July 2017 M.Sc. in Biochemistry Science and Technology RAHMAH HAMID KASSAR

UNIVERSITY OF GAZİANTEP

GRADUATE SCHOOL OF

NATURAL & APPLIED SCIENCES

RELATIONSHIP BETWEEN SERUM MAGNESIUM LEVEL AND INSULIN RESISTANCE IN OBESE NON-DIABETIC SUBJECTS AND OBESE TYPE2 DIABETIC PATIENTS

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IN

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BY

RAHMAH HAMID KASSAR

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Rahmah Hamid KASSAR

ABSTRACT

RELATIONSHIP BETWEEN SERUM MAGNESIUM LEVEL AND INSULIN RESISTANCE IN OBESE NON-DIABETIC SUBJECTS AND OBESE TYPE2 DIABETIC PATIENTS

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July 2017, 63 pages

Background and Aim: Type2 diabetes mellitus (T2DM) and obesity are multifactorial diseases including interactions between hereditary and environmental factors. A common feature shared between these two diseases is the existence of insulin resistance. Magnesium (Mg^{2+}) plays an important role in glucose homeostasis. Our study aimed to evaluate the relationship between serum Mg level and insulin resistance in obese non-diabetic subjects and obese T2DM patients then compared them with controls.

Patients and Methods: The present study consist of 120 subjects of both genders and aged range between (20-70) years. The subjects were divided into four groups: Group I including 30 healthy subjects as a control, group II consisted of 30 obese non-diabetic subjects, group III included 30 obese T2DM patients with disease history less than 1 year and group IV included 30 obese T2DM with disease history more than 5 years. Serum Mg, fasting lipids, glucose, insulin and creatinine were measured. BMI and HOMA-IR were calculated.

Results: Serum Mg level was significantly decreased in group IV (1.72±0.1 mg/dl) as compared to groups I (2.07±0.1 mg/dl) ($p<0.05$), while serum Mg level was slightly decreased in group II and group III (1.98±0.2 mg/dl) as compared to group I. The HOMA-IR was significantly increased in group IV (7.9±7.0) as compared to group I (1.03±0.3) ($p<0.05$), also a significant difference was found between group III (4.99±4.1) and group II (2.82±2.3) in compared to group I ($p<0.05$).

Conclusions: Low serum Mg level was found in obese T2DM patients and obese non-diabetic subjects. We suggest measuring serum Mg level regularly in obese T2DM patients especially in older age patients, and patients who require supplementation should consider.

Key Words: Type2 diabetes mellitus, obesity, insulin resistance, magnesium

ÖZET

OBEZ DİYABETİK OLMAYAN VE DİYABETİK HASTALARDA SERUM MAGNEZYUM DÜZEYİ VE İNSÜLİN DİRENCİ ARASINDAKİ İLİŞKİNİN İNCELENMESİ

KASSAR, Rahmah Hamid
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Giriş ve Amaç: Tip 2 diabetes mellitus (T2DM) ve obezite kalıtsal ve çevresel faktörler arasındaki etkileşimler de dahil olmak üzere multifaktöriyel hastalıklardır. Bu iki hastalık arasında paylaşılan bir ortak bir özelliği, insülin direnci varlığıdır. Magnezyum (Mg^{+2}), glikoz homeostazında önemli bir rol oynamaktadır. Çalışmada, obez diyabetik olmayan kişilerde ve obez T2DM hastalarda serum Mg düzeyleri ile insülin direnci arasındaki ilişkiyi değerlendirmek ve daha sonra kontrollerle karşılaştırmaktır.

Hastalar ve Yöntemler: Bu çalışma, hem cinsiyet hem de yaş aralığı (20-70) arasında değişen 120 kişiden oluşmaktadır. Kişiler dört gruba ayrıldı: Grup I, kontrol olarak 30 sağlıklı bireyi kapsamıştır, grup II 30 obez diyabetik olmayan bireyden oluşmuştur, grup III hastalığın öyküsü bir yıldan az olan 30 obez T2DM hastasını ve grup IV'te hastalığın öyküsü beş yıldan fazla olan 30 obez T2DM'yi kapsımıştır. Serum Mg, açlık glikozu, açlık insülin, açlık lipidleri ve kreatin ölçüldü, VKI ve HOMA-IR hesaplanmıştır.

