بسم الله الرحمن الرحيم

Republic of Sudan

Ministry of Higher Education and Scientific Research

Shendi University

Faculty of Graduate Studies and Scientific Research

Title:

Effect of Cigarette Smoking on Trace Elements (Magnesium, Zinc & Iron) of some resedents in Khartoum State, Sudan

A Thesis Submitted in Partial Fulfillment of the Requirements of Master Degree in Medical Laboratory Sciences (Clinical Chemistry)

Submitted By:

Sara Khalafalla Abdalgader Mohmmed

Supervisor:

Prof/Rashid Eltayeb Abdalla

March *2018*

بسم الله الرحمن الرحيم

قال تعالي :

" وفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٍ " (١)

" وَقُلْ رَبِّ زِدْنِي عِلْماً " (٢)

صدق الله العظيم

(١)سورة يوسف : الآية ٧٦

(٢) سورة طه : الآية ١١٤

Bibliographic Entry

Author: Sara Khalafalla Abdalgader Mohmmed

Thesis: Effect of Cigarette Smoking on Trace Element (Magnesium, Zinc & Iron) in Khartoum State, Sudan

Degree program: Master Degree.

Faculty: Medical Laboratory Sciences.

Field of study (specialty): Clinical Chemistry.

Supervisor: Prof. Rashid Eltayeb Abdalla

Duration: March June/2018

Number of pages:

Key words

Declaration and Statement

I Sara Khalafalla, under signed, declare and affirm that this Thesis is my own original work. I have followed all ethical and technical principles in the preparation, data collection, data analysis and compilation of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation. I, solemnly declare that this Thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Signature ....

Date ………………………………………………………………..

Dedication

To the everyone who taught me how to be available member in community?

Dear father

To the depth of be longings and rhythm of sympathy

Dear mother

To crown of pleasure and secret of existence

Dear husband

To the continuous encouragement and support

Friend and dear colleagues

And special dedication for every one that helped me to reach this place

Acknowledgment

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

And pray for Prophet Mohammed peace be upon him

I would like to acknowledge the contribution of my

Supervisor

Prof: Rashid Altayeb Abdallah

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 teacher

Abstract

This study was performed in Bahry and Alkalakla in Khartoum state, during the period March to June 2018. The aim of this study was to assess the effects of cigarette smoking on serum magnesium, iron and zinc in Sudanese male.

A 30 samples were collected from Sudanese smoker's males, the age of population studied ranged between (20–45) years. Samples were measured for the serum levels of magnesium, iron and zinc determined by atomic absorption spectrophotometer.

The study showed that there was significant decrease in the serum levels of magnesium (mean =15.0 mg/dl) when compared with normal range [17-27 mg/dl]. Also there was significant increase of serum iron (mean=3.1mg/dl) compared with normal range (0.5-1.5mg/dl), and significant decrease in serum level of zinc (mean 0.4mg/dl) compared with normal range (0.5-1.2mg/dl).

The study revealed that age of the smokers, duration of the smoking, marital status (with exception of zinc level which have significance difference), economic status their education, and that the number of cigarette smoked per day have no effect on the serum level of magnesium, iron and zinc.

المستخلص

أجريت هذه الدراسة في منطقه بحري والكلاكله القلعلة بولاية الخرطوم خلال الفترة من مارس إلى يونيو ٢٠١٨. تهدف هذه الدراسة إلى تقييم تأثير تدخين السجائر على نسبه الماغنيسيوم والحديد والزنك في الذكور السودانيين.، يتراوح عمر السكان المدروسين بين ٢٠-٤٥ سنة. تم قياسها لمستويات الماغنيسيوم والحديد والزنك التي يحددها مقياس الامتصاص الذري.

أوضحت الدراسة أن هناك نقصان معنوي(١٥مج\دسل متوسط)في مستويات المصل من الماغنيسيوم مقارنة بالمدى الطبيعي (١٧-٢٧ملجم\دسل). وارتفاع ملحوظ(٣٠١مجم \دسل \متوسط) في مستوى الحديد لدى مدخني السجائر مقارنة بالمدى الطبيعي (٠٥_١٠٥ملجم \دسل)٠وايضا هنالك انخفاض ملحوظ (٤٠ملجم \دسل متوسط) في نسبه الزنك مقارنه بالمدى الطبيعي (٠٥_١٠٢ملجم\دسل)٠

كما أشارت الدراسة إلى أن عمر المدخنين ،مدة التدخين ،عدد السجائر في اليوم الواحد،الحاله الاجتماعيه (باستثناء مستوى الزنك)،الحاله الاقتصاديه ،الوظيفه ، مستوى التعليم لا يوجد تأثير بينهم وبين مستوى الماغنيسيوم والحديد والزنك في المصل.

Table of contents

List of Figures

List of abbreviations

ADHD	Attention deficit hyperactivity disorder
ATP	Adenosine tri phosphate
COPD	Chronic obstructive pulmonary disease
Ca^{2+}	Calcium
DNA	Deoxyribonucleic acid
ETS	Environmental Tobacco Smoke
Fe^{2+}	Iron
HB	Hemoglobin
HH	Hereditary hemochromatosis
Mg^{2+}	Magnesium
OTC	Over-the-counter
RNA	Ribonucleic acid
RBCs	Red blood cells
TIBC	Total iron binding capacity
TCA	Tricolor acetic acid
Zn^{2+}	Zinc

1.1 Introduction

Smoking is a practice in which a substance, most commonly tobacco or cannabis smoke tasted or inhaled. The most common method of smoking today is through tobacco Use Leads Most Commonly to diseases affecting the heart and lungs, with smoking being a major risk 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 the exposure time and the level of dosage of tobacco. [(2)]
Minerals are very essential substances involved as catalysts in most cellular enzymatic reactions and assume a major role in metabolism. [(3)]

Fe^{2+}, Zn^{2+} Mg^{2+} and are examples of these essential minerals. Functions of Fe^{2+} include involvement in energy metabolism, gene regulation, cell growth and differentiation, oxygen binding and transport, muscle oxygen use and storage [(4)] [(5)] etc. Mg^{2+} is a critical cation and cofactor in numerous intracellular processes. it is involved in more than (300) essential metabolic reactions, some of which are: energy production, synthesis of essential molecules, structural roles, ion transport across cell membranes, cell signaling, and cell migration. [(6)]

Zn^{2+} is second only to iron in importance as an essential trace element. The main biochemical role of Zn^{2+} is its influence on the activity of more than (300) enzymes (from the classes of oxireductases, transferases, hydrolases, lyases, lsomerases, and ligases). Zn^{2+} can be essential for the structure, regulation, and catalytic action of an enzyme. Zn^{2+} occurs in enzymes that realize the synthesis and metabolism of DNA and RNA. Zn^{2+} influences the synthesis and metabolism of proteins, participates in glycolysis and cholesterol metabolism, maintains membrane structures, effects functions of insulin, and affects growth factor. [(1)]

