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Determination of Serum Iron Level in Sudanese Cigarette Smokers

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A Thesis Submitted in Partial Fulfillment of The Requirements
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الآية

قَالَ تَعَالَى:

﴿۞ وَقَضَىٰ رَبُّكَ أَلَّا تَعْبُدُوٓا إِلَّآ إِيَّاهُ وَبِٱلْوَٰلِدَيْنِ إِحْسَٰنًا إِمَّا يَبْلُغَنَّ عِندَكَ ٱلْكِبَرَ أَحَدُهُمَآ أَوْ كِلَاهُمَا فَلَا تَقُل لَّهُمَآ أُفٍّ وَلَا تَنْهَرْهُمَا وَقُل لَّهُمَا قَوْلًا كَرِيمًا ۝﴾

صَدَقَ اللهُ العَظِيمُ

سورة الإسراء: ٢٣

Dedication

I would like to dedicate dissertation to who gave me love, comfort throughout my life.

My mother

To who is always there for me and never let me need.

My father

To my

Brothers, sister, teachers

Acknowledgments

All thanks to Allah from the start to the end…..

And pray for Prophet Mohammed peace be upon him

I would like to express my sincere thanks and good respect to my supervisor

Dr. Munsour Mohammed

Who guide me throughout my way and helped me to make this research as accurate and useful as possible.

And I'm grateful to my friends and all those who contributed their time and helped me.

My thanks also extend to my college and my teachers

Abstract

Smoking is one of the biggest public health problem throughout the world. Cigarette smoking causes minerals disterbances which lead to serious consequences, this cross sectional study was carreid out to determine the effect of cigarette smoking on serum iron level . fifty male smokers aged (20 -50 years old as cases) and fifty male non smokers (controls were sex and age matched) were enrolled .Three mls of venous blood were collected from each volunteer ,serum obtained and analyzed colormetrically .statistical analysis was done using spss version 16 (in dependant t- test ,one way onova) it revealed that serum iron was insignificantly high in smokers compared to nonsmokers, (P.V=0.301), mean of serum iron in smokers (104.78µg/dl) ,(96.52µg/dl) in non smokers .Serum iron did not change due to age difference (P.V=0.766). There was insignificant difference between the mean of serum iron in smokers who smoked < 5 cigarette per day compared to those who smoked >15 cigarette per day (P.V= 0.772) , No correlation found between iron level and the number of cigarette per day , also no correlation found between iron level and duration of smoking (P.V=0.159).

ملخص البحث

التدخين هو واحد من أكبر مشاكل الصحة العامة في جميع انحاء العالم , التدخين بالسجائر يؤدي الى اضطراب المعادن مما يؤدي الى عواقب وخيمة .

اجريت هذه الدراسة المقطعية لتحديد تاثير تدخين السجائر علي مستوى مصل الحديد. خمسون من المدخنين الذكور الذين تتراوح أعمارهم بين(20- 50) سنة (كحالات وخمسون من غير المدخنين الذكور((المجموعتان متطابقتي العمر والجنس) تم . جمع ثلاثة مل من الدم الوريدي من كل متطوع ، تم الحصول على مصل الدم وتحليله بجهاز قياس الطيف المرئي .وكشف التحليل الإحصائي باستخدام الإصدار 16 , ارتفاعا ليس ذو دلالة معنوية في متوسط تركيز الحديد لدى المدخنين مقارنة بغير المدخنين ب= 0.301 وكان متوسط الحديد لدى المدخنبن (104.78) وغير المدخنين (96.52) ولم يتأثر متوسط تركيز الحديد في مصل الدم بإختلاف. العمر (ب=0.766) لم يكن هناك فرق ذو دلالة معنوية بين متوسط تركيز الحديد في . مصل الدم في المدخنين الذين يدخنون <5 سيجارة يوميا و الذين يدخنون >15 سيجارة يوميا(ب=0.772) لا يوجد ارتباط بين متوسط تركيز الحديد في مصل الدم وعدد السجائر في اليوم الواحد ، كما لم يتم العثور علي علاقة بين متوسط تركيز الحديد في مصل الدم ومده التدخين (ب=0.159).