Bulgular: Serum Mg düzeyi grup IV'de (1,72±0,1 mg/dl) olup grup I'e (2,07±0,1 mg/dl) göre anlamlı derecede azalmış iken ($p < 0.05$), serum Mg düzeyi grup I'e göre Grup II ve III'de (1,98 ± 0,2 mg/dl) hafifçe azalmıştır. Hesaplanan HOMA-IR, grup I'e (1,03±0,3) kıyasen grup IV'de (7,9±7,0) ($p < 0,05$) önemli derecede artmıştır. Ayrıca grup III (4,99±4,1) ile grup II (2,82±2,3) arasında de anlamlı şekilde fark bulunmuştur ($p < 0,05$).

Sonuçlar: Obez T2DM hastalarında ve diyabetik olmayan obezlerde serum Mg düzeyleri düşük bulunmuştur. Obez T2DM hastalarında özellikle yaşlı hastalarda serum Mg düzeyini düzenli olarak ölçmenizi öneririz ve takviyeye ihtiyaç duyan hastalar düşünülmelidir.

Anahtar Kelimeler: Tip 2 diabetes mellitus, obezite, insülin direnci, magnezyum

To my family thank you for the love and support over the years. Your belief in me supports me through the hardest days

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LIST OF SYMBOLS / ABBREVIATIONS

%	Percentage
ADA	American Diabetes Association
ATPase	Adenosine Triphosphatase
ADP	Adenosine Diphosphate
BMI	Body Mass Index
cm	centimetre
Ca^{2+}	Calcium Ion
CHE	Cholesterol Esterase
CHO	Cholesterol Oxidase
DM	Diabetes Mellitus
DM II	Diabetes Mellitus Type II
T2DM	Type2 Diabetes Mellitus
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra Acetic Acid
HOMA-IR	Homeostatic Model Assessment Insulin Resistance
HK	Hexokinase
H_2O_2	Hydrogen Peroxide
IR	Insulin Resistance
GTPase	Guanine Triphosphatase
G-6-P	Glucose-6-Phosphate
G6P-DH	Glucose-6-Phosphate Dehydrogenase
GEDTA	Glycoletherdiamine-N,N,N`, N`-Tetra Acetic Acid
GK	Glycerol Kinase
IFG	Impaired Fasting Glucose

IGT	Impaired Glucose Tolerance
IU/mL	International Units Per Millilitre
kg/m^2	kilogram Per Meter Square
KU/L	kilounits Per Liter
[Mg^{2+}]i	Intracellular Magnesium
HDL.C	High Density Lipoprotein. Cholesterol
LDL.C	Low Density Lipoprotein. Cholesterol
Mg	Magnesium
Mg^{+2}	Magnesium Ion
mg/dl	milligrams per Deciliter
mmol/L	millimoles per Liter
ml	milliliter
µl	microlitre
µU/ml	microunits Per Milliliter
Na^+/K^+-ATPase	Sodium/Potassium-Adenosine Triphosphatase
NADH	Nicotinamide Adenine Dinucleotide
NIDDM	Non-Insulin Dependent Diabetics Mellitus
N	Nitrogen
O_2	Oxygen
POD	Peroxidase
RNA	Ribonucleic Acid
US	United States
WHO	World Health Organization
WC	Waist Circumference

CHAPTER 1

INTRODUCTION

1.1. Definition of Type2 Diabetes Mellitus

Type2 diabetes mellitus (T2DM) is a metabolic and endocrinological disorder that associated with hyperglycemia related with both insulin resistance and defective insulin secretion (Sarkar et al., 2010). In 2013, American Diabetes Association (ADA) reported that T2DM is the greatest prevalent form of diabetes and counts approximately 90% toward 95% of all diabetes cases. T2DM is an increasing reason of morbidity and mortality around the world. T2DM influences 5.9% of the world's adult people among nearly 80% of the whole developing countries. It is a chronic disease needing routine medical care to avoid acute developments and lower their complications (Florez et al., 2009).