Literature survey showed that no sufficient work has been done to study the effect of cigarette smoking on serum minerals alterations, so this study was carried out to determine the influence of cigarette smoking on serum Fe^{2+}, Zn^{2+} and mg levels among Sudanese smokers and to determine the relationship between the levels of serum Fe^{2+}, Zn^{2+} and Mg^{2+} with age, number of cigarettes per day, and duration of smoking cigarette smoking causes minerals disturbances which lead to serious consequences, Smoking leads to tissue hypoxia which leads to inadequate oxygenation of blood circulation that results in erythropoiesis and consequent increased production of erythropoietin (7) which enhances erythropoiesis and increases red cell mass above normal level. (8) This leads to increase in the number of destroyed red cells in the normal turnover process which subsequently increases iron overload which causes hepatocellular damage. Chronic oxidative stress may modulate iron uptake and storage, leading to a self-sustained and ever increasing spiral of cytotoxic and mutagenic events. (10) Smoking causes Mg^{2+} deficiency due to decreased supply (lesser appetite) and reduced absorption caused by disturbances in the digestive system functions. (11) Nicotine –addicts usually have the risk of depletion / deficiency in important nutrients and minerals including zinc (12). Minerals disturbances may lead to sever and even life threatening metabolic abnormalities such as coronary heart disease, liver disease, lung infection, kidney failure, and disorders of endocrine system. (13)

1.2 Rationale

Cigarettes smoking had dangerous effects on essential biochemical mechanisms on the human body and this research will be conducted to determine its direct effects on some important minerals and to bridge the gap of information.

1.3 Objectives

1.3.1 General objective

To determine the effects of cigarette smoking on trace elements (Mg^{2+}, Fe^{2+} & Zn^{2+}).

1.3.2 Specific objectives

1. To determine direct effects of cigarette smoking on (Mg^{2+}, Fe^{2+} and Zn^{2+}) concentration among Sudanese population In Khartoum State.
2. To study the duration effect of cigarette smoking on level of minerals (Fe^{2+}, Mg^{2+}, Zn^{2+}) concentration.
3. To study the effect of cigarette smoking on level of minerals (Fe^{2+}, Mg^{2+}, Zn^{2+}) concentration according to age.
4. To correlate between the economic status effect of cigarette level smokers and minerals (Fe^{2+}, Mg^{2+}, Zn^{2+}).
5. To associate between the education effect of cigarette smokers and level minerals (Fe^{2+}, Mg^{2+}, Zn^{2+}).
6. To correlate between the social status effect of cigarette smokers and level minerals (Fe^{2+}, Mg^{2+}, Zn^{2+}).
7. To associate between the job of cigarette smokers and level minerals (Fe^{2+}, Mg^{2+}, Zn^{2+})

2 Literature Review

2.1 Smoking

Smoking is the inhalation of the smoke of burning tobacco encased in cigarettes. Pipes, and cigars. Casual smoking is the act of smoking only occasionally, usually in a social situation or to relieve stress. A smoking habit is a physical addiction to tobacco products. Many health experts now regard habitual smoking as a psychological addiction too, and one with serious health consequences

2.1.1 Health effects

Smoking is one of the leading causes of preventable death globally. In the United States about (500,000) deaths per year are attributed to smoking-related diseases and a recent study estimated that as much as (1/3) of China's male population will have significantly shortened life-spans due to smoking. Male and female smokers lose an average of (13.2 and 14.5 years) of life, respectively. (14) At least half of all lifelong smokers die earlier as a result of smoking. The risk of dying from lung cancer before age (85) is (22.1%) for a male smoker and (11.9%) for a female current smoker, in the absence of competing causes of death. The corresponding estimates for lifelong non-smokers are a (1.1%) probability of dying from lung cancer before age (85) for a man of European descent, and a (0.8%) probability for a woman. Smoking one cigarette a day results in a risk of heart disease that is halfway between that of a smoker and a non-smoker. The non-linear dose response relationship may be explained by smoking's effect on platelet aggregation. (15)Among the diseases that can be caused by smoking are vascular stenosis, lung cancer, heart attacks and chronic obstructive pulmonary disease. Smoking during pregnancy may cause anti diuretic hormone defiencey (ADHD) to a fetus. (16)

Many governments are trying to deter people from smoking with anti-smoking campaigns in mass media stressing the harmful long-term effects of smoking. Passive smoking, or secondhand smoking, which affects people in the immediate vicinity of smokers, is a major reason for the enforcement of smoking bans. This is a law enforced to stop individuals smoking in indoor public places, such as bars, pubs and many developing countries have not adopted anti-smoking policies, leading some to call for anti-smoking campaigns and further education to explain the negative effects of ETS (Environmental Tobacco Smoke) in developing countries. Tobacco advertising is also sometimes regulated to make smoking less appealing restaurants. The idea behind this is to discourage smoking by making it more inconvenient, and to stop harmful smoke being present in enclosed public spaces. A common concern among legislators is to discourage smoking among minors and many states have passed laws against selling tobacco products to underage customers (establishing a smoking age).

2.1.2 Society and culture

Smoking has been accepted into culture, in various art forms, and has developed many distinct, and often conflicting or mutually exclusive, meanings depending on time, place and the practitioners of smoking. Pipe smoking, until recently one of the most common forms of smoking, is today often associated with solemn contemplation, old age and is often considered quaint and archaic. Cigarette smoking, which did not begin to become widespread until the late 19th century, has more associations of modernity and the faster pace of the industrialized world. Cigars have been, and still are, associated with masculinity, power and is an iconic image associated with the stereotypical capitalist. In fact, some evidence suggests that men with higher than average testosterone levels are more likely to smoke. [(17)] Smoking in public has for a long time been something reserved for men and when done by women has been associated with promiscuity. In Japan during the Edo period, prostitutes and their clients would often approach one another under the guise of offering a smoke; the same was true for 19th century in Europe.[(18)]

2.2 Magnesium

(Mg^{2+}) is a chemical element with symbol Mg^{2+} and atomic number (12). It is a shiny gray solid which bears a close physical resemblance to the other five elements in the second column (group 2, or alkaline earth metals) of the periodic table: all (group II) elements have the same electron configuration in the outer electron shell and a similar crystal structure body and is essential to all cells and some (300) enzymes. Magnesium ions interact with polyphosphate compounds such as ATP, DNA, and RNA. Hundreds of enzymes require magnesium ions to function. Magnesium compounds are used medicinally as common laxatives, antacids (e.g., milk of magnesia), and to stabilize abnormal nerve excitation or blood vessel spasm in such conditions Mg^{2+} is the 11th most abundant element by mass in the human as eclampsia.[19]