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List of abbreviations

COPD	Chronic obstructive pulmonary disease
DNA	Deoxyribo nucleic acid
PH	Power of hydrogen
US	United state
MAO	Mono amine oxidase
ARDS	Adult respiratory distress syndrome
RBCs	Red blood cell
DMT-1	Divalent metal transporter -1
HFE	Hemochromatosis
BMP	Bone morphological protein
BMPR	Bone morphological protein receptor
SMAD	Sons of mothers against decapentaplegic
IL-6	Interlukin-6
m-RNA	Messenger ribo nucleic acid
STFR	Serum transferrin receptor
HH	Hereditary hemochromatosis
TIBC	Total iron binding capacity
CRF	Corticotrophin releasing factor
EPO	Erythropoietin
HuEPO	Human erythropoietin
SI	Serum iron
TSAT	Transferrin saturation
Hb	Hemoglobin
SPSS	Statistical pack for social science
PV	Probability value

Chapter I

Introduction

Chapter I

Introduction

Smoking

Smoking is the practice in which a substance most commonly tobacco or cannabis are burned,and the smoke tasted or inhaled. The most common method of smoking today is through cigarette. Tobacco use leads most commonly to diseases affecting the heart and the lungs. with smoking being a majorrisk factor for heart attacks , strokes , chronic obstructive pulmonary disease (COPD) , emphysema and cancer. It also causes peripheral vascular disease and hypertension, all developed due to exposure time and the level of dosage of tobacco. Minerals are very essential substances involved as catalysts in most cellular enzymatic reactions and assume amajor role in metabolism (Mudawi, 2011).

Serum iron

Iron are example of these essential minerals. Functions of iron include involvement in energy metabolism, gene regulation, cell growth and differentiation, oxygen binding and transport, muscle processes. It is involved in more than 300 essential metabolic reaction, some of which are: energy production,synthesis of essential molecules, structural roles, ion transport across cell membranes, cell signaling and cell migration (Mudawi, 2011).

Cigarette smoking causes minerals disturbances which lead to serious consequences,smoking leads to tissue hypoxia which leads to in adequate oxygenation of blood circulation that results in erythropoiesis and consequent increased production of erythropoietin. Which enhances erythropoiesis and increases red cell mass above normal level. This leads to increase in number of destroyed cells in the normal turn over process which subsequently increases iron over load which causes hepatocellular damage. Chronic oxidative stress may modulate iron uptake and storage, leading to self-sustained and ever increasing spiral of cytotoxic and mutagenic events (Mudawi, 2011).

Justifications

Cigarette smoking is an important and independent risk factor for atherosclerosis, coronary artery disease and peripheral vascular disease. Several studies have shown that cigarette contains carcinogen,irritant substance Carbone monoxide, and other gases which can harm lipid, proteins, and also cause DNA damage .cigarette smoking has increasing effect on hemoglobin concentration which proportional to the amount of cigarette smoked per day. Smoking leads to tissue hypoxia which leads to in adequate oxygenation of blood circulation that results in erythropoiesis and consequent increased production of erythropoietin, which enhances erythropoiesis and increases red cell mass above normal level. This leads to increase in number of destroyed cells in the normal turn over process which subsequently increases iron over load which causes hepatocellular damage ((Mudawi, 2011).

Chronic oxidative stress may modulate iron uptake and storage, leading to self-sustained and ever increasing spiral of cytotoxic and mutagenic events.Literature survey showed that no sufficient work has been done to study the effect of cigarette smoking on serum minerals alterations in Sudan, so this study was carried out to determine the influence of cigarette smoking on serum iron levels amongSudanese smokers and to determine the relationship between the level of serum iron with age, number of cigarette per day and duration of smoking (Mudawi, 2011).

Objectives

General objective

To determine the level of serum iron in Sudanese cigarette smokers .

Specific objectives

- To measure the concentration of serum iron in smokers and non-smokers.

-To correlate the level of serum iron with age.

-To correlate the level of serum iron with number of cigarettes per day.

-To correlate the level of serum iron with duration of smoking.

Chapter II

Literature Review

Chapter II
Literature Review

Smoking

Definition

Tobacco smoking is the practice where tobacco is burned and the vapors either tasted or inhaled.

The practice began as early as 5000–3000BC, many civilizations burnt incense during religious rituals, which was later adopted for pleasure or as a social tool and religious ceremonies.German scientists formally identified the link between smoking and lung cancer in the late 1920s leading the first anti-smoking campaign in modern history.The movement failed to reach across enemy lines during the Second World War, and quickly became unpopular thereafter.In 1950, health authorities again began to suggest a relationship between smoking and cancer.