1.1.1. The Pathogenesis of T2DM

T2DM pathogenesis is frequently related with decline insulin activity in target tissues e.g. liver, muscle and fat tissue (insulin resistance) and impaired insulin secretion through progressive beta cell dysfunction (Kahn et al., 2003; Sarkar et al., 2010). In general, 80 % of individuals suffering from T2DM are obese especially those with central visceral adiposity (Kim et al., 2009). Obesity, sedentary lifestyle, genetic predisposition, age, diet, lack of exercise and ethnicity are the most important risk factors for T2DM development as shown in Figure 1.1.

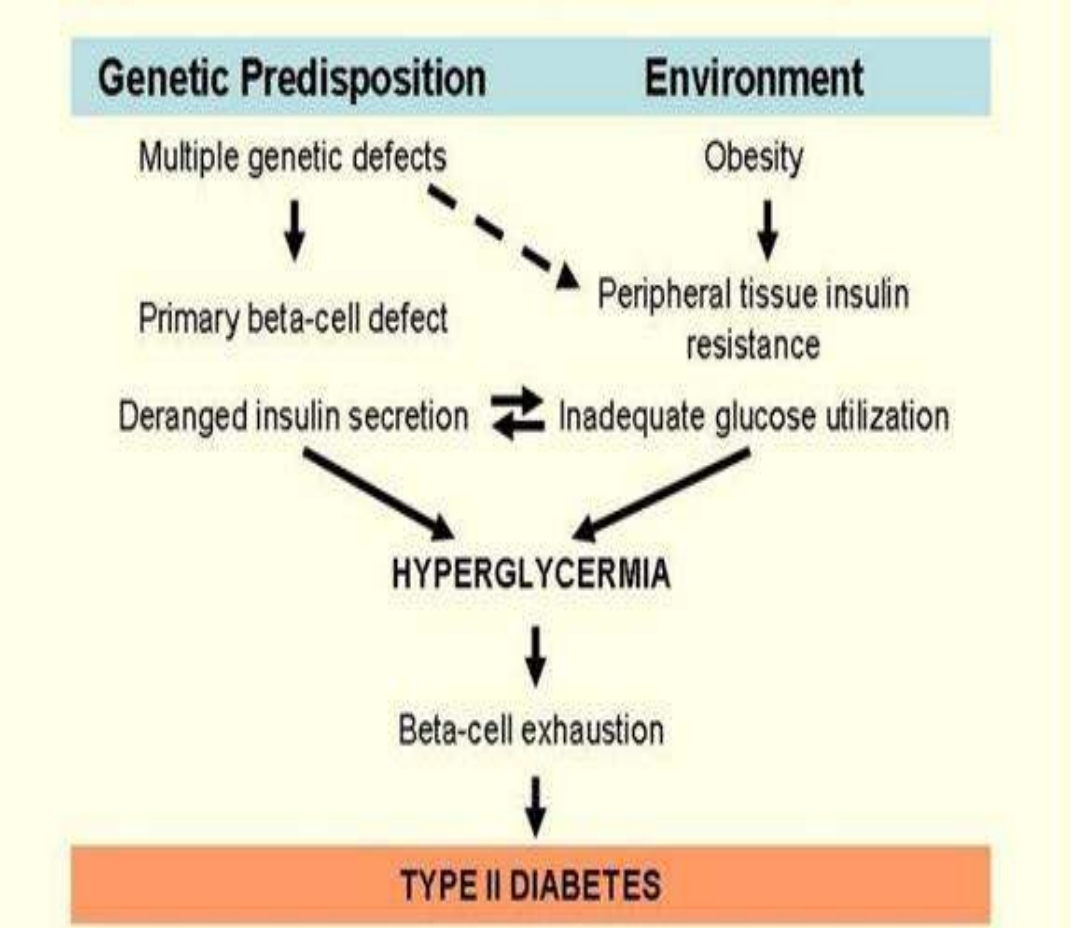

Figure 1.1. Pathophysiology of T2DM.

(http://ocw.tufts.edu/Content/51/lecturenotes/673764/674515).