2.2.1 Mechanism of action

The important interaction between phosphate and mg ions makes mg essential to the basic nucleic acid chemistry of all cells of all known living organisms. More than (300) enzymes require mg ions for their catalytic action, including all enzymes using or synthesizing ATP and those that use other nucleotides to synthesize DNA and RNA. The ATP molecule is normally found in a chelate with mg ion. [20]

2.2.2 Metabolism

An adult has (22–26) grams of Mg^{2+}, with (60%) in the skeleton, (39%) intracellular (20% in skeletal muscle), and (1%) extracellular. [18] Serum levels are typically (0.7–1.0 mmol/L) or (1.8–2.4 mEq/L). Serum Mg^{2+} levels may be normal even when intracellular magnesium is deficient. The mechanisms for maintaining the Mg^{2+} level in the serum are vary in gastrointestinal absorption and renal excretion. Intracellular Mg^{2+} is correlated with intracellular potassium. Increased Mg^{2+} lowers Ca^{2+} and can either prevent hypercalcemia or cause hypocalcemia depending on the initial level. [21] Both low and high protein intake conditions inhibit mg absorption, as does the amount of phosphate, phytate, and fat in the gut. Unabsorbed dietary Mg^{2+} is excreted in feces; absorbed magnesium is excreted in urine and sweat. [22]

2.2.3 Deficiency

Low plasma Mg^{2+} (Hypomagnesemia) is common: it is found in (2.5-15%) of the general population. The primary cause of deficiency is low dietary intake: fewer than (10% of people in the United States meet the recommended dietary allowance. Other causes are increased renal or gastrointestinal loss, an increased intracellular shift, and proton-pump inhibitor antacid therapy. Most are asymptomatic, but symptoms referable to neuromuscular, cardiovascular, and metabolic dysfunction may occur. Alcoholism is often associated with mg deficiency. Chronically low serum mg levels are associated with metabolic syndrome, DM type 2, fasciculation, and hypertension. [23]

2.3 Iron

(Fe^{2+}) is fourth most abundant element in the earth's crust. Pure Fe^{2+} is a soft, lustrous, ferromagnetic metal. Methods of extracting Fe^{2+} from ore have been known for centuries. The uses of Fe^{2+} are so widespread and well known that one can say that modern economies are based largely on the many uses of Fe^{2+}. The physical properties of Fe^{2+} alloys can be varied over an enormous range by appropriate alloying and heat treating methods, giving a range of strength, hardness, toughness, corrosion resistance (in the form of stainless steels), and magnetic properties and ability to take and hold a sharp edge.

2.3.1 Health effects

Of the (3 to 5 g) of Fe^{2+} in the body, approximately (2 to 2.5 g) of iron is in HB, 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 Fe^{2+} for full activity. These include peroxidases, 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. [24] Only (3 to 5 mg) of iron is found in plasma, almost all of it associated with transferrin, albumin, and free HB. [24]

2.3.2 Absorption, transport and excretion

Absorption of Fe^{2+} from the intestine is the primary means of regulating the amount of Fe^{2+} within the body. Typically, only about (10%) of the (1 g/day) of dietary iron is absorbed. To be absorbed by intestinal cells, Fe 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 Apo ferritin, then oxidized by ceruloplasmin to Fe (III) bound to ferritin. From there, Fe^{2+} is absorbed into the blood by Apo transferrin, which becomes transferrin as it binds two Fe (III) ions. In plasma, transferrin carries and releases Fe^{2+} to the bone marrow, where it is incorporated into HB of RBCs. After about (4 months) in circulation, red cells are degraded by the spleen, liver, and macrophages, which return Fe^{2+} to the circulation, where it is bound and carried by transferrin for reuse. Ferroprotein 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 ferroprotein. [25] Fe^{2+} regulation is primarily through modified absorption from the upper gastrointestinal tract. [25] 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 Fe^{2+}.

2.3.3 Deficiency

(Fe^{2+}) deficiency affects about (15%) of the worldwide population. Those with a higher than average risk of iron deficiency anemia include pregnant women, young children and adolescents, and women of reproductive age. [26] Increased blood loss, decreased dietary iron intake, or decreased release from ferritin may result in iron deficiency. Reduction in Fe^{2+} stores usually precedes both a reduction in circulating Fe^{2+} and anemia, as demonstrated by a decreased red blood cell count, mean corpuscular hemoglobin concentration, and microcytic RBCs.

2.3.4 Toxicity

(Fe^{2+}) overload states are collectively referred to as hemochromatosis, whether or not tissue damage is present. Primary Fe^{2+} overload is most frequently associated with hereditary hemochromatosis (HH). HH is a single-gene homozygous recessive disorder leading to abnormally high Fe^{2+} absorption, culminating in Fe^{2+} overload. Secondary Fe^{2+} overload may result from excessive dietary, medicinal, or transfusional Fe^{2+} intake or be due to metabolic dysfunction. Hemosiderosis has been used to specifically designate a condition of Fe^{2+} overload as demonstrated by an increased serum Fe^{2+} and total iron binding capacity (TIBC) or transferrin, but without demonstrable tissue damage.

(HH) causes tissue accumulation of Fe^{2+}, affects liver function, and often leads to hyperpigmentation of the skin. Some conditions associated with severe hemochromatosis include DM, 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 atransferrinemia.(26)

(Fe^{2+}) may play a role as a prooxidant, by contributing to lipid peroxidation, atherosclerosis, deoxyribonucleic acid (DNA) damage, carcinogenesis, and neurodegenerative diseases, (28, 29) 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 Fe^{2+}, intake of ascorbic acid may actually promote the generation of free radicals. (30) It seems likely that tissue damage caused by free iron is the underlying reason for the elaborate set of carrier proteins involved in Fe^{2+} transport and metabolism.

2.3.5 Laboratory evaluation of iron

Disorders of iron metabolism are evaluated primarily by packed cell volume, HB, red cell count and indices, total iron and TIBC, percent saturation, transferrin, and ferritin.[(31)].

2.4 Zinc

(Zn^{2+}) is a bluish white, lustrous metal. Zn^{2+} is stable in dry air and becomes covered with a white coating when exposed to moisture. Zn^{2+} is the fourth most used metal (after iron, aluminum, and copper). Zn^{2+} and its compounds are used in a production of alloys, especially brass (with copper), in galvanizing steel, in die casting, in paints, in skin lotions, in treatment of Wilson's disease, and in many over-the-counter (OTC) medications.[(32)].

