# Serum Concentrations of Magnesium Among Jordanians: Effect of Magnesium Level on Cardiovascular Diseases

**By**

**Sally Ziad Oumeish**

**Supervisor:** Prof. Tawfiq Arafat

**Co-supervisor:** Dr. Eyad Mallah

**A Thesis submitted in partial fulfilment of the requirements for the degree of**

**Master of Science**

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# ABSTRACT

Magnesium plays a role in a numerous enzymatic reactions, as such, it fulfils various intracellular physiological functions. Therefore, irregularity in magnesium status-mainly hypomagnesaemia as it is seen more often than hypermagnesaemia-might result in unwanted neuromuscular, cardiac or nervous disorders. Measuring total serum magnesium is a practical and affordable way to monitor changes in magnesium status, although it does not necessarily reflect total body magnesium content.

To date, we couldn’t find group has evaluated magnesium as a cardiovascular risk factor in Jordanian population. Thus, a simple colorimetric method (Xylidyl blue method), was used to determine the magnesium serum levels in a population of healthy students from university of Petra and cardiac patients from Jordan Hospital.

The blood sampling was conducted between September and November, 2014 .The experiment population was (151) subject .

There was no significant difference found between healthy and patient subjects (P value > 0.05), with Mg serum levels of healthy subjects showed low elevation compared to patient Mg serum levels (Cohen's d= 0.1007). Our findings indicate that we couldn’t prove a correlation between Mg serum levels and cardiovascular diseases among Jordanian . Similarly, no effect for gender or smoking magnesium.

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# Abbreviations

| | |
|---|---|
| AAS | Atomic absorption spectrophotometry |
| ATP | Adenosine triphosphate |
| ADP | adenosine diphosphate |
| °C | Celcius |
| Ca | Calcium |
| CAD | Coronary artery disease |
| CHF | congestive heart failure |
| CVD | Cardiovascular disease |
| DMSO | Dimethylsulfoxide |
| DNA | Deoxyribonucleic acid |
| DV | Daily Values |
| EDTA | Ethylenediaminetetraacetic acid |
| EGTA | Ethylene glycol tetraacetic acid |
| G | Gram |
| G/dl | Grame per dici liter |
| IHD | Ischemic heart disease |
| IOM | Inistitute of medicine |
| I.V. | Intravenous |
| K | Potassium |
| L | Liter |
| Mg | Magnesium |
| Mg/dl | Milligram per deci liter |
| Mmol | Milli mol |
| Mmol/L | Milli mol per liter |
| mN | Millinormality |
| mN/L | Millinormality per liter |
| Na | Sodium |
| Pg | Picogram |
| QT | Q wave to T wave |
| RBC | Red blood cells |
| RDA | Recommended dietary allowances |
| Rpm | Round per minute |
| SD | Standard deviation |
| ST | S wave to T wave |
| TALH | Thick ascending loop of henle |
| U/ml | Micro per milli liter |
| USDA | United states of daily allowences |
| W/V | Weight per volume |

# Chapter One
# Introduction

# Chapter one

## 1.1 Electrolytes

Electrolytes are minerals that can be found in blood and other body fluids which are known to have a role in carrying electrical charge, affecting body acid-base balance, muscle functions, metabolism, cellular functions including enzyme activities and electrical gradients and other essential processes (Lobo, 2004; Buckley *et al.*, 2010; Kaplan an Kellum, 2010 and Johnson, 2012).

The pool of electrolytes in human body is mainly consisted of sodium ($Na^{+}$),potassium ($K^{+}$), calcium ($Ca^{2+}$) and magnesium ($Mg^{2+}$) (Kraft *et al*., 2005; Buckley et al., 2010).

Electrolytes irregularities can cause major complications, the severity of these complications correlates with the scale of the disorder and the time frame in which the disorder occurred. (Kraft *et al*., 2005; Buckley *et al*., 2010 ; Kaplan & Kellum, 2010 and Johnson, 2012).

### 1.1.1 Sodium

Knowing that sodium is the most abundant extracellular electrolyte, it plays an essential role in serum osmolality regulation, and therefore water flow between body compartments as water moves from compartment of lower osmolality to compartments of higher osmolality until homeostasis is achieved (Adrogue & Madias, 2000; Buckley *et al.*, 2010).

Normal serum sodium concentration lies between 133 and 145 mM, this concentration determines the number of cations needed in intravascular space to achieve homeostasis with the interstitial and intracellular spaces. Another measure to total amount of sodium which is a major determinant of water balance ,therefore imbalances in sodium are best evaluated by serum sodium, followed by serum osmolality and then volume status(Kraft *et al.*, 2005; Sahay & Sahay, 2014)

### 1.1.2 Potassium

Potassium is the most abundant intracellular electrolyte in the body , 98% of body`s potassium is found in the intracellular space , while only 2% is found in the extracellular space. The sodium-potassium-adenosinetriphosphatase (Na-K-ATPase) pump is responsible for potassium entry into cells The normal concentration of potassium in serum is 3.5-5mN/L . Potassium is utilized for essential functions including regulation of action potentials across the membranes of excitable tissue, cellular metabolism, and glycogen and protein synthesis (Halperin & Kamel, 1998; Lobo, 2004).

The major route of potassium excretion is kidney. Many factors can alter potassium concentrations, including acid-base balance, kidney and organ dysfunction, trauma and malnutrition, furthermore, alternations in potassium level can cause severe cardiac abnormalities, therefore, close monitoring is required for critically ill patients (Buckley *et al.*, 2010; Wang *et al*., 2013).

### 1.1.3 Calcium

Calcium is one of the major cations in human body, it plays a major role in many bodily functions including bone metabolism, neuro-muscular conduction, action potentials in excitable tissues, coagulation, endocrine and exocrine glandular functions. Ninety nine percent of body calcium resides in bone while only 1% exists in the extracellular fluid. (Bushinsky & Monk, 1998; Kaplan & Kellum, 2010; Moon *et al.*, 2011; Johnson, 2012).

Because of this relation between albumin levels and total extracellular calcium, calcium level will decrease by 0.8mg/dL for every 1g/dL decrease in serum albumin concentrations below 4g/dL ,therefore corrected calcium concentrated should be calculated in order to assess calcium levels in patients with hypoalbuminemia using the following equation :

Corrected Calcium = (0.8 * (4 – albumin)) + serum calcium

Because the free ionized serum calcium represents the biologically active form of calcium, it is a more reliable indicator of calcium functional state .Normal serum concentrations of free active calcium lies between 1.12 and 1.3mmol/L. Furthermore, free active calcium levels poorly correlates with total serum calcium concentrations , therefore, free active calcium must be measured in critically ill patients in order to prevent serious complications of calcium irregularities including cardiac and blood pressure instabilities as these patients often present with acid-base imbalance and

hypoalbuminemia which will affect protein binding (Bushinsky & Monk, 1998;Lobo, 2004; Moon *et al*., 2011 and Wang *et al*., 2013).

### 1.1.4 Magnesium

Magnesium is the second most abundant intracellular cation after potassium and it is the fourth most abundant cation in human body. Normal magnesium levels lie between 1.5 and 2.4 mg/dL , with no correlation with weight (Wester, 1987 ; Rude, 1998; Ford & Mokdad, 2003 and Jahnen-Dechent & Ketteler, 2012). Thirty percent of magnesium in serum is bound to protein while 70% exists in the free form and therefore, can be excreted by kidney (Ford & Mokdad, 2003; Jahnen-Dechent & Ketteler, 2012).

Having two major unique characteristics; the ability to form chelates with major intracellular anions including ATP and the ability to compete with calcium on its binding sites, magnesium plays a major role in many physiological reactions (Table1). (Wester, 1987; Swaminathan, 2003; Jahnen-Dechent & Ketteler, 2012).

Furthermore, magnesium plays an important role in nucleic acids and proteins synthesis, and as a facilitator of more than 300 enzymes functions (Pasternak *et al.*, 2010; Jahnen-Dechent & Ketteler, 2012).

Magnesium influences enzymes activity by Ligand binding , Cofactor for the active site if enzyme, Causing conformational change during catalytic processes.

## 1.2 Distribution of Mg in the human body

Table (1) Magnesium functions in the body

| | |
|---|---|
| **Enzyme function** | As Enzyme substrate<br>Direct enzyme activation |
| **Membrane function** | Cell adhesion<br>Transmembrane electrolyte flux |
| **Calcium antagonist** | Muscle contraction/relaxation<br>Neurotransmission release<br>Action potential conduction |
| **Structural function** | Proteins<br>Polyribosomes<br>Nucleic acids<br>Multiple enzyme complexes<br>Mitochondria |

The total magnesium content of human body is ~20mmol/kg of fat free tissue. Therefore, total magnesium in an average 70 kg adult with 20%(w/w) fat in ~1000 to 1120 mmol (Ford & Mokdad, 2003; Swaminathan, 2003).

Ninety nine percent of body magnesium exists in bone, muscles and non-muscular soft tissue. Furthermore, 50-60% of magnesium resides as surface substituent of hydroxyapatite in bones (Swaminathan, 2003; Pasternak *et al.*, 2010).With aging, magnesium content in bones decreases making it less bioavailable for body in cases of magnesium deprivation. Nevertheless, bone still provides a huge exchangeable pool to buffer acute alternations in magnesium levels in serum because one third of skeletal magnesium is exchangeable (Pasternak *et al.*, 2010; Geiger & Wanner, 2012; Jahnen-Dechent & Ketteler, 2012).

Normal intracellular magnesium concentrations ranges from 5-20mmol/L. This amount of magnesium is further divided into free ionized form (1-5%) and bound form (95-99%) (Geiger & Wanner, 2012; Kupetsky-Rincon & Uitto, 2012).

One percent of total body magnesium exists in the extracellular space, specifically in RBCs and serum. Extracellular magnesium is divided into 3 main categories : Free/ionized form , protein bound form and anion bound form . Of these three forms, ionized magnesium has the greatest biological activity (Pasternak *et al*., 2010; Blaszczyk & Duda-Chodak, 2013).

## 1.3 Magnesium biological activity

Being a major intracellular cation, magnesium plays a major role in intracellular functions; it acts as a counter ion for energy rich compounds and nucleic acids (Elin, 1988; Jahnen-Dechent & Ketteler, 2012).Furthermore, magnesium is a critical stabilizer of many enzymes including ATP-generating enzymes (Saris *et al.*, 2000; Swaminathan, 2003).ATP is a major intracellular energy source and is generally required in many essential functions inside cells, including glucose metabolism, fat , proteins , nucleic acid and coenzyme synthesis , muscle contraction and many other processes. Knowing that , magnesium is an essential factor in these cellular functions. Furthermore, magnesium contributes to regulation of vascular tone , heart rhythm, platelets function, cell proliferation , cell adhesion , transmembrane transportation of ions including sodium and potassium and bone formation. It is also essential for structural proteins and mitochondrial functions (Wester, 1987;Aikawa, 1981 and Jahnen-Dechent & Ketteler, 2012).