1.1.2. Complications of Type2 Diabetes Mellitus

Type 2 diabetes mellitus is characterized by hyperglycaemia that leads to damage of nerves and blood vessels in several of body parts. Several years later, diabetics patient become suffering from many diabetes complications. Undiagnosed T2DM lead to severe complications such as renal failure and coronary artery disorders (Dhatariya, 2003; Smith, 2005). Furthermore, hypertension and dyslipidemia regularly are available in T2DM patients. In addition to acute diabetic completion such as hyperosmolar coma and ketoacidosis, patients with T2DM may have a chronic diabetic complication such as cardiovascular disease, retinopathy, nephropathy and polyneuropathy, as given in Figure 1.2.

Besides, though a clinical manifestation of diabetic complications is different, these signs are shared general pathophysiological symptoms. Between several factors that are needed for the study of diabetic complications is micronutrients play a significant role as preventive and treatment elements for common complications of diabetic. Magnesium is a micronutrient remarkable in this consideration. Some reviews demonstrate that T2DM distinguished by cellular and extracellular magnesium depletion that may result from a damaged tyrosine-kinase action at the insulin receptor level that responsible for the impedance in insulin activity and insulin resistance in T2DM (Maltezos et al., 1978; Johansson et al., 1982).

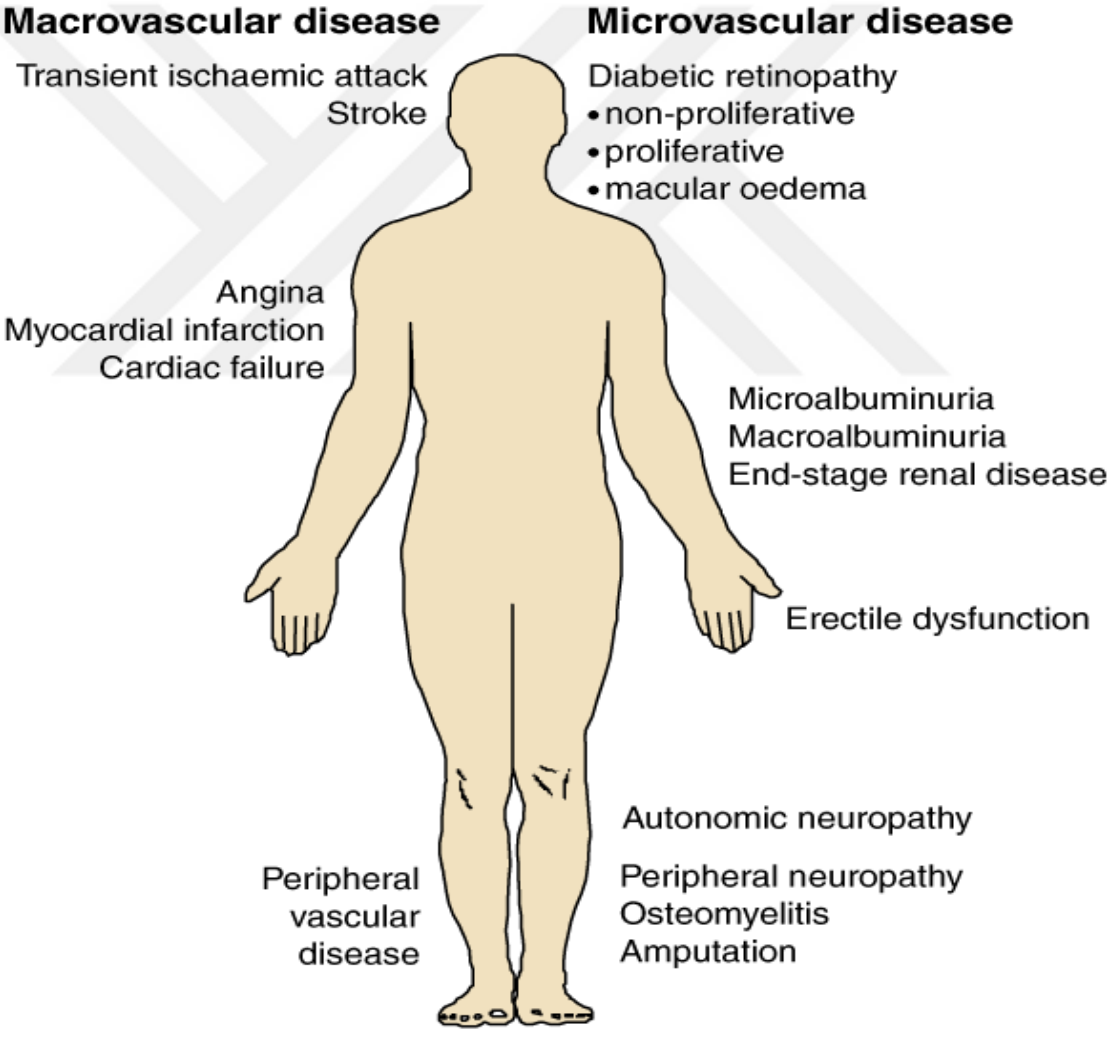

Figure 1.2. The diabetes mellitus complications (Bate and Jerums, 2003).

1.1.3. The Diagnosis of T2DM

T2DM is commonly diagnosed in children and adolescent when it is diagnosed the pancreas is still capable of producing adequate insulin and the body fails to utilize the insulin completely. Several years later, the pancreatic β-cells will be exhausted, insulin output decreases and glucose rise in the blood and the body is incapable of utilizing its main source of energy well. Moreover, the American Diabetes Association (ADA, 2017), diagnosis the type2 diabetes mellitus according to increased serum fasting glucose level or 2-hour serum glucose level after an oral glucose tolerance test, as shown in Table 1.1.

Table 1.1 Diabetes diagnostic criteria estimated by ADA (2017).

	Fasting glucose	**2-hour glucose**
	mmol/L	mmol/L
Normal	< 6.1	< 7.8