Scientific evidence mounted in the 1980s, which prompted political action against the practice.Rates of consumption from 1965 onward in the developed world have either peaked or declined (Gately, 2003), however; they continue to climb in the developing world.

Smoking is the most common method of consuming tobacco, and tobacco is the most common substance smoked. The agricultural product is often mixed with other additives, and then pyrolyzed.The resulting vapors are then inhaled and the active substances absorbed through the alveoli in the lungs.(Lloyd and Mitchinson , 2010)

The active substances trigger chemical reactions in nerve endings, which heighten heart rate, memory, alertness, and reaction time.Dopamine and later endorphins are released, which are often associated with pleasure.As of 2000, smoking is practiced by some 1.22 billion people.Men are more likely to smoke than women, though the gender gap declines with younger age.Many smokers begin during adolescence or early adulthood, usually

during the early stages, smoking provides pleasurable sensations, serving as a source of positive reinforcement. After an individual has smoked for many years, the avoidance of withdrawal symptoms and negative reinforcement become the key motivations to continue (West and Shiffmand,2016).

Early use

Smoking's history dates back to as early as 5000–3000 BC when the agricultural product began to be cultivated in South America; consumption later evolved into burning the plant substance either by accident or with intent of exploring other means of consumption (Gately, 2003).The practice worked its way into shamanistic rituals. Many ancient civilizations, such as the Babylonians, Indians and Chinese, burnt incense as a part of religious ritualsas did the Israelites and the later Catholic and Orthodox Christian churches(Leventhal *et.al*., 2008). Smoking in the Americas probably had its origins in the incense-burning ceremonies of shamans but was later adopted for pleasure or as a social tool the smoking of tobacco and various hallucinogenic drugs was used to achieve trances and to come into contact with the spirit world(Fadheel, 2012).

Eastern North American tribes would carry large amounts of tobacco in pouches as a readily accepted trade item and would often smoke it in pipes, either in defined ceremonies that were considered sacred, or to seal a bargain, and they would smoke it at such occasions in all stages of life, even in childhood.It was believed that tobacco was a gift from the Creator and that the exhaledtobacco smoke was capable of carrying one's thoughts and prayers to heaven (Lloyd and Mitchinson, 2010).

Method

Tobacco

Is an agricultural product processed from the fresh leaves of plants in the genus nicotiana, the genus contains a number of species, however

Nicoitanatabacum is the commonly grown.Nicotianarustica follows as second containing higher concentrations of nicotine. These leaves are harvested and cured to allow for the slow oxidation and degradation of caritenoid in tobacco leaf. This produces certain compounds in the tobacco leaves which can be attributed to sweet hay, tea, rose oil, or fruity aromatic flavors. Before packaging, the tobacco is often combined with other additives in order to: enhance the addictive potency, shift the products pH, or improve the effects of smoke by making it more palatable. In the United States these additives are regulated to 599 substances. The product is then processed, packaged, and shipped to consumer markets. Means of consumption has greatly expanded in scope as new methods of delivering the active substances with fewer by-products have encompassed or are beginning to encompass (US, 2001).

Cigar

Cigars are tightly rolled bundles of dried and fermented tobacco which are ignited so that smoke may be drawn into the smoker's mouth. They are generally not inhaled because the high alkalinity of the smoke, which can quickly become irritating to the trachea and lungs. The prevalence of cigar smoking varies depending on location, historical period, and population surveyed, and prevalence estimates vary somewhat depending on the survey method. The United States is the top consuming country by far, followed by Germany and the United Kingdom; the US and Western Europe account for about 75% of cigar sales worldwide.

As of 2005 it is estimated that 4.3% of men and 0.3% of women smoke cigars (US, 2001).

Cigarettes

Cigarettes, French for "small cigar", are a product consumed through smoking and manufactured out of cured and finely cut tobacco leaves and reconstituted tobacco, often combined with other additives, which are then

rolled or stuffed into a paper-wrapped cylinder.Cigarettes are ignited and inhaled, usually through a cellulose acetate filter, into the mouth and lungs (US, 2001).

Electronic cigarettes

Electronic cigarettes are an alternative to tobacco smoking, although no tobacco is consumed. It is a battery-powered device that provides inhaled doses of nicotine by delivering a vaporized propylene glycol/nicotine solution.

