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Studying Smoking and Progressive Age Effects on some Physiological Parameters in Adult Polycythemic Men in Maysan Province

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By

Marwah Ali Majeed

B.Sc. Biology (2010)

Supervised by

Prof. Dr. Zainab Abduljabbar Ridha Al-Ali

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Supervisor's Certificate

I certify that this thesis entitled "**Studying Smoking and Progressive Age Effects on some Physiological Parameters in Adult Polycythemic Men in Maysan Province** " has been prepared under our supervision at the college of science , university of Misan ; as a partial fulfillment of the requirements for the degree of master of biology.

Signature

Prof. Dr. Zainab Abduljabbar Ridha Al-Ali

Department of Biology

College of Science / Misan University

Date: / / 2023

Recommendation of Head of Biology Department

In view the available recommendations; I forward this thesis debate by examining committee.

Signature

Assist. Prof. Dr. Maytham A. Dragh

Head of Department of Biology

College of Science / Misan University

Date: / / 2023

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ﴾

صدق الله العليّ العظيم

سورة المجادلة، الآية (١١)

Dedication

To everyone who taught me a letter in this mortal world

To the example of devotion and sincerity... my beloved father

To whom I gave my happiness and comfort to her happiness

my virtuous mother

To those who have never been stingy in helping me... my dear

husband Ali

To the beats of my heart… my dear's children (Hussen and Hyder)

To the one who gave me advice and guidance... my dears

brother and sisters

To everyone who supported me even with a smile...

To everyone who called me well

I dedicate this humble work to you

Marwah

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Summary

Summary

The purpose of the current study was to known the impact of hypoxia in polycythemia subjects, by estimated the levels of hematological parameters, some hormones and lipid profile in adults men with polycythemia at Maysan province. The samples were collected from the main blood bank during the period from November 2022 to March 2023. The study included (100) men (75) with polycythemia, and (25) as control group, the average age was between (20-59) years. The men with polycythemia divided according to smoking into two groups (smokers and non-smokers). Also, they were divided according to age to two groups, the first (20-39) years and the second (40-59). Results showed the following:

There was a significant increased ($P<0.05$) in the values of red blood cells (RBCs) count , hemoglobin (Hb) and hematocrit (HCT) in both smoking and both age groups of men with polycythemia compared to the control. While, white blood cells (WBCs) did not show significant differences in the both smoking and age groups compared to control. The values of the RBC indices: Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were significantly increased ($P < 0.05$) in the smoking and first age group polycythemic men compared to control; while in the second age group, the values of MCH and MCHC not differ significantly between men with polycythemia and control. According to the smoking, the levels of MCV and MCH did not differ significantly for men with polycythemia compared to the control group.

The result of the present study showed a significant decrease in erythropoietin hormone (EPO) in the smoking and age groups of men with polycythemia compared to the control.

There is no significant difference in the levels of follicle stimulating hormone (FSH) and estradiol in the smoking and age groups of polycythemia compared to the control group. While, the luteinizing hormone (LH) increased considerably ($P<0.05$) in both age and smoking groups for men with polycythemia compared to the control group. Total testosterone (TT) didn't change significantly in the first age group (20-39) years for men with polycythemia compared to the control group, and increased considerably ($P<0.05$) in the second age group (40-59) years for men with polycythemia compared to the control group. Whereas, the TT showed no differences that were notable for men with smoking polycythemia patients compared to the control group.

Thyroid stimulating hormone (TSH) and thyroxine (T4) values did not differ significantly in both smoking and age groups of polycythemia compared with the control group. While, triiodothyronine (T3) increased significantly ($P < 0.05$) in the first age group (20-39) years, and did not show any significant variations in the second age group (40-59) years of polycythemia subjects when in compared to the control group. Also, the findings demonstrated a substantial increase ($P < 0.05$) in T3 in both smokers and non-smokers groups of polycythemia compared to the control.

The findings indicated a significant increase ($P < 0.05$) in the level of total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), and very low density lipoprotein-cholesterol (VLDV-C), and significant decreased ($P < 0.05$) in the level of high density lipoprotein-cholesterol (HDL-C) in the both smoking and age groups of polycythemia compared with the control group.

The physiological effect of the results which discussed according to the polycythemia and its relationship with the smoking which have harmful

substances that induce abnormalities in blood parameters, lipid profile and some hormones.

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List of Abbreviations

Abbrevations	Equivalences
17-OHP	17-Hydroxyprogesterone
2,3DPG	2,3 Diphosphoglycerate
AML	Acute Myeloid Leukemia
ABP	Androgen Binding Protein
Asn	Asparagine
BCSH	British Committee for standarde in Hematology
BFU-E	Burst Forming Unit Erythroid
CFU-E	Colony Forming Unit-Erythroid
CO	Carbon Monoxide
COHb	Carboxy Hemoglobin
CVD	Cardiovascular Disease
CNS	Central Nervous System
CSF	Cerebrospinl Fluid
CLIA	Chemiluminescent Immunoassy Analyzer
CHD	Chronic Heart Disease
CMS	Chronic Mountain Sickness
CBC	Complete Blood Count
CI	Confidence Interval
Cys	Cysteine
ESAs	Erythropoiesis-Stimulating agents
EPO	Erythropoietin
E2	Estradiol
EDTA	Ethylene Diamine Tetraacetic Acid
FSH	Follicle Stimulating Hormone
FDA	Food and Drug Administration
FT4	Free Thyroxine
Hct	Hematocrite
Hb	Hemoglobin
HDL-C	High Density Lipoprotein-Cholesterol
HPT	Hypothalamic Pituitary Thyroid axis
GnRH	Gonadotropin Releasing Hormone
HIF	Hypoxia-Inducible Factor
JAK2	Janus Kinase 2
KDa	Kello Daltone
LDL-C	Low Density Lipoprotein-Cholesterol
LH	Luteinizing Hormone
Lys	Lysine
MDA	Malondialdehyde

MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MF	Myelofibross
MPN	Myeloproliferative Neoplasms
OD	Optic Density
PCV	Packed Cell Volume
PV	Polycythemia Vera
PPV	Predictive Positive Value
PO_2	Pressure of Oxygen
rhEPO	Recombinant Human Erythopoietin
RBCs	Red Blood Cells
RCM	Red Cells Mass
RLUs	Relative Light Units
Ser	Serine
SPSS	Statistical Package for Social Science
TSH	Thyroid Stimulating Hormone
T4	Thyroxine
TC	Total Cholesterol
TT	Total Testosterone
TG	Triglycerides
T3	Triiodothyronine
VLDL-C	Very Low Density Lipoprotein-Cholesterol
WBCs	White Blood Cells
WHO	World Health Organization

Chapter One

Introduction

and

Literatures Review

1: Introduction

Polycythemia is an abnormally high level of hemoglobin and/or hematocrit in peripheral blood, which is primarily divided into primary and secondary polycythemia (Cınar *et al*., 1999).

The former includes polycythemia Vera (PV), the most prevalent cause of acquired primary polycythemia, and is characterized by autonomous erythrocyte production, as a result of a mutation represented by that of the Janus kinase 2 gene in erythrocyte progenitor cells. Secondary olycythemia is induced by an increase in erythropoietin (EPO), which is typically brought on by tissue hypoxia originate from the variety of circumstances such as, cardiovascular illnesses, high altitude, smoking, and sleep apnea. Erythropoietin-producing tumors such hepatocellular carcinoma, renal cell carcinoma, hemangioblastoma, pheochromocytoma, and uterine myoma can also result in polycythemia. People who have polycythemia are more likely to have blood that is viscous, which raises hemodynamic shear stress and raises the risk of thrombotic cardiovascular events (Alberti *et al*., 2005 ; Caliri *et al*., 2021).

Due to tissue hypoxia, smoking is a significant contributor to secondary polycythemia: Smokers have lower blood oxygen transport and tissue oxygen release than non-smokers. Smoking increases blood coagulability (Wouters *et al*., 2020), increases oxidative stress (Carneiro and Zanella, 2018), and worsens the blood lipid profile (Souresho *et al*., 2021), all of which are risk factors for atherosclerotic disorders.

As a result, smoking has a significant impact on the association between polycythemia and cardiovascular risk. However, it is yet unknown if polycythemia affects cardiovascular risk in the general population independently of smoking (Hsieh and Muto, 2006 ; Flack and Adekola, 2020).

Smoking reduces the amount of oxygen in the bone marrow, which prevents the production of red blood cells (RBCs), which transport oxygen. The effects of enhanced hematocrit caused by excessive erythrocytosis include increased RBC mass, increased blood viscosity, slower blood return through the venous system (slow blood flow), and increased platelet adhesion. Clot formation was caused by the blood's increased viscosity and the vessel wall's activation of the platelets (Alkhedaide, 2020).

Smokers' polycythemia (erythrocythemia) is an abnormally high plasma concentration of RBCs brought on by erythrocytosis (increased RBC count), and decreased plasma volume (hemoconcentration). Secondary polycythemia is a disorder of increased hemoglobin or hematocrit (increased RBC mass). States of carbon monoxide (CO) are most usually responsible for systemic hypoxia (Sung *et al*., 2022).

An abundance of RBCs or a rosy color is common in patients with high red blood cell mass. The symptoms are exceedingly ambiguous and diverse. The majority of people either experience no symptoms at all or may experience symptoms as a result of increased blood viscosity, and decreased tissue perfusion in various organs. Patients with poor blood flow to the central nervous system may display generalized weakness, reduced capacity for exercise, fatigue, headaches, blurred vision, lethargy, myocardial infarction, burning in the feet, legs, arms, and hands, or more severe manifestations such as myocardial infarction, deep vein thrombosis, thrombosis, and stroke (Jain *et al*., 2021).

Free radicals from tobacco smoke are known to cause a variety of intricate reactions such as increase DNA damage, inflammation, and oxidative stress (Sharifi-Rad *et al*., 2020). Smoking causes tar from

cigarettes to enter the bloodstream, where it thickens and hastens the clotting process. Additionally, it makes the heart work harder, raises blood pressure and heart rate, narrows the arteries, and increases blood pressure, all of which reduce the supply of oxygen-rich blood to the organs and increase the risk of stroke (Klein *et al*., 2021).

The EPO hormone is primarily produced by the kidneys, and is regarded as a crucial component of the feedback system that regulates the production of red blood cells (Koury, 2005).

Due to nicotine's effects on the hypothalamus-pituitary-gonad axis (HPG) and the metabolism of steroid sex hormones, a number of hormones, including sex steroid hormones, can change. Nicotine in cigarettes has also been shown to affect the prostate and seminal vesicle function (Heidary *et al*., 2012).

Smoking has long been known to have an impact on thyroid function, which may be related to exposure to the toxic metabolites, increased sympathetic nerve activity, or thyroid-focused autoimmune responses (Taylor *et al*., 2018).

The quitting of the smoking causes a rise in TSH, and a fall in T4 levels (Melander *et al.,* 1981). Since then, data has emerged showing that, with some exceptions, smoking is linked to lower TSH and higher FT4 levels, as well as higher or unaffected T3 (Arslan, 2015).

Smoking is a major modifiable risk factor for atherosclerosis and increasing morbidity and mortality of chronic heart diseases (CHD) (Howard *et al*., 1998). The liver absorbs the free fatty acids that are released as a result of lipolysis, which is induced by smoking's rise in plasma catecholamine (Benowitz *et al*., 1993). Atherosclerosis has been

described as an inflammatory condition that affects the arterial wall and is lipid driven (Tabas *et al*., 2007).

HDL-C is a preventive factor against coronary atherosclerosis, while LDL-C and VLDL-C are atherogenic factors (Stampfer *et al*., 1991).

There are links between smoking tobacco and higher levels of total cholesterol, triglycerides, LDL-cholesterol, and VLDL cholesterol, as well as, lower levels of HDL-cholesterol (Prabha *et al*., 2015). Additionally, it has been believed that the quantity of cigarettes smoked directly influences the chance of developing cardiovascular disease (CVD) (Burns, 2003).

The aim of the study

Based on was previously mentioned, and the fact that smoking is causative factor for hypoxia and relationship with polycythemia. Also, the role of the age. So that, this study aimed to estimate levels of some hematological, biochemical parameters and some hormones in polycythemia subjects:

1- **Hematological parameters:** RBCs, Hb, HCT, WBCs, MCV, MCH and MCHC.
2- **Hormonal parameters**: EPO, FSH, LH, TT, E2, TSH, T4 and T3.
3- **Biochemical parameters:** TC, TG, HDL-C, LDL-C and VLDL-C.

1.2: Literature review

1.2.1: Polycythemia

In 1892, Louis Henri Vaqueza French internist described a patient with cyanotic polycythemia; an autopsy showed that the spleen and liver had undergone hypertrophy. William Osler a Canadian physician in 1903, who was working at Johns Hopkins Hospital at the time, wrote a report on four polycythemia patients, two of whom exhibited splenomegaly. Until recently, this disorder was called Osler-Vaquez disease and is now referred to as Polycythemia Vera (PV) (Goldman, 2005).

In 1951, William Dameshek an American hematologist drew attention to the connections between essential thrombocythemia, PV, idiopathic myelofibrosis, along with chronic myeloid leukemia, and erythroleukemia should be included in the broad category of myeloproliferative syndromes (Dameshek, 1951).

Polycythemia derived from poly (many) and cythemia (cells in the blood), is a condition in which the body's overall number of blood cells, mostly red blood cells (RBCs), increases RBCs production may be high due to a bone marrow natural process, persistently low oxygen levels, or another condition. Health or lifestyle factors can also contribute to an elevated RBCs count. Hypoxia (low blood oxygen levels) and carbon monoxide exposure are two medical disorders that might result in an increase in RBCs (Abbas, 2011).

Erythrocytosis is brought on by either an increase in RBCs mass (absolute erythrocytosis) or a reduction in plasma volume (relative erythrocytosis). The cause of absolute erythrocytosis might be either primary or secondary. Primary erythrocytosis is caused by excessive RBC production that occurs on its own, such as when myeloproliferative

neoplasms (MPN) or rare genetic diseases are present. In situations when erythropoietin stimulates the production of additional RBC in the bone marrow (such as hypoxia or the presence of an erythropoietin-producing tumor), this condition is referred to as secondary erythrocytosis. PV is the most frequent cause of acquired primary erythrocytosis (McMullin, 2008).

1.2.2: Classification of the polycythemia

1- Relative Polycythemia:

Pseudo-polycythemia, as the relative polycythemia is also known, is a condition in which the HCT increases, but the total amount of red cells mass (RCM) remains within normal ranges. Dehydration, diarrhea, vomiting, the use of diuretics, capillary leak syndrome, and serious burns can contribute to a drop in blood plasma compared to cells (Vener *et al*., 2010).

2- Absolute polycythemia:

It means the proportion of HCT increasing, whether, it be primary or secondary, and the RCM really increasing (McMullin, 2009), and it is include two types:

A- Primary polycythemia or PV:

True polycythemia is caused by intrinsic factors such as red cell precursors and bone marrow with an increase in erythrocytes, leukocytes, and platelets. A latent thrombotic stage marks the beginning of the illness and is associated with an increased incidence of venous thromboembolism (Durmus *et al*., 2014).

A mutation in the Janus kinase 2 (JAK 2) gene, which is located in exon 14 of chromosome 9, has been identified as the cause of true PV illness. About 95% of individuals with PV, 50% of patients with primary myelofibrosis and critical thrombocytopenia have this mutation (Akalin *et*

al., 2013). Hematocrit (HCT) was the only laboratory parameter that showed a direct correlation with thrombosis and clinical signs of significantly increased erythropoiesis (Kilpivaara and Levine, 2008).

B-Secondary polycythemia:

EPO is essential for controlling RBC synthesis and is influenced by partial pressure of oxygen gas (PO_2), by increasing the bone marrow's sensitivity to this hormone.Another conditions, such as congenital heart disease, chronic respiratory disease,hypoventilation syndromes including obstructive sleep apnea, renal disease (local renal hypoxia and renal artery stenosis), smoking, and living at elevations where oxygen is scarce, can lead to secondary polycythemia (Nadeem *et al*., 2013 ; Durmus *et al*., 2014).

1.2.3: Epidemiology

Even while PV can happen at any age, its prevalence is highest between the ages of 5 and 7 years old. Men experience PV at a higher rate than women (Berglund and Zettervall, 1992). The natural history of PV varies. However, that people with PV have lower survival times than the general population (Hultcrantz *et al*., 2012).