Magnesium is considered to be a natural calcium antagonist owing to two facts; it was approved that magnesium and calcium competes with one another for the same binding site , also , magnesium inhibits calcium induced programmed cell death acting as an anti-apoptotic molecule antagonizing calcium-overload-triggered apoptosis (Eilat-Adar *et al*., 2013; Nicklas *et al*., 2014).

Inside nucleus, about 50% of magnesium is closely associated with nucleic acids and free nucleotides hence magnesium can neutralize negatively charged phosphate groups in these molecules as a cation (Wester, 1987; Pasternak *et al.*, 2010).

Owing to its capability to interact directly with proteins and therefore its ability to modulate histone phosphorylation , magnesium ions can affect cell cycle in the form of Mg-ATP (Pasternak *et al*., 2010).

Furthermore, magnesium is involved in essential processes by activating enzymes important for DNA repair (endonuclease), replication (topoisomerase II) , transcription , and it plays an essential role in maintaining the integrality of double stranded DNA molecules (Pasternak *et al*., 2010).

## 1.4 Magnesium intake and sources in diet

The recommended magnesium intake is 4.5mg/Kg/day, which is lower than previously recommended dose of 6-10mg/Kg/day , this recommended value increases in pregnant females (table 3) (Swaminathan, 2003) .

Table (2) Recommended Dietary Allowances (RDAs) for Magnesium *

| Age | Male | Female | Pregnancy | Lactation |
|---|---|---|---|---|
| Birth to 6 months | 30 mg* | 30 mg* | | |
| 7–12 months | 75 mg* | 75 mg* | | |
| 1–3 years | 80 mg | 80 mg | | |
| 4–8 years | 130 mg | 130 mg | | |
| 9–13 years | 240 mg | 240 mg | | |
| 14–18 years | 410 mg | 360 mg | 400 mg | 360 mg |
| 19–30 years | 400 mg | 310 mg | 350 mg | 310 mg |
| 31–50 years | 420 mg | 320 mg | 360 mg | 320 mg |
| 51+ years | 420 mg | 320 mg | | |

* Institute of Medicine (IOM). Food and Nutrition Board. Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D and Fluorideexternal. Washington, DC: National Academy Press, 1997

Magnesium sources in diet are mainly water , specifically "hard water" and green leafy vegetables, cereal, grain, nuts, cocoa, almonds , whole seeds.(table 4), (Swaminathan, 2003 and Alkurd, 2011)

Hard water provides a good source of magnesium hence it can provide up to 100 mg of magnesium daily. Furthermore, green leafy vegetables provide a good source of magnesium hence it is rich in chlorophyll (Alkurd ,2011 ; Eilat-Adar *et al.,* 2013 and Nicklas *et al.,* 2014) .

Magnesium content in food is largely dependent on its form. Unprocessed food contains much higher values of magnesium compared to processed food hence processing of food depletes magnesium content in foodstuff by 85% (Wester, 1987 and Swaminathan, 2003) .

Furthermore , magnesium concentration may be low in foodstuff where essential nutrients are depleted from soil. It is also known that new agricultural and food production technique often lead to reduced menirals content in plants (Rude, 1998 and Swaminathan, 2003).

However, recent studies show that the average magnesium intake in western countries is lower than the recommended dose allowance (Swaminathan, 2003).

Table (3): Selected Food Sources of Magnesium*

| Food | Milligrams (mg) | |
|---|---|---|
| | Preserving | Percent of DV |
| Almonds, dry roasted, 1 ounce | 80 | 20 |
| Spinach, boiled, ½ cup | 78 | 20 |
| Cashews, dry roasted, 1 ounce | 74 | 19 |
| Peanuts, oil roasted, ¼ cup | 63 | 16 |
| Cereal, shredded wheat, 2 large biscuits | 61 | 15 |
| Soymilk, plain or vanilla, 1 cup | 61 | 15 |
| Black beans, cooked, ½ cup | 60 | 15 |
| Edamame, shelled, cooked, ½ cup | 50 | 13 |
| Peanut butter, smooth, 2 tablespoons | 49 | 12 |
| Bread, whole wheat, 2 slices | 46 | 12 |

*U.S. Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 25. 2012

## 1.5 Magnesium absorption and metabolism

Generally, magnesium intake is directly related to energy intake, a well known exception is when the majority of energy comes from refined sugars or alcohol.

(Rude, 1998; Jahnen-Dechent & Ketteler, 2012; Altura *et al.*, 2013).

The exact mechanism involved in magnesium homeostasis is not well understood despite narrow maintenance of its level in serum. The average magnesium intake in normal adult is ~12 mmol/day. Additionally, 2 mmol/day of magnesium is secreted into intestine in bile, pancreatic and intestinal juice. Thirty percent of this intestinal pool is absorbed giving a net absorbtion of 4 mmol/day (Swaminathan, 2003; Pasternak *et al*., 2010), as seen in the Fig 1.1 Distribution of Magnesium in the body (Source:(Swaminathan, 2003)).

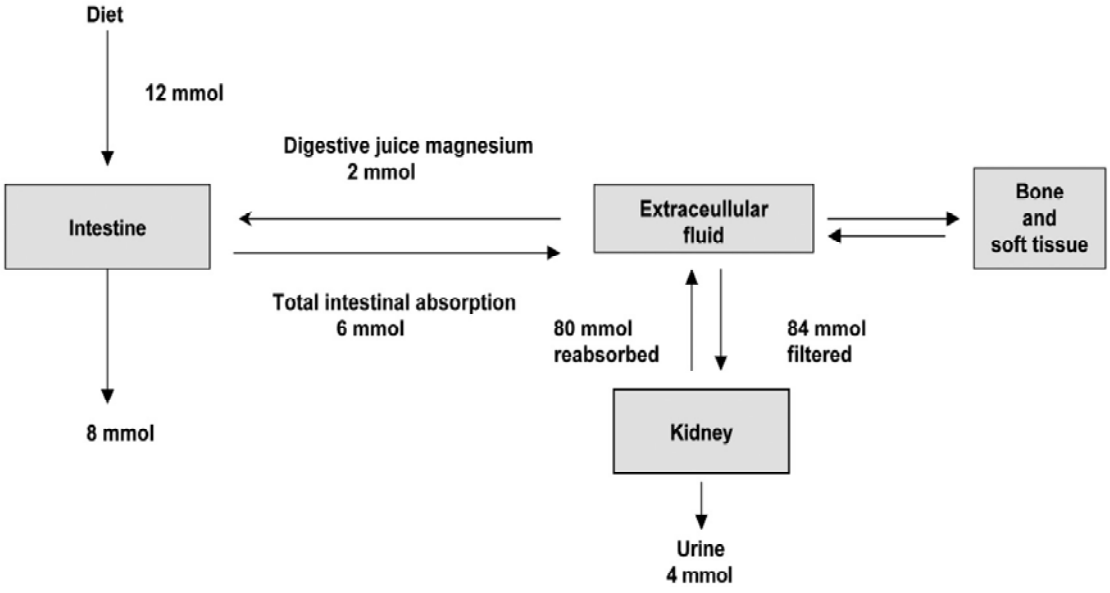


Fig 1.1 Distribution of Magnesium in the body

Being the major route of excretion , kidney plays a major role in magnesium homeostasis. Normally, when 80% of total plasma magnesium is ultrafiltrable, 84 mmol of magnesium is filtered daily, of this amount 95% is reabsorbed, of them 15-20% is reabsorbed in the proximal tubules, 65-70% in thick ascending loop of Henle and the rest in distal tubules (Swaminathan, 2003; Pasternak *et al.*, 2010; Jahnen-Dechent & Ketteler, 2012). In proximal tubules , magnesium is reabsorbed passively, this process mainly depends on sodium/water reabsorbtion and on luminal magnesium concentration .In thick ascending loop of Henle , magnesium reabsorbtion depends on sodium/chloride reabsorbtion and the positive luminal charge created by active cellular pumps and passive paracellular diffusion . On the other side, magnesium reabsorbtion is active and transcellular in the distal tubules . Magnesium reabsorbtion is inversely related to the rate of fluid flow in the tubules (Wester, 1987; Rude, 1998; Pasternak *et al*., 2010; Jahnen-Dechent & Ketteler, 2012).

## 1.6 Hypomagnesaemia

Magnesium deficiency , also known as hypomagnesemia is an electrolyte disorder that is frequently undetected owing to the fact that hypomagnesemia is often asymptomatic or a secondary disorder . Therefore, recent studies shows that hypomagnesemia is present more than previously thought (Swaminathan, 2003; Shah *et al*., 2014).In general, clinical manifestations of magnesium deficiency are usually not seen until serum magnesium concentration decreases to 0.5 mmol/L or lower, Table(5) shows a list of magnesium deficiency clinical manifistations (Rude, 1998; Jahnen-Dechent & Ketteler, 2012; Zittermann, 2013).

Table (4) Clinical Features of Hypomagnesaemia and magnesium deficiency

| | |
|---|---|
| Electrolyte disturbance | Hypokalemia<br>Hypocalcaemia |
| Neuromuscular and central nervous system | Carpopedal spasm<br>Convulsations Muscle cramps<br>Muscle weakness, fasciculations, tremors<br>Vertigo<br>Nystagmus<br>Depression, psychosis<br>Athetoid movements &choreform movements |
| Cardiovascular | Atrial tachycardias, fibrillation<br>Supraventricular arrhythmias<br>Ventricular arrhythmias<br>Torsade de pointes<br>Digoxin sensitivity |
| Complications of magnesium deficiency | Altered glucose homeostasis<br>Atherosclerotic vascular disease<br>Hypertension<br>Myocardial infarction<br>Osteoporosis |
| Miscellaneous | Migraine<br>Asthma<br>Chronic fatigue syndrome<br>Impaired athletic performance |

Hypomagnesemia can be caused by variety of mechanisms including redistribution, reduced intake or intestinal absorbtion , increased losses by intestinal and urinary tracts. Furthermore, an important cause of hypomagnesemia in clinical practice is drug use. A number of commonly used drugs can cause magnesium deficiency, including

antibioticas, chemotherapeutic agents and diuretics. Specifically, loop diuretics inhibit magnesium reabsorbtion in the thick ascending loop of Henle causing magnesium depletion, this complication of loop diuretics use is especially common in prolonged use of this type of diuretics. On the other hand, short-term use of thiazide diuretics which act on the distal convoluted tubules, where less than 5% of magnesium is reabsorbed, does not cause magnesium depletion (Feillet-Coudray *et al.,* 2002; Jahnen-Dechent & Ketteler, 2012; Blaszczyk & Duda-Chodak, 2013).

