppm	Normal value
Pat.	70	0.91 $\pm$ 0.20 b	
Cont.	50	1.85 $\pm$ 0.28 a	
***LSD		0.07	0.7 ppm ⁽¹²⁸⁾

Not:-Each value represents mean $\pm$ SD values with non-identical superscript (a and b) for vertical comparison are considered significantly differences ($p \leq 0.05$)

*:Number of sample

**: Standard deviation

***:Least Significant Difference

This study agrees with the result of (Guler, I., 2014, Zheng, G.; 2015). The association of zinc deficiency with PCOS is independent of obesity. Plasma zinc levels were significantly negatively correlated with TG/HDL ratio, which is one of the most important risk factors for cardiovascular disease (113).

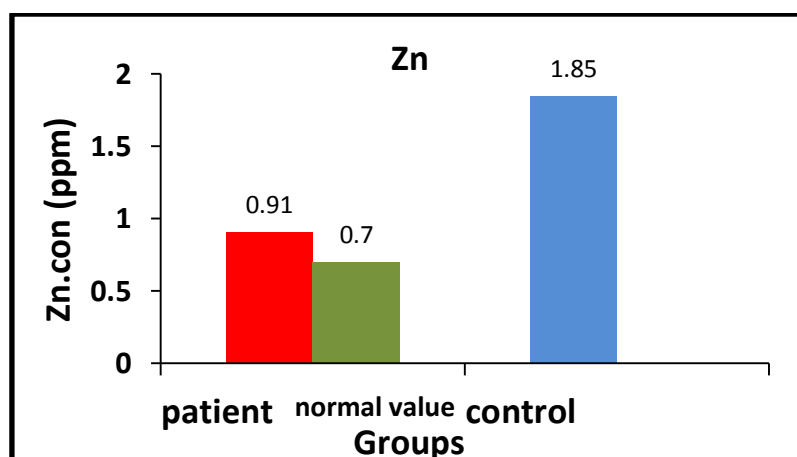


Figure 3.1.1 Zinc levels in patient and control groups

3.1.2: Determination Chromium concentration in Serum patient and control groups

The results showed a significant decrease ($p \leq 0.05$) in the concentration of Cr in patient serum in comparison with control groups.

Table 3.1.2 Chromium concentrations (ppm) in patient and control groups

Groups	No.	Cr (Mean $\pm$ SD) ppm	Normal value
Pat.	70	0.37 $\pm$ 0.09 b	
Cont.	50	0.40 $\pm$ 0.06 a	
LSD		0.02	0.1ppm ⁽¹²⁸⁾

-Legend as in table (3-1)

This result agree with the result (Milal, M.2017). Serum chromium deficiency may be associated with contemporary diets, which are poor in this essential trace element owing to soil depletion, refined foods, excessive sugar consumption, lack of exercise, and obesity ⁽¹¹⁶⁾.

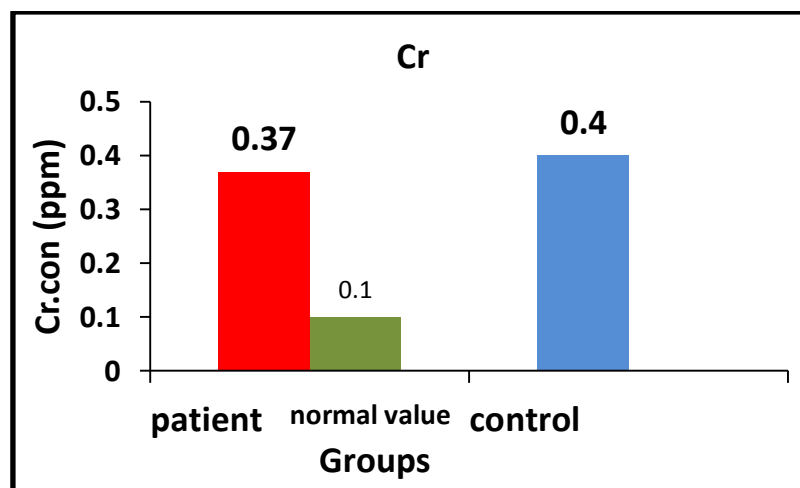


Figure 3.1.2 Chromium levels in patient and control groups

3.1.3: Determination iron concentration in Serum patient and control groups

The results showed a significant increase ($p \leq 0.05$) in the concentration of Fe in patient serum in comparison with control groups.

Table 3.1.3 Iron concentrations (ppm) in patient and control groups

Groups	No.	Fe (Mean $\pm$ SD)ppm	Normal value
Pat.	70	2.21 $\pm$ 0.51 a	
Cont.	50	1.48 $\pm$ 0.19 b	
LSD		0.10	0.5-1.5ppm ⁽¹²⁷⁾

-Legend as in table (3.1)

This result agree with the result of (Martínez-García, M.;2009). That Fe studied in the serum of patients with PCOS and the result was significantly higher in patients compared with control because found menstrual dysfunction to be the predictor of high serum ferritin in PCOS.

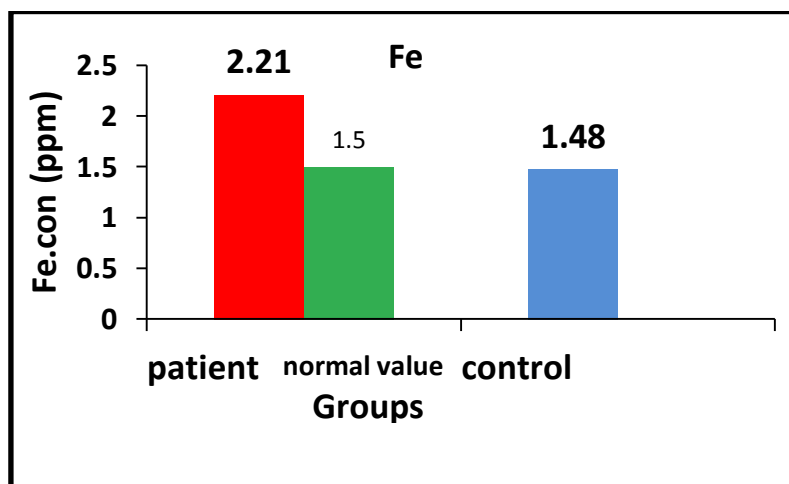


Figure 3.1.3 Iron levels in patient and control groups

3.1.4: Determination Copper concentration in Serum patient and control groups

The results showed a significant increase ($p \leq 0.05$) in the concentration of Cu in serum patient in comparison with control groups

Table 3.1.4 Copper concentrations (ppm) in patient and control groups

Groups	No.	Cu (Mean $\pm$ SD)ppm	Normal value
Pat.	70	2.28 $\pm$ 0. 58 a	
Cont.	50	1.56 $\pm$ 0.31 b	
LSD		0.13	0.8–1.6ppm ⁽¹²⁷⁾

-Legend as in table (3.1)

This result agree with the result of (Kurdoglu, Z.; 2012, Zheng, G.;2015). The main finding of this study was that Cu levels were found to be elevated in lean patients with PCOS in comparison to age and BMI matched healthy controls, suggesting that an alteration in Cu levels may play a role in cardiovascular disease or it may be a consequence of CV disease in PCOS. It has been recognized that PCOS is associated with cardiovascular disease risk factors such as obesity, dyslipidemia. However, all of these abnormalities are also common in obese subjects ^(118, 119).

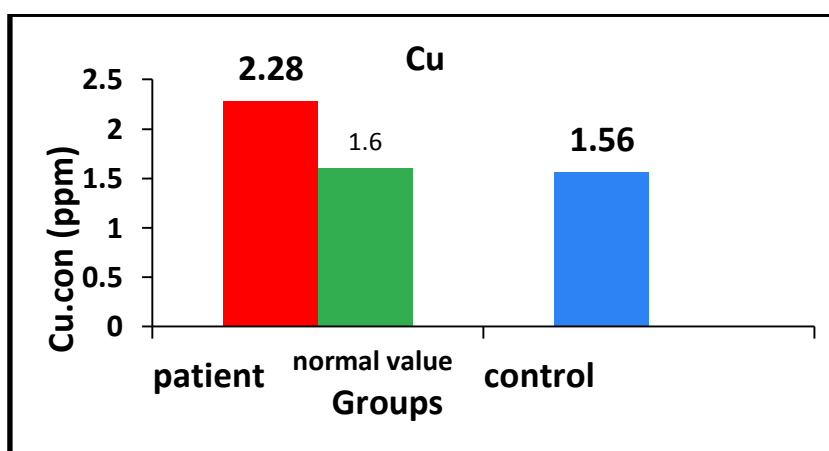


Figure 3.1.4 Copper levels in patient and control groups

3.1.5: Determination cobalt concentration in Serum patient and control groups

The results showed a non significant ($p \leq 0.05$) difference in the concentration of Co in serum patient in comparison with control groups.