The study on the incidence and prevalence of MPNs, including PV, are impeded by the disease's extremely sluggish behavior. The true annual frequency of these illnesses is underestimated since many patients experience prolonged periods of asymptomacity and do not seek medical care. The incidence and prevalence of PV have been examined across a number of investigations in recent literature-based reports. PV incidence rate ranged from 0.01 to 2.80 per 100,000 people annually, with a pooled incidence rate of 0.84 per 100,000 people (95% confidence interval (CI), 0.70-1.01). European research revealed a rate of1.05 per 100,000 (95% CI,

1.03-1.07), compared to North America's 0.94 per 100,000 (95% CI, 0.92-0.96).Male patients had crude annual incidence rates of 0.87 per 100,000 (95% CI : 0.58-1.30) and female subjects had crude annual incidence rates of 0.73 per 100,000 (95% CI : 0.46-1.15). PV prevalence ranged from 0.49 to 46.88 per 100,000 people (Moulard *et al*., 2014).

1.2.4: Pathophysiology

The physiological process that produces and maintains RBC mass is called erythropoiesis. Numerous hormones, receptors, and other elements control this process (Krantz, 1991). EPO is the most important of all the RBC mass regulators. It is produced mainly by the kidneys and liver. EPO is typically created in response to hypoxia and anemia. The creation of hypoxia-inducible factors is stimulated by hypoxia, which in turn increases the expression of the EPO gene (Prchal, 2001 ; Maran and Prchal, 2004).

RBCs are essential for oxygen transport. The capacity of the blood to carry oxygen will rise as RBC mass increases. For optimal tissue oxygen supply, patients with secondary polycythemia need larger RBC mass than usual for proper tissue oxygen delivery. It has been noted that hyper viscosity in normovolemic patients is related to HCT levels exceeding 45%. In these patients, there is a delicate balance between the hyper viscosity and normal tissue oxygenation; this is the therapeutic objective (Hocking and Golde, 1989).

1.2.5: Clinical symptoms

Polycythemia Vera (PV) is a myeloproliferative neoplasm (MPN) marked by erythrocytosis, leukocytosis, thrombocytosis, and splenomegaly (Stein *et al*., 2015). In the United States; for example, PV affects approximately 148,000 patients, with a prevalence ranging from 44

to 57 cases per 100,000 people and an incidence of 1 to 3 cases per 100,000 people (Johansson, 2006). PV patients are more likely to experience medical issues, such as thrombosis, leukemic change, and severe symptom burden (Tefferi and Vardiman, 2008 ; Mesa *et al*., 2016).

The probabilities of acute myeloid leukemia (AML) and post-PV myelofibrosis (MF) transformation ten years after diagnosis are 10% and 5% to 21%, respectively (Finazzi *et al*., 2005 ; Tefferi and Vardiman, 2008). A mutation in the JAK2 gene is present in the majority of PV patients. The JAK2 V617F mutation is present in 95% of patients, while 3% have the JAK2 exon 12 mutation (Baxter *et al*., 2005 ; Tefferi and Barbui, 2017).

At the time of diagnosis, symptoms associated with PV, such as exhaustion, headaches, visual abnormalities, and pruritus, are present in approximately 50% of patients (Ahn *et al*., 2007 ; Emanuel *et al*., 2012 ; Tefferi *et al*., 2013). Some PV sufferers are asymptomatic when they are first diagnosed and may only have their condition identified by chance results from laboratory blood tests (Passamonti *et al*., 2000 ; Raedler, 2014).

Patients frequently report worsening symptoms over time as PV advances, as well as new symptoms such fatigue, early satiety, and inactivity (Stein *et al*., 2015 ; Mesa *et al*., 2016). By interfering with everyday activities, family and social life, and work productivity, the symptom burden associated with PV may adversely influence patients' quality of life in terms of their health (Mesa *et al*., 2016 ; Harrison *et al*., 2017).

1.2.6: Treatment

The primary objective of PV treatment is to reduce the risk of developing thromboembolic complications. All patients should begin either antiplatelet or anticoagulation therapy, even if only high-risk patients need cytoreductive therapy. In most cases, low-dose aspirin is sufficient for thromboprophylaxis unless the patient has already experienced a thrombotic episode (Landolfi *et al*., 2004). Patients who are older than 60 or have a history of thrombosis are classified as high-risk (Marchioli *et al*., 2005).

Leukocytosis and cardiovascular risk factors, such as cardiovascular disease, diabetes, hypertension, hyperlipidemia, and smoking, are also believed to increase the risk of polycythemia (Gangat *et al*., 2007 ; Bonicelli *et al*., 2013).

Low-risk patients should take low-dose aspirin to prevent blood clots in veins and arteries, even without the need for cytoreduction. Periodic phlebotomy can be used to control the erythrocytosis, and keep the HCT of men and women at less than 45% and 42%, respectively (Marchioli *et al*., 2013).

Up to twice a week, phlebotomy can be performed, with 1 unit of RBCs removed each time. Patients may experience side effects such as tiredness, dizziness, orthostatic hypotension, and syncope, particularly if phlebotomy is frequently performed. Phlebotomy's primary goal is to deplete the body's iron stores, which reduces blood flow. Therefore, iron replacement should not be administered unless anemia results; even then, it must be done carefully to prevent the recurrence of polycythemia. The use of cytoreductive therapy with or without phlebotomy in high-risk

patients who are older than 60 or have a history of thromboembolic disease should be evaluated as indicated (Marchioli *et al*., 2005).

Other signs that cytoreduction is necessary include persistent symptoms despite phlebotomy, poor tolerance of phlebotomy, or platelet count of more than (1.5×10^6/μL). Hydroxyurea is first-line treatment for cytoreduction. Myelosuppression is hydroxyurea's main adverse impact, which is expectedly caused by it down regulating blood production. Unintended side effects such as fatigue, headaches, and rashes may also occur.In general, hydroxyurea is well-tolerated.It is crucial to gradually increase the dosage because hydroxyurea's impact on blood counts takes days or even weeks to manifest. The US Food and Drug Administration (FDA) authorized ruxolitinib in 2015 as a second-line treatment option for people with hydroxyurea intolerance or refractory illness. Ruxolitinib is a JAK1/JAK2 inhibitor and effective in symptom reduction and spleen size reduction, but it does not slow the progression of the disease (Vannucchi *et al*., 2015).

It does aid in blood count management, while phlebotomy may still be necessary to reach target RBC levels.Interferon alfa is effective, and some cases show long-term hematologic and molecular remissions. When hydroxyurea and ruxolitinib are not advised or when other treatments have not been successful during pregnancy, this medication plays a vital role.Busulfan, radioactive phosphorous-32, and pipobroman are therapies that are rarely used since, they have lost favor due to the possibility of causing leukemogenesis.A stem cell transplant is the last resort for treating PV (Kiladjian *et al*., 2008).

1.2.2: Secondary polycythemia due to hypoxia:

1.2.2.1: Smoking

Smoking is the primary cause of fatalities, disabling conditions, and avoidable diseases worldwide. In the United States, it causes more over 480,000 fatalities annually, or about one in every five deaths (Jamal *et al*., 2018).

Smoking cigarettes increases the risk of developing cardiovascular disorders, including peripheral vascular disease, atherosclerosis, myocardial infarction, stroke, and coronary artery disease. The exact process by which smoking causes these disorders in smokers is unknown, but it is believed that abnormalities in blood rheology, infections, inflammation, oxidative stress, changes in the fibrinolytic system, and antithrombotic systems are to blame (Malenica *et al*., 2017).

Smokers are exposed to a variety of dangerous compounds, such as nicotine, free radicals, carbon monoxide (CO), and other gaseous pollutants (Gitte, 2011). Nicotine and CO in tobacco have several effects on the body. They lead to an increase in Hb and HCT levels, stimulate bone marrow to produce more RBCs, and result in a very high RBC mass. This, in turn, decreases blood flow and elevates the risk of intravascular coagulation, coronary vascular resistance, decreased coronary blood flow, and a propensity for thrombosis (Roethig *et al*., 2010 ; Raval and Paul, 2010).

1.2.2.2: High altitude

Altitudes greater than 2400 meters above sea level are referred to as "high altitudes." 2% of the world's population, or more than 140 million people, reside in these areas (Moore, 2001).

The oxygen reduction availability caused by the low barometric pressure in these high-altitude environments is one of their hallmarks. People who live in these areas must therefore create adaptation strategies for the hypobaric hypoxia. There are various considerable high-altitude places that are inhabited around the world, and they are primarily concentrated in Africa (in the Ethiopian peaks), Asia (in the Tibetan plateau and along Himalayan mountains), and America (in the Andean highlands). The local inhabitants of these places have created adaptation mechanisms that enable them to survive in conditions with low oxygen levels (Beall, 2006).

Tibetans, who have lived at very high altitudes for millennia, exhibit normal hemoglobin levels despite arterial hypoxemia (Beall, 2007). The erythrocytosis and arterial hypoxemia in Andean populations are higher than in Ethiopian and Tibetan populations because they have lived at high altitudes for a shorter period of time. Some of these later people also develop chronic mountain sickness (CMS), also known as Monge's disease, where the erythrocytosis is so severe (hematocrit > 75) that the sufferers exhibit signs of pulmonary hypertension and hyper viscosity (Monge *et al*., 1992).

1.2.3: Erythropoietin (EPO)

The EPO, is a glycoprotein hormone primarily produced in the adult kidney and fetal liver, regulates erythropoiesis in response to hypoxia (Rey *et al*., 2019 ; Peng *et al*., 2020). EPO was first identified in the 1950s and then isolated from the urine of aplastic anemia patients in the 1970s. It was successfully cloned and produced in mammalian cells in 1984 (Simon *et al*., 2019).

Recombinant human EPO (rhEPO) was used to treat anemia in patients with end-stage renal failure, elevating the hemoglobin concentration in 9 out of 12 patients (Winearls *et al*., 1986).Chinese hamster ovary cells, commonly used for large-scale production of erythropoiesis-stimulating agents (ESAs), were able to produce biologically active rhEPO (Jelkmann, 2013).

The United States of America of FDA approved rhEPO in 1989, for the clinical treatment of anemia associated with chronic renal failure due to insufficient EPO production, and patients' quality of life improved as a result (Wright *et al*., 2015; Suresh *et al*., 2020).

Since it became clinically available in the 1990s, rhEPO has been widely utilized to treat chronic renal illnesses as well as, a variety of anemia brought on by many causes, including infection and chemotherapy for various cancers (Hernández *et al*., 2017; Simon *et al*., 2019).

1.2.3.1: Structure of EPO

Human EPO belongs to the type I cytokine superfamily and is found in all vertebrates (Kunze and Marti, 2019). It is made up of 166 amino acids and has a globular, three-dimensional structure and forms four amphipathic α-helices, two β-sheets, and two intra-chain disulfide bridges (Cys-7-Cys-161 and Cys-29-Cys-33) (Jelkmann, 2013; Ostrowski and Heinrich, 2018). Human EPO, like rhEPO, comprises approximately 40% carbohydrates, including three N-linked polysaccharide groups at positions Asn-24, Asn-38, and Asn-83, and one O-linked group at residue Ser-126 (Sinclair, 2013).

The amino acid residues in human EPO are Lys-24, Lys-38, and Lys-83, as shown in the figure (1-1).Notably, EPO's maturity is highly heterogeneous due to the glycosylation distribution, which also controls

the drug's pharmacokinetic and pharmacodynamics characteristics. In addition, the distribution of glycosylation regulates EPO's biological activity and influences its interaction with receptors (Castillo *et al*., 2018). Human EPO has a weight of 30.4 KDa, while rhEPO weighs approximately 34 KDa (Rama *et al*., 2019).

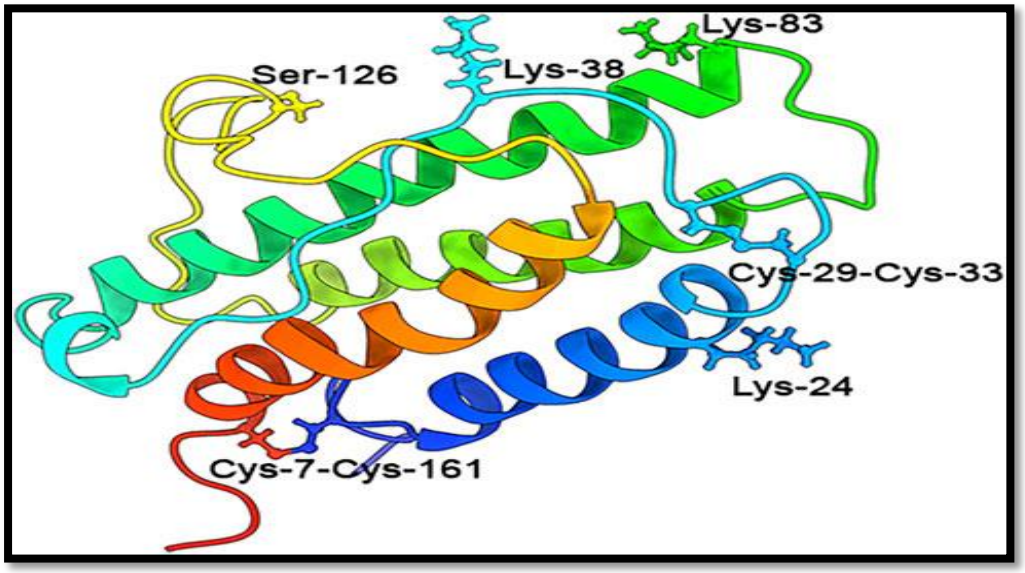


Figure (1-1): Human erythropoietin structure (Sinclair, 2013).

1.2.3.2: Production of EPO

Throughout a person's life, the primary EPO-producing organ changes (Rey *et al.*, 2019). EPO is initially produced in the liver, which is responsible for RBCs production during fetal development. However, as individuals mature, renal tubular interstitial cells in the kidney gradually become the primary site for EPO production and secretion (Jelkmann, 2001).

EPO may be created and released locally by cells of numerous organs and tissues, including the heart, spleen, bone marrow, lungs, testis, ovaries, retina, and central nervous system (CNS), in addition to fetal liver and adult kidney, which made up the majority of the circulating EPO in humans (Ostrowski and Heinrich, 2018 ; Suresh *et al*., 2020).

In the human brain, astrocytes, oligodendrocytes, neurons, and endothelial cells from regions like the cortex, hippocampus, amygdala, and midbrain are capable of producing EPO in a paracrine or autocrine manner (Simon *et al*., 2019). Furthermore, EPO has been detected in the cerebrospinal fluid (CSF) of both neonates and adults (Marti, 2004).

EPO synthesis may be stimulated by hypoxia. Under hypoxic conditions, binding of the hypoxia-inducible factor (HIF) to the hypoxic responsive element, boosted the production of EPO (Kobayashi *et al*., 2017; Anusornvongchai *et al*., 2018).

The HIF is a heterodimer composed of HIF-α (HIF-1α, HIF-2α, or HIF-3α) and HIF-1β (or ARNT). In renal EPO-producing cells, decreased oxygen levels lead to the stabilization of HIF-α and an increase in HIF-2α, which upregulates the expression of the EPO gene (Semenza, 2009).

A recent study demonstrated that a higher number of kidney cells producing EPO resulted in an increased response to hypoxia (Suresh *et al*., 2020). When the erythrocyte level dropped, the renal tubular interstitial cells detected relative hypoxia and, in a traditional endocrine manner, produced and released EPO into the plasma. The oxygen binding and transport capacity was then increased, as a result of EPO migrating to the bone marrow, and encouraging erythropoiesis (Peng *et al*., 2020).

Moreover, fractures in the elderly male population are associated with high serum EPO levels (Rauner *et al*., 2021). However, according to previous research, older rats express less EPO than their younger counterparts. In aging rats, oxidative stress may be the primary factor contributing to the decline in brain EPO, and the reduction in HIF-2α stability is involved in this decline (Li *et al*., 2016).

Recently, a clinical study reported that after 180 minutes of normobaric hypoxia, plasma EPO levels increased in both young and elderly individuals.During the same normobaric hypoxia, EPO levels in young individuals were higher than those in elderly adults (Torpel *et al*., 2019).

In addition to hypoxia, factors such as anemia, high altitude, mechanical damage, infection, metabolic stress, high temperature, intensive cerebral activity, enriched environment, and ischemia stress are some of the various challenges or events that can stimulate EPO synthesis (Pugh and Ratcliffe, 2017 ; Ostrowski and Heinrich, 2018). In humans, the circulatory half-life of kidney-derived EPO is 5–6 hours (Peng *et al*., 2020).

1.2.4: Polycythemia and hematological parameters

Polycythemia refers to elevated levels of Hb and HCT above the normal range. This complex process of erythropoiesis control is influenced by bone marrow susceptibility to oxygen and EPO. Indicators of polycythemia include the quantity of erythrocytes in peripheral blood, Hb levels, and HCT (Jeremic *et al*., 2021).

Diagnosing the disease involves analyzing blood through a complete blood count (CBC), which includes noting the HCT or PCV (percentage of red blood cells in the sample). Normal percentages vary among men (42% - 52%), women (37% - 47%), and children (36% - 44%). It's important to note that this percentage can vary depending on the analysis, laboratory, and units of measurement in use (Baxter *et al*., 2005; Kralovics *et al*., 2006). These HCT percentages can decrease or increase in the following conditions:

1- HCT or PCV is decreases in some cases such as leukemia, rheumatoid arthritis, bone marrow failure, starvation, and hemodialysis (delis blood).