Many legislation and public health investigations are currently pending in many countries due to its relatively recent emergence (US, 2001).

Physiology

The active substances in tobacco, especially cigarettes, are administered by burning the leaves and inhaling the vaporized gas that results. This quickly and effectively delivers substances into the bloodstream by absorption through the in alveoli the lung.The lungs contain some 300 million alveoli, which amounts to a surface area of over 70 m^2 (about the size of a tennis court).This method is inefficient as not all of the smoke will be inhaled, and some amount of the active substances will be lost in the process of combustion pyrolysis.Pipe and Cigar smoke are not inhaled because of its high alkalinity, which are irritating to the trachea and lungs. However, because of its higher alkalinity (pH 8.5) compared to cigarette smoke (pH 5.3), unionized nicotine is more readily absorbed through the mucous membrane in the mouth.Nicotine absorption from cigar and pipe, however, is much less than those from cigarette smoke.The inhaled substances trigger chemical reactions in nerve endings.The cholinergic receptors are often triggered by the naturally occurring neurotransmitter acetylcholine.Acetylcholine and nicotine express chemical similarities, which allow Nicotine to trigger the receptor as well The nicotinic acetylcholine receptors takes are located in the central nervous system and

at the nerve-muscle junction of skeletal muscles; whose activity increases heart rate, alertness, and faster reaction times, nicotine acetylcholine stimulation is not directly addictive. However, since dopamine-releasing neurons are abundant on nicotine receptors, dopamine is released, this release of dopamine, which is associated with pleasure, is reinforcing and may also increase working memory.Nicotine and cocaine activate similar patterns of neurons, which supports the idea that common substrates among these drugs.When tobacco is smoked, most of the nicotine is pyrolyzed, however, a dose sufficient to cause mild somatic dependency and mild to strong psychological dependency remains.There is also a formation of hormone (MAO inhibitor) from the acetaldehyde in tobacco smoke.This seems to play an important role in nicotine addiction—probably by facilitating a dopamine release in the nucleus acummbensas a response to nicotine stimuli,using rat studies, withdrawal after repeated exposure to nicotine results in less responsive nucleus accumbens cells, which produce dopamine responsible for reinforcement(Wilbert, 1987).

Diseases caused by smoking

Emphysema

Emphysema is a condition in which the alveoli are increased in size due to destruction of the lung parenchyma. It is frequently (but not invariably) caused by the smoking of tobacco.The path physiology of the disease is not entirely clear. Bronchial lavage shows that air spaces of the lungs of smokers become invaded by neutrophils. It is now thought that these cells secrete proteolytic enzymes that damage the parenchyma of the lungs.In addition, the smoke inhibits the movement of the bronchial cilia, slowing the removal of particulate matter from the airways.

The irritant effect of the smoke is the probable cause of the increased secretion of mucus in the larger airways.These effects combine to increase the chances of infection, which results in chronic inflammation of the

bronchiolar epithelium. As a result, the diameter of the airways is reduced and, as in asthma, it becomes difficult to exhale, leading to the entrapment of air (from which the disease takes its name). As a result of the loss of parenchymal tissue, the traction on the airways is reduced and their resistance is increased. By itself, this will limit ventilation, but the problem is compounded as the destruction of the alveoli also restricts the diffusing capacity of the lung. Moreover, the destruction is not uniform, the physiological dead space is increased. The result is inadequate gas exchange, hypoxemia (poor oxygenation of the blood), and chronic dyspenia (Umberager *et al.*, 2006).

Adult respiratory distress syndrome (ARDS)

This is a condition in which the lung parenchyma is severely damage. ARDS is characterized by a severe hypoxemia; Precipitating causes include septic shock .aspiration of the gastric contents, near drowning, and inhalation of toxic gases or smoke.The release of chemical mediators may result in further constriction of the pulmonary vasculature and the development of pulmonary hypertension.Within a week of the onset of the condition, the lungs become infiltrated by fibroblasts which lay down fibrous tissue in the pulmonary interstitium.There is a loss of elastic tissue and emphysema develops.This is reflected in an increase in the physiological dead space(Umberager *et al.*, 2006).