Table 3.1.5 Cobalt concentration (ppm) in patient and control groups

Groups	No.	Co (Mean $\pm$ SD) ppm	Normal value
Pat.	70	0.05 $\pm$ 0.01 a	
Cont.	50	0.05 $\pm$ 0.01 a	
LSD		0.02	1-1.2 ppm ⁽⁴⁰⁾

-Legend as in table (3.1)

This result agree with the result of (Kurdoglu, Z.; 2012, Zheng, G.;2015).

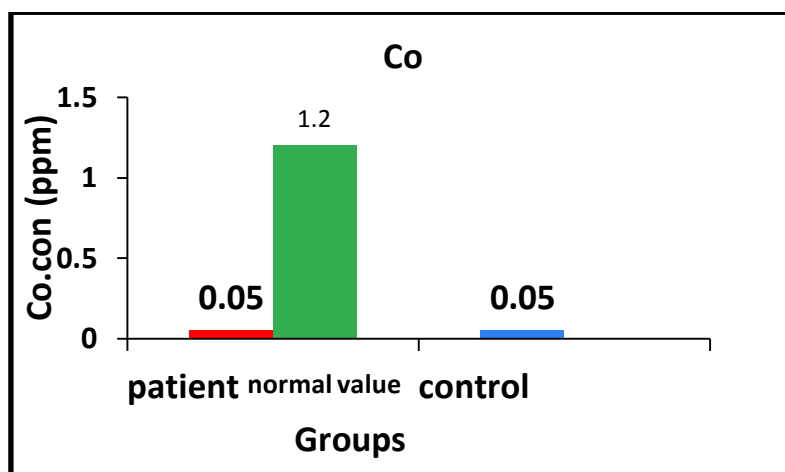


Figure 3.1.5 Cobalt levels of in patient and control groups

3.1.6: Determination Cadmium concentration in Serum patient and control groups

The results showed a significant increase ($p \leq 0.05$). in the concentration of serum Cd in patient in comparison with control groups. This study agrees with (Gallagher, C.;2010). Cd levels is associated with increase in early follicular phase estradiol (E2) levels ⁽¹²¹⁾.

Table 3.1.6 Cadmium concentrations (ppm) in patient and control groups

Groups	No.	Cd (Mean $\pm$ SD)ppm	Normal value
Pat.	70	0.06 $\pm$ 0.01 a	
Cont.	50	0.04 $\pm$ 0.01 b	
LSD		0.01	0.005ppm ⁽¹²⁸⁾

-Legend as in table (3.1)

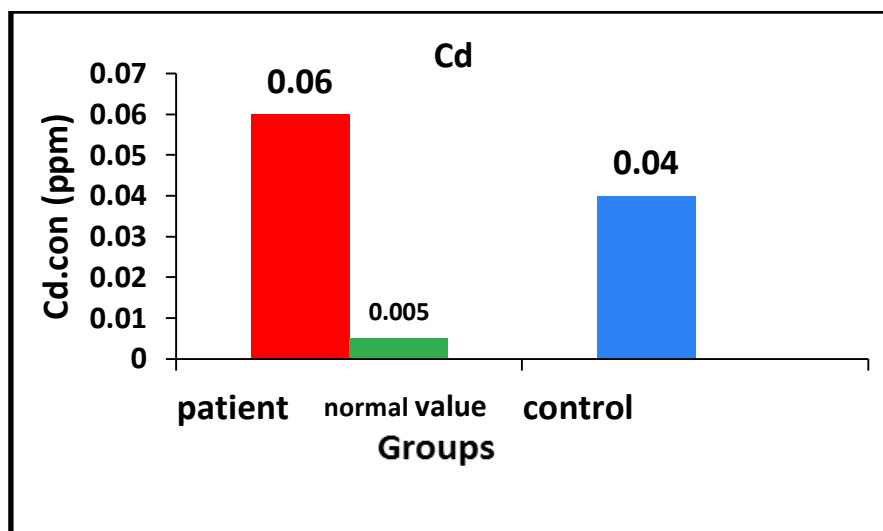


Figure 3.1.6 Cadmium levels in patient and control groups

3.1.7: Determination lead concentration in Serum patient and control groups

The results showed a significant increase ($p \leq 0.05$) in the concentration of Pb in serum patient in comparison with control groups because accumulation in the body with the progress the age and increase (FSH) and (LH) hormone concentrations with increase as the blood lead level in 35–60 years old women (Krieg, E. (2007)).

Table 3.1.7 Lead concentrations (ppm) in patient and control groups

Groups	No	Pb (Mean $\pm$ SD)ppm	Normal value
Pat.	70	0.48 $\pm$ 0.14 a	
Cont.	50	0.35 $\pm$ 0.09 b	
LSD		0.05	0.21ppm ⁽¹²⁸⁾

-Legend as in table (3.1)

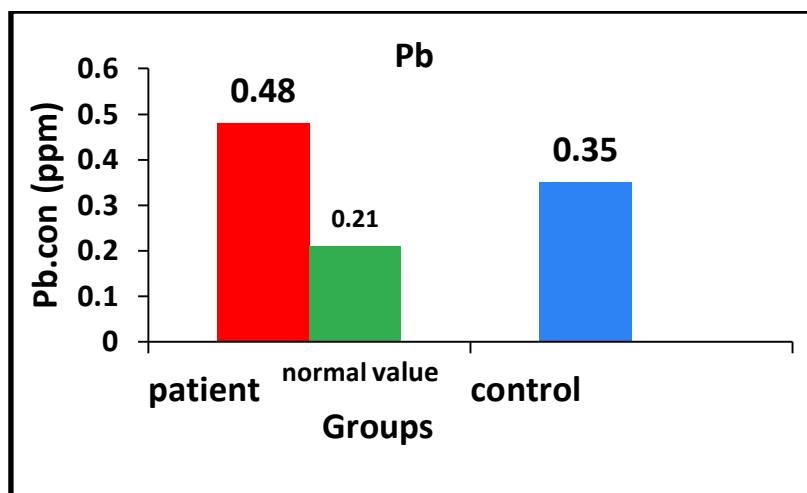


Figure 3.1.7 Lead levels in patient and control groups

3.1.8: Determination nickel concentration in Serum patient and control groups

The results showed a significant increase ($p \leq 0.05$). in the concentration of serum Ni in patient in comparison with control groups

Table 3.1.8 Nickel concentrations (ppm) in patient and control groups

Groups	No.	Ni (Mean $\pm$ SD)ppm	Normal value
Pat.	70	0.69 $\pm$ 0.19 a	
Cont.	50	0.33 $\pm$ 0.09 b	
LSD		0.04	0.01-0.05 ppm ⁽¹²⁸⁾

-Legend as in table (3.1)

This result agree with the result of (Zheng, G.;2015).Nickel levels are higher in PCOS in comparison to control women suggesting that nickel affects PCOS by increasing reproductive hormone levels related with oxidative stress ⁽¹¹⁴⁾.

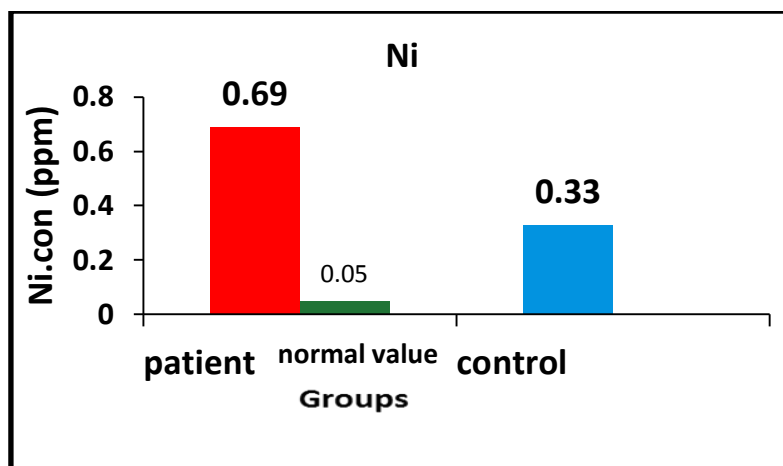


Figure 3.1.8 Nickel levels in patient and Control groups

3.2: Studying of concentrations elements in patient and control groups according to age groups

Divided into three groups to patient (A,B,and C) and control groups (AC,BC,and CC)

1- 10-20 years ,

2- 21-30 years

3- 31-40 years .

This study is shown in the following table:

3.2.1: Determination Zinc concentration in Serum patient and control groups according to age groups

Table 3.2.1 and figures 3.2.1 showed a significant decrease ($p \leq 0.05$) concentration of Zn serum in A, B and C groups in comparison with AC, BC and CC groups.

Also table 3.2.1 and figures 3.2.1 showed a significant increase in the concentration of Zn serum in group A in comparison with B and C groups , increase BC in comparison with AC, and CC) groups ($p \leq 0.05$).