2- Elevating PCV or HCT levels, the bone marrow produces a significant amount of red blood cells (RBCs), secreting more of them than the body needs. Consequently, levels of RBC, Hb, and HCT or PCV increase. (Barbui *et al*., 2014 ; Kroll *et al*., 2015)**.**

There are two methods to characterize erythrocytosis. The first method was based on the British Committee for Standards in Hematology (BCSH) and World Health Organization (WHO) 2008 categorization of myeloid neoplasms as Hb concentration 18.5 g/dL or HCT 52% in males, and in females is 16.5 g/dL or HCT 48% (McMullin *et al*., 2007 ; Tefferi and Vardiman, 2008).

The second method was based on the 2016 revision to the WHO classification of a Hb concentration.16.5 g/dl, or HCT.49% of men and a high Hb concentration.16.0 g/dl or HCT.48% of females (Arber *et al*., 2016).

Furthermore, a significant increase in CBC and RBC indices (MCV, MCH, MCHC) among smokers polycythemic patients aged (31-50) year, in province of Babylon in Iraq (Alkafaly, 2015).

1.2.5: Polycythemia and Erythropoietin

The WHO has established precise diagnostic criteria for PV, which include the following: Hb levels greater than 16.5 g/dL in males and 16 g/dL in females; panmyelosis in the bone marrow; the presence of the JAK2 mutation; and/or low serum EPO levels. Non-clonal causes of erythrocytosis encompass conditions that induce tissue hypoxia, such as kidney or heart conditions, or tumors that produce EPO as a result of paraneoplastic phenomena. Usually, in these acquired states, the serum EPO levels are high. Congenital causes of erythrocytosis are rare, and the

serum EPO levels in these conditions may be variable (Djulbegovic *et al*., 2018).

Subnormal values are still utilized by the WHO as a minor diagnostic criterion for PV diagnosis (Arber *et al*., 2016). Various studies have been carried out in the past to assess the sensitivity and specificity of EPO for polycythemia. In a study by Messinezy and his group (2002), they assessed the role of EPO in 125 patients with erythrocytosis and found that low EPO had 64% sensitivity and 92% specificity for diagnosing. In study about 241 erythrocytosis patients, 116 of whom had PV, the results showed a sensitivity of 97% and a predictive value of 97.8% for low EPO levels (Mossuz *et al*., 2004).

Ancochea and his group (2014), studied 99 patients with polycythemia and found the sensitivity and specificity were 79% and 97%, respectively. Davila-Gonzalez and his group (2021), found in study conducted over an 11-year period with 577 individuals, they demonstrated that EPO levels less than 2 mIU/mL, exhibited >99% specificity and only 12% sensitivity to predict PV.

Maslah and his team (2022), evaluated the diagnostic performance of EPO in 1090 individuals with polycythemia. Low EPO levels among patients with high HCT/Hb ratios exhibited a 92% predictive positive value (PPV), in their study, a verylow EPO level (≤1.99 mUI/mL) had a PPV of 100% for PV diagnosis.

1.2.5: Polycythemia and Reproductive hormones

The testosterone hormone itself induces the synthesis of erythropoietin and raises HCT (Bachman *et al*., 2014). Polycythemia is a documented unintended side effect consequence of testosterone therapy for hypogonadism (Jones *et al*., 2015). Additionally, high-altitude sickness is linked to elevated HCT and serum testosterone levels. (Gonzales, 2013).

Hypoxia and altitude-induced stress may have an impact on reproductive processes at various stages, including gametogenesis, the ovarian cycle and menstruation, birth weights, stillbirth rates, infant mortality, postpartum behavior, menopause and gonadal hormones. Key transcription factors known as (HIFs) mediate changes in gene regulation induced on by hypoxic stress in both adult and embryonic development (Tam *et al*., 2010).

The testicular tissue is significantly affected by high altitude, which has effecton physiological processes. The production of testosterone is reduced due to leydig cell malfunction brought on by hypoxia resulting from high attitude (Saxena, 1995).

The CMS, a lack of adaptation to high altitude (Xing *et al*., 2008) is characterized by excessive erythrocytosis brought on by hypoventilation (Reeves and Leon-Velarde, 2004). CMS is more common in men and increases in prevalence with age and altitude (Penaloza and Arias-Stella, 2007).

It most likely affects bone marrow at the level of polychromatophilic erythroblasts, where it increases the production of ribosomal RNA or its precursors and activates a nuclear ribonuclease (Zitzmann, 2008).

Additionally, testosterone increases the synthesis of RBC in males (Favier *et al*., 1997), and promotes erythropoiesis while, estradiol has the opposite effects (Horiguchi *et al*., 2005 ; Cristancho *et al*., 2007).

Previous study reported that administration of testosterone is associated with serum hepcidin suppression hepcidin is a liver-derived peptide that binds to and degrades the iron channel ferroportin, and low hepcidin is associatedwith increased iron absorption, systemic transport and stimulation erythropoiesis (Bachman *et al*., 2010).

1.2.6: Polycythemia and Thyroid hormones

The thyroid gland is classical endocrine gland that is essential for the body's healthy development, differentiation, metabolism, and physiological operation. In clinical practice, thyroid dysfunction is one of the most frequent disorders, and it has become more widespread globally in recent years, thus its risk factors have drawn a lot of attention (Garmendia *et al*., 2014 ; Taylor *et al*., 2018).

It may be brought on by anomalies in thyroid hormone receptors as well as the overproduction or under production of thyroid hormones. Age, sex, geography, and dietary variations in iodine consumptionall affect the occurrence of thyroid diseases (Ittermann *et al*., 2015).

It is easily diagnosable and treatable, but if left unnoticed or untreated, it may have serious implications. Thyroid dysfunction has a substantial negative impact on many aspects of health, such as CVD, metabolic problems, mentalhealth, and bone health (Diab *et al*., 2019).

Thyroid disorder can result in a variety of symptoms, such as the hypoplasia of erythroid cells in the bone marrow, the proliferation of immature erythroid progenitor cells (due to hypothyroidism), or the hyperplasia (related to hyperthyroidism) (Kawa *et al*., 2010).

Previous research showed that thyroid malfunction can cause anemia, erythrocytosis, leukopenia, thrombocytopenia, and, in rare cases, pancytopenia in blood cells (Davis *et al*., 1983).

Thyroid hormones stimulate the growth of erythroid colonies (BFU-E, CFU-E), increase erythrocyte 2, 3 Diphosphoglycerate(2,3 DPG) compactness, enhance erythropoiesis through a hyper proliferationof immature erythroid progenitors, increase EPO secretion by inducing EPO gene expression, and contribute to Hb production, effect on

megakaryocytes through modulation of bone marrow matrix proteins, such as fibronectin, increase the expression of fibronectin gene, an alter platelet function and affects hematopoiesis in many ways (Dorgalaleh *et al*., 2013).

1.2.7: Polycythemia and lipid profile

Information about the relationships between polycythemia and cardio metabolic risk factors such hypertension, dyslipidemia, and diabetesis still limited , The prevalence of polycythemia as a cardiovascular risk factor in the general population is not well understood (Wakabayashi and Daimon, 2015 ; Arber *et al*., 2016).

Due to tissue hypoxia, smoking is a leading cause of secondary polycythemia (Hsieh and Muto, 2006 ; Flack and Adekola, 2020). Smokers have decreased blood oxygen transport and tissue oxygen release compared to non-smokers. Smoking increases the risk of atherosclerotic diseases by causing oxidative stress, rise in blood coagulability and deterioration of the blood lipid profile (Carneiro and Zanella, 2018 ; Wouters *et al*., 2020 ; Souresho *et al*., 2021).

Previous research has suggested that smoking causes dyslipidemia that makes atherosclerosis more likely (Nagaraj and Paunipagar, 2014). Smoking and other blood lipid components both contribute important to structural and functional alterations in the arterial system, which ultimately result in CVD (Singh, 2016). Increased duration and daily cigarette smoking volume cause the alterations to become more pronounced (Nagaraj and Paunipagar, 2014).

Smokers showed higher levels of fasting TG, lower HDL-C levels, and increase percentage of tiny dense LDL-C particles (Eliasson *et al*., 1994). Craig and his group (1989) found that smokers exhibited lower levels of

HDL-C, higher levels of VLDL-C, and TC in their serum compared to non-smokers.

Smoking is thought to cause dyslipidemia, which manifests as elevated levels LDL-C levels, triglycerides level and decreased HDL-C level (Nagaraj and Paunipagar, 2014). Physiological changes brought on by both acute and long-term exposure to high altitudes can result in a wide range of disorders (Milledge, 2020).

The potential increased cardiovascular risk among those who live at high altitudes is one of the most contentious topics. The degree of adaptation to high altitudes that a population has achieved,as well as lifestyle factors,and genetic predisposition may all have an impact on the cardiovascular health of that population (Aryal *et al*., 2015).

Hyperlipidemia and hypercholesterolemia appear to be less common in highlanders, indicating a decreased risk of atherosclerosis and stroke (Ortiz-Prado *et al*., 2019).

According to the study on 158 individuals living at 4,100 meters revealed that the proportion of HDL-C and triglycerides is directly related to Hb value, and that rise is related to greater diastolic blood pressure. More specifically, high Hb levels were directly related to increased TC, LDL-C, HDL-C, and TG levels; there was no correlation between high Hb levels and glucose levels (Gonzales and Tapia, 2013).

Al Riyami *et al*., (2015), found that the most important factor influencing HDL-C was altitude, gender, serum TG, and the 2-hour post-meal plasma glucose.

Research has established that oxidative stress results in alterations to the serum lipid profile and that atherosclerosis and coronary heart disease are related in individuals with polycythemia (Durmus *et al*., 2014).

Oxidative stress is caused by an increase in reactive oxygen radicals within cells this in turn, leads to lipid peroxidation, thus causing cell damage and then death, measuring serum malondialdehyde (MDA) levels can help determine lipid peroxidation levels (Pérez-Rodríguez *et al*., 2015).

According to a study by Vener and his group (2010), patients with polycythemia have an increase in oxidative stress, which is correlated with a high homocysteine level. Both vascular events and mutagenesis, such as the JAK2V617F mutation in PV patients, have been associated with increased oxidative stress (Durmus *et al*., 2014).

Chapter Two

Materials and

Methods

2-1: Materials and Methods

2-1-1: The Materials

2-1-1-1: Subjects

The present study was carried out during the period from November 2022 to March 2023 in a main blood bank at Maysan province. All samples included (100) men aged between 20-59 years, (75) men with polycythemia and (25) men who were normally healthy as a control. The polycythemia patients were subdivided according to the smoking to smokers and non-smokers group. Also, according to their ages to two groups. First group (20–39) years and second group (40–59) years as illustrated in figure (2-1).

All subjects of the study were without other chronic diseases such as diabetes mellitus, thyroid disorders, cardiovascular disease, and renal disturbance. These data were obtained by questionnaire (Appendice, A). A questionnaire has been designed to obtain the actual information about the sample individuals in Maysan province.

2-1-1-2: Blood Samples

Six milliliters of venous blood were taken from all subjects at (9-11AM). The blood sample was divided into two tubes, 4 ml in gel tube and 2 ml in ethylene diamine tetraacetic acid (EDTA) tube for measuring the blood parameters. The serum tubes were centrifuged at 3000 rpm for 10 minutes. The serum was separated and divided into three aliquots and stored at (–20°C) until the time of use.

2.2: Experimental Design

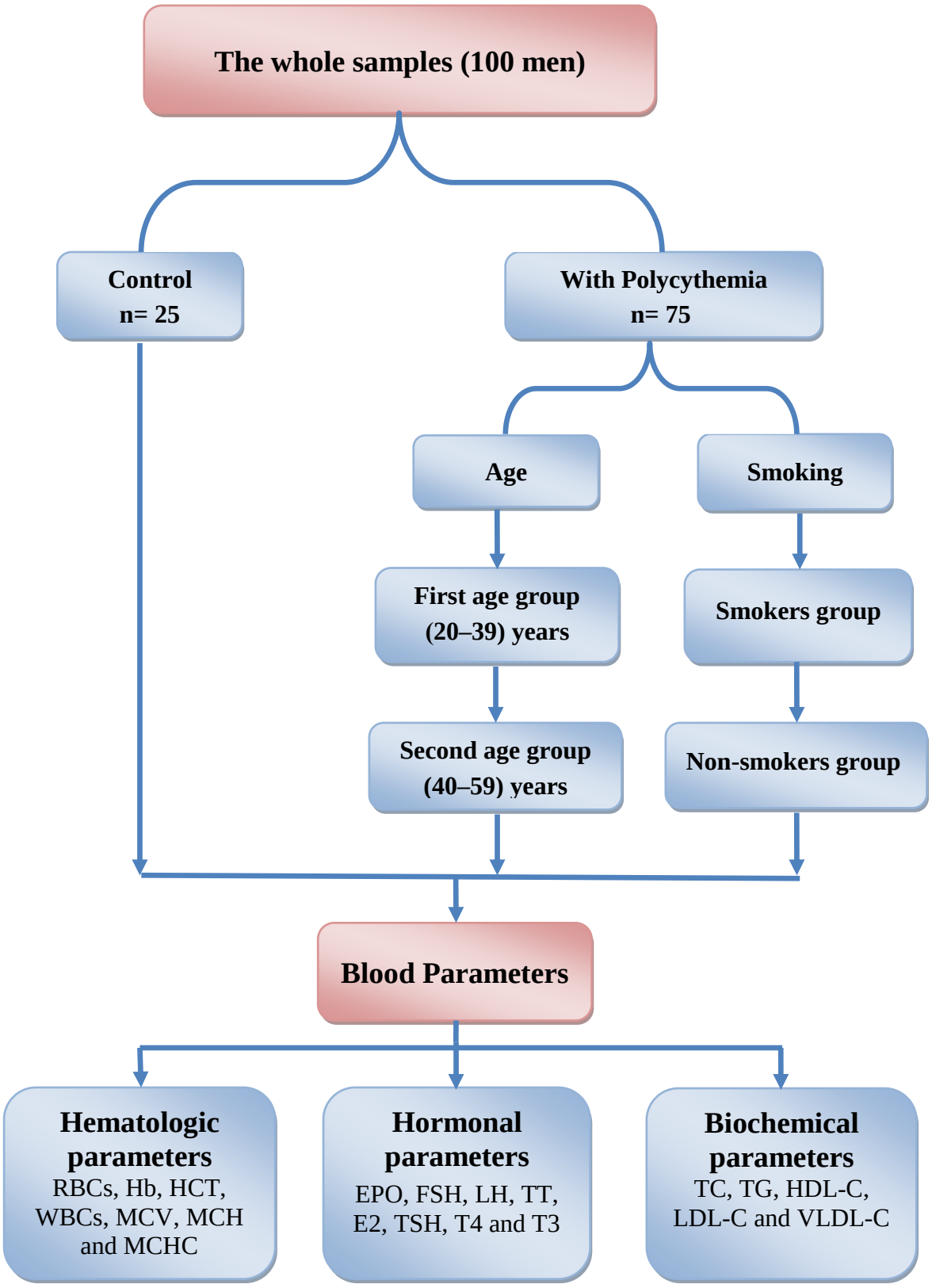


Figure (2-1): The experimental design

2-3: Instruments and Equipments:

The instruments and equipments used in this study with their companies and origin were shown in the table (2.1).

Table (2.1): The instruments and equipment used in the study.

No.	Instruments	Company (origin)
1	Automatic Hematology Analyzer Spincell 3	Spinreact (Spain)
2	BS-240Pro Chemistry Analyzer	Shenzhen Mindray Bio-medical Electronic (China)
3	Centrifuge	PLC (Taiwan)
4	Chemiluminescence-Iimmunoassay Analyzer	Mindray (China)
5	Disposable syringe	China
6	EDTA tube	AFCO (Jordan)
7	ELISA	Elisys Uno (Germany)
8	Eppendreff tube (different volumes)	Biobasic (Canada)
9	Freezer	Concord (Lebanon)
10	Gel tube	AFCO (Jordan)
11	Gloves	Galaxy (Malaysia)
12	Kan tube	AFCO (Jordan)
13	Micropipette (different volumes)	Eppendroff Research Plus (Germany)
14	Racks (different volumes)	China
15	Tips (different volumes)	China
16	Water path 1102	FANEM (Germany)

2.4: Laboratory Kits

The laboratory kits, used in this study are show in the table (2.2).