Hypomagnesemia is an uncommon electrolyte disturbance in healthy persons. Yet, it is seen in patients maintained on intravenous fluids or total parenteral nutrition, especially if these patients already have a marginal magnesium level to start off with (Ford & Mokdad, 2003; Blaszczyk & Duda-Chodak, 2013; Zittermann, 2013), Table (6) shows list of hypomagnesaemia causes (Rude, 1998; Swaminathan, 2003; Jahnen-Dechent & Ketteler, 2012).

Table (5). Causes of hypomagnesaemia

| | |
|---|---|
| Redistribution of magnesium | Refeeding and insulin therapy<br>Hungry bone syndrome<br>Correction of acidosis<br>Catecholamine excess<br>Massive blood transfusion |
| Gastrointestinal causes | Reduced intake<br>Mg free intravenous fluids<br>Dietary deficiency<br>Reduced absorption<br>Malabsorption syndrome<br>Chronic diarrhea<br>Intestinal resection |
| Renal loss | Reduced sodium reabsorption<br>Saline infusion<br>Diuretics |
| Renal disease | Post obstructive nephropathy<br>Post renal transplantation<br>Dialysis<br>Diuretic phase of acute renal failure<br>Inherited disorders |
| Endocrine causes | Hypercalcaemia<br>Primary hyperparathyroidism<br>Malignant hypercalcaemia<br>Hyperthyroidism<br>Hyperaldosteronism |
| Diabetes mellitus | |
| Alcoholism | |
| Drugs | Diuretics<br>Cytotoxic drugs<br>Antimicrobial agents<br>Beta adrenergic agonists |

## 1.7 Hypermagnesemia

Regularly, excess of magnesium will not cause a health risk in healthy individuals as the excessive amount will be eliminated by kidneys, and therefore, patients with impaired renal functions increases the risk of magnesium toxicity. However, high doses of magnesium from drugs and dietary products may cause clinical manifestations, most commonly diarrhea associated with nausea and abdominal cramps. Well known forms of magnesium that commonly cause diarrhea include magnesium carbonate, chloride, gluconate and oxide. The mechanism by which diarrhea occurs is the osmotic activity of unabsorbed salts in the intestinal lumen (Purvis & Movahed, 1992; Swaminathan, 2003 and Jahnen-Dechent & Ketteler, 2012).

Magnesium toxicity can be caused by large doses of magnesium-containing laxatives and antacids, typically providing more than 5,000 mg/day magnesium. Clinical manifestations of magnesium toxicity are usually seen when serum magnesium concentrations exceeds 1.74-2.61 mmol/L and include hypotension, nausea, vomiting, facial flushing, urinary retention, ileus, depression, and lethargy. Further more, these clinical manifestations may progress to more serious ones including muscle weakness, difficult breathing, extreme hypotension, arrhythmias and cardiac arrest (Wester, 1987; Geiger & Wanner, 2012; Jahnen-Dechent & Ketteler, 2012).

## 1.8 Determination of magnesium ion in serum

Traditionally, magnesium concentration in serum can be analysed using three main methods; colorimetric, potentiometric and atomic-absorption spectrometry (AAS) methods(Touyz, 2004).

### 1.8.1 Atomic-Absorption Spectrometry Method

ASS method is the most commonly used technique for measuring total magnesium. The sensitivity, defined as the concentration required for 1 per cent absorbance, is about 0.01 µ /ml for flame AAS at the 285 nm resonance line, while electrothermal (graphite furnace) AAS(ET-AAS) has a sensitivity of 0.17 pg(Uemoto, 2011). The sensitivity of magnesium measurement by flame atomic absorption is sufficiently high that the graphite furnace instrument is infrequently required for biological determinations (Vahl *et al.*, 2010; Uemoto, 2011).

A large number of interferences with this method exists with the worst interferences being observed with metals that form stable acid oxides at high temperature, including lithium, sodium, potassium, rubidium, chromium, selenium, beryllium, iron, vanadium, molybdenum, caesium, strontium, calcium and barium (Millart *et al*., 1995 and Vahl *et al*., 2010). Despite the large number of existing interferences, most are easily overcome. Sodium, potassium, calcium, phosphate and iron interferences can be prevented easily using air-acetylene flame. Furthermore, the presence of 0.1-1% (w/v) lanthanum chloride or strontium chloride eliminates the remaining interferences, a well known exception are interferences caused by chromium and titanium (Millart *et al*., 1995 and Vahl *et al.*, 2010).

### 1.8.2 Potentiometric method

The potentiometric determination of Mg is performed by complexometric titration using EDTA as titrant and murexide or Eriochrome black T as indicator. This method is affected by several other metal ions. The solution is stirred magnetically, until colour changes from pink to pure blue (Vahl *et al.*, 2010).

### 1.8.3 Colorimetric methods

There are three common colorimetric methods that are used in magnesium determination including Calmagite, Formazan dye and Xylidyl blue methods (Millart *et al*., 1995; Andrusishina, 2010). The Calmagite method, as its name implies, makes use of calmagite dissolved in water along with other chemicals dissolved together with it. Magnesium reaction with Calmagite produces a red-violet color that can be read at 532nm using a colorimeter. One restriction of this method is that the colored chelate is stable for only 30 minutes, therefore colored solution must be read immediately. The magnesium standard is made by dissolving 44.61g of magnesium iodate tetrahydrate in 1L of water. Well known interferences with this method can be prevented by adding chemicals to preparation including ethelyne glycol tetraacetic acid to prevent calcium interference caused by calcium binding to calmagite. Furthermore, cyanide is also added to preparation in order to prevent other metals interferences (Sharma *et al.*, 2007; Andrusishina, 2010).

Another method is the Formazan dye which is a dry-slide method which uses a multi-layered reagent that is magnesium sensitive. It is also imprinted with calcium chelators in order to prevent erroneous results. Magnesium, from the patient's sample, is

dropped on the slide and is evenly distributed to each layer. A reaction between magnesium and the dye in each layer creates a colour complex . The amount of colored complex formed is directly proportional to the magnesium present. It is now then measured and is read at 600nm (Elin, 1991; Millart *et al*., 1995).

Another regularly used method for serum levels, is the Xylidyl blue method. A color producing reaction occurs between magnesium and Xylidyl blue in an alkaline solution. The intensity of the colour is proportional tothe amount of magnesium present in the sample. By complexion with EGTA, calcium interference with this method is prevented (Samaie *et al*., 2012 and Kundu *et al*., 2013).

## 1.9 Therapeutic uses of Magnesium

For a very long time, magnesium has been used as a cathartic agent. Furthermore, the relationship between magnesium and cardiovascular diseases had been well studied, and will be extensively discussed below. Generally, magnesium has a well established role in treatment of some types of arrhythmias, specifically torsade de pointes (long QT syndrome), and thus it is used in the treatment of this cardiac disorder. However, magnesium role in other arrhythmias is not clear. Nevertheless, magnesium therapy is still considered in patients with refractory arrhythmias. Furthermore, magnesium is used in cardiac surgery for perioperative arrhythmias prophylaxis although the effectiveness of magnesium here is still unproved (Purvis & Movahed, 1992; Swaminathan, 2003; Geiger & Wanner, 2012).

With a strong evidence supporting its role in pre-eclampsia and eclampsia treatment, magnesium is used in these pregnancy disorders. Despite having no evidence to

approve the role of magnesium therapy in asthma treatment, magnesium has been used for years as a therapeutic agent for this respiratory disease (Swaminathan, 2003; Blaszczyk & Duda-Chodak, 2013; Qu *et al.*, 2013).

## 1.10 Magnesium and the metabolic syndrome

Metabolic syndrome is an increasing problem in both developed and developing countries and thus, is considered as a disease of modern times. This disorder is characterized by the simultaneous presence of several metabolic risk factors including obesity, hypertension and impaired glucose tolerance. Furthermore, dyslipidemias, prothrombotic state and active acute phase reactants (elevated c-reactive protein) may also contribute to this disorder. In 2002, it was estimated that one quarter of American adults is affected by this disorder. To dissect the factors responsible for each single condition, the various diseases underlying the metabolic syndrome will be discussed separately in detail (Swaminathan, 2003; Geiger & Wanner, 2012; Das, 2014 and Dibaba *et al*., 2014).

## 1.11. Magnesium and diabetes mellitus

Hypomagnesemia is often related to type II diabetes mellitus. Incidence rates of 13.5-47% have been reported. Many factors contribute to the development of magnesium deficiency in diabetic patients, these factors include hereditary factors, poor dietary intake, autonomic dysfunction, altered insulin metabolism, glomerular hyperfiltration, osmoticdiuresis, recurrent metabolic acidosis, hypophosphataemiaand hypokalaemia (Gonzalez *et al.*, 2013; Liamis *et al.*, 2014).

Furthermore, magnesium deficiency is linked to the development of type II diabetes mellitus, to the severity of the disease and to the deterioration of renal function. Moreover, hypomagnesemia correction using dietary magnesium supplement is approved to improve glucose handling and insulin response in elderly patients with type II diabetes mellitus. Owing to these facts, several investigators have addressed the topic of magnesium status and dietary magnesium intake, especially in diabetes mellitus (Rude, 1998; Geiger & Wanner, 2012; Gonzalez *et al.*, 2013).

## 1.12 .Magnesium and pre-eclampsia/eclampsia

Historically, it was approved that the occurrence of convulsions during pregnancy is associated with poor prognosis as eclampsia which was thought to be a simple convulsive disorder was associated with a 50% maternal mortality rate in early days (Roy *et al*., 2013; Jafrin *et al*., 2014).

After that, specifically during the $19^{th}$ century, an association between eclampsia, albuminuria and hypertension was noted, leading to an earlier diagnosis of the condition during the last century. Furthermore, seizure in eclampsia was distinguished from other types of seizures by the absence of past history of convulsions before pregnancy (Geiger & Wanner, 2012; van Dijk *et al*., 2013).

Pre-eclampsia is defined as a condition with a triad of hypertension, proteinuria, and pathologicaledema. Statistically, pre-eclampsia occurs in about 6-8% of gestations above 20 weeks and is more commonly seen in nulliparous women and is known to regress rapidly postpartum. Pathophysiologically, pre-eclampsia is characterized by

heamoconcentration and vasoconstriction causing increased systemic peripheral resistance and reduction in cardiac output and plasma volume.