Table 3.2.1 Zinc concentration (ppm) in patient and control groups according to age

Pat.	Age	Zn (Mean $\pm$ SD)* ppm	Cont.	Age	Zn (Mean $\pm$ SD) ppm	LSD
A	10-20	1.01 $\pm$ 0.25 ^{B, a}	AC	10-20	1.73 $\pm$ 0.10 ^{A, b}	0.12
B	21-30	0.90 $\pm$ 0.24 ^{B, b}	BC	21-30	1.96 $\pm$ 0.41 ^{A, a}	0.10
C	31-40	0.84 $\pm$ 0.11 ^{B,b}	CC	31-40	1.68 $\pm$ 0.34 ^{A, b}	0.12
**LSD		0.08	LSD		0.11	

Not:-Each value represents mean $\pm$ SD values with non-identical superscript (a and b) for vertical comparison and (A,B) for horizontal comparison are considered significantly differences ($p \leq 0.05$)

*SD: Standard deviation

**LSD: Least Significant Difference

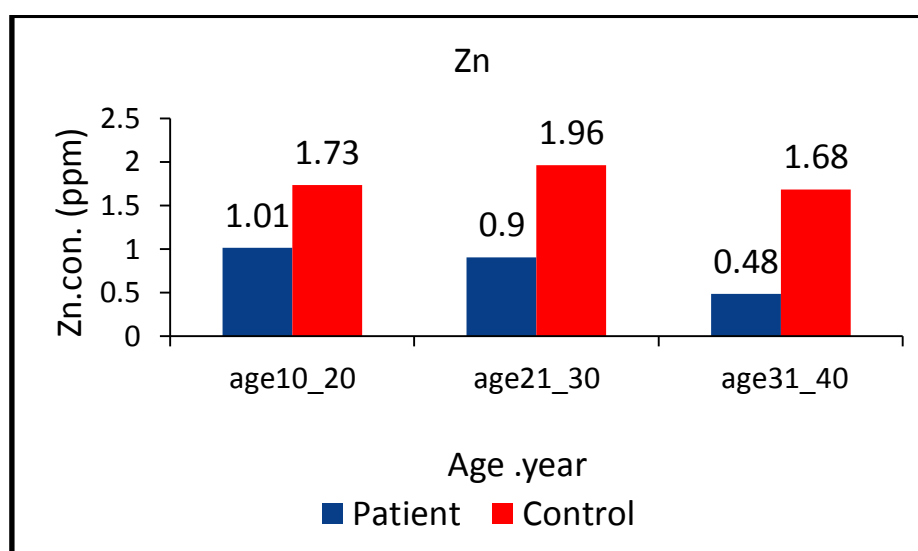


Figure 3.2.1 Zinc levels in patient and control groups according to age

3.2.2 : Determination Chromium concentration in Serum patient and control groups according to age groups

Table 3.2.2 and figures 3.2.2 showed a significant decrease A, B and C groups in the concentration of Cr serum in comparison with AC, BC and CC groups ($p \leq 0.05$).

Also table 3.2.2 and figures 3.2.2 showed a significant increase in the concentration of Cr serum in B, C in comparison with A and increase BC and CC in comparison with AC groups ($p \leq 0.05$).

Table 3.2.2 Chromium concentration (ppm) in patient and control according to age

Pat.	Age	Cr (ppm)	Cont.	Age	Cr(ppm)	LSD
A	10 -20	$0.32 \pm 0.06^{B,b}$	AC	10 -20	$0.40 \pm 0.14^{A,b}$	0.04
B	21 - 30	$0.36 \pm 0.07^{B,a}$	BC	21 - 30	$0.47 \pm 0.08^{A,a}$	0.02
C	31- 40	$0.38 \pm 0.06^{B,a}$	CC	31 -40	$0.45 \pm 0.03^{A,a}$	0.05
	LSD	0.03		LSD	0.03	

-Legend as in table (3.2.1)

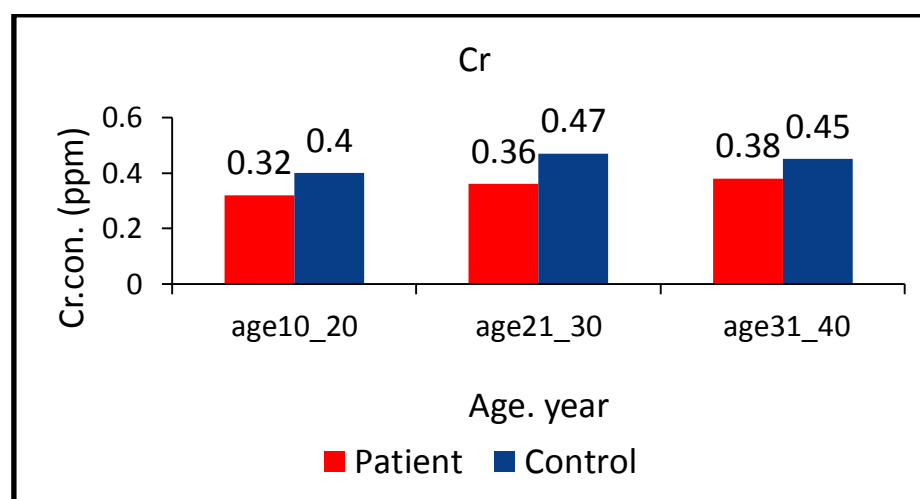


Figure 3.2.2 Chromium levels in patient and control according to age

3.2.3: Determination Iron concentration in Serum patient and control groups according to age groups

Table 3.2.3 and figures 3.2.3 showed a significant increase A,B and C groups in the concentration of Fe serum in comparison with AC, BC and CC groups ($p \leq 0.05$). In the same table it found a significant increase in the concentration of Fe serum in group CC in comparison with AC and BC groups ($p \leq 0.05$). It was non significant differences found in A,B, and C groups ($p \leq 0.05$).

Table 3.2.3 Iron concentrations (ppm) in patient and control according to age

Pat.	Age	Fe (ppm)	Cont.	Age	Fe (ppm)	LSD
A	10 -20	$2.08 \pm 0.28^{A,a}$	AC	10 -20	$1.36 \pm 0.20^{B,b}$	0.13
B	21 - 30	$2.26 \pm 0.56^{A,a}$	BC	21 -30	$1.42 \pm 0.22^{B,b}$	0.16
C	31- 40	$2.15 \pm 0.30^{A,a}$	CC	31-40	$1.59 \pm 0.19^{B,a}$	0.25
	LSD	0.15		LSD	0.10	

-Legend as in table (3.2.1)

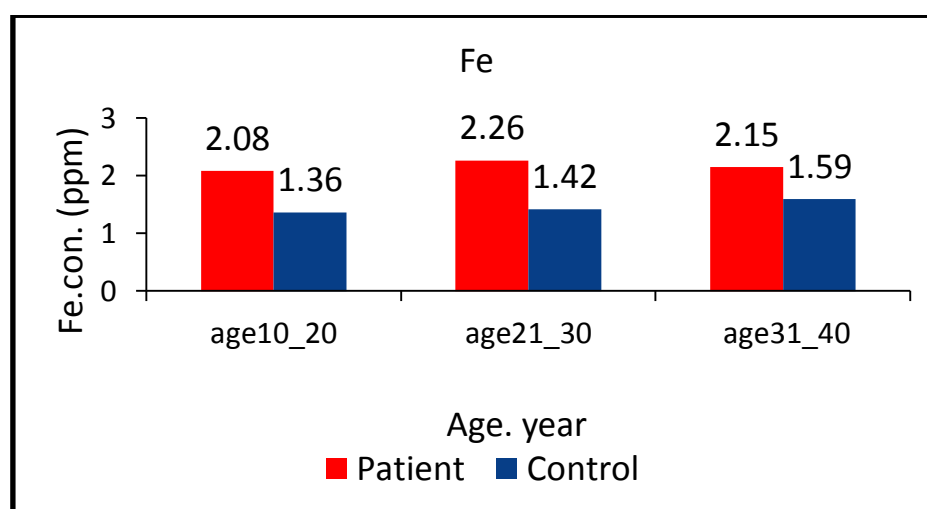


Figure 3.2.3 Iron levels in patient and control according to age

3.2.4: Determination Copper concentration in Serum patient and control groups according to age groups

The results showed a significant increase A, B and C groups in the concentration of Cu serum in patient in comparison with AC, BC and CC control groups ($p \leq 0.05$). In the same table it found a significant increase in the concentration of serum Cu in BC in comparison with AC and CC groups ($p \leq 0.05$). Also increase in C and A with comparison B

Table 3.2.4 Copper concentrations (ppm) in patient and control according to age

Pat.	Age	Cu (ppm)	Cont.	Age	Cu (ppm)	LSD
A	10 -20	2.25 $\pm$ 0.38 ^{A,b}	AC	10 -20	1.45 $\pm$ 0.19 ^{B,b}	0.24
B	21 - 30	2.07 $\pm$ 0.28 ^{A,c}	BC	21 - 30	1.68 $\pm$ 0.25 ^{B,a}	0.15
C	31 -40	2.92 $\pm$ 0.44 ^{A,a}	CC	31 -40	1.40 $\pm$ 0.17 ^{B,b}	0.25
	LSD	0.15		LSD	0.20	

-Legend as in table (3.2.1)