Table (2.2): The laboratory kits used in the study with their origin

No.	Kits	Company (Origin)
1	CBC	Spinreact (Spain)
2	Cholesterol	BS-240pro (China)
3	Erythropoietin	Shanghain YL Biotech (China)
4	Estradiol	Mindray (China)
5	FSH	Mindray (China)
6	HDL	BS-240pro (China)
7	LDL	BS-240pro (China)
8	LH	Mindray (China)
9	T3	Mindray (China)
10	T4	Mindray (China)
11	Testosterone	Mindray (China)
12	Triglyceride	BS-240pro (China)
13	TSH	Mindray (China)

2.5: The Methods:

2.5.1: Hematological parameters:

The red blood cells (RBCs) count, hemoglobin concentration (Hb), Hematocrite (HCT), white blood cells (WBCs) count, and estimation of red blood indices: mean corpuscular volume (MCV), mean corpuscular hemogloin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were obtained directly form automatic hematological analyzer (Spincell 3) apparatus.

2.5.2: EPO

This kit uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human Erythropoietin (EPO).

Principle of Assay

EPO to the wells was added, are pre-coated with EPO monoclonal antibody and then incubated. After that, anti-EPO antibodies labeled with biotin to unite was added with streptavidin-HRP, which forms an immune complex. Unbound enzymes were removed after incubation and washing. Substrates A and B were added. Then the solution will turn blue and change into yellow due to the acidic conditions. The shades of solution and the concentration of Human EPO are positively correlated.

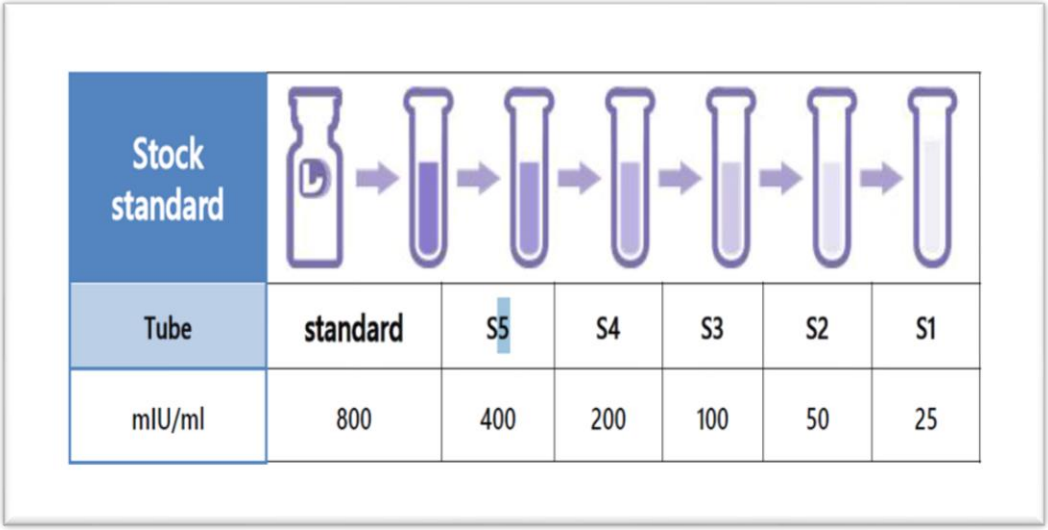
Table (2.3): Content of the Erythropoietin of Reagent

Reagents	Quantity
Anti EPO antibodies labeled	1ml
Chromogen solution A	6ml
Chromogen solution B	6ml
Coated ELISA plate	12-Well * 8 Tubes
Hermetic bag	1
Instruction	1
Seal plate membrane	2
Standard dilution	3ml
Standard solution(800mIU/ml)	0.5ml
Stop solution	6ml
Streptavidin-HRP	6ml
Washing concentrate (30X)	20ml

Assay Procedure:

1- Dilution of standard solutions: (This kit has a standard original concentration, which could be diluted in small tubes by the user independently following the instructions):

400mIU/ml	Standard No.5	120µl Original Standard + 120µl Standard diluents
200mIU/ml	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
100mIU/ml	Standard No.3	120µl Standard No.4 + 120µl Standard diluents
50mIU/ml	Standard No.2	120µl Standard No.3 + 120µl Standard diluents
25mIU/ml	Standard No.1	120µl Standard No.2 + 120µl Standard diluents



The number of stripes needed is determined by that of samples to be tested and added by that of standards. It is recommended to arrange each standard solution and blank well with at least three or more adjacent wells whenever possible.

2- Sample injection: 1) Blank well: Only Chromogen solutions A and B were added, and was stopped the solution. 2) Standard solution well: Add 50µl standard and 50µlstreptavidin-HRP. 3) Were added sample well to be tested: 40µl sample and then 10µl EPO antibodies, were added 50µl streptavidin-HRP. Then was covered it with a seal plate membrane. They were shaked gently to mix them up. Incubate at 37℃ for 60 minutes.

3- Preparation of washing solution: The washing concentrate (30X)was diluted with distilled water for later use.

4- Washing: The seal plate membrane was removed carefully, drain the liquid and shaked off the remaining liquid. Each well was filled with washing solution. The liquid was drained after 30 secondsof standing. This procedure was repeated five times and blot the plate.

5- Color development: 50µl chromogen solution A was added first to each well and then 50µl chromogen solution B was added to each well as

well. Shake gently to mix them up. Incubated for 10 minutes at 37℃away from light to allow for color development.

6- Stop: 50µl Stop Solution was added to each well to stop the reaction (At that moment, the blue color instantly changes to yellow).

7- Assay: Consider the blank well as the zero-reference point, the absorbance (Optical Density OD) of each well was measured one by one under wavelength of 450nm, which should be carried out within 10 minutes after having added the stop solution.

8- According to standards' concentrations and the corresponding OD values, the linear regression equation of the standard curve was calculated. Then according to the OD value of samples, the concentration of the corresponding sample was calculated. Special software could be employed to calculate accordingly (Appendix, B).

2.5.3: Determination of Reproductive Hormones Assay

The CL-series FSH, LH, TT and E2 assay is a Chemiluminescent Immunoassay Analyzer (CLIA) of quantitatively determining of FSH, LH, TT and E2 in human serum.

2.5.3.1: FSH

Principle of Assay:

The CL-series FSH assay is a two-site sandwich to determine the level of FSH (Beastall *et al*., 1987).

1- In the first step, a sample, paramagnetic microparticles coated with monoclonal anti-FSH antibody (mouse) and monoclonal anti-follicle stimulating hormone antibody (mouse)-alkaline phosphataselabeled conjugate are added into a reaction vessel. After incubation, FSH present in the sample binds to both anti-FSH antibody coated microparticle and

anti-FSH antibody alkaline phosphatase-labeled conjugate to form a sandwich complex. Microparticlesare magnetically captured while other unbound substances are removed by washing.

2- In the second step, the substrate solution is added to the reaction vessel. It is catalyzed by anti- FSH antibody (mouse)-alkaline phosphatase conjugate in the immunocomplex retained on the microparticle. The resulting chemiluminescent reaction is measured in terms of relative light units (RLUs) by a photomultiplier built into the system. The amount of FSH present in the sample is proportional to the light units (RLUs) generated during the reaction. The FSH concentration can be determined via a calibration curve.

Table (2-4): Contents of the FSH Kit - Reconstitution of Reagents

Ra	Paramagnetic microparticles coated with monoclonal anti-FSH antibody (mouse) in TRIS buffer with preservative.
Rb	Monoclonal anti-FSH antibody (mouse) alkaline phosphatase conjugate in MES buffer wit preservative

3.5.3. 2: LH

Principle of Assay:

The CL-series LH assay is a two-site sandwich assay to determine the level of LH (Beastall *et al*., 1987).

1- In the first step, sample and paramagnetic microparticles coated with mouse monoclonal anti-LH antibody was added into a reaction vessel. After incubation, LH in the sample binds to the mouse monoclonal anti-LH antibody coated microparticles. Microparticles are magnetically captured while other unbound substances are removed by washing.

2- In the second step, mouse monoclonal anti-LH antibody alkaline phosphatase labeled conjugate was added to the reaction vessel. The mouse monoclonal anti-LH antibody alkalinephosphatase labeled

conjugate binds to anotherreaction site of the LH that has bound to theparamagnetic microparticles coated with LHantibody to form a sandwich complex.Microparticles are magnetically captured whileother unbound substances are removed bywashing.

3- In the third step, the substrate solution was addedto the reaction vessel. It is catalyzed by mousemonoclonal anti-LH antibody-alkalinephosphatase conjugate in the immunocomplex retained on the microparticle. The resultingchemiluminescentreaction is measured asrelative light units (RLUs) by a photomultiplierbuilt into the system. The amount of LH presentin the sample is proportional to relative lightunits (RLUs) generated during the reaction. TheLH concentration can be determined via acalibration curve.

Table (2.5): Contents of the LH Kit- Reconstitution of Reagents

Ra	Paramagnetic microparticles coated with monoclonal anti-LH antibody (mouse) in TRIS buffer with preservative
Rb	Monoclonal anti-LH antibody (mouse)-alkaline phosphatase conjugate in MES buffer with preservative
Rc	MES Buffer with preservative.

2.5.3.3: TT

Principle of Assay:

The CL series TT assay is an immunoenzymatic competitive binding assay (Wheeler ,1995).

1-In the first step, paramagnetic microparticles coated with a monoclonal anti-testosterone antibody (of mouse origin) and testosterone alkaline phosphatase conjugate was introduced into the reaction vessels. Following an incubation period, the sample treatment solution facilitates the release of endogenous testosterone from the sample. This released testosterone competes with the

testosterone-alkaline phosphatase conjugate for binding to the anti-testosterone antibody. Subsequently, the microparticles coated with monoclonal anti-testosterone antibody are magnetically captured, while any unbound substances are eliminated through a washing process.

2-In the second step, the substrate solution was added to the reaction vessel. It is catalyzed by the testosterone alkalinephosphatase conjugate in the immunocomplex retained on the microparticle. The resulting chemiluminescent reaction is measured as relative light units (RLU) by a photomultiplier built into the system. The amount of testosterone present in the samplesInversely proportional to the relative light units (RLU) generatedduring the reaction. Testosterone concentration can be determined using a calibration curve.

Table (2-6): Contents of the TT Kit- Reconstitution of Reagents

Ra	paramagnetic microparticles-coated with monoclonal anti-testosterone antibody (mouse) in a TRIS buffer with preservative.
Rb	Testosterone-alkaline phosphatase conjugate in TRIS buffer with preservative
Rc	sample treatment solution with preservative

2.5.3.4: E2

Principle of Assay:

The CL-series E2 assay measures the concentration of estradiol via a competitive binding immunoenzymatic assay (Hall, 1993).

1- In the first step, a sample, paramagnetic microparticle coated with goat anti-rabbit IgG, sample treatment solution, and polyclonal anti-estradiol antibody (rabbit) was added into a reaction vessel. After incubation, estradiol inthe sample binds to an anti-estradiol antibody.

2- In the second step, estradiol-alkaline phosphatase conjugate was added to the reaction vessel. Estradiol in the sample competes with the estradiol-alkaline phosphatase conjugate for binding sites on the anti-estradiol antibody. The resulting antigen: antibody complexes are bound to goat anti-rabbit IgG on the microparticle, which is magnetically captured while other unbound substances are removed by washing.

3- In the third step, the substrate solution was added to the reaction vessel. It was catalyzed by estradiol-alkaline phosphatase conjugate in the immunocomplex retained on the microparticle. The resulting chemiluminescent reaction was measured as relative light units (RLUs) by a photomultiplier built into the system. The amount of estradiol present in the sample is inversely proportional to the relative light units (RLUs) generated during the reaction. The estradiol concentration can be determined via a calibration curve.

Table (2.7): Contents of the E2 Kit- Reconstitution of Reagents

Ra	Paramagnetic microparticles coated with goat anti-rabbit IgG in TRIS buffer with preservative.
Rb	Estradiol-alkaline phosphatase conjugate in MES buffer with preservative.
Rc	Polyclonal anti-estradiol antibody (rabbit) in TRIS buffer with preservative
Rd	Sample treatment solution with preservative.

Assay Procedure:

For optimal performance of this assay, operators should read the related system operation manual carefully, to get sufficient information such as operation instructions, sample preservation and management, safety precaution, and maintenance. All required materials for the assay as well was prepared. Before loading the FSH, LH, TT and E2 (CLIA)

reagent kit on the machine for the first time, unopened reagentbottle should be inverted gently for at least 30 times to resuspend the microparticles that have settled during shipment or storage. Visually inspect the bottle to ensure the microparticles have been resuspended. If the microparticles remain adhered to the bottle, continue inverting until the microparticles have been completely resuspended. If the microparticles cannot be resuspended, it is recommended not to use this bottle of reagent. Mindray Customer Service was contacted for help. Do not invert opened reagent bottle.

This assay requires 25 µL of FSH, 15 µL of LH, 20 µl of TT and 35 µL of E2 sample for a single test.This volume does not include the dead volume of the sample container. Additional volume is required when performing additional tests from the same sample. Operators should refer to the system operation manual and specific requirements of the assay to determine the minimum sample volume.

2.5.4: Determination of Thyroid Hormones Assay

The CL-series TSH, T4 and T3 assay is a Chemiluminescent Immunoassay Analyzer (CLIA) for the quantitative determination of TSH, T4 and T3 in human serum.

2.5.4.1: TSH

Principle of Assay:

The CL-series TSH test is a sandwich assay with two sites to measure TSH level (Keffer, 1996).

1- In the first step, a sample, paramagneticmicroparticle coated with monoclonal anti-TSH antibody (mouse) and monoclonal anti-TSH antibody (mouse)-alkaline phosphatase conjugate were added into a reaction vessel. After incubation, TSH in the sample was bound to both

anti-TSH antibody coated microparticle and anti-TSH antibody alkaline phosphatase-labeled conjugate to form a sandwich complex. Microparticle is magnetically captured while other unbound substances are removed by washing.

In the second step, the substrate solution was added to the reaction vessel. It is catalyzed by anti-TSH antibody (mouse)-alkaline phosphatase conjugate in the immunocomplex retained on the microparticle. The resulting chemiluminescent reaction was measured as relative light units (RLUs) by a photomultiplier built into the system. The amount of TSH present in the sample is proportional to relative light units (RLUs) generated during the reaction. The TSH concentration can be determined via a calibration curve.

Table (2.8): Contents of the TSH Kit - Reconstitution of Reagents

Ra	Paramagnetic microparticles coated with monoclonal anti-TSH antibody (mouse) in MES buffer with preservative.
Rb	Monoclonal anti-TSH antibody (mouse)-alkaline phosphatase conjugate in MES buffer with preservative.

2.5.4.2: T4

Principle of Assay

The CL-series T4 assay uses a competitive binding immunoenzymatic assay to measure the concentration of total T4 (Bermudez *et al*., 1975).

1- In the first step, thesample and stripping agent were added to a reaction vessel. After incubation, the stripping agent dissociates T4 from the binding proteins.

2- In the second step, paramagnetic microparticles coated with monoclonal anti-T4 antibody (mouse) and T4 alkaline phosphatase conjugate were added into a reaction vessel. T4 in the sample competes with the T4

alkaline phosphatase conjugate. After incubation, the T4 alkaline phosphatase binds to the T4 antibody coated microparticle to form a sandwich complex. Microparticles are magnetically captured while other unbound substances are removed by washing.

3- In the third step, the substrate solution was added to the reaction vessel. It is catalyzed by T4 alkaline phosphatase conjugate in the immunocomplex retained on themicroparticle. The resulting chemiluminescent reaction is measured as relative light units (RLUs) by a photomultiplier built into the system. The amount of total T4 present in the sample is inversely proportional to the light units (RLUs) generated during the reaction. The total T4 concentration can be determined via a calibration curve.

Table (2-9): Contents of the T4 Kit- Reconstitution of Reagents.

Ra	paramagnetic microparticle coated with monoclonal anti-T4 antibody (mouse) in TRIS buffer with preservative
Rb	T4 alkaline phosphatase conjugate in TRIS buffer with preservative.
Rc	8-Anilino-1-Napthalenesulfonic Acid (ANS) in TRIS buffer with preservative

2.5.4.3: T3

Principle of Assay:

The CL-series T3 test uses competitive binding to measure the concentration of total T3 (Fisher, 1996).

1- In the first step, the sample, stripping agent and monoclonal anti-T3 antibody (mouse)-alkalinephosphatase conjugate were added into a reaction vessel. After incubation, the stripping agent dissociates T3 from the binding proteins.

2- In the second step, the streptavidin coated microparticle and biotinylated T3 were added into the reaction vessel. T3 in the sample competes with the biotinylated T3 for anti-T3 alkaline phosphatase conjugate. The biotinylated T3-antibody complexes are bound to the streptavidin coated microparticle. Microparticlesaremagnetically captured while other unbound substances are removed by washing.

3- In the third step, the substrate solution was added to the reaction vessel. It is catalyzed by anti-T3 antibody (mouse)-alkaline phosphatase conjugate in the immunocomplex retained on the microparticle. The resulting chemiluminescent reaction was measured as relative light units (RLUs) by a photomultiplier built into the system. The amount of total T3 present in the sample is inversely proportional to the relative light units (RLUs) generated during the reaction. The total T3 concentration can be determined via a calibration curve.