The cause behind these changes is the shift in balance of thromboxane/prostacyclin caused by a decrement in prostacyclins synthesis which are potent vasodilators and inhibitors of platelets aggregation. Therefore, the disturbance in prostacylin/thromboxane ratio might end-up favoring vasoconstriction and platelet aggregation causing the pathophysiological changes of the disorder. Additionally, pre-eclampsia is associated with glomerular lesions causing protienuria commonly seen in affected women. Furthermore, circulating angiogenic factors such as growth factor type one receptor, also known as Fms-like tyrosine kinase 1 (sFlt 1) are suggested to be contributors to this disorder development (Roy *et al*., 2013; Jafrin *et al*., 2014).

## 1.13 Magnesium and cardiovascular diseases

More and more studies are conducted to study the effect of magnesium in cardiovascular system showing increasing evidence that magnesium status is important in the pathogenisis, prevention and treatment of cardiovascular disorders. The role of magnesium in cardiovascular system is explained by the essential role of magnesium in activating ATP which is essential for normal cell membrane function and is the energy source for Na-K ATPase pump (Rude, 1998; Geiger & Wanner, 2012; Qu *et al.*, 2013).

Earlier studies conducted on animals showed a relationship between low magnesium intake and high blood pressure (Joffres *et al*., 1987). Furthermore, other studies showed a linkage between low magnesium levels and atherosclerosis. (Joffres *et al*., 1987).

Later studies conducted in humans showed a correlation between low magnesium levels and cardiovascular diseases and deaths, based on data from the National Health and Nutrition Examination Survey Epidemiologic Follow-up Study (NHANES) in the USA Likewise, another study conducted on northern German population showed that hypomagnesemia is a significant independent risk factor of all-cause and cardiovascular mortality after adjustment of other well-known cardiovascular risk factors including hypertension and diabetes (An *et al.*, 2014).

Therefore, $Mg^{+2}$ supplementation can bring about a significant decrease in blood pressure and a stabilization of cardiac arrhythmias and acute myocardial infarction (Chakraborti *et al.*, 2002).

A more recent stratified study that investigated a pool of other studies and included a total of 313,041 individuals in whom 4106 CVD,3215 IHD, and 1528 fatal IHD events were documented for circulating magnesium and 7889 CVD, 4319 IHD, and 1158 fatal IHD events for dietary magnesium, provides the most robust evidence to date of the associations between circulating and dietary magnesium across their usual physiologic ranges and CVD risk. The study showed a significant association between both circulating and dietary magnesium levels and risk of CVD. Specifically, circulating magnesium (per 0.2-mmol/L increment) was associated with a 30% decrement in the risk of CVD, with trends toward a lower risk of IHD and fatal IHD. Additionally, dietary magnesium was shown to be associated with a 22% lower risk of IHD and showed a non-linear association with fatal IHD, with a 27% lower risk up to a threshold of w250 mg/d, compared with lower intakes (Del Gobbo *et al*., 2013).

### 1.13.1 Coronary artery disease

Data from different studies supported magnesium effect on the incidence of fatality in CAD patients. Moreover, studies showed an evidence of a significant correlation between hypomagnesemia and IHD (Ford, 1999; Ueshima, 2005).

Magnesium protective role in CAD is explained by multiple factors, including reducing calcium levels, which is essential for CAD, dilate coronary arteries, reduce the total peripheral resistance, and inhibition of platelet functions (Ford, 1999; Ueshima, 2005; Del Gobbo *et al.*, 2013).

Therefore, an increased intake of dietary magnesium can offer protection against cardiovascular deaths as magnesium may protect cardiac cells from the effects of CAD and improves cardiac cells ability to resist CAD effects (Ueshima, 2005).

### 1.13.2 Hypertension

Several studies have shown an inverse relationship between magnesium levels and blood pressure. However, the exact mechanism behind this association is still unclear (Purvis & Movahed, 1992 and Cunha *et al.*, 2012).

Magnesium has an essential role in the regulation of electrolytes movement across cell membrane because it is significant in stimulating Na/K ATPase pump that accelerates potassium movement into the cell and sodium movement out of the cell. Additionally, as calcium antagonist, magnesium is vital in decreasing calcium entry into cell, thus, magnesium deficiency can lead to increased intracellular sodium and calcium concentrations leading to an increased peripheral vascular resistance and vasospasm,

which have been noticed in experimental animals (Fazekas *et al.*, 1993; Cunha *et al.*, 2012).A decline in retinal spasm with magnesium supplementation has also been observed in a group of eight hypertensive patients with elevated renin levels although the systemic arterial pressure did not change.

The relationship between hypertension and intake of dietary cations was reported in many studies, of them, a number of studies reported a strong significant inverse correlation between magnesium levels and both systolic and diastolic blood pressure values. Nevertheless, other studies have found varying relations. Other studies were conducted to assess magnesium levels in untreated hypertensive patients in comparison with normotensive individuals, likewise, increased, decreased and same magnesium levels were observed (Joffres *et al.*, 1987; Chakraborti *et al.*, 2002; Eilat-Adar *et al.*, 2013).

However, a comprehensive review of the epidemiologic and clinical evidence studying the correlation between magnesium deficiency and blood pressure concluded that the evidence was insufficient to prove a link between magnesium status and hypertension. Furthermore, studies have shown no overall improvement in the systemic arterial pressure of otherwise untreated hypertensive patients with magnesium supplementation. Nevertheless, there is an evidence that suggests that magnesium status may be important in some hypertensive patients. Another approved correlation was the inverse correlation between systemic arterial pressure and intracellular magnesium levels in patients with a family history of hypertension, nevertheless, this result was not found in patients with no family history of hypertension (Chakraborti *et al.*, 2002; Qu *et al.*, 2013; Nicklas *et al.*, 2014).

Additionally, some studies contradicted correlation between hypomagnesemia and hypertension and found increased in Mg levels in hypertension (Geiger & Wanner, 2012). Furthermore, some studies find no difference in Mg levels between hypertension patients and healthy patients (Touyz, 2004). This indicates that not all personnel with hypomagnesaemia suffer from hypertension, and that there are other important factors that affect the correlation between magnesium and hypertension, including obesity, diabetes, pregnancy and race (Geiger & Wanner, 2012).

Magnesium supplementation has been shown to benefit some patients receiving other anti-hypertensives, particularly, Patients on traditional non-potassium-sparing diuretics who tend to have a potentially dangerous magnesium deficiency. In these patients, studies have shown a decrement of systolic blood pressure by approximately 10mmHg, nevertheless, another study could find no benefit. It is of interest that Resnick et al found that, as hypertension was controlled, the levels of intracellular free magnesium rose, regardless of which antihypertensives was used (Del Gobbo *et al.*, 2013; An *et al.*, 2014; Shah *et al*., 2014).

### 1.13.3 Coronary Artery Spasm

The relation between coronary artery spasm and magnesium deficiency is shown in several studies. Magnesium is considered as a naturally occurring calcium channel blocker as it controls calcium movement across cardiac muscles cell (Fazekas *et al.*, 1993; Guerrera *et al*., 2009). In vitro studies have shown that the arteries are more likely to constrict when incubated in solutions with low concentrations of magnesium. Furthermore, contractile responses to norepinephrine, acetylcholine, serotonin,

angiotensin, and potassium of both large and small coronary arteries are significantly increased. Another evidence of the correlation between hypomagnesemia and coronary artery spasm is that variant angina has been treated successfully by intravenous magnesium sulfate (Purvis & Movahed, 1992; Fazekas *et al.,* 1993; Rude, 1998; Ho, 2008; Guerrera *et al.,* 2009; An *et al*., 2014).

### 1.13.4 Thrombosis

Since the 1950s, it was suggested that parenteral magnesium treatment reduces coronary thrombosis occurrence. Furthermore, recent animal experiments have shown that magnesium salts inhibit adenosine diphosphate (ADP)-induced platelet aggregation, while magnesium infusions have been shown to reduce clotting in preeclamptic patients by reducing certain clotting factors. Another studies have been able to reduce the increased platelet aggregation of diabetic patients by oral supplementation of magnesium. These studies suggests that magnesium supplementation can reduce thrombosis in some patients (Purvis & Movahed, 1992; Rude, 1998; Eilat-Adar *et al*., 2013; An *et al.,* 2014).

### 1.13.5 Myocardial Infarction

Magnesium deficiency has been linked with induction of severe vascular damage in the heart, acceleration of the development of atherosclerosis, vasoconstriction of the coronary arteries, increase in blood pressure and enhanced platelet aggregation. Hypomagnesaemia looks to be involved in the pathogenesis of ischemic heart disease by changing lipoprotein composition, predisposing individuals to atherosclerosis. In

animal studies, magnesium administration prior to reperfusion led to a reduction in infarct size (Touyz, 2004; Geiger & Wanner, 2012; Mhaskar *et al*., 2013).

In recent years, the use of intravenous (I.V.) magnesium can be considered as a major breakthrough in the treatment of myocardial infarction. It is found that in patients of myocardial infarction, who became critical and who died suddenly, had low serum magnesium levels. Similarly, life-threatening arrhythmias were found to be more frequent in patients with acute myocardial infarction with low serum magnesium levels. It was also shown that the magnesium content of the infracted/ischemic myocardium was much lower (about 40-50%) as compared to that of normal heart muscle. It has been shown that magnesium depletion modifies coronary blood flow, blood clotting, and atherogenesis. Magnesium lowers systemic vascular resistance, dilate coronary arteries, decrease platelet aggregation, improve myocardial metabolism, protect against catecholamine-induced myocardial necrosis, and stabilize cell membranes. It is also cheap and easy to handle. Thus, it would appear to be an excellent contender for a place in the routine treatment of myocardial infarction, but it has not achieved this status yet. Therefore, the use of magnesium in myocardial infarction is a worthy topic of serious consideration. We, therefore, decided that we would evaluate the effect of I.V. magnesium supplement therapy in patients admitted for acute myocardial infarction and if this would be helpful in reducing the morbidity and mortality in patients.

Magnesium deficiency appears to be extremely important in the peri-infarction period.In addition to previous uncontrolled studies,several more recent controlled studies have evaluated the effect of magnesium infusion in the peri-infarction

period. Magnesium infusion in patients with suspected myocardial infarction could reduce myocardial oxygen demand and limit the infarct size. The preinfarction magnesium status is unclear, because serum and intracellular magnesium levels have been found to be decreased, no different from,or increased, compared to patients without myocardial function (Fazekas *et al.*, 1993; Altura *et al.*, 2013; An *et al.*, 2014).