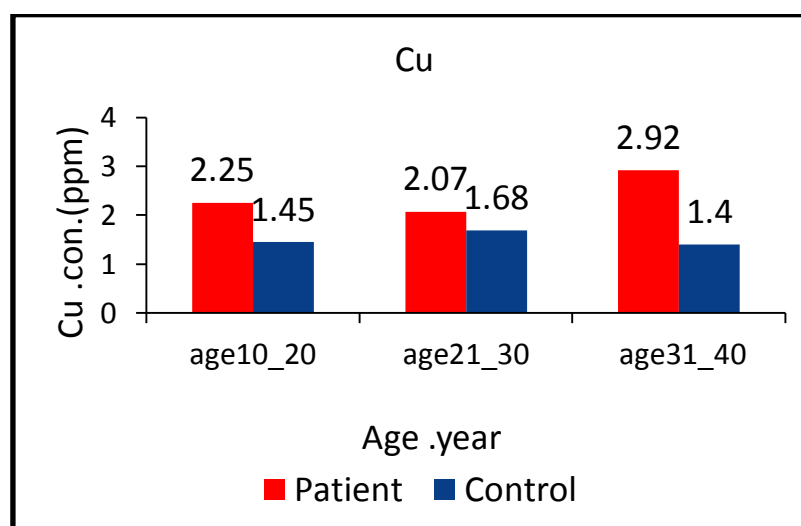


Figure 3.2.4 Copper levels in patient and control according to age

3.2.5: Determination Cobalt concentration in Serum patient and control groups according to age groups

The results showed non significant differences found in A, B and C groups in patient groups in comparison with AC, BC and CC control groups ($p \leq 0.05$).

Also table 3.2.5 and figures 3.2.5 showed a significant increase in the concentration of Co in B in comparison with A and C groups ($p \leq 0.05$). It was non significant in AC, BC, and CC groups ($p \leq 0.05$).

Table 3.2.5 Cobalt concentrations (ppm) in patient and control according to age

Pat.	Age	Co (ppm)	Cont.	Age	Co (ppm)	LSD
A	10 -20	$0.05 \pm 0.01^{A,b}$	AC	10 -20	$0.04 \pm 0.01^{A,a}$	0.02
B	21 - 30	$0.06 \pm 0.01^{A,a}$	BC	21 -30	$0.06 \pm 0.01^{A,a}$	0.01
C	31 -40	$0.05 \pm 0.01^{A,b}$	CC	31 -40	$0.04 \pm 0.01^{A,a}$	0.02
	LSD	0.02		LSD	0.04	

-Legend as in table (3.2.1)

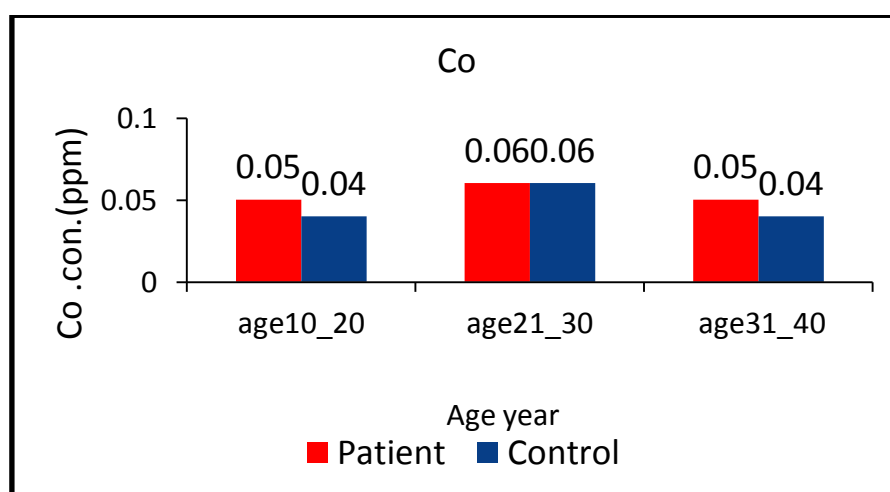


Figure 3.2.5 Cobalt levels in patient and control according to age

3.2.6: Determination Cadmium concentration in Serum patient and control groups according to age groups

Table 3.2.6 and figures 3.2.6 showed a significant increase the concentration in A and B groups in comparison with AC and BC ,also non significant differences found between C and CC groups ($p \leq 0.05$) , also non significant differences found between A,B,and C groups ($p \leq 0.05$). It was a significant increase the concentration in CC in comparison with AC, BC groups ($p \leq 0.05$).

Table 3.2.6 Cadmium concentrations (ppm) in patient and control according to age

Pat.	Age	Cd (ppm)	Cont.	Age	Cd (ppm)	LSD
A	10 -20	$0.07 \pm 0.01^{A,a}$	AC	10 -20	$0.04 \pm 0.01^{B,a}$	0.02
B	21 - 30	$0.06 \pm 0.01^{A,a}$	BC	21 - 30	$0.03 \pm 0.01^{B,a}$	0.01
C	31- 40	$0.08 \pm 0.01^{A,a}$	CC	31 -40	$0.04 \pm 0.02^{A,a}$	0.02
	LSD	0.03		LSD	0.02	

-Legend as in table (3.2.1)

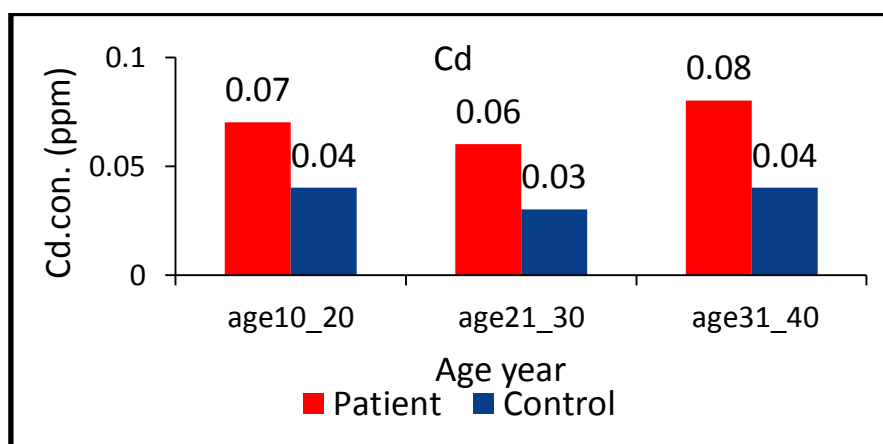


Figure 3.2.6 Cadmium levels in patient and control according to age

3.2.7: Determination Lead concentration in Serum patient and control groups according to age groups

The results showed a significant increase in A, B and C in patient groups in comparison with AC, BC and CC control groups ($p \leq 0.05$).

It was a significant decrease in the concentration of Pb between A and B comparison with C groups ($p \leq 0.05$). In the same table it was non significant differences found in the concentration of serum Pb in group AC, BC and CC groups $p \leq 0.05$.

Table 3.2.7 Lead concentrations (ppm) in patient and control groups according to age

Pat.	Age	Pb (ppm)	Cont.	Age	Pb (ppm)	LSD
A	10 -20	$0.46 \pm 0.12^{A,b}$	AC	10 -20	$0.28 \pm 0.04^{B,a}$	0.06
B	21 - 30	$0.47 \pm 0.11^{A,b}$	BC	21 - 30	$0.35 \pm 0.08^{B,a}$	0.08
C	31 - 40	$0.58 \pm 0.13^{A,a}$	CC	31 - 40	$0.37 \pm 0.09^{B,a}$	0.10
LSD		0.06		LSD	0.08	

-Legend as in table (3.2.1)

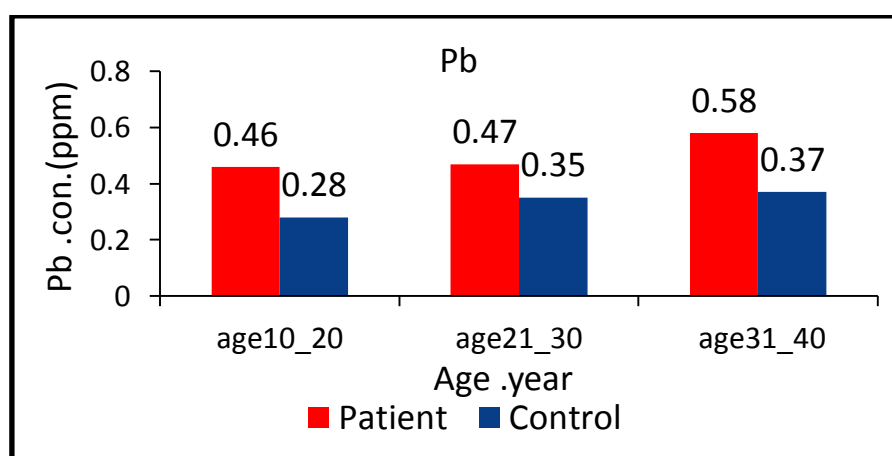


Figure 3.2.7 Lead levels in patient and control according to age

3.2.8: Determination Nickel concentration in Serum patient and control groups according to age groups

The results showed a significant increase in the concentration of Ni in A, B and C in patient in comparison with AC, BC and CC control groups ($p \leq 0.05$). It was a significant increase in the concentration of serum Ni between BC and CC in comparison with AC groups ($p \leq 0.05$). In the same table found a significant increase in C comparison B and C groups ($p \leq 0.05$).