Table (2-10): Content of The T3 Kit - Reconstitution of Reagents

Ra	Paramagnetic microparticles coated with streptavidin in HEPES buffer with preservative.
Rb	Monoclonal anti-T3 antibody (mouse)-alkaline phosphatase conjugate in MES buffer with preservative.
Rc	Biotinylated T3 in PBS buffer with preservative.
Rd	8-Anilino-1-Napthalenesulfonic Acid (ANS) in MES buffer with preservative

Assay Procedure:

For optimal performance of this assay, operators should read the related system operation manual carefully, to get sufficient information such as operation instructions, sample preservation and management,

safety precautions, and maintenance. Prepare all required materials for the assay as well.

Before loading the TSH, T4 and T3 (CLIA) reagent kit on the machine for the first time, the unopened reagent bottle should be inverted gently for at least 30 times to resuspend the microparticles that have settled during shipment or storage. Visually the bottle to ensure the microparticles have been resuspended was inspected. If the microparticles remain adhered to the bottle, continue inverting until the microparticles have been completely resuspended. If the microparticles cannot be resuspended, it is recommended not to use this bottle of reagent. Contact Mindray Customer Service for help. Do not invert opened reagent bottle.

This assay requires 110 μL of TSH, 30 μL of T4 and 20 μL of T3 sample for a single test.This volume does not include the dead volume of the sample container. Additional volume is required when performing additional tests from the same sample. Operators should refer to the system operation manual and specific requirements of the assay to determine the minimum sample volume.

2.5.5: Lipid Profile Assay

2.5.5.1: Total cholesterol (TC)

In vitro test for the quantitative determination of TC, TG, LDL-C and HDL-C concentration in serum on photometric systems**.**

Principle of Assay

$$\text{Cholestrolesters}+H_2o \xrightarrow{\text{CHE}} \text{Cholestrol}+\text{Fatty acid}$$

$$\text{Cholestrol}+o_2 \xrightarrow{\text{CHO}} \Delta\ 4\text{-cholestrol} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrin}+\text{phenol} \xrightarrow{\text{POD}} \text{Quinoneimie}+H_2O_2$$

By the catalysis of CHE and CHO, Cholestrol ester was catalyzed to yield H_2O_2, which oxidates 4-Aminoantipyrine with phenol to form a colored dye of quinoneimine. The absorbency increase is directly proportional to the concentration of cholesterol (Pisani *et al*., 1995).

2.5.5.2: Triglycerides (TG)

Principle of Assay

$$\text{Triglycerides}+3H_2O \xrightarrow{\text{lipase}} \text{Glycerol}+\text{Fatty acid}$$

$$\text{Glycerol}+\text{ATP} \xrightarrow{\text{GK}} \text{Glycerol}+3\text{Phosphate}+\text{ADP}$$

$$\text{Glycerol}+3\text{Phosphate}+O_2 \xrightarrow{\text{GBO}} \text{Dihydroxyacetone Phosphate}+H_2o$$

$$H_2O_2+ \text{4-Aminoantipyrin}+4\text{cholestrol} \rightarrow \text{Quinoneimine}+HCl+2H_2O$$

Through a sequence of enzymatic catalysis steps by lipase, Gk and GPD, triglycerides was catatlyzed to yield H2O2, which oxidize 4-Aminoantipyrine to yield a colored dye of quinoneimine. The absorbency increase is directly proportional to the concentration of triglycerides (Pisani *et al*., 1995).

2-5-4-3: Low Density Lipoprotein-Cholestsrol (LDL-C)

Principle of Assay

$$(1)\text{HDL ,VLDL ,Chylomicrons} \xrightarrow{\text{CHE+CHO}} \text{Cholestenone}+H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O2$$

$$(2)\text{LDL} \xrightarrow{\text{CHE+CHO}} \text{Cholestenone}+H_2O_2$$

$$H_2O_2+ \text{TOOS}+\text{4-Aminoantipyrin}+\text{4-Chlorophen} \xrightarrow{\text{POD}} \text{Quinoneimine}+HCL+2H_2o$$

The system monitors the change in absorbance at 600 nm. This change in absorbance is directly proportional to the concentration of

cholesterol in the sample and is used by the system to calculate and express the LDL-cholesterol concentration (Friewald *et al*.,1972).

2.5.5.4: High Density Lipoprotein-Cholesterol (HDL-C)

Assay of principle

$$(1)\text{LDL , VLDL , Chylomicrons} \xrightarrow{\text{CHE+CHO}} \text{Cholestenone+}H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O_2 + O_2$$

$$(2)\text{HDL} \xrightarrow{\text{CHE+CHO}} \text{Cholestenone+}H_2O_2$$

$$H_2O_2\text{+ HDAOS +4Aminoantipyrin} \rightarrow \text{Quinoneimine}$$

Change in absorbance is directly proportional to the concentration of cholesterol in the sample and is used by the system to calculate and express the HDL-cholesterol concentration (Lopes-Virella *et al*., 1977).

2.5.5.5: Very Low Density Lipoprotein-Cholesterol (VLDL-C)

Concentration of VLDL was determined according to method by (Friewaled *et al*., 1972 ; Tietz, 1999).

VLDL = TG/5

2.6: Statistical Analysis

Data were analyzed by two ways ANOVA by general liner model procedure using statistical package for social science (SPSS) version 23. The comparisons between means scores were made using least significant differences (LSD) using Genstat3statistic program. The differences were considered to be significant at ($P<0.05$) using multivariate model in SPSS. The data are presented as mean ± S.E. The correlation coefficient Pearson was calculated to examine association among parameters (Kirkpatrick, 2015).

Chapter Three

Results

3.1: According to the Smoking

3.1.1: Hematological study

3.1.1.1: Red Blood Cells (RBCs) count

The RBCs values in the smoker ($5.08 \pm 0.05\ 10^6/mm^3$) and non-smoker groups ($5.03 \pm 0.05\ 10^6/mm^3$) showed a significant increase ($P < 0.05$) compared to the control group ($4.71 \pm 0.08\ 10^6/\ mm^3$). However, the smoker and non-smoker groups showed no significant difference ($P > 0.05$) between them as illustrated in table (3-1).

3.1.1.2: Hemoglobin (Hb)

The Hb values in the smoker (17.21 ± 0.10 g/dL) and non-smoker groups (16.74 ± 0.10 g/dL) showed a significant increase ($P < 0.05$) compared to control group (14.34 ± 0.06 g/dL). Additionally, there was a significant difference ($P < 0.05$) between the smoker and non-smoker groups as shown in table (3-1).

3.1.1.3: Hematocrite (HCT)

The HCT values in the smoker (55.07 ± 0.28 %) and non-smoker groups ($53.\ 6 \pm 0.25$ %), showed a significant increase ($P < 0.05$) compared to control group (46.50 ± 0.33 %). Furthermore, there was a significant difference ($P < 0.05$) between the smoker and non-smoker groups as shown in table (3-1).

3.1.1.4: White Blood Cells (WBCs) count

No significant ($P > 0.05$) variation was recorded in the values of WBCs in the smoker and non-smoker groups (7.63 ± 0.27, $7.5 \pm 0.40\ 10^3/\mu L$, respectively) compared to the control group ($6.86 \pm 0.31\ \ 10^3/\mu L$), as presented in table (3-1).

Table (3-1): Complete blood count (CBC) for polycythemic smokers and non-smokers men compared to control group**.**

Parameters / Groups	RBC_S (10^6/ mm^3)	Hb (g/dL)	HCT (%)	WBC_S (10^3/µL)
Control group n= 25	**a 4.71± 0.08**	**a 14.34 ± 0.06**	**a 46.50 ± 0.33**	**a 6.86 ± 0. 31**
Smokers group n= 50	**b 5.08 ± 0.05**	**b 17.21± 0.10**	**b 55.07± 0.28**	**a 7.63 ± 0.27**
Non smokers group n= 25	**b 5.03 ± 0.05**	**c 16.74± 0.10**	**c 53.6 ± 0.25**	**a 7.5 ± 0.40**

Values are means ±Standard Error (SE).

The different letters refer to the significant differences among the groups at a level of ($p < 0.05$)

The similar letters refer to non-significant differences among the groups at a level of ($p<0.05$).

3.1.1.5: Mean corpuscular volume (MCV)

The MCV values in the smoker group (107.90 ± 0.75 fL) showed a significant increase ($P < 0.05$) compared to the control group (103.76 ± 2.27 fL), and showed no significant difference ($P > 0.05$) compared to non-smoker group (105.74 ± 0.96 fL). The non-smokers group did not significantly ($p > 0.05$) compared to the control group as illustrated in table (3-2).

3.1.1.6: Mean corpuscular hemoglobin (MCH)

The MCH values in the smoker group (33.83 ± 0.29 pg) showed a significant increase ($P < 0.05$) compared to the control group (32.05 ± 0.82 pg), and showed no significant difference ($P > 0.05$) with the non-smoker group (33.26 ± 0.37 pg). While, the non-smoker group did not significantly ($p > 0.05$) differ from the control group as shown in table (3-2).

3.1.1.7: Mean corpuscular hemoglobin concentration (MCHC)

The MCHC values in the smoker (31.36 ± 0.01 g/dL) and non-smoker groups (31.48± 0.21 g/dL) showed a significant difference ($P < 0.05$) compared to control group (30.83 ± 0.19 g/dL). While, smoker and non-smoker groups showed no significant difference ($P < 0.05$) between them as shown in table (3-2).

Table (3-2): Red blood corpuscular indices for polycythemic smokers and non-smokers men compared to control group**.**

Parameters / Groups	MCV (fL)	MCH (pg)	MCHC (g/dL)
Control group n= 25	**a 103.76 ± 2.27**	**a 32.05 ± 0.82**	**a 30.83 ± 0.19**
Smokers group n= 50	**b 107.90 ± 0.75**	**b 33.83 ± 0.29**	**b 31.36± 0.01**
Non Smokers group n= 25	**ab 105.74 ± 0.96**	**ab 33.26 ± 0.37**	**b 31.48 ± 0.21**

Values are means ±Standard Error (SE).

The different letters refer to the significant differences among the groups at a level of ($p < 0.05$).

The similar letters refer to non-significant differences among the groups at a level of ($p<0.05$).

3.1.2: Hormonal study

3.1.2.1: Erythropoietin (EPO)

The levels of EPO in the smoker and non-smoker groups (58.90 ± 4.95 , 99.28 ± 5.16 mlU/ml, respectively) showed a significant decrease ($P < 0.05$) compared to the control group (113.93 ± 3.33 mlU/ml). While, the EPO levels in non-smoker group was increased significantly ($p < 0.05$) compared to the smoker group as shown in table (3-3).

Table (3-3): EPO level for polycythemic smokers and non-smokers men compared to control group**.**

Parameters / Groups	EPO (mlU/ml)
Control group n= 25	**a 113.93 ± 3.33**
Smokers group n= 50	**b 58.90 ± 4.95**
Non Smokers group n= 25	**c 99.28 ± 5.16**

Values are means ±Standard Error (SE).
The different letters refer to the significant differences among the groups at a level of ($p < 0.05$)
The similar letters refer to non-significant differences among the groups at a level of ($p<0.05$).

3.1.2.2: Follicle stimulating hormone (FSH)

There were no significant ($P > 0.05$) changes recorded in FSH levels in both the smoker and non-smoker groups (5.32 ± 0.39, 6.53 ± 0.82 mlU/ml, respectively) in comparison with the control group (6.18 ± 0.54 mlU/ml) table (3-4).

3.1.2.3: Luteinizing hormone (LH)

The levels of LH in the smoker (7.63 ± 0.41 mlU/ml) and non-smoker groups (8.95 ± 0.92 mlU/ml) showed a significant increase ($P < 0.05$) compared to the control group (5.01 ± 0.24 mlU/ml). While, there was no significant ($P > 0.05$) difference between the smoker and non-smoker groups as shown in table (3-4).

3.1.2.4: Total Testosterone (TT)

There were no significant ($P > 0.05$) changes recorded in the levels of TT in the smoker and non-smoker groups (2.65 ± 0.15 , 2.72 ± 0.23 ng/ml, respectively) compared to the control group (2.45 ± 0.16 ng/ml) as illustrated in table (3-4)

3.1.2.5: Estradiol (E2)

There were no significant (P > 0.05) changes recorded in the levels of E2 in the smoker and non-smoker (45.76 ± 1.47, 44.76 ± 2.36 pg/ml, respectively) compared to the control group (43.40 ± 2.17 pg/ml) as shown in table (3-4).

Table (3-4): Reproductive hormones levels for polycythemic smokers and non-smokers men compared to control group**.**

Parameters / Groups	FSH (mlU/ml)	LH (mlU/ml)	TT (ng/ml)	E2 (pg/ml)
Control group n= 25	**a 6.18 ± 0.54**	**a 5.01 ± 0.24**	**a 2.45 ± 0.16**	**a 43.40 ± 2.17**
Smokers group n= 50	**a 5.32 ± 0.39**	**b 7.63 ± 0.41**	**a 2.65 ± 0.15**	**a 45.76 ± 1.47**
Non Smokers group n= 25	**a 6.53 ± 0.82**	**b 8.95 ± 0.92**	**a 2.72 ± 0.23**	**a 44.76 ± 2.36**

Values are means ±Standard Error (SE).
The different letters refer to the significant differences among the groups at a level of ($p < 0.05$)
The similar letters refer to non-significant differences among the groups at a level of ($p<0.05$).

3.1.2.6: Thyroid Stimulating hormone (TSH)

There was no significant (P > 0.05) change recorded in the levels of TSH in the smoker and non-smoker groups (2.75 ± 0.23, 2.54 ± 0.21 µlU/ml, respectively) compared to the control group (2.95 ± 0.22 µlU/ml) as shown in table **(**3-5).

3.1.2.7: Thyroxine (T4)

There was no significant (P > 0.05) change recorded in the levels of T4 in the smoker and non-smoker groups (8.05 ± 0.21, 7.74 ± 0.33 Ug/dl, respectively) compared to the control group (8.36 ± 0.27 µg/dl) as shown in table **(**3-5).

3.1.2.8: Triiodothyronine (T3)

The T3 levels in the smoker (0.86 ± 0.02 ng/dl) and non-smoker groups (0.88 ± 0.02 ng/dl) showed a significant increase ($P < 0.05$) compared to the control group (0.80 ± 0.02 ng/dl). While, it showed no significant difference ($P > 0.05$) between smoker and non-smoker group as illustrated in table **(**3-5).

Table (3-5): Thyroid hormones levels for polycythemic smokers and non-smokers men compared to control group**.**

Parameters / Groups	TSH (µlU/ml)	T4 (µg/dl)	T3 (ng/dl)
Control group n= 25	**a 2.95 ± 0.22**	**a 8.36 ± 0.27**	**a 0.80 ± 0.02**
Smokers group n= 50	**a 2.75 ± 0.23**	**a 8.05 ± 0.21**	**b 0.86 ± 0.02**
Non Smokers group n= 25	**a 2.54 ± 0.21**	**a 7.74 ± 0.33**	**b 0.88 ± 0.02**

Values are means ±Standard Error (SE).
The different letters refer to the significant differences among the groups at a level of ($p < 0.05$)
The similar letters refer to non-significant differences among the groups at a level of ($p<0.05$).

3.1.3: Biochemical study

3.1.3.1: Total Cholesterol (TC)

The values of TC in the smoker (175.50± 4.07 mg/dl) and non-smoker groups (185.81 ± 6.93 mg/dl) showed a significant increase ($P < 0.05$) compared to the control group (148.09 ± 3.28 mg/dl). While, both groups, the smoker and non-smoker showed no significant difference ($P > 0.05$) between them as shown in table (3-6).

3.1.3.2: Triglyceride (TG)

The values of TG in the smoker (182.27 ± 11.93 mg/dl) and non-smoker groups (201.15 ± 22.25 mg/dl) were increased significantly ($P < 0.05$) compared to the control group (129.76 ± 7.83 mg/dl). While, smoker and non-smoker showed no significant difference ($P > 0.05$) between them as shown in table (3-6).

3.1.3.3: High Density Lipoprotein-Cholesterol (HDL-C)

The values of HDL-C in the smoker (38.54 ± 1.29 mg/dl) and non-smoker groups (37.96 ± 1.58 mg/dl) were decreased significantly ($P < 0.05$) compared to the control group (44.49 ± 0.75 mg/dl). While, the smoker and non-smoker groups showed no significant difference ($P > 0.05$) between them as appeared in table (3-6).

3.1.3.4: Low Density Lipoprotein-Cholesterol (LDL-C)

The values of LDL-C in the smoker (119.26 ± 3.91 mg/dl) and non-smoker groups (130.03 ± 5.70 mg/dl) were increased significantly ($P < 0.05$) compared to the control group (101.01 ± 3.33 mg/dl), while the smoker and non-smoker groups stated no significant difference ($P > 0.05$) between them as presented in table (3-6).