2.4.1 Health effects

(ZN^{2+}) is second only to iron in importance as an essential trace element. The main biochemical role of Zn^{2+} is its influence on the activity of more than (300) enzymes (from the classes of oxireductases, transferases, hydrolases, lyases, isomerases, and ligases). Zn^{2+} can be essential for the structure, regulation, and catalytic action of an enzyme. Zn^{2+} occurs in enzymes that realize the synthesis and metabolism of DNA and RNA. Zn^{2+} influences the synthesis and metabolism of proteins, participates in glycolysis and cholesterol metabolism, maintains membrane structures, effects functions of insulin, and affects growth factor. [(33)]

2.4.2 Absorption, transport and excretion

The body content in a normal individual is about (2.5 g) Zinc, which is mainly in muscles (60%) and skeleton (30%). The remaining (10%) is distributed in all tissues with highest concentrations in eyes, prostate, and hair. All tissue levels depend on age. Zn^{2+} absorption mainly occurs in the small intestine and especially in the jejunum. In blood, the absorbed Zn^{2+} is distributed between RBCs (80%), plasma (17%), and white blood cells (3%). Different factors modify the absorption of Zn. The factors increasing Zn^{2+} absorption include: presence of animal proteins and amino acids in a meal, intake of Ca^{2+}, and unsaturated fatty acids. The factors decreasing Zn^{2+} absorption include intake of Fe^{2+}, taking Zn^{2+} on empty stomach, presence of copper at high levels, and age. In normal dietary circumstances, about (90%) of Zn^{2+} is excreted in feces.(34)

2.4.3 Deficiency

Nutritional Zn^{2+} deficiency is widespread all over the world. Zn^{2+} deficiency causes growth retardation, slows skeletal maturation, causes testicular atrophy, and reduces taste perception. Old age, pregnancy, lactation, and alcoholism are also associated with poor Zn^{2+} nutrition.

Infants with acrodermatatisenteropathica (Zn^{2+} malabsorption) usually first develop characteristic facial and diaper rash. Untreated, symptoms progress and include growth retardation, diarrhea, impaired T-cell immunity, insufficient wound healing, infections, delayed testicular development in adolescence, and early death. Zn^{2+} deficiency in adolescents is manifested by slow growth or weight loss, altered taste, delayed puberty, dwarfism, impaired dark adaptation, alopecia, emotional instability, and tremors. In severe cases, lymphopenia may occur; death follows an overwhelming infection. (35)

2.4.4 Toxicity

(Zn^{2+}) is relatively nontoxic. Nevertheless, high doses (1 g) or repetitive doses of (100 mg/day) for several months may lead to disorders, especially gastrointestinal tract symptoms, decrease in heme synthesis due to an induced copper deficiency, and hyperglycemia. Exposure to Zn^{2+} fumes and dust may cause "zinc fume fever." The symptoms include chemically induced pneumonia, severe pulmonary inflammation, fever, hyperpnea, coughing, pains in legs and chest, and vomiting. (36)

2.5 Previous study

A previous study was done by Sulafa Ali Abdalla Mudawi, Samia Mahdi Ahmed, et al, in Sudanese (2013) for assessment of the levels of serum Fe^{2+} and Mg^{2+} in Sudanese cigarette smokers, and the result were as follows; Serum Fe^{2+}: The analysis results showed that, there was a significant difference in the mean of serum Fe^{2+} between smokers and non-smokers (140±52) µg/dl versus (90±22) µg/dl, p value 0.000, <0.05.

There was insignificant difference in serum Fe^{2+} in the different age groups, p value 0.2, >0.05.There was insignificant difference between the mean of serum Fe^{2+} in smokers who smoked (≤15) cigarettes per day (138±50) µg/dl compared to those who smoked ≥15 cigarettes per day (145± 55) µg/dl, p value 0.6. Also there was no correlation between serum Fe^{2+} and number of cigarettes per day. There was insignificant difference in serum Fe^{2+} with different smoking durations; p value 0.7, also there was no correlation between serum Fe^{2+} and the duration of smoking. Serum Mg^{2+}: The analysis results showed that, there was a significant difference in the mean of serum mg between smokers and non-smokers (1.4±0.40) mg/dl versus (2.2±0.39) mg/dl, p. value 0.000, <0.05.

There was insignificant difference in serum Mg^{2+} in different age groups, p value 0.4, >0.05.There was significant difference between the mean of serum Mg^{2+} in smokers who smoked ≤15 cigarettes per day (1.5±0.36) mg/dl compared to those who smoked ≥15 cigarettes per day (1.3±0.35) mg/dl, p value 0.03. And there was a weak negative correlation between serum Mg^{2+} and the number of cigarettes per day, correlation coefficient r = -0.2.There was a weak negative correlation between serum Mg^{2+} and the smoking duration, correlation coefficient r = -0.3.

3 Materials and Methods

3.1 Study design

Prospective, laboratory based analytical study.

3.2 Study duration

The study was carried out during the period from March to June (2018).

3.3 Study area

This study was conduct in Khartoum state at Bahry and Alkalakla localities.

3.4 Sample size

A 30 Blood samples were collected from cigarette smokers.

3.5 Study population

Persons presenting to Bahry and Alkalakla were heavy cigarette smokers.

3.6 Selection criteria

3.6.1 Inclusion criteria

Heavy cigarette smokers from different ages more than (15 years) old and free from any disease that may affect the results.

3.6.2 Exclusion criteria

Any Sudanese individual that have disease my effect the result of Mg^{2+}, Fe^{2+} and Zn^{2+}.

3.7 Sampling

Two and half ml of blood was collected into the syringe after which the needle was detached and the collected blood was delivered carefully from the syringe into the heparin container, down the side of the container. To prevent coagulation, the blood was mixed promptly and thoroughly, yet gently, with the anticoagulant. A piece of cotton was then placed on the site of vein puncture and the patient was told to raise their arm upwards for a minute so as to apply pressure to the site.

3.8 Data collection

By prepared questionnaire.

3.9 Data analysis

The data was analyzed by using the application of SPSS (statistical package for social sciences).

3.10 Sample collection

Under a septic condition, about (2.5ml) of venous blood were collected from each volunteer by venipuncture technique, and were placed in anticoagulant containers, and then centrifuged at (3000 rpm) for (5 minutes) to obtain plasma which kept in eppindorff tubes for measurements of Fe^{2+}, Zn^{2+} and Mg^{2+}.

3.11 Ethical consideration

Permission to carry out the study was taken from health administration, Shendi University committee and the smokers were informed for the purpose of the study before collection of samples and verbal consent will be taken.

3.12 Methods

3.12.1 Preparation of sample

Iron: In a polyethylene tube, dilute a minimum of a (1.0 Ml) serum sample with an equal volume of a 20% (w/v) TCA solution. Cap the tube loosely, mix and heat in a heating block at (90 °C) for (15 minutes). Cool and centrifuge.

For the determination of magnesium dilute the serum or plasma sample 1:50 with deionized water.

For the determination of serum Zn^{2+}, dilute the sample 1:5 with deionized water. Samples are diluted with deionized water. The analysis is performed against standards prepared in glycerol to approximate the viscosity characteristics of the diluted samples. The dilution ratio can be adjusted to insure that concentrations fall within a suitable absorbance range.