Impaired fasting glucose (IFG)	6.1≤ IFG <7.0	< 7.8
Impaired glucose tolerance (IGT)	< 7.0	≥ 7.8
Diabetes	≥ 7.0	≥ 11.1

1.2. Definition of Obesity

Obesity considers as a main epidemic problem in the world due to increases the number of obese individuals in a worldwide. Obesity defined as irregular or extra fat accumulation in adipose tissue this may lead to impair the health (Cummings et al., 1997). Being overweight or obesity constitutes a health risk and is considered as the main risk factor for several comorbidities including T2DM, dyslipidemia, hyperuricaemia cardiovascular diseases, etc. (Thomas et al., 2004). Due to its association with various complex diseases, increased mortality (Calle et al., 1999) and morbidity (Eckel et al., 1998). Obesity induced insulin resistance is a most important etiological feature in the pathogenesis of T2DM. Obesity particularly central obesity (visceral obesity) is the main risk factor for T2DM (Modan et al., 1986; Bjo¨rntorp, 1994; Boyko et al., 2000). Obesity is define based on weight in

kilograms -for- height in meters, so-called (BMI) body mass index (Yoon et al., 2006). BMI used as an indicator of obesity and is the main gauge in medicine for evaluation of the healthy weight, obesity, and overweight. BMI is counted by weight kg/ (height)² m² (Kopelman, 2000). Recent epidemiological reviews have revealed that death rate increases significantly with expanding body weight. Consequently, the risk of developing diabetes increases with increasing body weight (Gregg et al., 2004).

1.3. The 'Diabesity' the Obesity-Diabetes Link

T2DM and obesity are multifactorial disorders including interactions between genetic and environmental factors. Obesity is the main risk for subsequent development of T2DM. The link between diabetes and obesity is so noticeable 97% of T2DM patients are obese that some health authorities coined the term Diabesity (Zimmet et al., 2001). As explained by (Ford et al., 1997) the risk of diabetes rises between (4.5 - 9) percent for each kilogram of weight expansion. The progress from obesity to diabetes is due to defect in insulin secretion that follows the increase of insulin resistance (Warram et al., 1990). The defects in insulin secretion and resistance perform in the first stages of obesity development, which become worse as the patient becomes diabetic.