Table 3.2.8 Nickel concentrations (ppm) in patient and control groups according to age

Pat.	Age	Ni (ppm)	Cont.	Age	Ni (ppm)	LSD
A	10 -20	0.42± 0.09 ^{A,c}	AC	10 -20	0.30± 0.06 ^{B,b}	0.02
B	21 - 30	0.70± 0.10 ^{A,b}	BC	21 - 30	0.38± 0.07 ^{B,a}	0.03
C	31- 40	0.91±0.05 ^{A,a}	CC	31- 40	0.39 ±0.09 ^{B,a}	0.05
	LSD	0.09		LSD	0.06	

-Legend as in table (3.2.1)

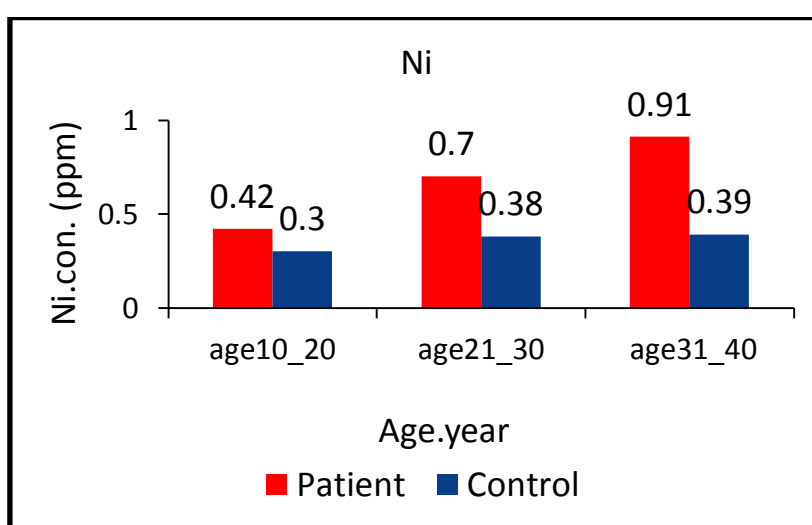


Figure 3.2.8 Nickel levels in patient and control according to age

3.3: Study of elements concentrations in patient and control groups according to body mass index (BMI)

Divided into three groups:

1-Non obese range of the group (18-24) Kg

3-Over weight range of the group(25-29) Kg

2- Obese range of the group (30-40) Kg

The present study has shown a significant increased in BMI in Iraqi PCOS patients compared with control group. Obesity is one of clinical characteristics of the PCOS along with oligomenorrhea ,hirsutism and infertility .However ,not all obese females have PCOS and not all PCOS patients are obese, the different increased in BMI in PCOS patients might be due to the different nutritional habits in Iraq then other countries were significantly elevated ($p < 0.05$) in the PCOS patients when compared to the control group ^(123,124,125). This study is shown in the following tables:

3.3.1 : Determination Zinc concentration in Serum patient and control groups according to body mass index (BMI)

The results showed a significant decrease in the concentration of Zn in A,B,and C patient groups in comparison with AC,BC, and CC control groups ($p \leq 0.05$).

Also table 3.3.1 and figures 3.3.1 showed a significant increase in the concentration of Zn in A and C in comparison with B groups ($p \leq 0.05$). In the same table it was found a significant increase in the concentration of Zn in BC and CC in comparison with AC groups ($p \leq 0.05$). serum zinc concentrations are negatively correlated with BMI in one study⁽¹²⁶⁾.

Table 3.3.1 Zinc concentrations (ppm) in patient and control groups according to BMI (Kg/m²)

Pat.	Groups	Zn (ppm)	Cont.	Groups	Zn (ppm)	LSD
A	Non- obese	0.93±0.26 ^{B,a}	AC	Non- obese	1.67±0.30 ^{A, b}	0.22
B	Over wight	0.96±0.24 ^{B,a}	BC	Over wight	1.99±0.50 ^{A,a}	0.11
C	Obese	0.83±0.18 ^{B,b}	CC	Obese	1.91±0.49 ^{A, a}	0.11
	LSD	0.09		LSD	0.17	

-Legend as the table (3.2.1)

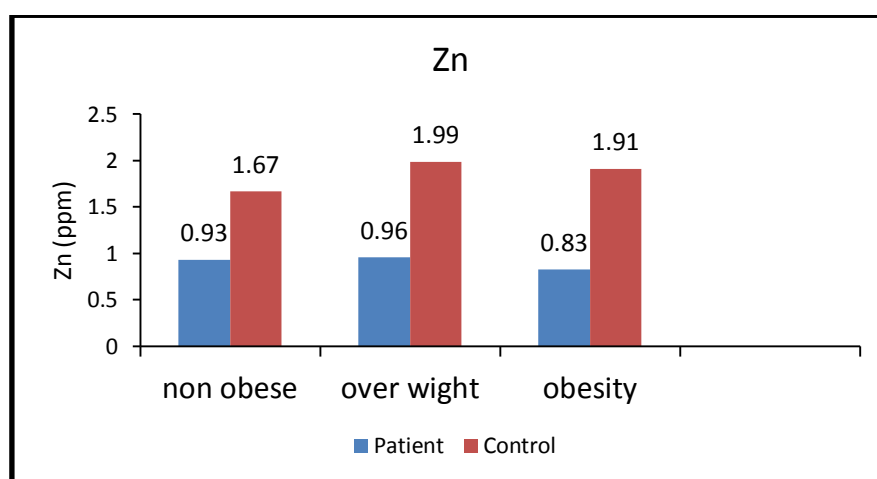


Figure 3.3.1 Zinc levels (ppm) in patient and control groups according to BMI (Kg/m²)

3.3.2 : Determination Chromium concentration in Serum patient and control groups according to body mass index (BMI)

The table results showed significant decrease in the concentration of Cr serum in A,B and C patient in comparison with AC,BC,and CC control groups ($p \leq 0.05$).

Also table 3.3.2 and figures 3.3.2 showed non significant differences found in the concentration of Cr (A , B,and C) groups ($p \leq 0.05$), and non significant differences found in the AC, BC, and CC) groups ($p \leq 0.05$).

Table 3.3.2 Chromium concentrations (ppm) in patient and control groups according to BMI (Kg/m²).

Pat.	Groups	Cr (ppm)	Cont.	Groups	Cr (ppm)	LSD
A	Non-Obese	0.33±0.07 ^{B,a}	AC	Non-Obese	0.39 ±0.07 ^{A, a}	0.05
B	Over wight	0.32±0.09 ^{B,a}	BC	Over wight	0.40 ±0.06 ^{A, a}	0.02
C	Obese	0.35±0.07 ^{B,a}	CC	Obese	0.38±0.04 ^{A, a}	0.06
	LSD	0.03		LSD	0.03	

-Legend as the table (3.2.1)

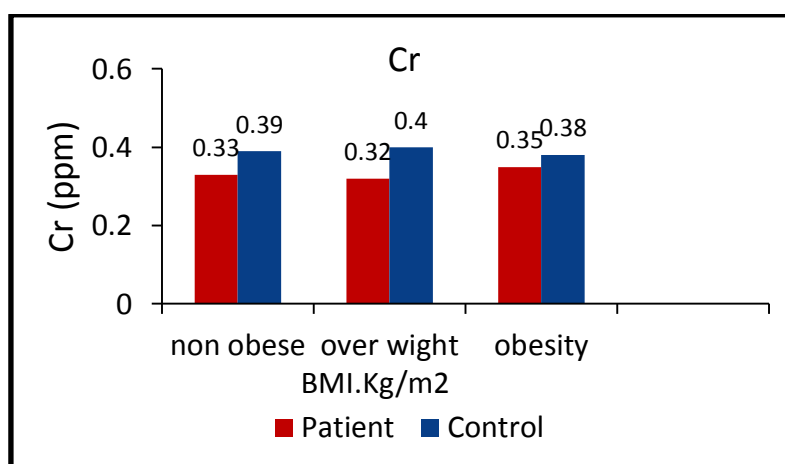


Figure 3.3.2 Chromium levels (ppm) in patient and control groups according to BMI (Kg/m²)

3.3.3: Determination Iron concentration in Serum patient and control groups according to body mass index (BMI)

The results showed a significant increase in the concentration of Fe in A, B, and C patient in comparison with AC, BC, and CC control groups ($p \leq 0.05$). In the same table 3.3.3 and figures 3.3.3 showed non significant differences found in the concentration of serum Fe between (A, B, and C)

groups ($p \leq 0.05$), also non significant differences found in the concentration of Fe AC, BC, and CC) groups ($p \leq 0.05$).