An association between hypomagnesemia and postinfarction ventricular arrhythmias has been revealed in most,but not all, studies. Supraventricular tachycardia and atrial fibrillation have been found more frequently in hypomagnesemic patients, and atrioventricular blocks and supraventricular bradycardias have been found more frequently in hypermagnesemic patients (Purvis & Movahed, 1992; Rude, 1998; Kupetsky-Rincon & Uitto, 2012).

### 1.13.6 Cardiomyopathy

Magnesium deficiency has been suggested as the cause of cardiomyopathy. Pathologic relations as well as epidemiologic, histological, and animal studies, have implicated magnesium deficiency in a variety of cardiomyopathies (Purvis & Movahed, 1992; Rude, 1998; Kupetsky-Rincon & Uitto, 2012).

For instance, patients with hypoparathyroidism can manifest a cardiomyopathy which responds to calcium and magnesium replacement. Alcoholic patients are known to have both cardiomyopathy and magnesium deficiency (Purvis & Movahed, 1992; Kupetsky-Rincon & Uitto, 2012; An *et al*., 2014).

Several different experimental animal models have shown that magnesium deficiency is cardiotoxic, resulting in gross pathologic changes and histologic changes from injury. Postmortem evaluation has shown that with a variety of insults, magnesium and potassium leave cardiac tissue and calcium and sodium accumulate in cardiac tissue. Any of these changes could cause necrosis (Purvis & Movahed, 1992; Kupetsky-Rincon & Uitto, 2012; An *et al*., 2014).

### 1.13.7 Congestive Heart Failure

Patients with congestive heart failure (CHF) regularly have magnesium due to increased urinary excretion. Magnesium deficiency worsens hyperaldosteronism, which may lead to fluid retention. Magnesium loss also compounds hypokalemia, which could theoretically produce ventricular arrhythmias and hemodynamic deterioration in CHF. Magnesium depletion may worsen cardiac function by weakening contractility, increasing vasoconstriction,or by depleting energy stores (Douban *et al*., 1996; Qu *et al*., 2013).

Not all evidence supports the importance of magnesium in CHF. Studies have shown that in ambulatory patients with dilated cardiomyopathy and CHF, the incidence of hypomagnesemia is fairly low and serum, circulating mononuclear cell, skeletal muscle, and myocardial magnesium concentrations relate poorly with each other (Douban *et al.*, 1996; An *et al.*, 2014).

### 1.13.8 Arrhythmias

The importance of magnesium in ventricular, supraventricular and digitalis-related arrhythmias has been the matter of extensiveattention. Magnesium deficiency which is regularly accompanied by hypokalemia produces prolongation of QT interval,-ST-segment depression, and low-amplitude T waves (Brugada, 2000).

Increased magnesium levels lead to bradycardia, increased conduction time, and diminished automatism. Magnesium possibly effects transport of potassium,sodium,and calcium across the cell.

Magnesium may eradicate ventricular arrhythmia during acute myocardial ischemia due to prevention of conduction slowing by an anti-ischemic action (Fazekas *et al.*, 1993; Ho, 2008). While hypomagnesemia is related with hyponatremia, hypocalcemia, and hypophosphatemia, the relationship with hypokalemia is the best known link.

Both potassium and magnesium tend to be depleted with thiazide diureticsand spared with potassium-sparing diuretics.

This is particularly true for the elderly and for patients on high doses of diuretics, and may be true for others already at risk for electrolyte abnormalities, including alcoholics, diabetics, those with congestive heart failure, or those with a recent myocardial infarction. It has been shown that although serum potassium rises with replacement therapy, the level of potassium in muscle will not increase unless magnesium is replaced as well. The benefit of replacing potassium and magnesium in patients with deficits in both seems to be larger than for either alone, particularly in patients treated with non-potassium-sparing diuretics. Patients on non-potassium-

sparing diuretics have more frequent ventricular arrhythmias. Even more ominous is the finding in a recent prospective study that 66% of patients in cardiac arrest had magnesium abnormalities and none of these patients were successfully resuscitated (Fazekas *et al.*, 1993; Brugada, 2000; Ho, 2008).

## 1.14 Aim of the study

1- To Determine the level of serum magnesium among healthy and cardiovascular patients in Jordan.

2- To Examine the effect of some demographic factors ( gender and smoking) on Mg levels .

3- To Examine the association between magnesium levels and presence of cardiac diseases.

# Chapter Two
# Experimental Part

# Chapter 2

## Experimental Part

## 2.1 Clinical part

### 2.1.1 Study population

The blood sampling was conducted between September and November, 2014, from healthy students at Petra University and cardiovascular diseases patients at Jordan Hospital . The experiment population is (151) subject, divided into (101) healthy and (50) patient.

The study case report form was approved by Faculty of pharmacy; Petra University. The case report forms were used to gather demographics, height, weight, profession, and food intake. (Appendix A)

### 2.1.2 Blood sampling

Blood samples of 5 ml were drawn by trained nurse, collected in plane tubes stand for clotting for 5-10 minutes and were centrifuged at 5000 rpm for 5 minutes, then serum was collected and stored at -70C until analysis.

Before collecting samples, many questions and information had been collected by self-reporting from volunteers using case report form, then classified according to study requirements as shown in table 2.1 and 2.2.

Table 2.1: Demographics of the study healthy population

**Healthy population**

| Parameter | Mean ± SD | Range | Median |
|---|---|---|---|
| **Total** | | | |
| **Age (yrs)** | 21.5 ± 3.8 | 18-42 | 20 |
| **Males** | | | |
| **Age (yrs)** | 21.2 ± 3.75 | 19-42 | 20 |
| **Females** | | | |
| **Age (yrs)** | 21.73 ± 3.77 | 18-40 | 20 |
| **Smoker** | | | |
| **Age (yrs)** | 22.33 ± 5.12 | 19-40 | 21 |
| **Non-smoker** | | | |
| **Age (yrs)** | 21.395 ± 3.479 | 18-42 | 20 |

Table 2.2: Demographics of the study patient population

**Patient population**

| Parameter | Mean ± SD | Range | Median |
|---|---|---|---|
| **Total** | | | |
| **Age (yrs)** | 63.14 ± 13.73 | 20-89 | 68 |
| **Males** | | | |
| **Age (yrs)** | 63.41 ± 14.16 | 20-78 | 70 |
| **Females** | | | |
| **Age (yrs)** | 62.83 ± 13.52 | 34-89 | 64 |
| **Smoker** | | | |
| **Age (yrs)** | 42.33 ± 13.78 | 20-57 | 44 |
| **Non-smoker** | | | |
| **Age (yrs)** | 65.98 ± 11.14 | 44-89 | 70 |

## 2.2 Magnesium analysis assay

Quantitative analysis of magnesium in human serum is conducted using Xylidyl Blue-I Method. A Magnesium kit was used (Mindray Bio-Medical Electronics Co.,Ltd, China). Mg serum samples were analyzed according to the manufacturer procedures. Briefly, collected samples were centrifuged before the assay, to get rid of any possible precipitates, then 10µl mixed thoroughly with 1ml reagent, at 37C˚. The reagent is a combination of xylidyl blue 0.1mM, EGTA 0.13mM, DMSO 1.4M, Buffer, and surfactants. EGTA is used to eliminate calcium interference, while the surfactant system is included to remove protein interference.A blank was prepared by mixing 10µl distilled water with 1ml reagent. The absorbance was read five minutes after the sample preparations, using Mindray BS 200 chemical analyzer (Mindray Bio-Medical Electronics Co.,Ltd, China).

## 2.3 Data analysis

Data were expressed as Mean ± SD. Comparisons of variables were assessed by using t-test using SPSS (version 17.0). P value of $<0.05$ is considered statistically significant. Mg normal serum level was set as (1.5 to 2.4 mg/dL) (Geiger & Wanner, 2012; Kupetsky-Rincon & Uitto, 2012).

# Chapter Three
# Results and Discussion

# Chapter 3
# Results and Discussion

### 3.1. Mg analysis and distribution

#### 3.1.1 Demographics of the study

A total of 151 person participated in the study. Of these, 101 person were healthy (34% males, 66% females), and 50 patient (54% males, 46% females). Healthy participants ranged from 18 to 42 years old, with an average of 21.5 ± 3.8 years, while patient participants ranged from 20 to 89 years old, with an average of 63.14 ± 13.73 years (Table 3.1).

#### 3.1.2 Mg serum levels in healthy and patient population

The mean of Mg serum levels in the study population, healthy and patient subjects, was within the normal range (1.5 to 2.4 mg/dL) (Table 3.1).

- From the 101 healthy population,2 (2%) were above the normal Mg serum levels (1 male, 1 female).
- From the 50 patient population, 3 (6%) were above the normal Mg serum levels (1 male, 2 female).

Table (3.1) Mg serum levels in healthy and patient subjects

| Healthy Statistics | | | | |
|---|---|---|---|---|
| | **N** | **Mean (mg/dl)** | **Std. Deviation** | **Std. Error Mean** |
| **Total Healthy** | 101 | 2.0372 | 0.18336 | 0.01825 |
| **Healthy Males** | 37 | 2.0322 | 0.19208 | 0.03158 |
| **Healthy Females** | 64 | 2.0402 | 0.17962 | 0.02245 |
| **Healthy Smokers** | 15 | 2.0047 | 0.13643 | 0.03523 |
| **Healthy Non-Smokers** | 86 | 2.0429 | 0.19045 | 0.02054 |
| **Patients Statistics** | | | | |
| | **N** | **Mean (mg/dl)** | **Std. Deviation** | **Std. Error Mean** |
| **Total Patients** | 50 | 2.0130 | 0.28586 | 0.04043 |
| **Patient Males** | 27 | 2.0519 | 0.22424 | 0.04316 |
| **Patient Females** | 23 | 1.9674 | 0.34431 | 0.07179 |
| **Patient Smokers** | 6 | 2.0583 | 0.28358 | 0.11577 |
| **Patient Non-Smokers** | 44 | 2.0068 | 0.28886 | 0.04355 |