At '21st Century' the World Health Organization has described the increased prevalence of diabetes and obesity. The epidemic status of obesity accompanied with an increasing number of children and adolescents with T2DM (Caterson and Gill, 2002). The term diabesity derived from the diseases diabetic and obesity that is a group of a metabolic disorder associated with hyperglycemia result from a defect in insulin action and insulin secretion.

Diabesity is the main cause of heart disease, dementia, cancer, and premature death and affects about over 1billion people worldwide (Stumvoll et al., 2005), both diabetes and obesity are emerging pandemics that related with possibly life-threatening comorbidities and huge economic costs. Consequently, statistics presented that approximately 60 to 90 percent of totally T2DM patients are overweight/obese therefore the terms 'obesity-dependent diabetes' and 'diabesity' are generally utilized (Farag et al., 2011).

1.4. Epidemiology of T2DM and Obesity

The incidence of T2DM rising rapidly in the recent decades and expected to increase further so it becomes a common health problem in a world (Guariguata et al., 2014). The incidence of T2DM has increased around the world in adolescents and children equally with the increase in the rate of obesity. Epidemiological data in the US shows that diabetes accounts for over 77 billion US dollars in healthcare costs (Tracey, 2003). The prevalence T2DM identical to obesity. An increased incidence of obesity has taken attention in a world and any recent epidemiological studies have recorded the rapid increase in their prevalence that was recorded among all age, gender and racial/ethnic groups (Baskin et al., 2005).

In the other hand, in 2005 a data estimated by World Health Organization indicates that 1.6 billion adults were obese in a world. Newly information from the Third National Health and Nutrition Examination Survey (NHANES III) show that two-thirds of adults, both women as well as men, have BMI more than 27 kg/m^2 (Flegal and Troiano, 2000). The prevalence of diabesity becomes epidemic extends worldwide so the medical care costs for diabesity become a significant economic difficulty for patients, families, and society (Tunceli et al., 2005; Gustafson, 2006). At the start of this century, in 2014 an expected that 422 million adults were living with diabetes, as compared in 1980 to 108 million adults.

In 2016 the World Health Organization, reported that the worldwide incidence of diabetes has almost doubled over since 1980. That reflects a rise in related risk factors such as being obese or overweight.

1.5. Mechanisms Link Obesity To T2DM

Insulin resistance is a main pathogenic mechanism in T2DM and obesity (Scheen et al., 2003). At a recent time, the ‘twin cycle hypothesis’ has suggested by Taylor et al, for explaining the etiology of T2DM in obese patients as shown in Figure1.3.

Liver instead of muscle insulin resistance is included in an appearance of hyperglycemia in diabetes (Taylor et al., 2012). Insulin resistance in the muscle is associated with genetic and natural components that prompt to expanded serum insulin levels that with expanded a caloric overload will extend cumulating of fat in the liver. Thus, an increase of liver fat is causing hepatic insulin resistance leading

failure of insulin to restrain liver glucose creation. Subsequently, expanded plasma glucose and expanded insulin secretion will develop this lead to worsening hepatic statuses. This is the primary cycle (the liver cycle). Liver fat will be infiltrated, and related extraordinary fatty acids turnover then prompts an over-contact of the beta cells to fatty acids, which will damage insulin secretion in response to ingested food and this promote to postprandial hyperglycemia. Hyperglycemia will be promoted excite insulin secretion, which consequently increases liver lipogenesis, thus linking the liver cycle with the pancreas cycle.