3.1.3.5: Very Low Density Lipoprotein (VLDL-C)

The values of VLDL-C in the smoker (36.45 ± 2.39 mg/dl) and non-smoker groups (40.23 ± 4.45 mg/dl) were increased significantly ($P < 0.05$) compared to the control group (25.95 ± 1.57 mg/dl). While, the smoker and non-smoker groups showed no significant difference ($P > 0.05$) between them as shown in table (3-6).

Table (3-6): The values of Lipid profile for polycythemic smokers and non-smokers men compared to control group**.**

Parameters / Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Control group n= 25	**a 148.09 ± 3.28**	**a 129.76 ± 7.83**	**a 44.49 ± 0.75**	**a 101.01 ± 3.33**	**a 25.95 ± 1.57**
Smokers group n= 50	**b 175.50 ± 4.07**	**b 182. 27 ± 11.93**	**b 38.54 ± 1.29**	**b 119.26 ± 3.91**	**b 36.45 ± 2.39**
Non Smokers group n=25	**b 185.81 ± 6.93**	**b 201.15 ± 22.25**	**b 37.96 ± 1.58**	**b 130.03 ± 5.70**	**b 40.23 ± 4.45**

Values are means ±Standard Error (SE).

The different letters refer to the significant differences among the groups at a level of ($p < 0.05$)

The similar letters refer to non-significant differences among the groups at a level of ($p<0.05$).

3.2: According to the age

3.2.1: Hematological study

3.2.1.1: Red Blood Cells count (RBCs)

The RBCs values in the first ($5.05 \pm 0.04\ 10^6/mm^3$) and the second age groups ($5.10 \pm 0.07\ 10^6/mm^3$) showed a significant increase ($P < 0.05$) compared to the control group ($4.71 \pm 0.08\ 10^6/mm^3$), while the first and the second group showed no significant difference ($P > 0.05$) between them as shown in table (3-7).

3.2.1.2: Hemoglobin (Hb)

The Hb values in the first (17.13 ± 0.10 g/dL) and the second age groups (16.97 ± 0.13 g/dL) were increased significantly ($P < 0.05$) compared to the control group (14.34 ± 0.06 g/dL), while the first and the second age groups showed no significant difference ($P > 0.05$) between them as shown in table (3-7).

3.2.1.3: Hematocrite (HCT)

The HCT values in the first (54.65 ± 0.27 %) and the second age groups (54.65 ± 0.40 %), were increased significantly ($P < 0.05$) compared to the control group (46.50 ± 0.34 %), while the first and the second age groups showed no significant difference ($P > 0.05$) between them as shown in table (3-7).

3.2.1.4: White Blood Cells count (WBCs)

There were no significant ($P > 0.05$) changes recorded in the values of WBCs in the first and the second age groups (7.73 ± 0.29, 7.27 ± 0.30 10^3/µL, respectively), compared to the control group (6.86 ± 0.31 10^3/ µL) as illustrated in table (3-7).

Table (3-7): Complete blood count (CBC) in different ages in men with polycythemia compared to the control group**.**

Parameters / Age groups	**RBC_S (10^6/mm^3)**	**Hb (g/dL)**	**HCT (%)**	**WBC_S (10^3/µL)**
Control (20-59) years n= 25	**a 4.71± 0.08**	**a 14.34 ± 0.06**	**a 46.50 ± 0.34**	**a 6.86 ± 0. 31**
First group (20-39) years n= 50	**b 5.05 ± 0.04**	**b 17.13± 0.10**	**b 54.65± 0.27**	**a 7.73 ± 0.29**
Second group(40-59) years n= 25	**b 5.10 ± 0.07**	**b 16.97± 0.13**	**b 54.65 ± 0.40**	**a 7.27 ± 0.30**

Values are means ±Standard Error (SE).
The different letters refer to the significant differences among the groups at a level of ($p < 0.05$)
The similar letters refer to non-significant differences among the groups at a level of ($p<0.05$).

3.2.1.5: Mean corpuscular volume (MCV)

The MCV values in the first (107.61 ± 0.73 fL) and the second age groups (106.55 ± 1.10 fL) were increased significantly ($P < 0.05$) compared to the control group (99.52 ± 4.66 fL), while first and second

groups showed no significant difference (P > 0.05) between them as shown in table (3-8).

3.2.1.6: Mean corpuscular hemoglobin (MCH)

The MCH values in the first age group (33.83 ± 0.28 pg) was significantly increased (P < 0.05) compared to the control group (32.05 ± 0.82 pg) and showed no significant difference (P > 0.05) with the second age group (33.32 ± 0.43 pg). Also, the second group showed no significant difference (P > 0.05) compared to the control group as shown in table (3-8).

3.2.1.7: Mean corpuscular hemoglobin concentration (MCHC)

The MCHC values in the first age group (31.45 ± 0.10 g/dL) was increased significantly (P < 0.05) compared to the control group (30.83 ± 0.19 g/dL) and showed no significant difference (P > 0.05) with the second age group (31.27 ± 0.21 g/dL). While, the second age group showed no significant difference (P > 0.05) compared to the control group as shown in table (3-8).

Table (3-8): Red blood corpuscular indices in different ages in men with polycythemia compared to the control group.

Parameters / Age group	MCV (fL)	MCH (pg)	MCHC (g/dL)
Control (20-59) years n= 25	**a 99.52 ± 4.66**	**a 32.05 ± 0.82**	**a 30.83 ± 0.19**
First group (20-39) years n= 50	**b 107.61 ± 0.73**	**b 33.83 ± 0.28**	**b 31.45± 0.10**
Second group(40-59) years n= 25	**b 106.55 ± 1.10**	**ab 33.32 ± 0.43**	**ab 31.27 ± 0.21**

Values are means ±Standard Error (SE).

The different letters refer to the significant differences among the groups at a level of (p < 0.05)

The similar letters refer to non-significant differences among the groups at a level of (p<0.05).

3.2.2: Hormonal study

3.2.2.1: Erythropoietin (EPO)

The levels of EPO in the first (67.83 ± 5.31 mlU/ml) and the second age groups (79.85 ± 9.61 mlU/ml) were decreased significantly (P > 0.05) compared to the control group (113.93 ± 3.33 mlU/ml), while first and second groups did not differ significantly (P > 0.05) between them, table (3-9).

Table (3-9): EPO level in different ages in men with polycythemia compared to the control group**.**

Parameters / Groups	EPO (mlU/ml)
Control (20-59) years n= 25	**a 113.93 ± 3.33**
First group (20-39) years n= 50	**b 67.83 ± 5.31**
Second group(40-59) years n= 25	**c 79.85 ± 9.61**

Values are means ±Standard Error (SE).

The different letters refer to the significant differences among the groups at a level of ($p < 0.05$)

The similar letters refer to non-significant differences among the groups at a level of ($p<0.05$).

3.2.2.2: Follicle stimulating hormone (FSH)

There was no significant (P > 0.05) change recorded in the levels of FSH in the first and second age groups (5.38 ± 0.49, 6.21 ± 0.50 mlU/ml, respectively), compared to the control group (6.18 ± 0.54 mlU/ml), as shown in table (3-10).

3.2.2.3: Luteinizing hormone (LH)

The levels of LH in the first and the second age groups (7.10 ± 0.47 , 8.00 ± 0.73 mlU/ml, respectively) were increased significantly (P < 0.05)

compared to the control group (5.01 ± 0.24 mlU/ml), while first and second groups did not differ significantly (P > 0.05) between them, as shown in table (3-10).

3.2.2.4: Total Testosterone (TT)

The levels of TT in the table (3-10) showed the first age group (2.43 ± 0.13ng/ml) did not differ significantly (P > 0.05) in comparison with control group (2.45 ± 0,16 ng/ml).While, the level of TT was increased significantly (P < 0.05) in second age group (3.15 ± 0.26ng/ml)in comparison with first age and control groups.

3.2.2.5: Estradiol (E2)

There was no significant (P > 0.05) change recorded in the levels of E2 in the first and second age groups (45.67 ± 1.51 , 45.09 ± 2.24 pg/ml, respectively), in comparison with the control group (43.40 ± 0.18 pg/ml) as shown in table (3-10).

Table (3-10): Reproductive hormones levels in different ages in men with polycythemia compared to the control group**.**

Parameters / Groups	FSH (mlU/ml)	LH (mlU/ml)	TT (ng/ml)	E2 (pg/ml)
Control (20-59) years n= 25	**a 6.18 ± 0.54**	**a 5.01 ± 0.24**	**a 2.45 ± 0,16**	**a 43.40 ± 0.18**
First group (20-39) years n= 50	**a 5.38 ± 0.49**	**b 7.10 ± 0.47**	**a 2.43 ± 0.13**	**a 45.67 ± 1.51**
Second group(40-59) years n= 25	**a 6.21 ± 0.50**	**b 8.00 ± 0.73**	**b 3.15 ± 0.26**	**a 45.09 ± 2.24**

Values are means ±Standard Error (SE).
The different letters refer to the significant differences among the groups at a level of ($p < 0.05$)
The similar letters refer to non-significant differences among the groups at a level of ($p<0.05$).

3.2.2.6: Thyroid Stimulating hormone (TSH)

There was no significant (P > 0.05) change in the levels of TSH in the first and second age groups (2.75 ± 0.23, 2.58 ± 0.26 µlU/ml, respectively)

compared to the control group (2.95 ± 0.23 µlU/ml) as shown in table (3-11)**.**

3.2.2.7: Thyroxine (T4)

There was no significant (P > 0.05) change in the levels of T4 in the first and second age groups (7.81 ± 0.22 , 8.28 ± 0.28 µg/dl, respectively) compared to the control group (8.36 ± 0.27 µg/dl) as shown in table (3-11).

3.2.2.8: Triiodothyronine (T3)

The T3 levels in the first age group (0.88 ± 0.02 ng/dl) was increased significantly (P < 0.05) compared to the control group (0.86 ± 0.02 ng/dl). However, showed no significant difference (P > 0.05) in the second age group (0.81 ± 0.02 ng/dl). Moreover, the second group showed no significant difference (P > 0.05) compared to the control group as appeared in table **(**3-11).

Table (3-1): Thyroid hormones levels in different ages in men with polycythemia compared to the control group**.**

Parameters / Groups	TSH µlU/ml	T4 µg/dl	T3 ng/dl
Control (20-59) years n= 25	**a 2.95 ± 0.23**	**a 8.36 ± 0.27**	**a 0.86 ± 0.02**
First group (20-39) years n= 50	**a 2.75 ± 0.23**	**a 7.81 ± 0.22**	**b 0.88 ± 0.02**
Second group(40-59) years n= 25	**a 2.58 ± 0.26**	**a 8.28 ± 0.28**	**b 0.81 ± 0.02**

Values are means ±Standard Error (SE).

The different letters refer to the significant differences among the groups at a level of (p < 0.05)

The similar letters refer to non-significant differences among the groups at a level of (p<0.05).

3.2.3: Biochemical study

3.2.3.1: Total Cholesterol (TC)

The values of TC in the first (175.77 ± 4.28 mg/dl) and second age groups (184.41 ± 6.17 mg/dl) were increased significantly ($P < 0.05$) compared to the control group (148.09 ± 3.28 mg/dl), while the first and the second groups showed no significant difference ($P > 0.05$) between them as shown in table (3-12).

3.2.3.2: Triglyceride (TG)

The values of TG in the first (177.03 ± 11.69 mg/dl) and the second age groups (211.59 ± 21.90 mg/dl) were increased significantly ($P < 0.05$) compared to the control group (129.77 ± 7.83 mg/dl). While, the first and second groups showed no significant difference ($P > 0.05$) between them as shown in table (3-12)

3.2.3.3: High Density Lipoprotein-Cholesterol (HDL-C)

The values of HDL-C in the first (39.12 ± 1.39 mg/dl) and second age groups (36.71 ± 1.08 mg/dl) were decreased significantly ($P < 0.05$) compared to the control group (44.50 ± 0.75 mg/dl), while the first and second groups showed no significant difference ($P > 0.05$) between them as illustrated in table (3-12).

3.2.3.4: Low Density Lipoprotein-Cholesterol (LDL-C)

The values of LDL-C in the first (119.62 ± 4.14 mg/dl) and second age groups (128.41 ± 4.93 mg/dl) were increased significantly ($P < 0.05$) compared to the control group (101.01 ± 3.33 mg/dl). While, the first and second groups showed no significant difference ($P > 0.05$) between them as shown in table (3-12).

3.2.3.5: Very Low Density Lipoprotein (VLDL-C)

The values of VLDL-C in the first (35.41 ± 2.34 mg/dl) and second age groups (42.32 ± 4.38 mg/dl) were increased significantly ($p < 0.05$)

compared to the control group (25.95 ± 1.57 mg/dl), while the first and second groups showed no significant difference ($p > 0.05$) between them as shown in table (3-12)

Table (3-12): The values of lipid profile in different ages in men with polycythemia compared to the control group**.**

Parameters / Age group	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Control (20-59) years n= 25	**a 148.09 ± 3.28**	**b 129.77 ± 7.83**	**a 44.50 ± 0.75**	**a 101.01 ± 3.33**	**a 25.95 ± 1.57**
First group (20-39) years n= 50	**b 175.77 ± 4.28**	**b 177.03 ± 11.69**	**b 39.12 ± 1.39**	**b 119.62 ± 4.14**	**b 35.41 ± 2.34**
Second group (40-59) years n= 25	**b 184.41 ± 6.17**	**b 211.59 ± 21.90**	**b 36.71 ± 1.08**	**b 128.41 ± 4.93**	**b 42.32 ± 4.38**

Values are means ± S.E.

The different latters refer to difference between groups that is statistically significant at the level of ($p < 0.05$).

The smaller latters refer to differences between groups that are not statistically significant at level of ($p<0.05$).

Chapter Four

Discussion

4.1: According to the smoking

4.1.1: Hematological Study

4.1.1.1: Red Blood Cells (RBC_S), Hemoglobin (Hb), Hematocrit (HCT) and White Blood Cells (WBCs):

The results of the present study showed that the total count of RBC, Hb and HCT levels were increased significantly ($p< 0.05$) in smokers with polycythemia, when compared with control (Table, 3-1).

The results of this study is consistent with the study conducted by Raval and Paul (2010), they showed a significant elevation of RBC mass and high quantity of RBCs in smokers polycythemia. Tarazi and his groups (2008), found that smokers had significantly higher Hb concentrations. Higher Hb concentrations are associated with increased RBC counts or increase RBC sizes. RBC levels were noticeably higher in smokers compared to non-smokers. According to another study, higher RBC and HCT levels are associated with higher blood viscosity and coagulation in smokers (Ho, 2004).

In comparison to non-smokers, smokers' Hb values were noticeably higher (Kume *et al*., 2009). Increased HCT levels have been linked to polycythemia and a higher risk of atherosclerosis progression and CVD (Ferro *et al*., 2004).

The important experimental disparities revealed in the blood parameters (RBCs, Hb, and HCT) in subjects 'smokers in comparison with non-smokers , according to the study by Asif *et al*., (2013).

Other study agrees with the results of our study, they showing that Hb and HCT levels were significantly higher in smokers compared to non-

smokers, and that polycythemia was observed in 7.02% of all individuals (8.42% of smokers versus 6.54% of non-smokers) (Kung *et al*., 2008).

The effect of smoking on the blood parameter may be due to CO released from smoke binds with Hb to form carboxyhemoglobin (COHb), which affects tissue hypoxia and increases EPO production and erythropoiesis. Plasma volume decreases in response to CO elevation of capillary permeability, like relative polycythemia (Jain *et al*., 2014).

Smokers exhibit elevated levels of COHb, which cause progressive hypoxia and changes to the hematological parameters (Lakshmi *et al*., 2014). CO is produced by the inefficient use of carbon-containing materials, as in the state of cigarette smoking in comparison to oxygen, CO is thought to have an affinity for hemoglobin that is around 200 times higher (Carallo *et al*., 1998). As a result, COHb complex which inhibits the release of oxygen from hemoglobin to tissue, forms when CO combines with hemoglobin rather than oxygen in the erythrocyte (Cronenberger *et al*., 2008).

An increase in the number of cigarettes smoked per day is associated with a gradual rise in mean Hb concentration and HbCO levels. Additionally, the duration of chronic exposure to HbCO is associated with the onset of polycythemia (Leifert, 2008).

Smoking has been observed to generate a unique state of linked polycythemia to chronic hypoxia, increasing the release of erythrocytes due to an increase in HbCO, and causing a decrease in plasma volume (Raval and Paul, 2010), this causes an increased in HCT and Hb values, which are thought to be a compensation strategy for CO exposure (Roethig *et al*., 2010).