3.12.2 Principle

The technique makes use of absorption spectroscopy to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert Law.

In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer-Lambert Law.

4.1 Results

Direct effect of cigarette smoking on Mg^{2+} /Fe^{2+} and Zn^{2+} concentration among Sudanese population In Khartoum State. The result of Fe^{2+} denoted high concentration with mean (3.1mg/L) compared with normal range (0.5-1.5mg/L). But result of Mg^{2+} indicated mean (15 mg/L) which was low concentration compared with normal range (17-28mg/L), also result of Zn^{2+} showed low concentration with mean (0.4 mg/L) compared with normal range 0.5-1.2mg/L.

Table 1: Mean and Std. Deviation. of Fe^{2+}, Mg^{2+} and Zn^{2+}.

	Fe^{2+}	Mg^{2+}	Zn^{2+}
Mean	3.1mg/L	15.0mg/L	0.4mg/L
Std. Deviation	0.8	1.7	0.1

Table (1) revealed that high Mg^{2+} and low Fe^{2+}, & Zn^{2} levels.

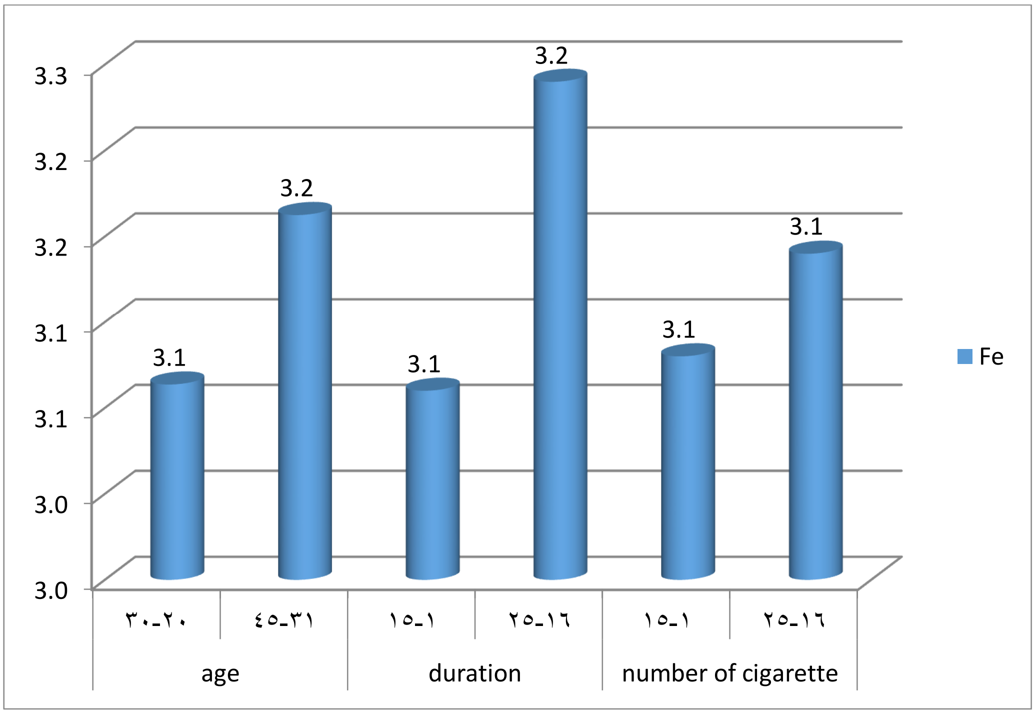

Figure1: Mean of Fe^{2+}, with age (p-value =0.70), duration (p-value= 0.60) and number of cigarette (p-value =0.80), revealed insignificance difference in compare with normal p-value (0.05).

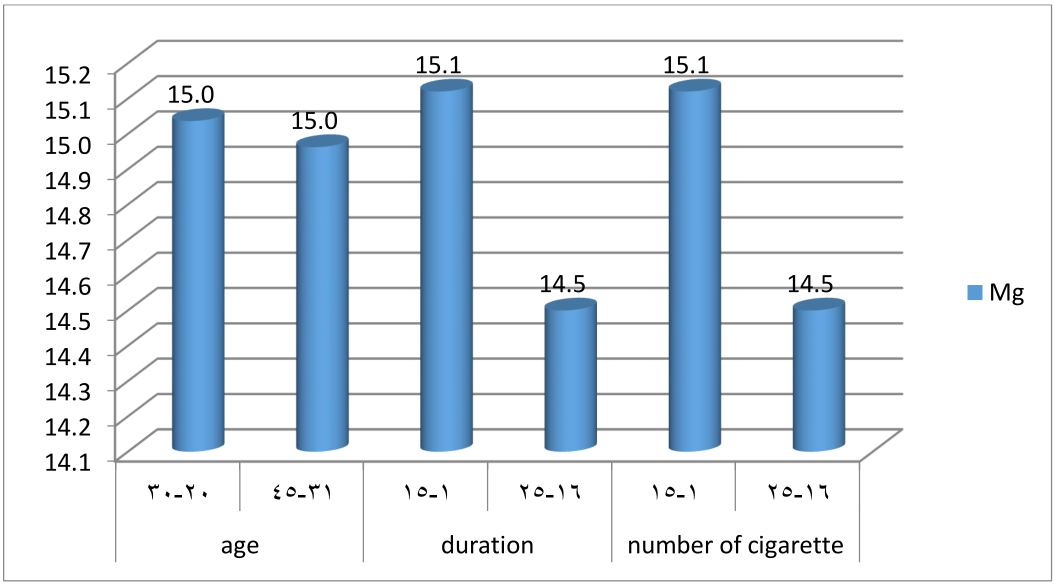

Figure 2: Mean of Mg^{2+} with age (p-value=0.90), duration (p-value=0.40) and number of cigarette (p-value=0.40), revealed insignificance difference in compare with normal p-value (0.05).