Chronic obstructive pulmonary disease (COPD)

Is one of the most common lung diseases, affecting -10% of the population older than 40 years worldwide, while a recent Korean nation surveillance indicated its higher prevalence in the Korean population. An imbalance between oxidants and anti-oxidants is considered to play a critical role in COPD pathogenesis, while cigarette is akey risk factor for COPD development, and most COPD patients are current or former smokers (Ghio and Hilborn, 2017.).

Serum iron

Health Effects

Of the 3 to 5g of iron in the body, approximately 2 to 2.5 g of iron is in hemoglobin, mostly in RBCs and red cell precursors. A moderate amount of iron (130 mg) is in myoglobin, the oxygen-carrying protein of muscle. A small (8 mg), but extremely important, pool is in tissue where iron is bound to several enzymes that require iron for full activity. These include peroxidase, cytochromes, and many of the Krebs cycle enzymes. Iron is also stored as ferritin and hemosiderin, primarily in the bone marrow, spleen, and liver. This critical pool of iron may be the first to become diminished in iron deficiency states.48 Only 3 to 5 mg of iron is found in plasma, almost all of it associated with transferrin, albumin, and free hemoglobin (Sambyal *et al.,* 2015).

Iron chemistry

The metabolic functions of iron depend on its ability to change its valence state from reduced ferrous (Fe^{+2}) iron to the oxidized ferric (Fe^{+3}) state. Thus it is involved in oxidation and reduction reactions such as the electron transport with in mitochondrial cytochromes. In cells ferrous iron can react with peroxide via the Fenton reaction, forming highly reactive oxygen molecules .

$Fe^{+2} + H_2O_2 = Fe^{+3} + OH^{-} + OH.$

The resulting hydroxyl radical (OH) also known as free radical. Isespecially reactive as a short – lived but potent oxidizing agent able to damage proteins,lipids and nucleic acids (Rodak *et al.,* 2013.)

Iron kinetics

Systemic body iron regulation

Systemic body iron is regulated by absorption in to the body, because there is no mechanism for excretion. ferrous iron in the lumen of the small intestine is carried across the luminal side of the enterocyte by divalent

metal transporter-1 (DMT-1) once iron has been absorbed in to enterocytes, it requires another transporter , ferroprotein , to carry it across the basolaminal enterocyte membrane in to blood stream thus truly absorbing it in to the body .ferroprotein is the only known protein that export iron across cell membranes. When the body has adequate stores of iron, the gepatocytes sense that and will increase production of hepicidin. A protein able to bind to ferroprotein leading to it is inactivation. As a result, iron absorption in to body decreases hepicidin production. As a result, ferroprotein is once again active and able to transport iron into the blood. Thus homeostasis of iron is maintained by modest fluctuation in liver hepicidin production in response to body iron status. Themeganism by which the hepatocytes are able to sense iron levels and produce hepicidin is highly complex, with multiple stimulatory pathways likely involved. Although this system is not yet fully elucidated, a number of critical molecules have been identified. The proteins involved include at least the hemochromatosis receptor (HFE), transferrin receptor 2, hemojuvelin, bone morphogenic protein(BMP) and its receptor (BMPR)and sons of mothers against decapentaplegic (SMAD) (Pugliese and Faver, 2001).

Haemosidrin

Is an insoluble protein –iron complex contain approximately 37% iron by weight. It is derived from partiallysosomal digestion of aggregatesof ferritin molecules and is visible in macrophages and other cells by light microscopy after staining by perls (Prussian blue) –reaction.Iron in ferritin and haemosidrin is in the ferric form, it's mobilized after reduction to the ferrous form .vitamin C being involve. A coppercontain enzyme, caeruloplasmin, catalusesoxidation of iron to the ferric form for binding to plasma transferring, iron is also present in muscle as myoglobin and in mast cells of the body in iron containing enzymes (Mehta and brand, 2009).

Hepicidin

Hepicidin is a 25- amino acid polypeptide produced by liver cells. It is both an acute phase protein and the major hormonal regulator of iron homeostasis. It is inhibits iron release from macrophages, intestinal epithelial cells and from placental syncytiotrophoblasts by interaction with the transmembrane iron exporter ferroprotein, accelerating deregulation of ferroprotein mRNA. Increased production of hepicidin is induced by inflammation via interleukin 6 (IL-6). hepicidin synthesis and secretion are controlled by three proteins : HFE, hemojuvelin and transferrin receptor 2 .decreased production of hepicidin occurs in response to iron deficiency , hypoxia and ineffective erythropoiesis (Mehta and brand, 2009).