The results of the present study showed that the total count of WBCs did not significantly in polycythemic (smokers and non-smokers) when compared with control (Table, 3-1).

The results of this study agree with a study done by Nadia *et al*., (2015), there was showed no significant change in WBCs count in smokers compared with non-smokers.

On contrary, Al Dayyeni and his group (2023), showed that the WBCs count in smokers with polycythemia significantly increased compared to non-smokers. Another study suggested that the elevated leukocyte count may be caused by nicotine's catabolic release of catecholamine's, which raises blood lymphocyte numbers. The irritating effects of cigarette smoke on the respiratory system, and the ensuing inflammation may also be contributing factors to a higher WBCs count. Additionally, it has been proposed that inflammation-stimulating the bronchial tract causes blood circulation to produce more inflammatory markers (Calapai *et al*., 2009).

4.1.1.2: Red Blood Cells (RBCs) Indices: MCV, MCH and MCHC:

The current results showed a significant increase ($p< 0.05$) in the values of MCV, MCH, and MCHC in polycythemic smokers when compared with the control, While MCV and MCH did not differ significantly between non-smoker and control group (Table 3-2).

In a study about the effects of smoking on hematological parameters, smokers' MCV values were higher than MCV in non-smokers' (Inal *et al*., 2014). Also, Kung and his groups (2008), noted that smokers' MCV levels of RBC macrocytosis were higher than those of non-smokers. Moreover, it is shown that smokers have higher levels of MCV, MCH, MCHC, and COHb, potentially leading to increased blood viscosity and vascular loading, resulting in RBC macrocytosis and hyperchromia, and these results agree with our results.

In contrast, Jain and his group (2014), found no significant differences in MCV and MCH between smokers and non-smokers, but they found a significant decline in MCHC values among smokers. Conversely, Salamzadeh's study (2004), reported that the values of MCH and MCHC in the smoker group were markedly decreased in compared to those of the non-smoker group.

4.1.2: Hormonal study

4.1.2.1: Erythropoietin (EPO)

The results of the present study showed a significant decrease ($P < 0.05$) in the levels of EPO in both polycythemia groups (smokers and non-smokers) when compared to the control group (Table 3-3).

Our results are consistent with the findings of Lupak and his team (2020), when studying 75 patients with PV, 51of them had serum EPO below the lower limit and 24 patients had a level within the normal limit. Also, they found that EPO was below the normal limit in four patients with secondary polycythemia.

Al Dayyeni and his group (2023), observed that patients smokers with polycythemia had low level of EPO than non-smokers. Another, study that done by Alkhedaide (2020), has shown a significant reduction in EPO levels in smokers serums compared to the non-smokers.

Furthermore, smoking causes low serum levels of EPO, because of the negative feedback from increased Hb and RBC levels (Eisenga *et al*., 2018). It is allegedly assumed that secondary erythrocytosis associated with smoking occurs owing to tissue hypoxia, which consequently increases secretion of EPO ,and augments erythropoiesis to increase oxygen delivery (Gleiter *et al*., 1996).

In a study conducted by Eisenga and his group (2018), they identified that smoking is associated with lower, not higher, EPO levels. They hypothesize that smokers may have elevated EPO levels during the day, leading to erythrocytosis, which through a negative feed back loop, will inhibit EPO production at night during smoking cessation. With a reported half-life of endogenous circulating EPO in the order of 6 to 8 hours, this could then result in low EPO levels in the morning when blood samples are drawn.

4.1.2.2: Reproductive hormones

The results of the present study showed no significant differences in the levels of FSH, TT and E2 in polycythemic smokers and non-smokers when compared to the control group (Table 3-4).

Our findings were consistent with another study done by Halmenschlager and his team (2009), when analyzing 255 men, in a cross-sectional study, they found no significant effect of smoking on FSH levels.

According to a prior study, those who smoked more than 10 cigarettes per day saw a 37% reduction in FSH level compared to those who smoked fewer than 10 cigarettes per day **(**Florescu *et al*., 2009**)**. Contrary to findings from another study, they noted that non-smokers' FSH levels were lower than those of smokers (Trummer *et al*., 2002**).**

Our findings regarding TT are in agreement with the study conducted by Harman and his group (2001), in which they analyzed 890 men in a population-based study and found no significant effect of smoking on TT levels. Furthermore, Saadat (2009) observed no influence of cigarette smoking on TT levels.

Conversely, another study noted higher TT levels in smokers (Wu *et al.*, 2008). Trummer and his group (2002), analyzed 1,104 men and found higher levels of TT in smokers compared with non- and ex-smokers. Additionally, Corona and his group (2005), studied 1,150 men in an outpatient clinic for sexual dysfunction and they found higher TT levels in smokers.

In cultured rat embryonic olfactory placode, nicotine stimulates gamma-aminobutyric acid release, which, in turn, inhibits GnRH secretion (Kimura, 2004). Additionally, nicotine stimulates the release of opioid peptides in the brain, which inhibits GnRH secretion (Pomerleau, 1998). According to the study by Yardimci and his group (1997), the cigarette smoking causes degeneration of Leydig cells, as well as a decrease in cell population, which can lead to a decrease in testosterone production.

Our findings were like to another study by Osadchuk and his team (2023), they found no significant differences between smokers and non-smokers for serum levels of E2. These conflicting results may be explained at least partially by differences in study designs, and sample volume as well as by inadequate methods to quantify tobacco exposure.

The results of the present study showed a significant increase ($p< 0.05$)in the levels of LH in polycythemic smokers and non-smokers when comparison with the control group (Table 3-4).

The results in this study agree with a study performed by Bassey and his group (2018), that revealed a elevations in the level of LH hormone in smoking compared to control. The increase in LH concentration is consistent with a previous study (Ramlau-Hansen *et al*., 2007).

But, contrary to the study done by Halmenschlager and his team, (2009), they found no significant effect of smoking on LH levels in adult men. According to Araujo and Wittert (2011), the higher levels of LH observed in smokers may be attributed to testicular dysfunction. Elevated levels of LH in males usually reflect a lack of male steroid hormone-negative feed back (Sansone *et al*., 2018).

Smoking was thought to promote LH secretion by activating Leydig cells (Trummer *et al*., 2002). In addition, it has been proposed that nicotine in cigarettes may stimulate the nicotinic acetylcholine receptors in the neurons of the mesolimbic pathway, hence increasing dopamine release.This could affect the release of gonadotropins and alter the feedback mechanism of the hypothalamus hypophysisgonads to alter testosterone production (Ciccone *et al*., 2010).

4.1.2.3: Thyroid hormones

The results of the present study showed no significant differences in the levels of TSH and T4 in polycythemic smokers and non-smokers when compared to the control group (Table 3-5).

This result is consistent with previous study, which indicate that smoking has an impact on the functioning of the anterior pituitary gland and increases the levels of several of its hormones, such as growth hormone (GH) and adrenocorticotropin (ACTH), but has no impact on TSH levels (Kapoor and Jones, 2005). Furthermore, the level of TSH in smokers' serum are lower than those no smokers (Jorde and Sundsfjord, 2006).

Our results agree with a study performed by Fisher and his group (1997), they found no significant effect of smoking on T4 levels. In contrast to the results of another investigation, they noted that smokers'

T4 levels were higher than those of non-smokers (Kadkhodazadeh *et al*., 2020).

The results of the present study showed a significant increase ($p< 0.05$) in the levels of T3in polycythemic smokers and non-smokers when compared to the control group (Table 3-5).

Ahmadi and his team (2012), found a significant increase in serum level of T3 in smokers when compared to the control. Furthermore, smoking has been shown to affect thyroid hormone levels, with smokers having higher T3 levels than non-smokers (Gruppen *et al*., 2020). The results of previous studies are in agreement with our study.

The people who smoke heavily, the serum level of T3 compared to those non-smoking, has significantly decreased according to a study by (Pradhan*et al*., 2020).

As a thyroid stimulant, nicotine has been recognized. The hypothalamic-pituitary-thyroid axis is another highly active system that nicotine can stimulate. It imitates acetylcholine's effects at specific central nicotine acetyl cholinergic receptors, activating the sympathetic nervous system, which then stimulates the thyroid gland and increases T3 output. Lower serum levels of TSH in smokers result from its suppression due to elevated serum T3 levels as a result of negative feedback regulation (Balhara and Deb, 2014).

Moreover, suggested that smoking reduces thyroid autoimmune processes resulting in alterations in TSH and T3 levels (Wiersinga, 2013).

4.1.3: Biochemical study

4.1.3.1: Lipid profile

In the current study, the result showed that TC, TG, LDL-C and VLDL-C which increased significantly ($p< 0.05$). While, HDL-C decreased significantly in both groups of polycythemia smokers and non-smokers, compared with the control group (Table 3-6).

The results of this study agree with study done by Abbas (2011), they showed high levels of TC and TG and low HDL-C in polycythemic smokers patient's compared to the control. Furthermore, our findings are consistent with a previous study (Moustafa, 2010), that reported significantly lower levels of HDL-C in heavy smokers with polycythemia.

These findings support the findings of Altaher and his group (2019), who demonstrated that cigarette smoking increases serum TC, TG, LDL-C, and decreases serum HDL-C levels in smokers compared to non-smokers.The reason for this increase may be attributed to the fact that smoking works on the presence of different mechanisms that lead to a change in the level of fats, the most important of which are: Nicotine stimulates the sympathetic nerves in the adrenal gland, the primary pharmacologically active component of cigarettes, which lead to the secretion of its hormones (epinephrine and norepinephrine), which causes to an increase in the lipase enzyme ,and the concentration of free fatty acids (FFA) and causes to high levels of TC and TG, and low level of HDL-C in the blood stream (Shai *et al*., 2004 ; Lee *et al*., 2017).

Smoking reduces the distensibility of blood vessel walls, and induces impairment of lipoprotein metabolism, pro-thrombotic and proinflammatory state (Al-Dahr, 2010).

Tobacco smoke contains large numbers of gas and tar phase radicals and other oxidants. It has been estimated that a single puff of a cigarette contains as much as 1015 gas phase radicals and 1014 tar phase radicals potentially capable of modifying endogenous macromolecules including lipids (Pryor and Stone, 1993).

4.2: According to the age

4.2.1: Hematological Study

4.2.1.1: Red Blood Cells (RBC_S), Hemoglobin (Hb), Hematocrit (HCT) and White Blood Cells (WBCs):

The results of the present study showed that the total count of RBCs, Hb and HCT levels were increased significantly ($p< 0.05$), While WBCs count did not differ significantly in both age groups of polycythemia compared with the control group (Table 3-7).

This study agrees with a study by Qasim and Al-Yasiri (2021), they showed a significant increase in the values of RBC_S, Hb and HCT in polycythemia subjects compared to the control group, and the incidence of polycythemia**,** increased with age, they found the peak in the age group between (41- 50) year, whereas, the incidence was decrease in the age group between (61-70) year. As well as, the results of this study also agree with McMullin (2009), which noted that polycythemia, often affects adults over the age of fifty. Also, it can affect people between the ages of fifteen to ninety.

The capacity of the blood to transport oxygen and the rate of gas transport remains nearly constant. It is always adequate for the body's requirements, even though red blood cells are influenced by a variety of

variables, such as age, gender, height above sea level, nutritional status, and health status (Eroschenko and Di Fiore, 2013).

The results of the current study agree with a study by Yang and his group (2020), they observed the number of WBCs no significantly differ at high altitude ,when analysing the data of 467 males, and an average age between (18–78) years.

The results of the current study are disagreement with the study by Parasuraman and his group (2020), they observed the number of WBCs increased in patients with polycythemia with age,when analysis the data of 1565 patients, and average age between (18–60) years.

4.2.1.2: Red Blood Cells (RBCs) Indices (MCV, MCH and MCHC):

The results showed a significant increase ($P<0.05$) for the levels of MCV, MCH, and MCHC in men with polycythemia in the first age group compared to the control, while MCH and MCHC did not differ significantly in second age group (Table 3-8).

Our findings were similar to another study by Chen and his group (2021), which noted in a group of men with ages<60 and >60 year, the level of MCV and MCH increased significantly. Moreover, found the level of MCHC in 168 men with age (25-80) years increased significantly. While, the level of MCV and MCH were not significant (Basak *et al*., 2021).

Risso and his team (2007), studied the high altitude acclimatizationon blood parameters in mountain climbers after and before 53 days of acclimate, the age range between (28-43) years, which found that the RBCs count increased and values of MCV, MCH, and MCHC did not differ before and after acclimatization depending on the age of the climbers, and this results disagree with our results.

4.2.2: Hromonal study

4.2.2.1: Erythropoietin (EPO)

The results of the present study showed a significant decrease in the levels of EPO in both age groups of polycythemia when compared to the control group (Table 3-9).

The current results agree with a study by Gunes and his team (2019), their research included 243 patients of polycythemia under the age of 50 years, all of them were tested for serum EPO levels.

In a study conducted by Khodnapur and Das (2021), on 204 male participants aged (20-95) years, they noticed a decrease in the level of EPO in serum in the age group (60-69) and 70 years, this is consistent with results of our study.These results differ from our study may be due to the differences in the number of samples and in ages.

The results of our study differed from those of Musso and his team (2004), who studied 74 males: 22 adults, 30 old, and 22 very old, they found that among the three groups no change in EPO levels; they concluded that normal senescence does not alter EPO levels, even during advanced ageing. Furthermore, involving 143 individuals aged above 20 years, found a significant increase in EPO levels (Ershler *et al*., 2005).

High Hb levels and JAK2 mutation status are key indicators for PV diagnosis. The specificity of low EPO for diagnosing PV is limited, although EPO values are supplementary and aid in diagnosis. However, given that normal or high EPO has a high negative predictive value for excluding PV, it is still a crucial test parameter in the diagnostic procedure for distinguishing PV from other secondary causes of erythrocytosis. In addition, it is worth noticing that low serum EPO may be observed in other unusual/rare causes of erythrocytosis, where in there is the absence of leukocytosis and thrombocytosis or other features

suggestive of a MPN. Therefore, an integrated approach including the history, physical examination, complete blood counts, EPO levels, and JAK2 mutational workup is necessary for an accurate diagnosis in a case of polycythemia (Gupta *et al*., 2023).

The increase in serum EPO with aging may be compensating for increased RBC turnover or increased EPO resistance of red cell precursors. It is possible that the compensatory process fails in those who are very old or have impaired renal function (such as those who have diabetes or high blood pressure) (Ershler*et al*., 2005).

4.2.2.2: Reproductive hormones

The results of the present study showed no significant differences in the levels of FSH and E2 in polycythemia age groups when compared to the control group (Table 3-10).

Furthermor, our findings were agree with study by Gonzales and his team (2009), which study seventy adult men aged between (35–65) years old, and observed levels of FSH in men with polycythemia did not differ from the control men**.** Also, Benso and his team (2007), found no differences in levels of E2 in persons with polycythemia at different age.

The results of this study showed no significant differences in the levels of TT in first age group, while second age group of polycythemia was higher significant ($P < 0.05$) when compared to the control group (Table 3-10).

Moreover, our findings were agree with study by Gonzales and his team (2009) which studied seventy adult men aged between (35–65) years old, and observed the level of TT was high in men with polycythemia.

The current results disagree with the results by Von Wolff and his group (2018), where observed in twenty-one males aged between (18-70) years the level of TT did not differ significantly in adult men with polycythemia.

Testosterone is generated by the Leydig cells, from one its precursors, 17-hydroxyprogesterone (17- OHP) (Amory *et al*., 2008). The process of androgenesis indicates that 17- OHP is converted in several steps to dehydropeiandrosterone sulfate, and then after one step more it is converted to testosterone. Because ,17- OHP is increased at hypoxia, this elevation indicates that the process of producing testosterone at hypoxia is elevated since the precursor, 17- OHP, was elevated (Hwang *et al*.,2007).

The results of the present study showed a significant increase ($P< 0.05$) in the levels of LH in both age groups of polycythemia when compared to the control group (Table 3-10).

The present results agree with a study performed by He and his group (2015), on 58 males, their age was ranging from (18–21) years and they showed high levels of LH in people who lived in high altitudes compared to the control. Another study agrees with our study, which included 17 men and a mean age of (37.1 ± 14.2), showed that the level of LH increased significantly with progressive age (Bribiescas, 2005). However, the present results disagree with a study performed by Richalet and his group (2010), in men with a mean age of 28 years (range 23-34 years), they showed no significant differences in LH level.

The positive associations of LH with age suggest that an increase in GnRH production with age are the most likely causal mechanism for greater LH levels as males (22-73) years. However, clinical investigations

showed that hypothalamic function and gonadotropin secretion are less sensitive to changes in energetic status in older men (Bergendahl *et al.*, 1998).

Androgen levels have been shown to peak at approximately 30 years of age with a steady decline of about 1–2% per year thereafter (Araujo *et al*. 2004). Furthermore, confirmed these results by determining that GnRH secretion is lower in older men, but LH secretion is relatively stable (Veldhuis *et al*., 2012).