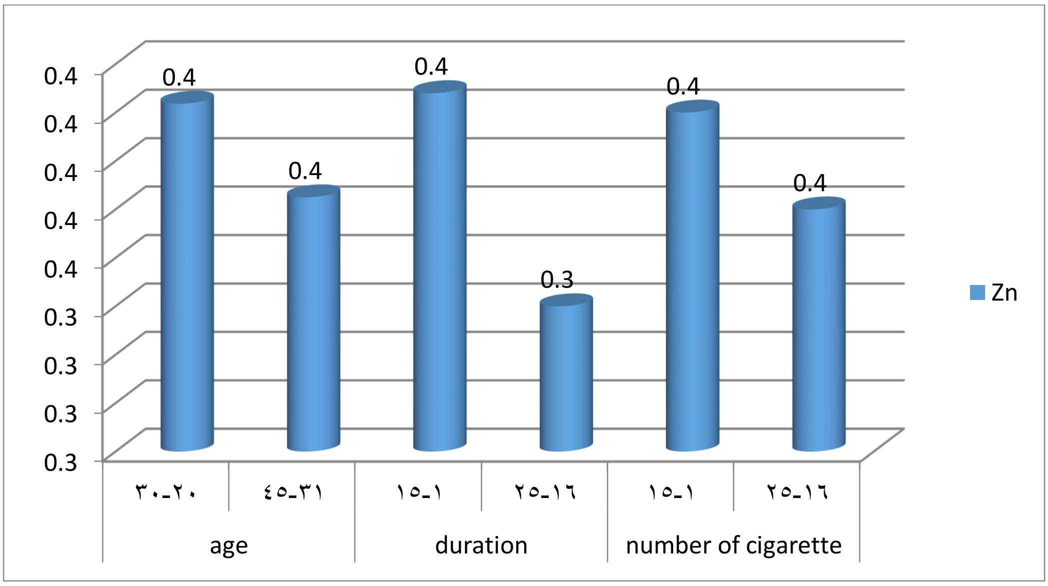

Figure 3: Mean of Zn^{2+} with age (p-value= 0.60), duration (p-value =0.30) and number of cigarette (p-value =0.60), revealed insignificance difference in compare with normal p-value (0.05).

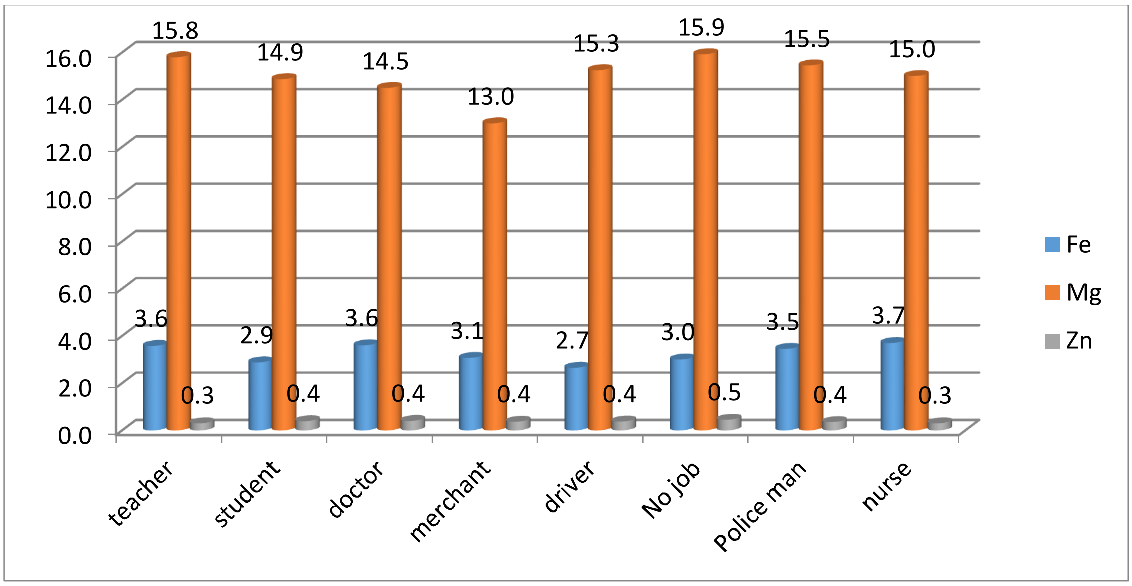

Figure 4: Mean of Fe^{2+} (p-value =0.40), Mg^{2+} (p-value= 0.50), Zn^{2+} (p-value= 0.80) with job, revealed insignificance difference in compare with normal p-value (0.05).

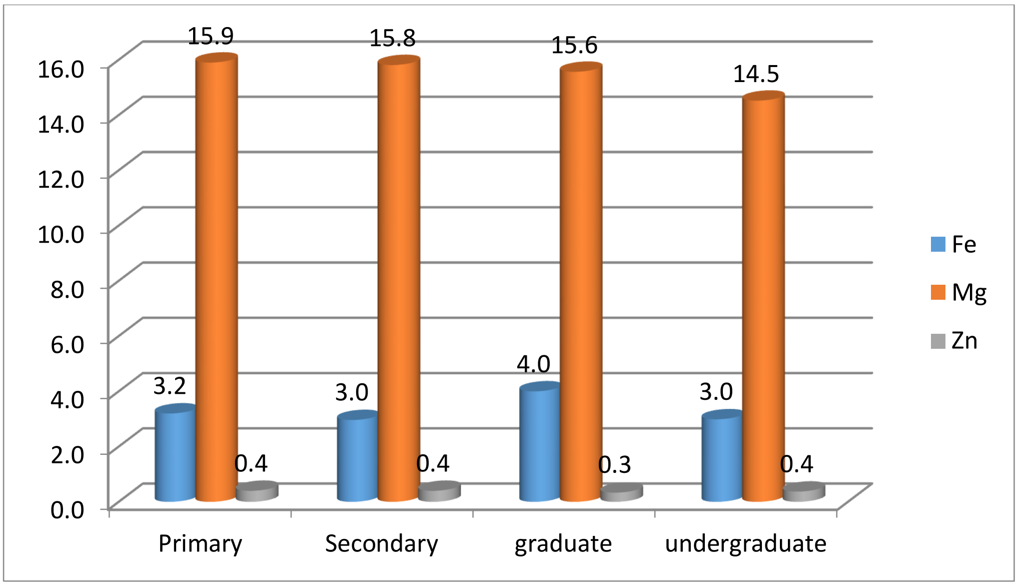

Figure 5: Mean of Fe^{2+} (p-value=0.70). Mg^{2+} (p-value=0.50), and Zn^{2+} (p-value =0.30) with education revealed insignificance difference in compare with normal p-value (0.05).