Table 3-3-3 Iron concentrations (ppm) in patient and control groups according to BMI (Kg/m^2)

Pat.	Groups	Fe (ppm)	Cont.	Groups	Fe (ppm)	LSD
A	Non-Obese	$2.34 \pm 0.40^{A,a}$	AC	Non Obese	$1.55 \pm 0.31^{B,a}$	0.11
B	Over wight	$2.21 \pm 0.51^{A,a}$	BC	Over wight	$1.52 \pm 0.21^{B,a}$	0.17
C	Obese	$2.11 \pm 0.42^{A,a}$	CC	Obese	$1.45 \pm 0.17^{B,a}$	0.16
	LSD	0.14		LSD	0.10	

-Legend as the table (3.2.1)

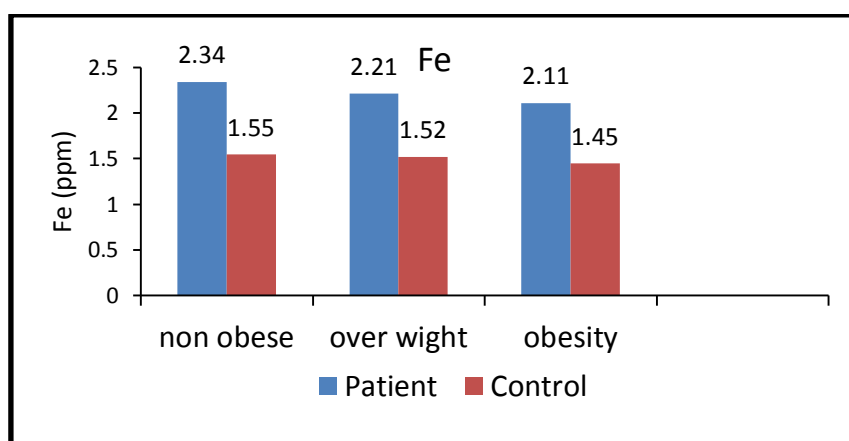


Figure 3.3.3 Iron levels (ppm) in patient and control groups according to BMI (Kg/m^2)

3.3.4 : Determination Copper concentration in Serum patient and control groups according to body mass index (BMI)

The results showed a significant increase in the concentration of Cu in A,B, and C patient in comparison with AC, BC, and CC control groups ($p \leq 0.05$). Also table 3.3.4 and figures 3.3.4 showed non significant differences found in the concentration of Cu between A,B, and C, groups

($p \leq 0.05$), also non significant differences found in the concentration of Cu in AC, BC, and CC) groups ($p \leq 0.05$).

Table 3.3.4 Copper concentrations (ppm) in patient and control groups according to BMI (Kg/m^2)

Patient	Groups	Cu (ppm)	Control	Groups	Cu (ppm)	LSD
A	Non Obese	$2.30 \pm 0.63^{A,a}$	AC	Non Obese	$1.59 \pm 0.38^{B,a}$	0.37
B	Over wight	$2.17 \pm 0.55^{A,a}$	BC	Over wight	$1.45 \pm 0.50^{B,a}$	0.22
C	Obese	$2.28 \pm 0.62^{A,a}$	CC	Obese	$1.69 \pm 0.29^{B,a}$	0.17
	LSD	0.20		LSD	0.20	

-Legend as the table (3.2.1)

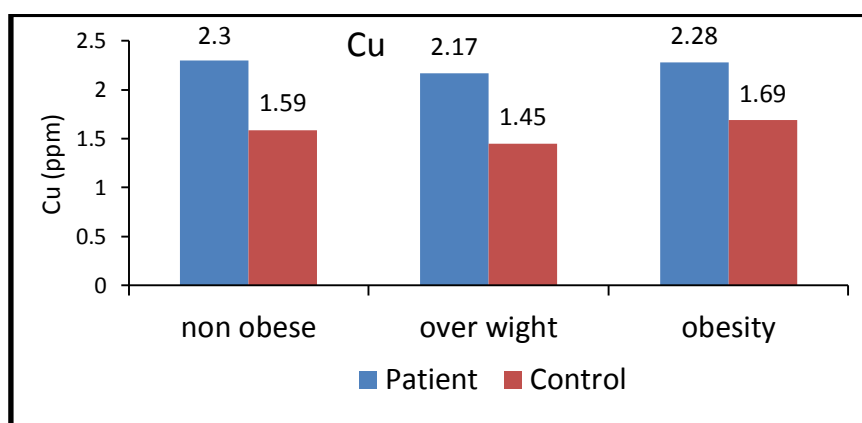


Figure 3.3.4 Copper levels (ppm) in patient and control groups according to BMI (Kg/m^2)

3.3.5 : Determination Cobalt concentration in Serum patient and control groups according to body mass index (BMI)

The results showed non significant in the concentration of Co in A, B and C patient group in comparison with AC, BC and CC control groups ($p \leq 0.05$). Also table 3.3.5 and figures 3.3.5 showed non significant differences found in the concentration of Co between A, B, and C groups

($p \leq 0.05$), also non significant differences found in the concentration AC, BC, and CC groups ($p \leq 0.05$).

Table 3.3.5 Cobalt concentrations (ppm) in patient and control groups according to BMI (Kg/m^2).

Pat.	Groups	Co(ppm)	Cont.	Groups	Co(ppm)	LSD
A	Non- Obese	$0.05 \pm 0.01^{A,a}$	AC	Non -Obese	$0.04 \pm 0.01^{A,a}$	0.02
B	Over wight	$0.06 \pm 0.02^{A,a}$	BC	Over wight	$0.05 \pm 0.01^{A,a}$	0.03
C	Obese	$0.05 \pm 0.02^{A,a}$	CC	Obese	$0.04 \pm 0.01^{A,a}$	0.03
	LSD	0.02		LSD	0.02	

-Legend as the table (3.2.1)

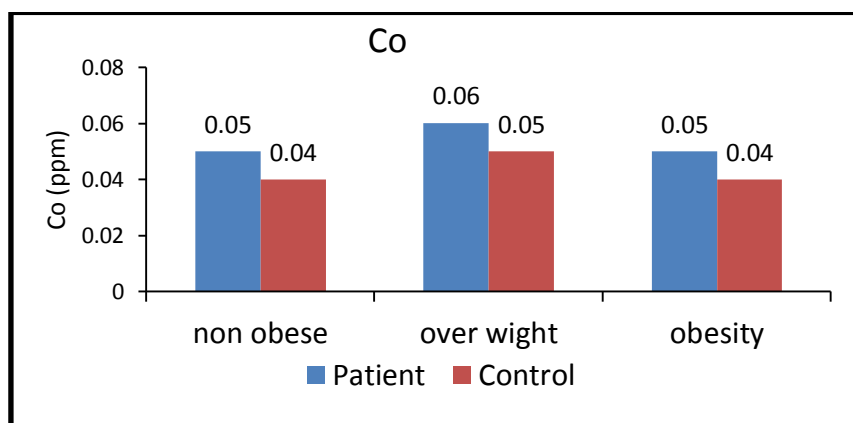


Figure 3.3.5 Cobalt levels (ppm) in patient and control groups according to BMI (Kg/m^2).

3.3.6: Determination Cadmium concentration in Serum patient and control groups according to body mass index (BMI)

The results showed a significant increase in the concentration of Cd in A, B, and C patient in comparison with AC, BC, and CC control groups ($p \leq 0.05$). Also table 3.3.6 and figures 3.3.6 showed non significant differences found in the concentration of Cd between A, B, and C groups

($p \leq 0.05$), also non significant differences found in the concentration of Cd AC, BC, and CC groups ($p \leq 0.05$).

Table 3.3.6 Cadmium concentrations (ppm) in patient and control groups according to BMI (Kg/m^2).

Pat.	Groups	Cd (ppm)	Cont.	Groups	Cd (ppm)	LSD
A	Non -Obese	$0.06 \pm 0.01^{A, a}$	AC	Non- Obese	$0.03 \pm 0.01^{B, a}$	0.02
B	Over wight	$0.06 \pm 0.01^{A, a}$	BC	Over wight	$0.04 \pm 0.01^{B, a}$	0.02
C	Obese	$0.07 \pm 0.01^{A, a}$	CC	Obese	$0.03 \pm 0.01^{B, a}$	0.01
	LSD	0.03		LSD	0.02	

-Legend as the table (3.2.1)

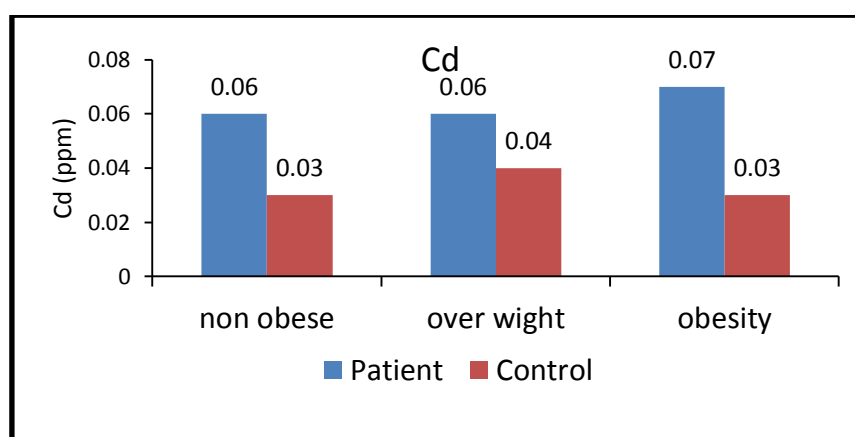


Figure 3.3.6 Cadmium levels (ppm) in patient and control groups according to BMI (Kg/m^2).