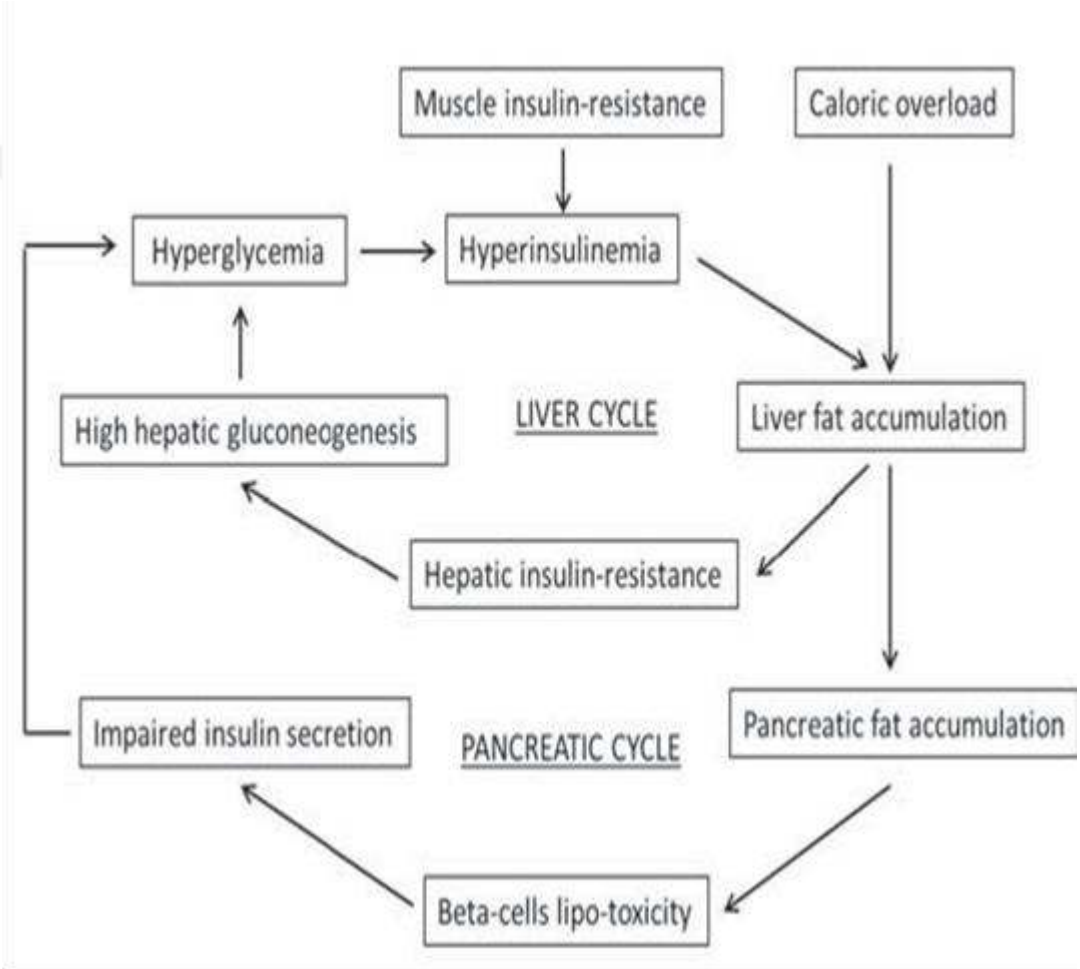

Figure 1.3. Twin cycle hypothesis. Muscle insulin resistance and the parts of muscle, liver, and pancreas in the incidence of T2DM in obesity (Cătoi et al., 2015).

1.6. Insulin Resistance

Insulin resistance (IR) is the main pathogenic factor in numerous metabolic disorders such as T2DM, obesity and it is a significant reason for cardiovascular disease and early death (DeFronzo et al., 1991). Which consequences are recognized by clinicians extend from endocrinologists to cardiologists (Krentz et al., 1996). IR

characterized by weakened affect ability to insulin of its target organs that are adipose tissue, liver, and muscle. At last decade, Gerald Reaven's was hypothesized that IR to the tissue is a component connecting T2DM, coronary heart disease and hypertension by which this three diseases are responsible for considerable morbidity and developed mortality worldwide (Reaven et al., 1988). HOMA-IR is an uncomplicated method for assessing insulin sensitivity. The HOMA-IR is widely used in large epidemiological studies (Matthews et al., 1985). Measurement of insulin resistance provides an early predictor of T2DM (Eriksson et al., 1989). The index of HOMA-IR is calculated by fasting glucose * fasting insulin / 22.95 (Gelloneze et al., 2006).