Absorption, Transport, and Excretion

Absorption of iron from the intestine is the primary means of regulating the amount of iron within the body typically; only about 10% of the 1g/day of dietary iron is absorbed. To be absorbed by intestinal cells, iron must be in the Fe (II) (ferrous) oxidation state and bound to protein.Because Fe (III) is the predominant form of iron in foods, it must first be reduced to Fe (II) by agents such as vitamin C before it can be absorbed.In the intestinal mucosal cell, Fe (II) is bound by apoferritin, and then oxidized by cereloplasmin to Fe (III) bound to ferritin. From there, iron is absorbed into the blood by apotransferrin, which becomes transferrin as it binds two Fe (III) ions. In plasma, transferrin carries and releases Fe to the bone marrow, where it is incorporated into hemoglobin of RBCs. After about 4 months in circulation, red cells are degraded by the spleen, liver, and macrophages, which return Fe to the circulation, where it is bound and carried by transferrin for reuse. Ferroportin controls the release of iron from cells. The recently discovered peptide hormone hepcidin largely controls iron metabolism by its ability to modulate the release of iron from cells by inhibiting ferroportin. Iron regulation is primarily through modified

absorption from the upper gastrointestinal tract. Absorption and transport capacity can be increased in conditions such as iron deficiency, anemia, or hypoxia. Iron is lost primarily by desquamation and red cell loss to urine and feces. With each menstrual cycle, women lose approximately 20 to 40 mg of iron (Sambyal et al., 2015)

Iron requirements

The amount of iron required each day to compensate for losses from the body and for growth varies with age and sex; it is highest in pregnancy, adolescent and menstruating females therefore these groups are particularly likely to develop iron deficiency if there is additional iron loss or prolonged reduced intake. Bone marrow iron Bone marrow examination is not essential to assess iron stores except in complicated cases. In iron deficiency anemia there is a complete absence of iron from stores (macrophages) and from developing erythroblasts. The erythroblasts are small and have a ragged cytoplasm. Serum transferrin receptor is shed from cells into plasma. The level of serum transferrin receptor (STFR) is increased in iron deficiency anemia but not in the anemia of chronic disease or Thalassemia trait. The level is also raised if the overall level of erythropoies is increased (Neufeld and Cerrato, 2008).

Deficiency

Iron deficiency affects about 15% of the worldwide population. Those with a higher than average risk of iron deficiency anemia include pregnant women, young children it is frequently caused by dietary deficiency because milk has low iron content (Burtis *et al.,* 2012).

Adolescents, and women of reproductive age.51 Increased blood loss, decreased dietary iron intake, or decreased release from ferritin may result in iron deficiency. Reduction in iron stores usually precedes both a reduction in circulating iron and anemia, as demonstrated by a decreased

red blood cell count, mean corpuscular hemoglobin concentration, and microcytic RBCs (Sambyal *et al*., 2015).

Toxicity

Iron overload states are collectively referred to as haemochromatosis, whether or not tissue damage is present. Primary Fe overload is most frequently associated with hereditary hemochromatosis (HH). HH is a single-gene homozygous recessive disorder leading to abnormally high Fe absorption, culminating in Fe overload. Secondary Fe overload may result from excessive dietary, medicinal, ortransfusional Fe^{+2} intake or be due to metabolic dysfunction. Hemosiderosis has been used to specifically designate a condition of iron overload as demonstrated by an increased serum iron and total iron binding capacity (TIBC) or transferrin, but without demonstrable tissue damage. HH causes tissue accumulation of iron, affects liver function, and often leads to hyper pigmentation of the skin. Some conditions associated with severe hemochromatosis include diabetes mellitus, arthritis, cardiac arrhythmia or failure, cirrhosis, hypothyroidism, impotence, and liver cancer. Treatment may include therapeutic phlebotomy or administration of chelators, such as deferoxamine, transferrin can be administered in the case of lipidperoxidation, atherosclerosis, deoxyribo nucleic acid (DNA) damage, carcinogenesis, and neurodegenerative diseases, Fe (III), released from binding proteins, can enhance production of free radicals to cause oxidative damage. In iron-loaded individuals with Thalassemia who are treated with chelators to bind and mobilize iron, intake of ascorbic acid may actually promote the generation of free radicals. It seems likely that tissue damage caused by free iron is the underlying reason for the elaborate set of carrier proteins Involved in iron transport and metabolism(Sambyal *et al.*, 2015)**.**