4.2.2.3: Thyroid hormones

The results of this study showed no significant differences for the levels of TSH and T4, but the level of T3 showed an increase significantly($P < 0.05$) in the first age groups of polycythemia which comparison with the control group (Table 3-11).

These results agree with study done by Alhazmi and his group (2018), on 420 individual with polycythemia with age means (42.57 ± 5.45), they showed no significantly difference for the levels of TSH. Also, our results agrees with the study done by Gonzales and his group (2009), they noted did not effect of age in T4 levels, when studied seventy adult men with polycythemia in high altitudes, aged between (35–65) year.

Regarding about the levels of T3, our study agree with Alhazmi and his group (2018), who observed high levels of T3 in people with polycythemia. While, previous study found in nine male (age mean 40.2 ± 1.4) an increase only in the T4 values and decrease in the T3 values (Benso *et al*., 2007). Richalet and his colleague (2010) stated that the increase in catecholamine may be the factor that influences T3 elevation.

In a study by Chaurasia and his team (2011), a cross sectional study included 198 patients age <20 and >60 year, data on age, TSH, T3 and T4

levels were analyzed. The study population was classified into different age groups. The serum thyroid hormones and TSH levels for male of different age groups shows that TSH level is highest in the age group (20-40) years and lowest in >60 year, while in <20 and (41-60) year, it is almost similar. The T3 was observed lowest in <20 and highest in (20-40) year. In 41-60 year and >60 years it remained nearly stable. The T4 value is highest in <20 which remained nearly stable in other groups.

Moreover, was conducted on a population aged between (18-80) years, a tendency toward increased TSH levels in adults was observed in the 80 years, and FT3 levels in males showed a gradual decrease as TSH increased with age. The FT4 level in males was basically the same. FT3/FT4 ratios remained stable until the age of 60 years followed by a rapid decline (Chen *et al*., 2019).

The age-related TSH increase was caused by changes in TSH or by decreased bioactivity (Bremner *et al.*, 2012). These modifications may also be influenced by genetic factors (Atzmon *et al.*, 2009).

Studies have shown that the hypothalamic-pituitary-thyroid axis response to thyroxine (T4) and triiodothyronine (T3) levels has changed due to modifications in the set points by age (Hine *et al*., 2017 ; Chen *et al*., 2019). The thyroid gland affected by age might modify the set point of response to FT4 or FT3 level, resulting in changed thyrotropin (TSH) levels in older adults (Surks and Boucai, 2010 ; Bano *et al*., 2019).

Serum rT3, an inactive metabolite of T4, seems to increase with age (Bano *et al*., 2019).

4.2.3: Biochemical study

4.2.3.1: Lipid profile:

The results showed a significant increase (P< 0.05) in the TC, TG, LDL-C, and VLDL, and a significant decrease in the HDL-C values in both age groups of polycythemia, compared with the control group (Table 3-12).

The current results agree with result of Hassan (2016), high levels of TC, TG, LDL-C and low levels HDL-C in polycythemia in men aged (20-35) years.

Also, our results agree with study done by Qasim and Al-Yasiri (2021), who showed high values of TC, TG, LDL-C and low values in HDL-C in the secondary polycythemia group when compared with true polycythemia and control groups in men aged (20- 70) year.

In Chinese population-based cross-sectional study for a group of men and wide age range (18–99) years, they recorded that TC levels increased at age (36–40) years, and plateaued between 41 and 60 years, and then decreased in those >80 years. Also, displayed increasing, plateau and decreasing phases in men ≤ 40, between 41 and 55, and ≥ 56 years. Regarding LDL-C levels, the trends for men displayed roughly an increasing phase (56–60) year group, followed by a decreasing phase >80-year group.In contrast, the trends in HDL-C levels were relatively unique, mainly manifesting as irregular trends with age. Nonetheless, increasing and decreasing phases were also found roughly in men for the ≤ 55 and ≥ 56 year groups. Age was found to be an independent risk factor associated with an elevation in TC, TG, LDL-C and HDL-C levels in men ≤40, ≤60 and ≥56 years, respectively. In addition, age was independently associated with declining TC, TG, LDL-C and HDL-C levels in men, ≥41, ≥61 and ≤55 years (Feng *et al*., 2020).

Another study found that TC, LDL-C and TG levels increase up to middle age and then decrease (Park *et al*., 2015). A study about 2222 men aged (20–79) years found that plasma TC levels climbed in unison among younger age groups, but levels fell in those who were older (Wilson *et al*., 1994).

Also, a study of 269 healthy white participants 126 men, aged (40–60) years reported a significant, linear age-related increase in TG and curvilinear effects of age on LDL-C and TC (Schubert *et al*., 2006).

In polycythemia the blood flow slowly, TC was stable without use, TG would be concentrated in the blood, the tissue cannot get TG due to slowing of the bloodstream, and HDL-C was decreased as a result of increased in LDL-C, the parameters of the lipid profile are connected together (Hassan, 2016). According to Pérez-Rodríguez and his group (2015), the higher level of TG was neutralized the lowers levels of HDL-C.

Chapter Five

Conclusions and Recommendations

5-1: Conclusions

1-The polycythemia caused an increase in hematological parameters in both groups (smoking, non-smoking and age) due to a change in the production of red blood cells.

2-The current study showed that the levels of EPO decrease in men in the smoking and age groups for polycythemia.

3-Polycythemia did not negatively affect men's fertility because it did not change the values of some hormones and some increased, such as LH and TT.

4-The appearance of contrast in some thyroid hormones, as it was observed that the T3 hormone increased, while the T4 and TSH hormones were not affected in patients with Polycythemia in age and smoking groups.

5-Dyslipidemia is over in men with polycythemia in both groups (smoking and age).

5-2: Recommendations

1-Future studies with more number of samples, both sexes and other parameters such as trace element and antioxidant agent in polycythemia subjects.

2-Future studies on effect shisha smokers in men and women with and without polycythemia.

3-Measurement the levels of clotting factors, and other parameters related to blood clotting in subjects with polycythemia in smokers and non-smokers.

4-Comparative study of some hematological, hormones and biochemical pararmeters study between polycythemiaVera and non-polycythemia Vera or in primary polycythemia and secondary polycythemia.

5- Genetic study of genes responsible for polycythemia Vera in male and female.

6- Study relationship between polycythemia and other diseases such as diabetes, cardivascular diseases, high blood pressure and obesity in men.

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Appendices

Appendice (A)
Questionnaire

الاسم (اختياري) : رقم الهاتف :
العمـر: المواليد:
الوزن : الطـول :
الحالة الزوجية متزوج ☐ غير متزوج ☐

هل تعاني من مشاكل في الغدة الدرقية ؟ نعم ☐ لا ☐
هل تعاني من مرض تصلب الشرايين ؟ نعم ☐ لا ☐
هل تعاني من مشاكل عضوية في الخصوبة او الجهاز التكاثري؟ نعم ☐ لا ☐

هل تعاني من مشاكل في القلب ؟ نعم ☐ لا ☐
هل تعاني من مشاكل في الكلية ؟ نعم ☐ لا ☐

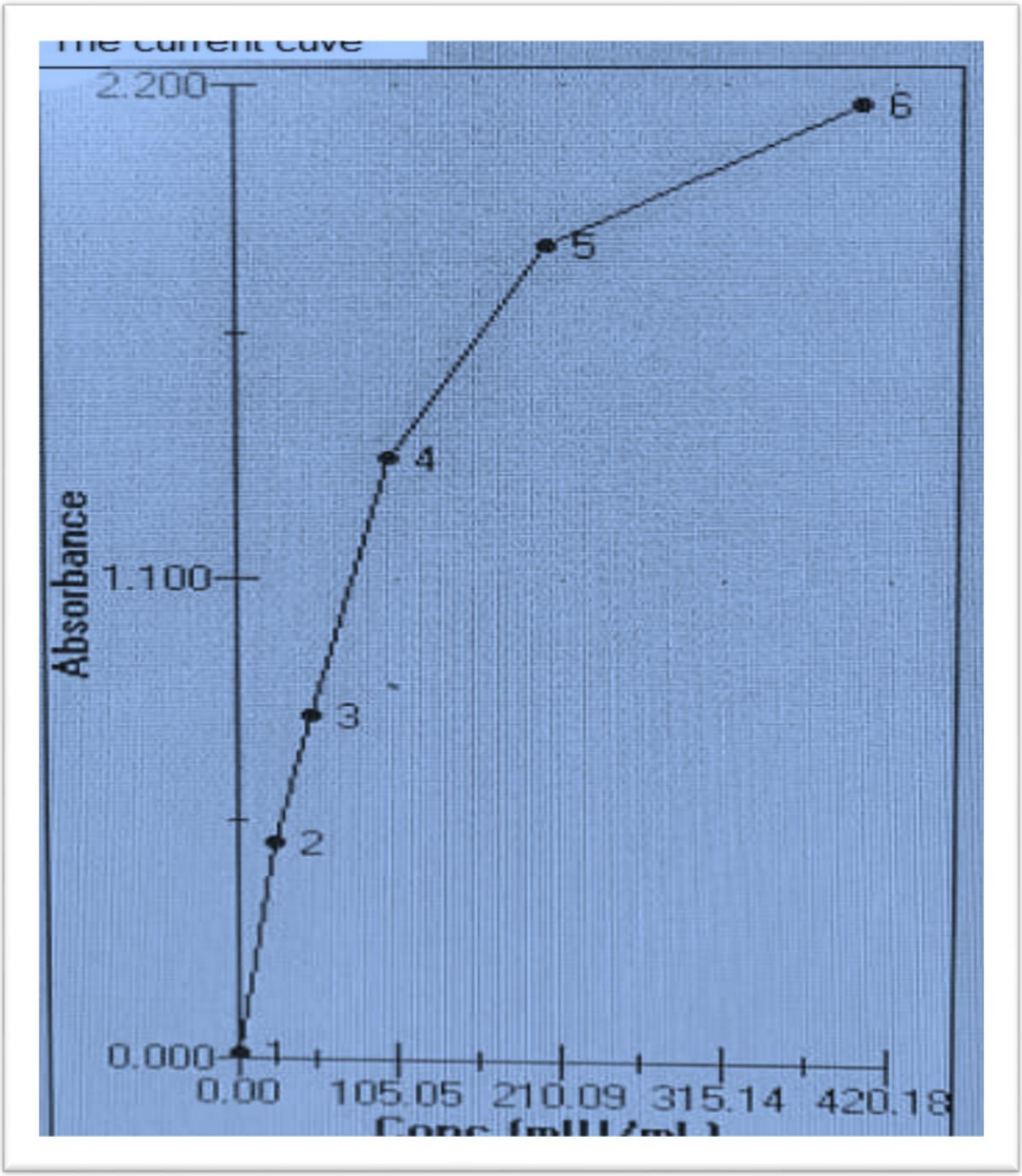
هل تعاني من امراض أخرى نعم ☐ لا ☐
اذا كنت تعاني من امراض أخرى اذكرها

تاريخ اول تشخيص بالمرض : .....
مدة الإصابة: .....
تاريخ الإصابة : .....
نوع العلاج المستخدم لعلاج الزيادة : .....
إصابة وراثية .....
تاريخ عائلي .....
هل تتناول هرمون الارثروبويتين .....

هل انت مدخن؟ نعم ☐ لا ☐
هل انت متبرع بالدم سابقاً؟ نعم ☐ لا ☐
هل قمت بالحجامة سابقاً؟ نعم ☐ لا ☐

Appendice (B)

The curve of EPO



الخلاصة

هدفت الدراسة الحالية الى بيان تأثير نقص الأكسجة على الاصابة بكثرة كريات الدم الحمر وذلك من خلال تقدير مستويات بعض المعايير الدموية ، الهرمونات وصورة الدهون لدى الرجال المصابين بكثرة الكريات الحمر في محافظة ميسان، جمعت العينات من مركز الدم خلال الفترة من تشرين الثاني ٢٠٢٢ الى اذار ٢٠٢٣ ، شملت الدراسة (١٠٠) رجل (٧٥) مصاب بكثرة الكريات الحمر و(٢٥) غير مصاب بكثرة الكريات الحمر (مجموعة الضابطة) والذين تراوحت أعمارهم بين (٢٠-٥٩) سنة ،تم تقسيم المصابين بكثرة الكريات حسب التدخين الى مجموعتين (مدخنين وغير مدخنين) ، وايضا قسموا حسب العمر الى مجموعتين الأولى من ٢٠الى ٣٩سنة والثانية من ٤٠الى ٥٩ سنة اظهرت النتائج ما يأتي :

أرتفاع معنوي عند مستوى احتمالية ($P<0.05$) في قيمة كل من عدد كريات الدم الحمر وخضاب الدم وحجم الخلايا المكدسة عند المصابين بكثرة الكريات ولكل من مجموعتي التدخين و العمر، بينما لم يظهر خلايا الدم البيض فروق معنوية ($P>0.05$) بين المصابين بفرط الكريات والمجموعة الضابطة ، بينت قيم دلائل كريات الدم الحمراء (متوسط حجم الكريات ومتوسط خضاب الكرية ومتوسط تركيز خضاب الكروي) ارتفاعا معنويا ($P<0.05$) في مجموعتي المدخنين والمجموعة العمرية الأولى، بينما في المجموعة العمرية الثانية فأن قيم متوسط خضاب الكرية و متوسط تركيز الخضاب الكروي لم تختلف معنوياً لمرضى كثرة الكريات الحمر مقارنة مع مجموعة الضبط ، كذلك قيم متوسط حجم الكريات و متوسط خضاب الكرية أيضاً لم تختلف معنوياً لمرضى كثرة الكريات الحمر غير المدخنين مقارنة مع مجموعة الضابطة .

أظهرت النتائج انخفاضاً معنوياً ($P>0.05$)في مستوى هرمون الاريثروبويتين في المجاميع التدخين و العمر لمرضى كثرة الكريات الحمر مقارنة مع مجموعة الضبط.

لا يوجد فرق معنوي ($P>0.05$) في الهرمون المحرض للجريبات و الاستراديول في مجموعتي التدخين و العمر لمرضى كثرة الكريات الحمر، بينما الهرمون اللوتيني فقد ارتفع معنوياً ($P<0.05$) في مجموعتي التدخين و العمر لمرضى كثرة الكريات الحمر مقارنة بالمجموعة الضابطة ،اما التستوستيرون الكلي في مجاميع التدخين و المجموعة العمرية الأولى لم يظهر أي فروق معنوية ($P>0.05$) لمرضى كثرة الكريات الحمر مقارنة مع المجموعة الضابطة ، بينما ارتفع معنوياً ($P<0.05$) في المجموعة العمرية الثانية لمرضى كثرة الكريات الحمر مقارنة بمجموعة الضبط ،

لم تختلف قيم الهرمون المحفز للدرقية و الثيروكسين اختلافاً معنوياً ($P>0.05$) في مجموعتي مرضى كثرة الكريات الحمر مقارنة مع مجموعة الضابطة حسب التدخين و العمر، بينما بينت النتائج ارتفاعا معنويا ($P<0.05$) في ثلاثي يودوثيرونين في مجموعتي المدخنين وغير المدخنين والمجموعة العمرية الاولى لمرضى كثرة الكريات الحمر مقارنة بالمجموعة الضابطة ، بينما لم تظهر المجموعة العمرية الثانية فروق معنوية ($P>0.05$) عند مرضى كثرة الكريات الحمر مقارنة بمجموعة الضبط .

أظهرت النتائج ارتفاعاً معنويا ($P<0.05$) في قيم الكوليسترول والدهون الثلاثية والدهون واطئة الكثافة والدهون واطئة الكثافة جداً و انخفاضاً معنويا ($P>0.05$) في مستوى الدهون عالية الكثافة في مجاميع التدخين و العمر لمرضى كثرة الكريات الحمر مقارنة مع المجموعة الضابطة .

ان التأثير الفسيولوجي لهذه النتائج نوقش على اساس كثرة الكريات الحمر وعلاقته مع التدخين، لاحتوائه على مواد ضارة يمكن ان تحدث تغييرات غير طبيعية في معايير الدم، بعض الهرمونات و صورة الدهون .

وزارة التعليم العالي والبحث العلمي
جامعـــــــة ميســـــــان
كليـــــة العلـــــــوم
قســـم علـوم الحيـاة

دراسة تأثير التدخين و التقدم بالعمر على بعض المعايير الفسيولوجية في الأشخاص البالغين المصابين بكثرة الكريات الحمر في محافظة ميسان

رسالة مقدمة
الى مجلس كلية العلوم / جامعة ميسان
وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل
مـــروة علي مجيـــــد
بكالوريوس علوم / علوم الحياة ٢٠١٠

أشراف
أ.د زينب عبد الجبار رضا

أيلول ٢٠٢٣ م
ربيع الأول ١٤٤٥ هـ