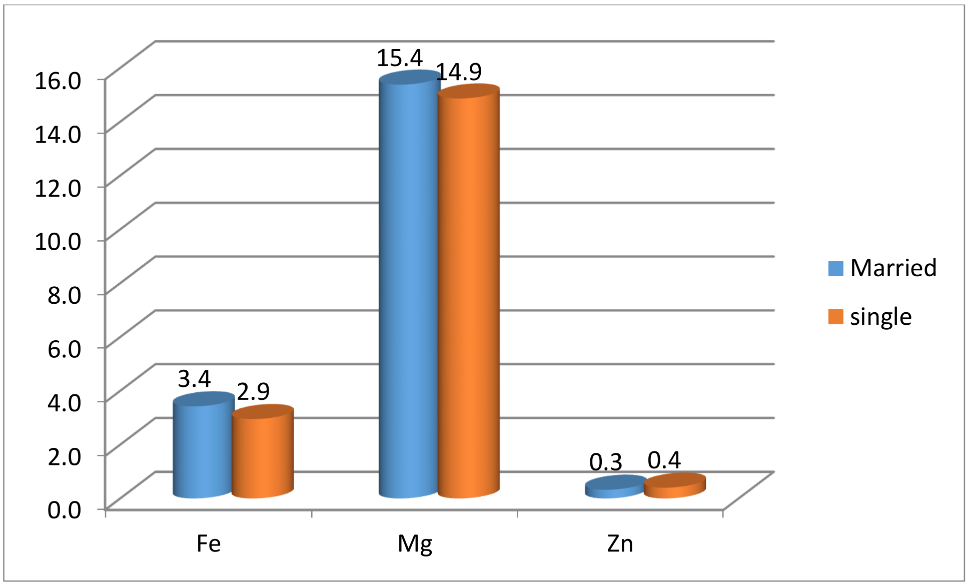

Figure 6: Mean of Fe^{2+}, (p-value= 0.10) Mg^{2+} (p-value=0.40) with marital status revealed insignificant difference. Zn^{2+}show significance difference (p-value=0.02).in compare with normal p-value (0.05).

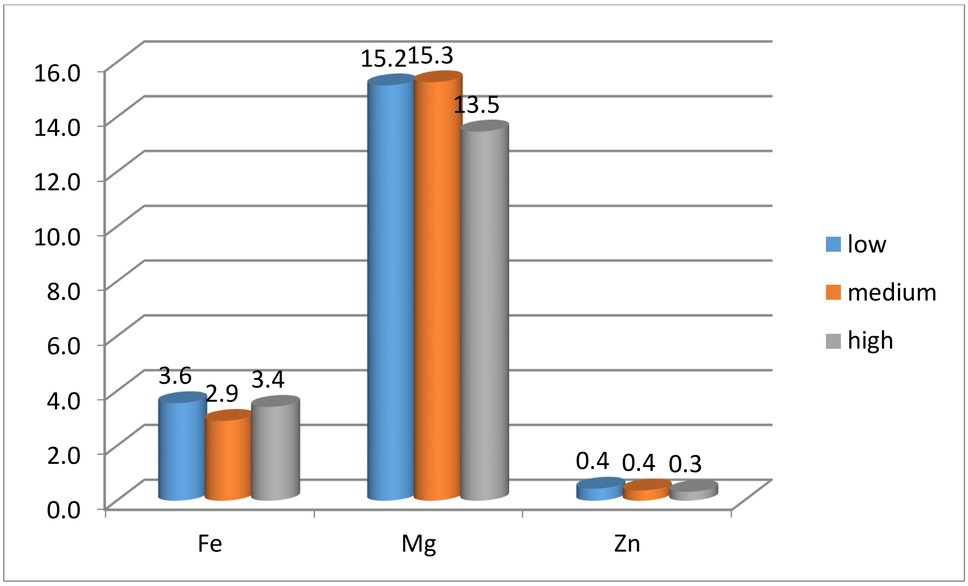

Figure 7: Mean of Fe^{2+}, (p-value=0.50), Mg^{2+} (p-value =0.40) & Zn^{2+} (p-value=0.20) with economic status, revealed insignificant difference in compare with normal p-value (0.05).

5.1 Discussion

The present study was carried out to investigate the trace element (magnesium, iron and zinc) among Sudanese people of cigarette smokers in Bahry and Alkalakla cities, in Khartoum state in the Sudan during period from March to June 2018; 30 blood samples were collected from Sudanese male smokers.

The present study showed that high concentration of Fe^{2+} with mean (3.1mg/dl) compared with normal range (0.5-1.5mg/dl). The serum level of Mg^{2+} is low mean (15 mg/L) which was low concentration compared with normal range (17-28mg/L) and also result of Zn^{2+} showed low concentration with mean (0.4 mg/L) compared with normal range 0.5-1.2mg/L. This agreed with (Sulafa Ali and Samia Mahdi et al, 2013) whom were reported statistically significant changes in the serum levels of Mg^{2+} and Fe^{2+} between test and control group, the level of Mg^{2+} was high and was Fe^{2+} low in smokers compared to nonsmokers.

The findings of this study also prevailed a non-significant difference between the serum levels of Mg^{2+}, Fe^{2+} and Zn^{2+} of the test group according to the duration (P-value =0.4/0.6/0.3), and to the age (P-value =0.9/0.7/0.6) respectively. The number of cigarettes smoked per day have no effect on the level of serum Mg^{2+}, Fe^{2+} and Zn^{2+} (P-value =0.4/0.8/0.6), this agreed with (Sulafa Ali and Samia Mahdi et al, 2013) whom were reported that there was statistically no significant influence of age, duration and number of cigarette per day on Mg^{2+}, Fe^{2+} levels, when compared with serum Mg^{2+}, Fe^{2+} with age, duration and number of cigarette per day with study group.

The results of the recent study presented non-significant difference between the serum levels of, Mg^{2+}, Fe^{2+} and Zn^{2+} of the test group according to the job (P-value =0.5/0.4/0.8) respectively, and non-significance difference to the education (P 0.5/0.7/0.3) subsequently, also to social status (p 0.4/0.1) in which Zn^{2+} has significant difference with (p 0.02) and showed non-significant difference between the serum level of Mg^{2+}, Fe^{2+} and Zn^{2+} according to economic status (p 0.4/0.5/0.2) respectively.

5.2 Conclusion

From this study the following can be concluded:

The serum level of magnesium iron and zinc was affected by the smoking, the serum level of magnesium and zinc are decrease and iron increased in smoking. The age, duration of the smoking, number of cigarette, social status, economic status, job and education of smokers have no effect on the serum level of magnesium, iron and zinc.

5.3 Recommendations

It is recommended that:

1. To conduct other researches on magnesium, iron and zinc with larger sample size to known other effect of smoking.
2. To communicate with smokers to make available information's about smoking and its complication.
3. It is strongly recommended to avoid smoking for the benefit of cardiac health. It is important to establish a visible and audible communication aid and through schools and colleges explaining risks of smoking on the cardiovascular system and other system.