Haemochromatosis

Is condition in which the amount of total body iron is increase; the excess iron is deposited in/and cause damage to several organ(Böcske *et al.,* 1992)

Hereditary Haemochromatosis(primary)

In this case iron is deposited through the body and total body iron may reach 20-60g. The important organ involve: liver/endocrine gland /heart (Böcske *et al.,* 1992)**.**

Secondary haemochromatosis

Many condition / including chronic hemolytic disorder/ sidroblasticanemia / and other condition requiring multiple blood transfusion and dietary iron over load / are associated widespread secondary siderosis . The feature are similar primary haemochromatosis/ but history and clinical findings point to the true diagnosis (Böcske *et al.,* 1992).

Low or decreased serum iron status may be a result of increased level of circulating cytokine capable of inducing macrophages of reticuloendothelial system that hold on iron both groups of patients in the present study. Some of these reasons may include platelet dysfunction which is common in CRF. Others may include high may lead to a decrease in the endogenous EPO production or decreased responsiveness of erythroid precursor cells to endogenous or exogenous EPO. Various reasons could have caused the reduced platelet counts in e uremia, hereditary thrombocytopenia and drugs. There were few limitations of the present study. Some data were missing from the clinical records of patients. This suggests proper documentation is very important. The short study period only allowed evaluation of r HuEPO treatment up to three months. However, as patients are treated on a long-term basis with rHuEPO, it would be useful to track their hemoglobin and iron status over a longer period of time. Other confounding factors that may affect hemoglobin and iron status included patients' diet, compliance to dietary supplement pills

from hospital, other underlying chronic diseases such as hypertension, diabetes, blood loss during menstruation in women and blood loss due to other conditions. Anemia is common in renal failure. Serum iron (SI), and the percentage of transferrin saturation (TSAT) reflect the amount of iron immediately available for hemoglobin (Hb) synthesis. Serum ferritin level reflects total body iron stores. Adequate iron stores are essential for achieving maximum benefit from recombinant human erythropoietin (Epo). A low level of either of these indices may indicate the need for supplement iron to support erythropoiesis (Gupta *et al.,* 2017).

Chapter III

Materials and Methods

Chapter III
Materials and Methods

Study design

A cross sectional descriptive study, conducted in Khartoum State in the period between March and October 2019 to evaluate serum iron levels in Sudanese smokers.

Study population

Sudanese smokers and none smokers male (fifty smokers and fifty non smokers).

Data collection tools

Data were collected by observation of laboratory test results and per-coded questionnaire used to obtain demographic, style life and smoking behavior.

Blood Sampling

Venous blood samples were collected using sterile disposable plastic syringe after cleaning the vein puncture area with 70% ethanol, the blood samples were left on bench for half hour to clot and centrifuged at 3000 rpm for 10 min then serum was separate into a new eppendorf tube for investigate.

Inclusion criteria

- Cases: adult Sudanese healthy cigarette smoker's males.
- Control: adult Sudanese none smokers males.

Exclusion criteria

Adult Sudanese males smoke other than cigarette, females or any person suffer from chronic abnormalities.

Ethical considerations

Written consent was obtained from each participant in the study before blood sample collection after being informed with all detailed objectives of the study and its health benefit in the future.

Data analysis

The collected data were analyzed using SPSS computer software version 16.(in dependent t- test , one way onova)

Method

Serum Iron measurement

Principle of method

Transferrin bound ferric iron in the sample are release by guanidinium and reduced to ferrous by mean of hydroxylamine. Ferrous iron reacts with ferrozine forming colored complex that can be measured by spectrophotometer.

Contents and composition

Reagent A: guanidinium 1mol/l, hydroxylamine 0.3mol/l, acetate buffer0.4mol/l, pH 4.0.

reagent B: ferrozine 8mol/l.

iron standard. Iron 200µg/dl (35.8 µmol/l).

Equipment

-plain container

-centrifuge

-glass tube

-spectrophotometer.

Procedure

The reagents were brought to room temperature, pipetted into labeled test tube, mixed thoroughly and let to stand the tube for 5 minute at room temperature, the absorbance (a) of the sample blank read at 560 nm against distilled water ,The absorbance (a) of the sample and standard Read at 560 nm against the reagent blank.