3.3.7: Determination Lead concentration in Serum patient and control groups according to body mass index (BMI)

The results showed a significant increase in the concentration of Pb in A, B, and C patient in comparison with AC, BC and CC control groups ($p \leq 0.05$). Also table 3.3.7 and figures 3.3.7 showed non significant differences found in the concentration of Pb (A, B, and C groups ($p \leq 0.05$),

also non significant differences found in the concentration of Pb in AC, BC, and CC) groups ($p \leq 0.05$).

Table 3.3.7 Lead concentrations (ppm) in patient and control groups according to BMI (Kg/m^2).

Pat.	Groups	Pb (ppm)	Cont.	Groups	Pb (ppm)	LSD
A	Non- Obese	$0.46 \pm 0.11^{A,a}$	AC	Non- Obese	$0.30 \pm 0.06^{B,a}$	0.11
B	Over wight	$0.50 \pm 0.09^{A,a}$	BC	Over wight	$0.34 \pm 0.04^{B,a}$	0.05
C	Obese	$0.47 \pm 0.12^{A,a}$	CC	Obese	$0.40 \pm 0.09^{B,a}$	0.07
	LSD	0.08		LSD	0.09	

-Legend as the table (3.2.1)

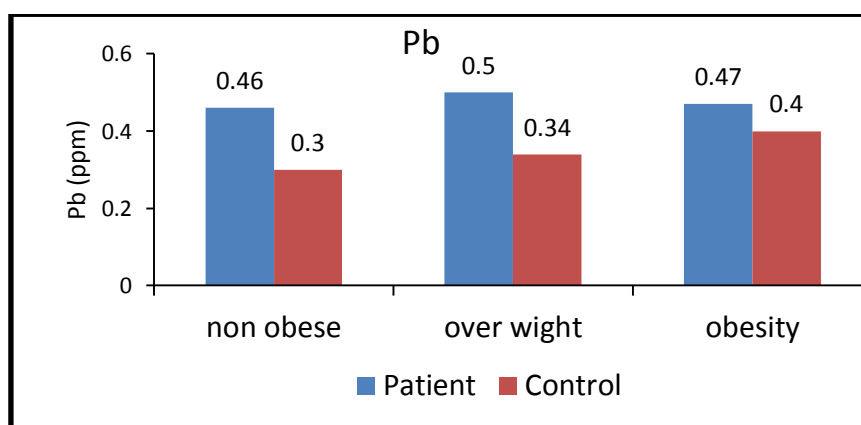


Figure 3.3.7 Lead levels (ppm) in patient and control groups according to BMI (Kg/m^2).

3.3.8: Determination Nickel concentration in Serum patient and control groups according to body mass index (BMI)

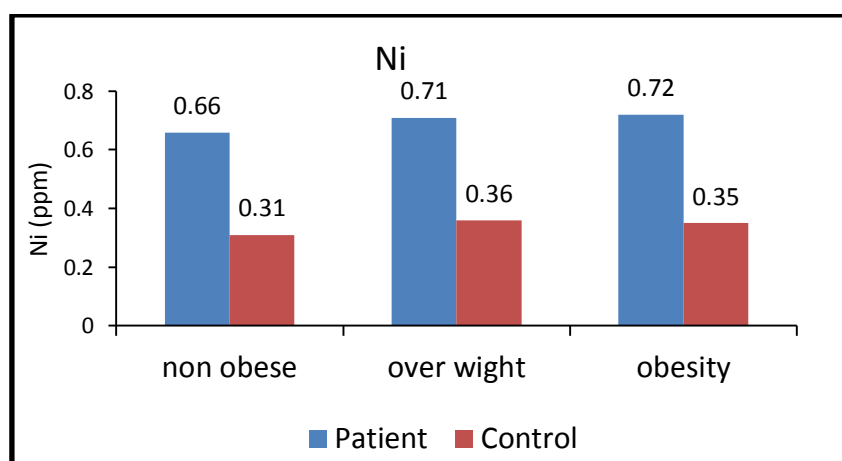
The results showed a significant increase in the concentration of Ni in A, B, and C patient in comparison with AC, BC, and CC control groups ($p \leq 0.05$). Also table 3.3.8 and figures 3.3.8 showed non significant differences found in the concentration of Ni (A, B, and C groups ($p \leq 0.05$),

also non significant differences found in the concentration of Ni AC , BC and CC) groups ($p \leq 0.05$).

Table 3.3.8 Nickel concentrations (ppm) in patient and control groups according to BMI (Kg/m^2)

Pat.	Groups	Ni (ppm)	Cont.	Groups	Ni (ppm)	LSD
A	Non- Obese	$0.66 \pm 0.14^{A,a}$	AC	Non-Obese	$0.31 \pm 0.01^{B,a}$	0.10
B	Over wight	$0.71 \pm 0.31^{A,a}$	BC	Over wight	$0.36 \pm 0.01^{B,a}$	0.13
C	Obese	$0.72 \pm 0.15^{A,a}$	CC	Obese	$0.35 \pm 0.01^{B,a}$	0.18
	LSD	0.09		LSD	0.08	

-Legend as the table (3.2.1)



Figures 3.3.8 Nickel levels (ppm) in patient and control groups according to BMI (Kg/m^2)

3.4: Study of elements concentrations in patient and control groups according to waist

3.4.1: Determination Iron, Copper, Nickel, Cadmium, and Lead concentration in Serum patient and control groups according to waist

From the following table. It was found significant increase in waist and concentration of elements (Fe, Cu, Ni, Cd and Pb) in the patient than control groups $p < 0.05$.

Table 3.4.1 Iron, Copper, Nickel, Cadmium, and Lead concentration (ppm) in patient and control groups according to Waist (cm).

Groups	Waist (cm) Mean $\pm$ SD	Fe (ppm) Mean $\pm$ SD	Cu (ppm) Mean $\pm$ SD	Ni (ppm) Mean $\pm$ SD	Cd (ppm) Mean $\pm$ SD	Pb (ppm) Mean $\pm$ SD
Pat.	34.87 $\pm$ 4.70a	2.21 $\pm$ 0.5a	2.28 $\pm$ 0.58a	0.69 $\pm$ 0.1a	0.06 $\pm$ 0.0a	0.48 $\pm$ 0.1a
Cont.	34.48 $\pm$ 4.01b	1.48 $\pm$ 0.1b	1.56 $\pm$ 0.31 b	0.33 $\pm$ 0.0b	0.04 $\pm$ 0.0b	0.35 $\pm$ 0.0b
LSD	0.13	0.10	0.13	0.04	0.01	0.05

-Legend as the table (3.2.1)

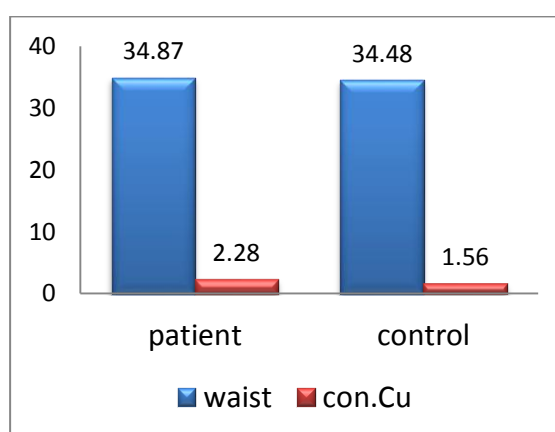
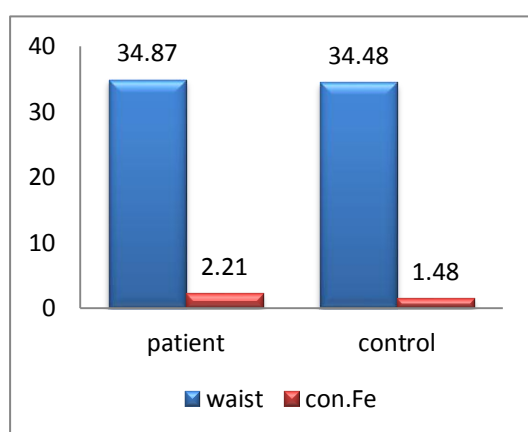


Figure 3.4.1 Iron levels (ppm) according to Waist (cm) Figure 3.4.1 Copper levels (ppm) according to Waist (cm)