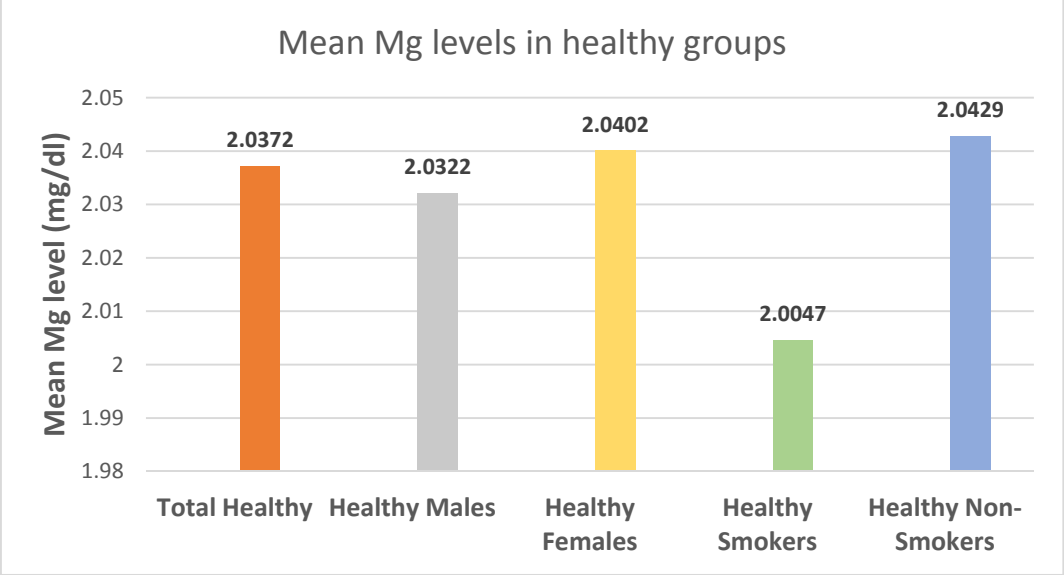


**Figure 3.1 : Comparison between mean magnesium levels in healthy groups.**

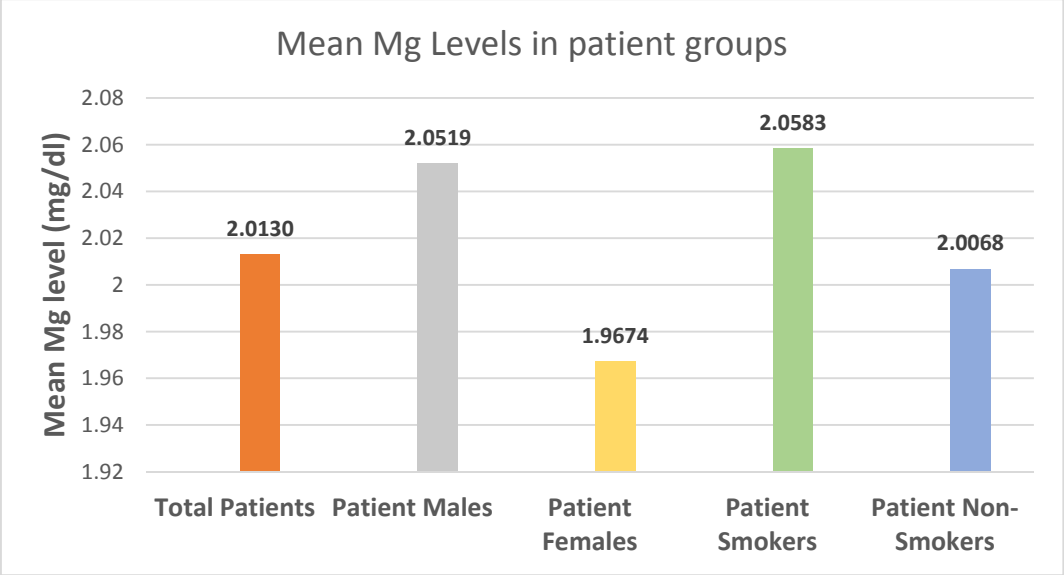


**Figure 3.2 : Comaprison between mean magnesium levels in patient groups.**

### 3.1.2 Biostatical analysis of Mg serum level

- There is no significant difference found between healthy and patient subjects (P value > 0.05), with Mg serum levels of healthy subjects showed a little elevation compared to patient Mg serum levels (Cohen's d= 0.1007) (Table 3.2).

**Table (3.2): Biostatistical analysis of Mg serum levels in healthy and patient subjects**

| Healthy Vs Patients | N | Mean | P-value | Description | Cohen's d |
|---|---|---|---|---|---|
| Healthy | 101 | 2.0372 | 0.587 | Insignificant | 0.10077 |
| Patient | 50 | 2.0130 | | | |

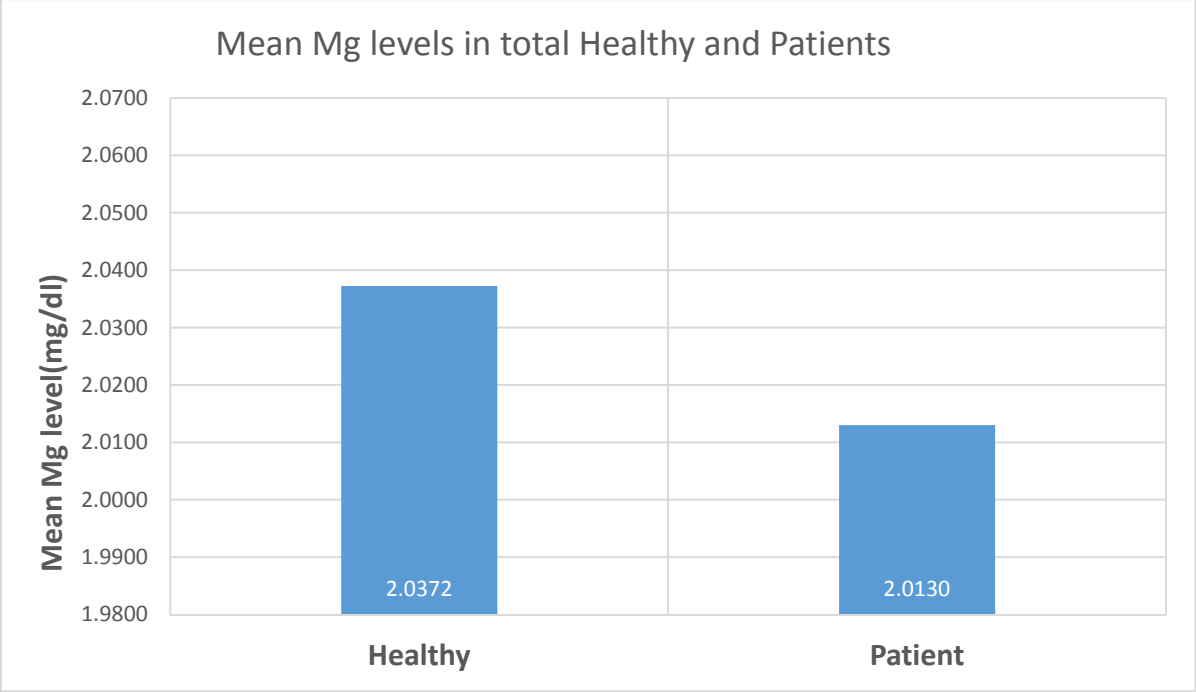


**Figure 3.3: Comparison between mean magnesium level in healthy and patients.**

From the results above, we conclude that there is no significant difference in Mg serum levels between healthy and cardiovascular Jordanian patients. These findings can be related to the Jordanian dietary intake of Mg, which is believed to be slightly below the recommended daily intake (Alkurd, 2011). This might indicate that the Mg intake levels within the Jordanian community is sufficient enough to prevent hypomagnesemia.

Few previous studies indicated similar observations, such as Khatami et al study, there was also no correlation between Mg serum levels and CVDs risk factors in hemodialysis patients (Khatami *et al.*, 2013).

### 3.2 Effect of gender on Mg levels

- There is no significant effect for subject gender on the Mg serum levels (P value >0.05). Both males and females, healthy or patient, have Mg serum levels within the normal range, and without any significant differences between healthy and patient subjects.
- Mg serum levels of patient males showed a little elevation compared to healthy males Mg serum levels (Cohen's d= -0.09435) (Table 3.3). While Mg serum levels of healthy females showed medium elevation compared to patient females Mg serum levels (Cohen's d= 0.2651) (Table 3.4).

**Table (3.3): Biostatistical analysis of Mg serum levels in males**

| Healthy Males vs Patient Males | N | Mean | P-value | Description | Cohen's d |
|---|---|---|---|---|---|
| Healthy<br>Patient | 37<br>27 | 2.0322<br>2.0519 | 0.714 | Insignificant | -0.09435 |

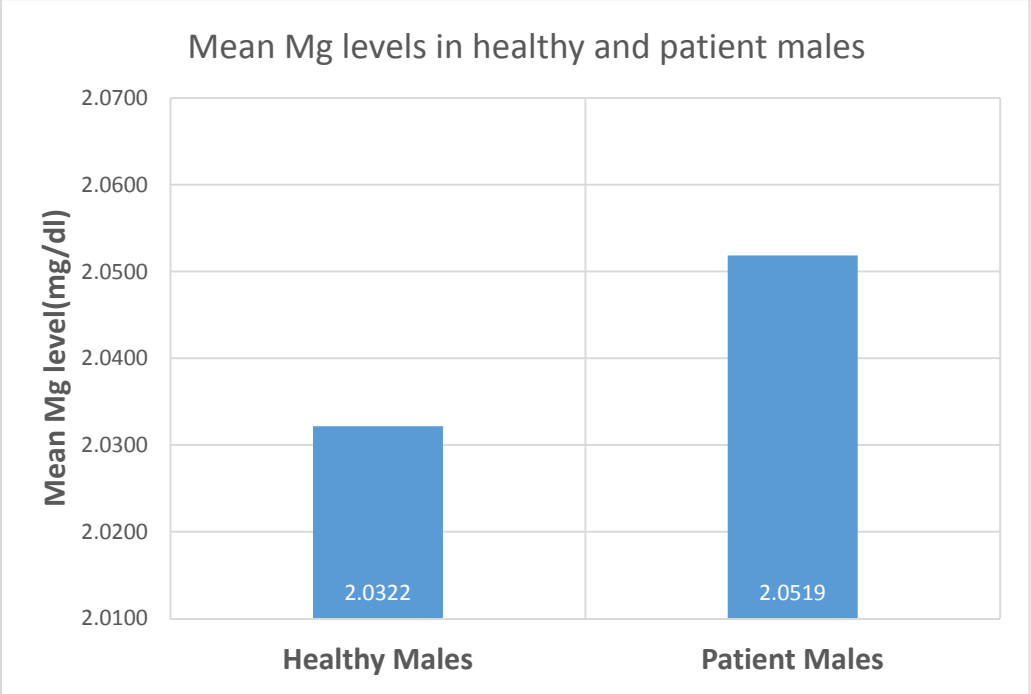


**Figure 3.4: Comaprison between mean magnesium levels in males ( healthy and patients ).**

| Table (3.4): Biostatistical analysis of Mg serum levels in females | | | | | |
|---|---|---|---|---|---|
| Healthy Females vs Patient Females | N | Mean | P-value | Description | Cohen's d |
| Healthy<br>Patient | 64<br>23 | 2.0402<br>1.9674 | 0.34 | Insignificant | 0.2651 |

We can conclude from these results that there is no effect for gender on the Mg serum levels. These results confirm previous studies that suggest that there is no effect of gender on Mg serum levels (Augusta CN *et al.*, 2006; Mohammed *et al.*, 2012).