Calculations

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

A sample - a sample blank/a standard) * c standard =c sample.

Reference Values:

Serum level in males: 65-175ug/dl.

Quality Control

The reliability of a methods used to measure biochemical parameters were evaluated by assaying quality control samples.

Chapter IV

Results

Chapter IV

Results

Table (4-1) show the mean of serum iron in smoker and non-smoker.

	Number	Mean(μg/dl)	P. value
Smoker	50	104.78	
Non smoker	50	96.52	0.301

Table (4.2): Show mean of serum iron among different age group.

age group(Years)	N	Mean(µg/dl)	%	P. Value
20-30	60	102.82	60	
31-40	28	98.57	28	
41-50	12	94.67	12	0.766
Total	100	100	100	

Table (4.3): show the mean of serum iron in smokers according to duration of smoking.

Duration (years)	N	%	Mean(µg/dl)	P. Value
< 5	12	12	107.67	
5-10	17	17	101.24	
10-15	7	7	115.29	0.159
>15	14	14	101.36	
Total	50	50		

Table (4.4): show the mean of serum iron in smokers according to number of cigarette per day.

Cigarettes /day	N	%	Mean(µg/dl)	P. Value
< 5	10	10	89.2	
5-10	15	15	95.4	
10-15	8	8	127.5	0.772
>15	17	17	111.5	
Total	50	50		

Chapter V
Discussion, Conclusion and Recommendations

Chapter V

Discussion

This is a cross-sectional study conducted in Khartoum State during period from March 2019 and November 2019. Hundred volunteers were recruited to participate in this study, fifty of them were smokers and the remaining were non-smokers considered as control group. All of the participants were males, the ages of smokers and non-smokers were matched (20-50)yrs. The study aimed to measure serum iron levels among Sudanese smokers.

This study shows that the mean of serum iron in study group is (104.78μg/dl), while in control group the mean of serum iron is (96.52μg/dl). Statistical analysis revealed that there is insignificant variation with (p. value of 0.301). This result agreed with a study done in Turkey by Ismail *et al.,* 2012. They concluded that smoking does not affect the serum iron levels (*P* value more than 0.05).

This finding were different with results obtained by Mudawi*et al*, 2011 in a study showed that serum iron was significantly increased in smokers when compared to non-smokers (p value =0.000). Different results were obtained by Meera*et al.,* 2017, who found a significant increase in serum iron level in smokers compared to non-smokers (p value= 0.001). The age group 20-30 years old were found to be with higher frequency in the study population.

In the study group with age of (20-30) years have mean of serum iron (102.82μg/dl) while in group with age of (31-40) years is (98.57μg/dl) and in group with age of (41 -50) years the mean is (94.67μg/dl) Statistical analysis revealed that there is insignificant variation between serum iron and age ,(p. value=0.766). Results showed in table (4-3) indicate that the mean of serum iron is not increased with increasing the duration of the smoking and indicate that no significant variation, p. value (0.159).

Result showed in table (4-4), found that there is insignificant variation with P. value (0.772).

Conclusion

On the bases of the findings the study conclude that smoking does not affect the serum iron level in the study population. The age, duration and number of cigarette are do not affect the level of serum iron.

Recommendations

The study recommend the followings:

- Further study should be done including large sample size so as to confirm results.

- Different parameters should be investigated in order to find out the real effects of cigarette smoking and other type of smoke on serum iron level.

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Appendix

Questionnaire NO(   )

Questionnaire about the effect of smoking on serum iron level

- **Name:…………………………………………**

- **Phone NO: …………………………………………..**

- **Age (Years) :**

  - 20-30 {   }   b. 31-40 {   }   c. 41-50 {   }

- **Duration of smoking:-**

  a. less 5 year {   }   b. 5 – 10years {   }   c. 10-15years {   }

  d. more than 15years

- **Number of cigarette per day?**

  a. less than 5 {   } b. 5-10 times   {   }

  c.10-15cig   {   } d-more than 15 cig{   }

- **Do you suffering from any chronic   diseases?**

  a. Yes   {   }   b. No {   }**.**

- **Do you take any treatment:**

  a. Yes   {   }   b. No   {   }

  If yes mention …………………………………..…………………

- **Serum iron concentration=** ………………………..µg/dl**.**