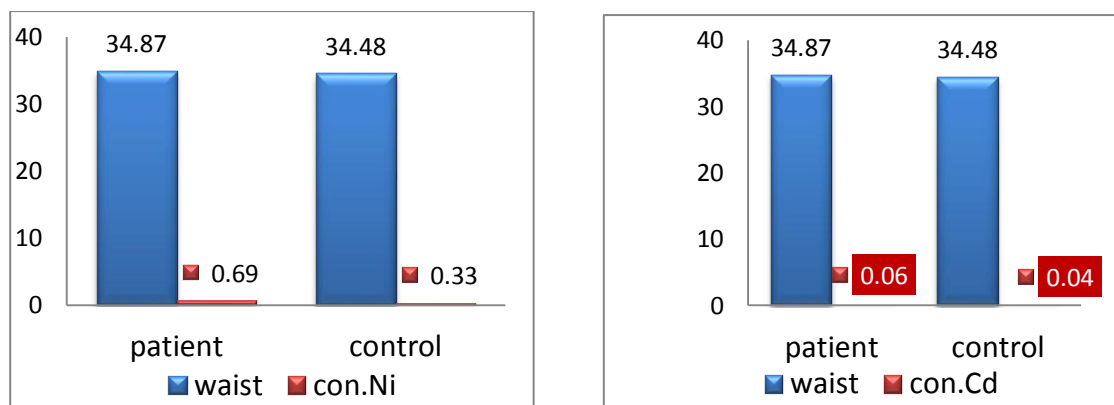


Figure 3.4.1 Cadmium levels (ppm) according to Waist(cm) Figure 3.4.1 Nickel levels (ppm) according to Waist(cm)

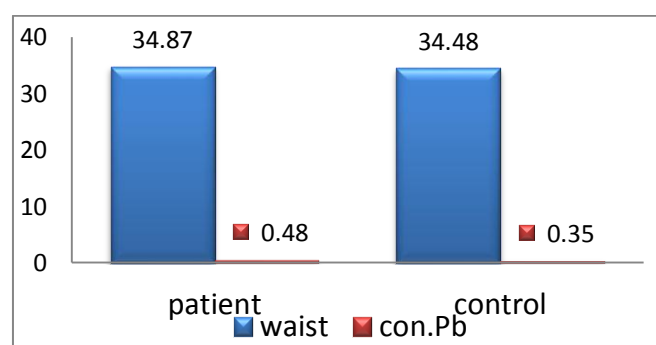


Figure 3.4.1 Lead levels (ppm) according to Waist(cm)

3.4.2: Determination Zinc and Chromium concentration in Serum patient and control groups according to waist

From the following table. It was found significant decrease concentration of Zn and Cr in comparison with the waist increase in the patient and control groups $p \leq 0.05$.

Table 3.4.2 Zinc and Chromium concentrations (ppm) in patient and control groups according to Waist(cm).

Groups	Waist (Mean±SD)cm	Zn (Mean ±SD)ppm	Cr(Mean ±SD)ppm
Pat.	34.87±4.70 a	0.91±0.20 b	0.37± 0.09 b
Cont.	34.48±4.01 b	1.85± 0.28 a	0.40±0.06 a
LSD	0.13	0.07	0.02

-Legend as the table (3.2.1)

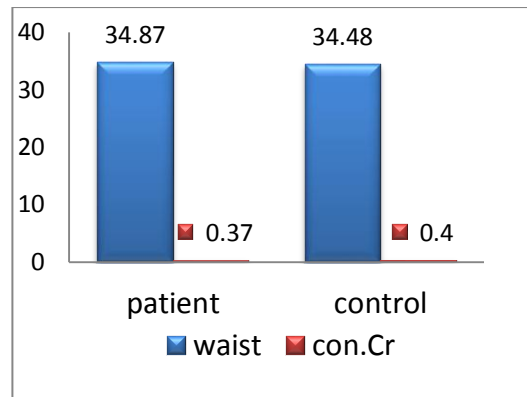
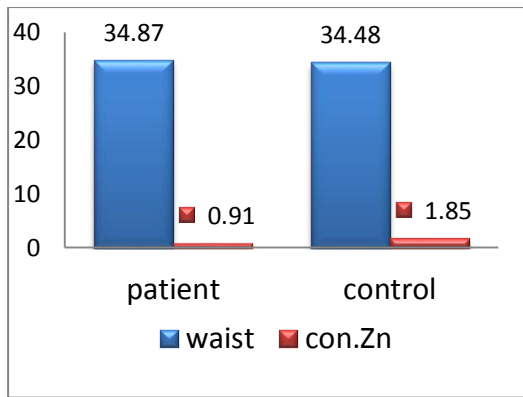


Figure 3.4.2 Zinc levels (ppm) according to Waist(cm) Figure 3.4.2 Chromium levels (ppm) according to Waist(cm)

3.4.3: Determination Cobalt concentration in Serum patient and control groups according to waist

The results showed a non significant difference found in the concentration of Co in comparison the waist increase in patient and control groups ($p \leq 0.05$).

Table 3.4.3 Cobalt concentrations (ppm) in patient and control groups according to Waist (cm).

Groups	Waist (Mean $\pm$ SD)cm	Co (Mean $\pm$ SD)ppm
Pat.	34.87 $\pm$ 4.70 a	0.05 $\pm$ 0.01 a
Cont.	34.48 $\pm$ 4.01 b	0.05 $\pm$ 0.01 a
LSD	0.13	0.02

-Legend as the table (3.2.1)

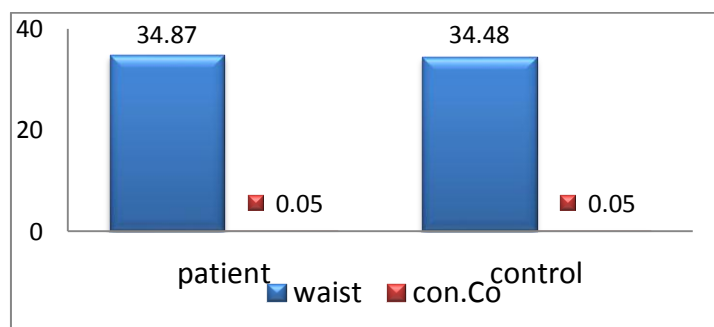


Figure 3.4.2 Cobalt levels (ppm) according to waist (cm)

Conclusion and Recommendations

Conclusions

In the present study, we examined 8 trace elements concentrations in the PCOS and control groups' female serum, which was consistent with previous studies with the data in this study, it was concluded the following points relevant to patients with polycystic ovary syndrome :-

- 1- Patients with PCOS had increased Ni, Fe, Pb, Cd and Cu with decreased Zn and Cr concentrations than the controls $P \leq 0.05$ when divided concentration according to age, waist and body mass
- 2- increasing of Ni, Cu, Fe, Pb, Cd, Cu and decreasing Zn and Cr may have effects on the PCOS group by or reproductive hormone levels related to oxidative stress.

Recommendations

For future work the investigations are recommended:

1. Measurement FSH, LH, DHEAS, TT, TSH, Cholesterol, Triglycerides, LDL-C, HDL-C, Fasting insulin, Fasting glucose levels in PCOS women.
2. Patient monitoring to determine the effect of treatment on the levels of the elements and the extent of improvement in the situation
3. Avoiding exposure to resources of trace elements for PCOS women
4. Must be diets recommended for obese PCOS patients are low in calories with a reduced carbohydrate intake

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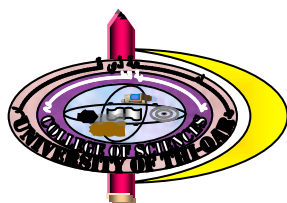
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الخلاصة

تم تصميم هذه الدراسة لتحديد مستويات العناصر النزرة (Ni، Pb، Fe، Cr، Cd، Co، Zn، Cu) في سيرم (70) مرضى تكيس المبايض (PCOS) و(50) كونترول. النتائج: عند دراسة تراكيز العناصر في حالة التقسيم العام وتقسيم الفئات العمرية من (10-20 و 21-30 و 31-40) وتقسيم حسب كتلة الجسم إلى ثلاث مجموعات (الغير سمينه والسمينه والبدينه) بالاضافه الى التقسيم حسب الخصر وجدنا تراكيز العناصر (النحاس والنيكل والرصاص والكاديوم والحديد) في المرضى أعلى من الكونترول بينما تراكيز كل من الزنك والكروم أقل في المرضى مقارنة بالكونترول وكذلك اتضح انه لا يوجد فرق معنوي في تركيز الكوبالت بين المرضى والكونترول. واستندت هذه النتائج على المتوسط الحسابي وكذلك الانحراف المعياري ($\text{Mean} \pm \text{SD}$) تحت مستوى الاحتمال 0.05 هذه النتيجة يمكن أن تتغير مع التغيرات البيئية والعادات الغذائية ومدة التعرض لهذه العناصر او استخدامها في الحياة اليومية



وزارة التعليم العالي والبحث العلمي

جامعة ذي قار

كلية العلوم

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رساله مقدمه الى

مجلس كلية العلوم - جامعة ذي قار

وهي جزء من متطلبات نيل درجه ماجستير علوم في علوم الكيمياء

تقدمت بها

نبا نعمة حميد

بكالوريوس علوم كيمياء - جامعة ذي قار / 2015

اشراف

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