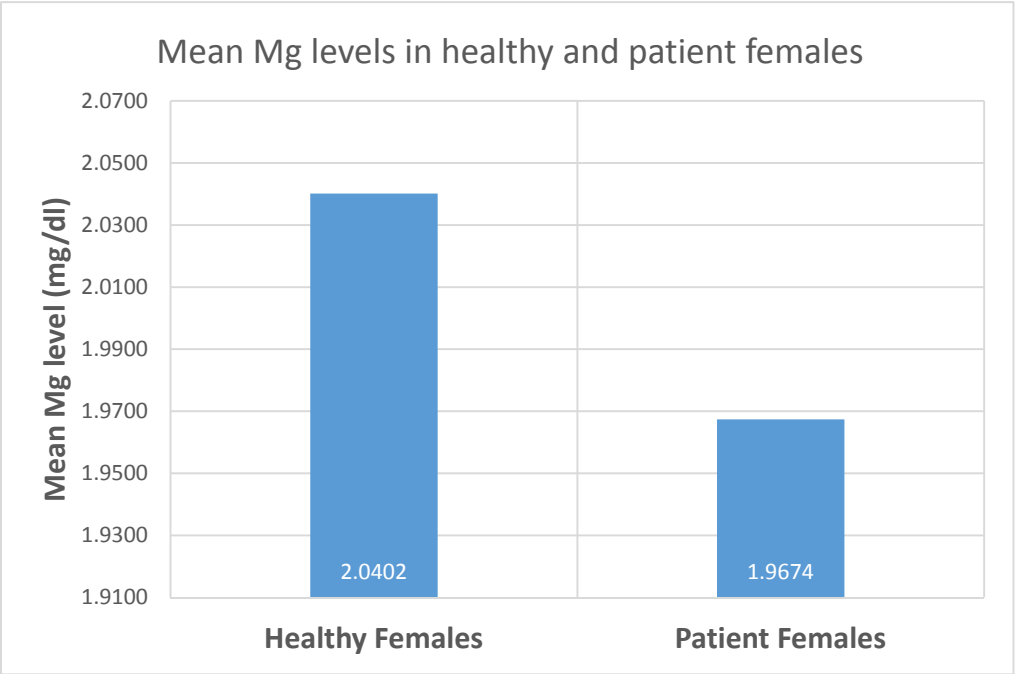


**Figure 3.5: Comparison between mean magnesium levels in healthy and patients female**

### 3.3 Effect of smoking on Mg levels

- No significant effect for subject smoking on the Mg serum levels (P values > 0.05). Both smokers and non-smoker, healthy or patient, have Mg serum levels within the normal range, and without any significant differences between healthy and patient subjects.
- Mg serum levels of patient smokers showed medium elevation compared to healthy smokers Mg serum levels (Cohen's d= -0.2408) (Table 3.5). While

Mg serum levels of healthy non-smoker showed small elevation compared to healthy non-smoker Mg serum levels (Cohen's d= 0.2651) (Table 3.6).

**Table (3.5): Biostatistical analysis of Mg serum levels in smokers**

| Healthy Smokers vs Patient Smokers | N | Mean | P-value | Description | Cohen's d |
|---|---|---|---|---|---|
| Healthy | 15 | 2.0047 | 0.673 | Insignificant | -0.2408 |
| Patient | 6 | 2.0583 | | | |

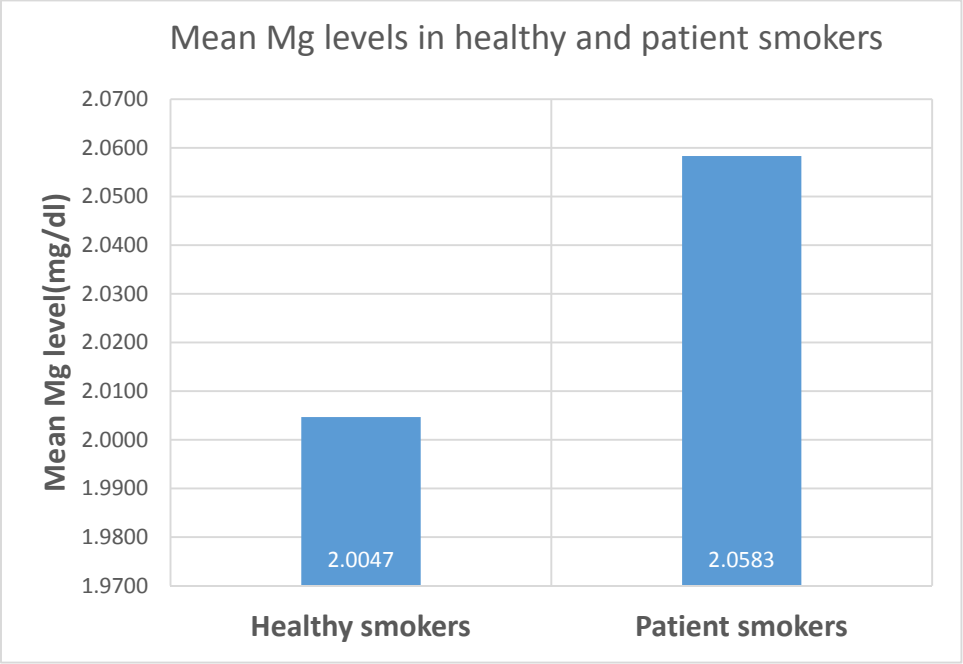


**Figure 3.6 : Comparison between mean magnesium levels in healthy and patients smokers .**

| Table (3.6): Biostatistical analysis of Mg serum levels in non-smokers | | | | | |
|---|---|---|---|---|---|
| Healthy Non-Smokers vs Patient Non-Smokers | N | Mean | P-value | Description | Cohen's d |
| Healthy | 86 | 2.0429 | .456 | Insignificant | 0.1475 |
| Patient | 44 | 2.0068 | | | |

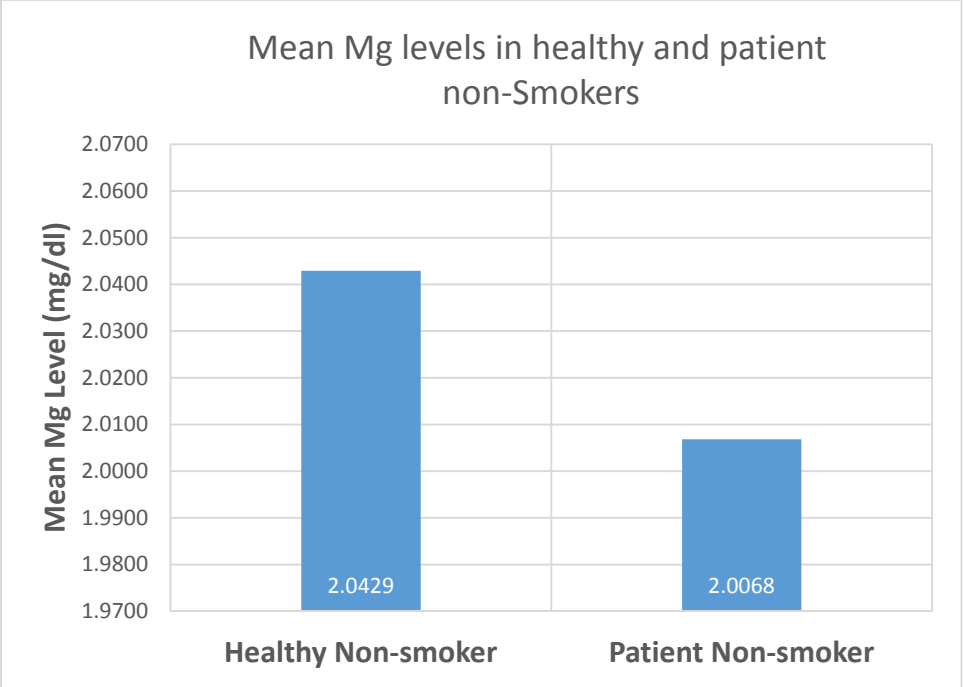


**Figure 3.7 : Comaprison between mean magnesium levels in healthy and patients non smokers.**

From these results we conclude that there is no relation between smoking and Mg serum levels. Previous studies have indicated that low Mg serum levels lead to development for nicotine addiction(M. Nechifor *et al*., 2004; Mihai Nechifor, 2012).

Mg role in CVD has been studied for the past decades, and several researchers indicated a correlation between low serum Mg and CVD, especially in IHD and some type's arrhythmia. Yet in our study, there was no significant difference between Mg serum levels in patients and healthy subjects, with the mean of both groups relay in the normal range. Further on, there was no correlation between Mg level and sex or smoking.

We hypothesize that low Mg serum levels caused by low intake of dietary Mg, might be a leading cause or a risk factor for a CVD, but not necessary a sign for an existing CVD condition. This has been previously implied in a follow up studies, were low Mg serum levels predicted future CV complications (Reffelmann *et al.*, 2010).

Our results are not consistent with those observed by other authors who suggest a correlation between hypomagenesmia and cardiovascular diseases here in Jordan.

Some electrolytes levels can affect the homeostasis of magnesium, in our study, normalization of potassium levels may be caused by such abnormalities .

However, as most of the studies that prove the presence of correlation between magnesium levels and cardiovascular diseases are conducted in western countries, diet

variations between societies may be a cause of normalized magnesium levels in Jordanian population.

Additionally, aging may play an important role in normalizing our results, as it is a well known factor that disturbs magnesium levels in patients as aging decreases dietary intake and is associated with other functional abnormalities that could normalize magnesium levels in our sample.

# Chapter Four
# Conclusion and Recommendations

# Chapter Four
# Conclusion and Recommendations

Magnesium appears within the normal range among Jordanian people , sex and smoking didn't affect the magnesium levels in those population .

Further studies investigating the role of Mg in CVD is required, to find if there is a correlation between Mg and specific types of cardiac diseases

**Recommendations:**

1- We recommend more studies about the links among cardiovascular diseases, diabetes, renal failure ,hypothyrodism, depression with magenesium level.