6.1 References

1. Shiffman S, Robert S (2007) Fast Facts: Smoking Cessation. Oxford: Health Press Ltd.
2. Nichter ME, Cartwright (1991) Saving the children for the tobacco industry. Med Anthropol Q 5: 236–256.
3. John AK (2007) Disorders of acid-base balance. Crit. Care Med 35: 2630-2636.
4. Provan D (1999) Mechanisms and Management of iron deficiency anaemia. Br J Haematol 105: 19-26.
5. Beard JL (2001) Iron biology in immune function, muscle metabolism and neuronal functioning. J Nutr 131: 568S-579S.
6. Rude RK, Shils ME. Magnesium. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ, eds. Modern Nutrition in Health and Disease.10th ed. Baltimore: Lippincott Williams Wilkins; 2006:223-247.
7. El -Zayadi AR (2006) Heavy smoking and liver. World J Gastroenterol 12: 6098-6101.
8. Balcerzek SP, Bromberg PA (1975) Secondary polycythemia. Semin. Hematol 12: 339-351.
9. Bacon BR, Britton RS (1990) The pathology of hepatic iron overload: A free radical-mediated process? Hepatology 11: 127-137.
10. Emrit J, Beaumont C, Trivin F (2001) Iron metabolism, free radicals, and oxidative injury. Biomed. Pharmacother 55: 333-539.
11. Winiarczyk AU, Bagniuk A, Lalkowska KG, Szubartowska E (2008) Calcium, Magnesium, Iron, Zinc and Copper Concentration In the Hair of Tobacco Smokers. Biol Trace Elem Res 128: 152-160.
12. John AK. Disorders of acid base balance. Crit. Care Med.2007; 35:2630-2636.
13. L. H.A.Al-Azzawy. The Impact of Cigarette Smoking on Levels of Sex Hormones and Zinc in Blood of Smokers14, December, 2010؛IBN AL-HAITHAM J. FOR PURE & APPL. SCI; VOL.24 (2) 2011.page 2.

14. Centers for Disease Control and Prevention (CDC) (2002). Annual smoking-attributable mortality, years of potential life lost, and economic costs--United States, 1995-1999. MMWR Morb. Mortal. Wkly. Rep.51 (14): 300–3. PMID 12002168.
15. Law MR, Morris JK, Wald NJ; Morris; Wald (1997). "Environmental tobacco smoke exposure and ischaemic heart disease: an evaluation of the evidence". BMJ. 315(7114): 973–80.
16. Berlinger (May 5, 2016). "California raises smoking age to 21". CNN. Retrieved May 5, 2016.
17. CNN Health (Dec 1999). "Testosterone The good and the bad". CNN.
18. Timon Screech, "Tobacco in Edo Period Japan" in Smoke, pp. 92-99.
19. Dietary Supplement Fact Sheet: Magnesium". Office of Dietary Supplements, US National Institutes of Health. 11 February 2016. Retrieved13 October 2016.
20. Magnesium in diet". Medline Plus, U.S. National Library of Medicine, National Institutes of Health. 2 February 2016. Retrieved 13 October 2016.
21. Magnesium | University of Maryland Medical Center". Umm.edu. 7 May 2013. Retrieved 19 September 2013.
22. Wester PO (1987). "Magnesium". Am. J. Clin. Nutr. 45 (5 Suppl): 1305-12.PMID 3578120.
23. Geiger H; Wanner C (2012). "Magnesium in disease" (PDF). Clin Kidney J. 5 (Suppl1): i25–i38. doi:10.1093/ndt plus/sfr165.
24. Meneghini R. Iron homeostasis, oxidative stress, and DNA damage. Free Radic Biol Med 1997;23:783–792.
25. Rossi E. Hepcidin: The iron regulatory hormone. Clin Biochem Rev 2005:26:47–49.
26. Higgins T, Beutler E, Doumas BT. Hemoglobin, iron, and bilirubin. In Tietz textbook of clinical chemistry and molecular diagnostics. 4th ed. St. Louis, Mo.: Elsevier, 1187–1188.

27.Beard J, Dawson B, Pinero D. Iron metabolism: A comprehensive review. Nutr Rev 1996;54:295–317.

28.Smith MA, Perry G. Free radical damage, iron, and Alzheimer's disease. J NeurolSci 1995;134S:92–94.

29.Herbert V, Shaw S, Jayatilleke E. Vitamin C-driven free radical generation from iron. J Nutr 1996;126;1213S–1220S

30.Herbert V, Shaw S, Jayatilleke E. Vitamin C-driven free radical generation from iron. J Nutr 1996;126;1213S–1220S.

31.Tofaletti JG. Trace elements. In Bishop ML, Fody EP, Schoeff LE, eds. Clinical chemistry: Principles, procedures, correlations. 5th ed. Philadelphia, Pa.: Lippincott, Williams & Wilkins, 2004.

32.Hambridge KM, Casey CE, Krebs NF. Zinc. In Mertz W, ed. Trace elements in human and animal nutrition (Vol. 2). 5th ed. St. Louis, Mo.: Academic Press, 1986:1–137.

33.Kaplan LA, Pesce AJ.Examination of urine. In: Kaplan LA, Pesce AJ, eds. Clinical chemistry: theory, analysis, and correlation. 4th ed. St. Louis, Mo.: CV Mosby, 2003:1092–1109.

34.DuFour DR. Professional practice in clinical chemistry: a companion text, water and electrolyte balance. Washington, D.C.: AACC, 1999.

35.Russell PT, Sherwin JE, Obernolte R, et al.Nonprotein nitrogenous compounds. In: Kaplan LA, Pesce AJ, eds. Clinical chemistry: theory, analysis, and correlation. 2nd ed. St. Louis, Mo.: CV Mosby, 1989:1005.

36.Kleinman LI, Lorenz JM. Physiology and pathophysiology of body water and electrolytes. In: Kaplan LA, Pesce AJ, eds. Clinical chemistry: theory, analysis, and correlation. 4th ed. St. Louis, Mo.: CV Mosby, 2003:441–461

6.2 Appendix

Shendi University

Faculty of Graduate Studies and Scientific Research

Research questionnaire

Effect of cigarette Smoking on trace element (magnesium iron & zinc) in Khartoum State.

The patient's questionnaire includes the following sections:

Section 0 – Questionnaire identification data (6) codes
Section 1 – Background characteristics (5) Questions

0 QUESTIONNAIRE IDENTIFICATION DATA

001 QUESTIONNAIRE IDENTIFICATION NUMBER |___|___|___|

002 CITY--------------------------------

002NAME ---------------
003 DATE OF INTERVIEW: __\ ____ \ _____
004 CHECKED BY SUPERVISOR: Signature ___________________ Date ____________

Section 1: Background characteristics

No.	Questions and filters	Coding categories
Q101	Duration of smoking	□1-15year □16-25 year
Q102	Age	□ up to 20-30 year □31_45 year
Q103	Job	□doctor □teacher □merchant □police □driver □student □ no job □other
Q104	Social status	□married □ married
Q105	Economic status	□low □medium □high
Q106	Education	□primary □secondary □graduate □undergraduate
Q103	Number of cigarette per day	□ up to 1_15 r □16_25 □No Response
Q104	History of disease	□ Yes □ No

If no escape the following questions; but if yes, go to the following questions:

Q105	Other disease	□ Liver disease □GIT disease Thyroid disease □Respiratory disease other

If there is positive history of any of the above mentioned diseases we excluded this patient from our study.

Blood test result:

Iron Level: ……mg/L Magnesium Level: ………mg/L. zinc level………mg\dl