2- We recommend a test for water in Jordan .

3-We recommend to use another method of analysis like Atomic Absorption Spectrometry.

# Chapter Five

# References

# Chapter 5

## References


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# Appendix

**Petra University**
**( Private Accredited University )**
**Faculty of Pharmacy &Medical Sciences .**

جامعة البترا

**جامعـة البتــرا**
**( جامعة خاصة معتمدة)**
**كلية الصيدلة والعلوم الطبية**

# Case Report Form

**Volunteer/ Patient name and number :**

**Date of Examination : day/month/year**

**Demographic Data** :

- Age:
- Gender : Female Male
- Weight ( kg ):
- Height ( cm ) :
- BMI:
- Mobile number :

## Section A - Diet/Lifestyle

| | |
|---|---|
| Smoking more than three cigarettes per day | 5 |
| Strenuous exercise or training more than 3 times per week (running, sports, gym etc) | 5 |
| More than 3 coffees daily | 4 |
| High sugar containing foods daily | 2 |
| Eat processed food or junk food daily | 1 |
| Consume a diet high in fatty foods daily (fried foods, butter, hamburgers, bacon, ice-cream, cheese) | 4 |
| A diet low in green leafy vegetables, seeds and nuts (less than one serve every day) | 4 |
| Consume soft drinks / fizzy drinks daily | 4 |
| | |
| Score for section **A** | |

## Section B - Health conditions:

| | |
|---|---|
| Chronic headaches or migraines | 15 |
| Asthma | 5 |
| Diabetes | 10 |
| Glaucoma | 4 |
| Osteoporosis | 5 |
| Chronic kidney disease | 5 |
| High blood pressure | 5 |
| An overactive thyroid, or underactive thyroid | 2 |
| An endocrine condition such as Hyperaldosteronism or Hyperparathyroidism | 5 |
| Very fast heart beats, irregular heartbeats, or arrhythmia (or have experienced these symptoms in the last 12 months) | 10 |
| Chronic intestinal disease, Ulcerative colitis, Crohn's disease or Irritable bowel syndrome | 5 |
| Frequent diarrhea or constipation | 4 |
| PMS or menstrual cramps | 5 |
| Frequent lethargy or fatigue | 3 |
| Known parasites (eg. Pinworm) | 2 |
| Currently pregnant | 5 |
| Recently pregnant (in the last 12 months) | 1 |
| Breastfeeding or recently breastfed for longer than 12 months | 3 |
| Have diagnosed Haemochromatosis (Iron overload) | 5 |
| In a previous pregnancy had high blood pressure or pre-eclampsia | 5 |
| Recent traumatic stress, physical or emotional (in the last 12 months) | 5 |
| Score for Section **B** | |

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## Section C Nervous System

| | | | | |
|---|---|---|---|---|
| Muscle spasms in hands or feet | 1 | 2 | 3 | 4 |
| Difficulty sleeping | 1 | 2 | 3 | 4 |
| Irritability, or being easily provoked | 1 | 2 | 3 | 4 |
| Feeling restless, or agitated | 1 | 2 | 3 | 4 |
| Small muscle twitching around your eyes, facial muscles | 1 | 2 | 3 | 4 |
| Small muscle twitching anywhere else in your body | 1 | 2 | 3 | 4 |
| Convulsions | 1 | 2 | 3 | 4 |
| Experience long or intense periods of stress | 1 | 2 | 3 | 4 |
| Shakiness or tremor in your hands | 1 | 2 | 3 | 4 |
| Muscle cramps | 1 | 2 | 3 | 4 |
| Chronic lack of interest, indifference, or apathy | 1 | 2 | 3 | 4 |
| Poor memory | 1 | 2 | 3 | 4 |
| Experience physical or mental fatigue | 1 | 2 | 3 | 4 |
| Loss of concentration | 1 | 2 | 3 | 4 |
| Anxiety | 1 | 2 | 3 | 4 |
| Mood swings | 1 | 2 | 3 | 4 |
| Depression for no apparent reason | 1 | 2 | 3 | 4 |
| Feelings of disorientation as to time or place | 1 | 2 | 3 | 4 |
| Experience emotional stress | 1 | 2 | 3 | 4 |
| Feelings that people are against you | 1 | 2 | 3 | 4 |
| Cold hands or feet | 1 | 2 | 3 | 4 |
| Numbness in face, hands, or feet | 1 | 2 | 3 | 4 |
| Experience tingling or 'pins and needles' anywhere in your body | 1 | 2 | 3 | 4 |
| Score for Section **D** | | | | |

1 = Almost never 2 = Sometimes 3 = Fairly often 4 = Very often

*Total Magnesium Status Score

A + B + C =

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**Interpretive Guide to your Total Magnesium Status Score:**

**Score 0 – 25**
Suggests it is unlikely there is a Magnesium deficiency; however it is important to maintain a healthy diet rich in Magnesium by consuming nutritious whole foods including nuts and seeds, whole grains and green leafy vegetables. During times of physical or emotional stress, your practitioner may advise you to take a clinically proven, bioavailable Magnesium supplement.

**Score 26- 50**
Suggests it is likely your magnesium levels are low. Your practitioner may advise you to take a regular Magnesium supplement which has been clinically trialled and is proven to improve intracellular Magnesium levels quickly. It is also important to consume Magnesium rich foods such as nuts and seeds, whole grains and green leafy vegetables.

**Score over 50**
Suggests it is highly likely you are suffering from low Magnesium levels and may be experiencing Magnesium deficiency symptoms. It is advisable that your practitioner prescribe a clinically trialled, bioavailable Magnesium, proven to improve Magnesium status quickly. It is also important to consume Magnesium rich foods such as nuts and seeds, whole grains and green leafy vegetables.

**Name of person who completed the form:……**

**Signature …….**

**aATotal Healthy vs Patient**

**Group Statistics**

| | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Type | Healthy | 100 | 2.0372 | .18336 | .01825 |
| | Patient | 50 | 2.0130 | .28586 | .04043 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| | Equal variances assumed | 16.424 | .000 | .630 | 149 | .530 | .02423 | .03845 | -.05175 | .10020 |
| | Equal variances not assumed | | | .546 | 69.580 | .587 | .02423 | .04435 | -.06424 | .11270 |

**Healthy Males vs Patient Males**

**Group Statistics**

| | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Type | Healthy | 37 | 2.0322 | .19208 | .03158 |
| | Patient | 27 | 2.0519 | .22424 | .04316 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| | Equal variances assumed | .578 | .450 | -.377 | 62 | .707 | -.01969 | .05219 | -.12401 | .08463 |
| | Equal variances not assumed | | | -.368 | 50.781 | .714 | -.01969 | .05347 | -.12706 | .08768 |

**Healthy Females vs Patient Females**

**Group Statistics**

| | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Type | Healthy | 64 | 2.0402 | .17962 | .02245 |
| | Patient | 23 | 1.9674 | .34431 | .07179 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| | Equal variances assumed | 26.783 | .000 | 1.281 | 85 | .204 | .07276 | .05680 | -.04018 | .18571 |
| | Equal variances not assumed | | | .967 | 26.425 | .342 | .07276 | .07522 | -.08173 | .22726 |

**Healthy Smokers vs Patient Smokers**

**Group Statistics**

| | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Type | Healthy | 15 | 2.0047 | .13643 | .03523 |
| | Patient | 6 | 2.0583 | .28358 | .11577 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| | Equal variances assumed | 5.695 | .028 | -.595 | 19 | .559 | -.05367 | .09021 | -.24248 | .13515 |
| | Equal variances not assumed | | | -.443 | 5.950 | .673 | -.05367 | .12101 | -.35037 | .24303 |

**Healthy Non-Smokers vs Patient Non-Smokers**

**Group Statistics**

| | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Type | Healthy | 86 | 2.0429 | .19045 | .02054 |
| | Patient | 44 | 2.0068 | .28886 | .04355 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| | Equal variances assumed | 11.006 | .001 | .853 | 128 | .395 | .03609 | .04231 | -.04764 | .11981 |
| | Equal variances not assumed | | | .750 | 62.684 | .456 | .03609 | .04815 | -.06013 | .13231 |

**تراكيز امصال المغنيسيوم لدى الاردنيين :**

**تأثير مستوى المغنسيوم على أمراض القلب**

**اعداد : سالي زياد عميش**

**المشرفون :**

**د. توفيق عرفات**

**د. اياد الملاح**

**د. رياض عوض**

**د. وائل ابو دية**

**د. فراس الحجي**

## ملخص

يؤدي المغنيسوم دوراً هاماً في تفاعلات انزيمية عديدة, حيث يقوم بعدة مهام وظيفيةٍ داخل الخلية، ولذلك تكون التغيرات في تركيز المغنيسيوم – وبالاخص هبوط مستواه حيث انه اكثر شيوعاً من ارتفاع مستواه– سبباً في تغيرات غير مرغوبة على مستوى العصبي– العضلي, وعلى مستوى القلب والجهاز العصبي.

ان قياس تركيز المغنيسيوم في بلازما الدم هي الطريقة العملية والمتوافرة لقياس التغيرات الطارئة على حالة المغنيسيوم داخل الجسم, على الرغم من عدم ضرورة كونها تعكس كمية المغنيسيوم الكاملة داخل الجسم.

الى الآن, لم يُقيَّمْ تأثير تركير المغنيسيوم كعامل خطورةٍ مؤدٍّ الى أمراض القلب والاوعية الدموية على مستوى الاردن، لذلك قامت الباحثة باستخدام (colorimetric method) لدراسة تركيز المغنيسيوم في عينةٍ تكونت من طلاب جامعة البتراء الأصحاء, بالاضافةِ الى عينةٍ أخرى تتكون من مرضى مصابين بأمراض القلب من مستشفى الاردن.

وقد تم أخذ عينات الدم خلال الفترة الممتدة ما بين أيلول و تشرين ثاني من عام ٢٠١٤. وتكونت العينة من ١٥١ شخص خضع للدراسة.

لم توجِد الدراسة فارقاً في تركيز المغنيسيوم بين عينة الاصحاء وعينة مرضى القلب ($P\ value > 0.05$) , بالاضافة الى أن العينة من الاصحاء أظهرت ارتفاعاً طفيفاً في المستوى مقارنةً بنظيرتها من المرضى (Cohen's $d= 0.1007$) . تظهر دراستنا وما نتج عنها من مشاهدات عدمَ وجود رابطِ بين مستويات المغنيسيوم في الدم وأمراض القلب في الاردنيين. وكذلك, فان المشاهدات لم تُظهِر تأثيراً للجنس أو للتدخين على مستويات المغنيسيوم.