1.6.2. Mechanism Link Insulin Resistance to T2DM and Obesity

IR is a main characteristic of the metabolic disease and frequently developments to T2DM. The pathogenesis of T2DM associated by hyperglycemia causes by impair β-cell function and insulin resistance (Kahn, 2003; Abdul-Ghani et al., 2006). Furthermore, the earliest detectable abnormality in T2DM is probably the failure of target tissues to respond to insulin, known as insulin resistance, require extra insulin secretion in an attempt to stimulate the desensitized cells (Schrinner et al., 2005). About 80-90% of all types of diabetes accompanied by IR that generally precedes the first symptoms of the disease (Juhan-Vague et al., 1993; Hochholdinger et al., 1999). IR is caused by inadequate production or failure of insulin to stimulate target sites in a liver, muscle and adipose tissues due to reduced insulin levels or its activity. Consequently, this leads to extraordinary of glucose levels in the blood and finally to T2DM that named as non-insulin dependent diabetes mellitus (NIDDM), as summarized in Figure 1.6.

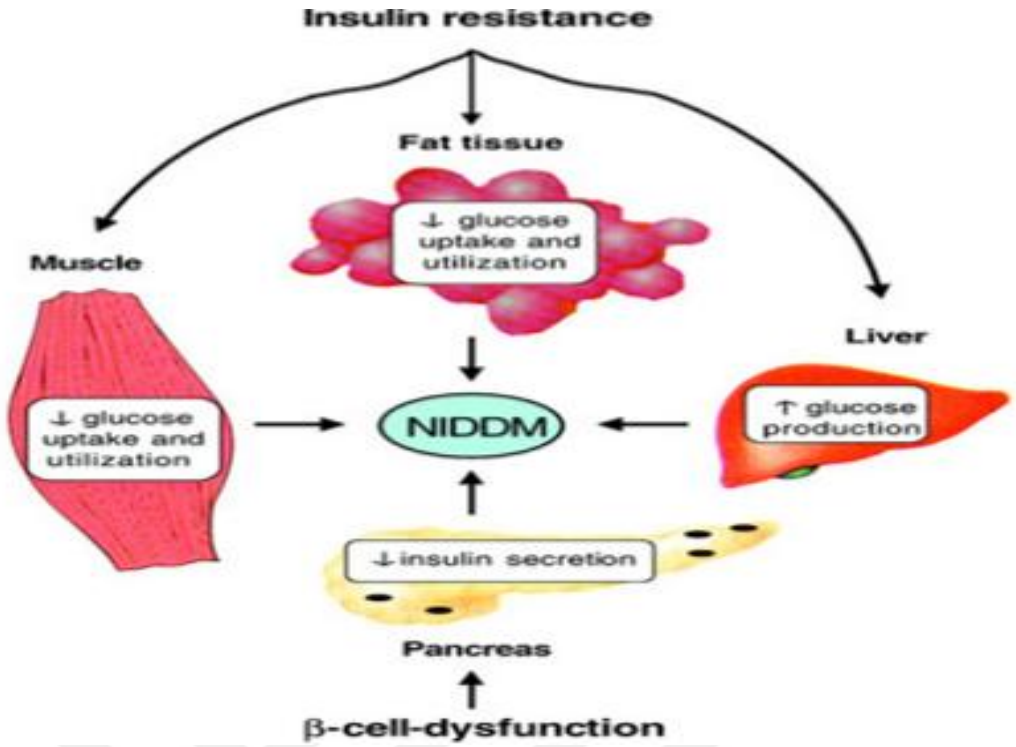

Figure 1.4. The resistance to insulin in target tissues that are liver, muscle and fat tissue, besides with failure of β-cell cause NIDDM (Baudry et al., 2002).

Insulin resistance is the main important link between obesity and T2DM. Furthermore, a variety of molecular and cellular defects has been included in the causation of IR. Obesity is considered as the most common underlying causative environmental risk factor for IR, aggravating the metabolic disturbance in T2DM (Kahn et al., 2001). IR correlates with obesity in diabetics as well as in non-diabetics developing gradually with weight gain (Boyko et al., 1996; Odeleye et al., 1997). IR is categorized by an alteration in glucose metabolism that results from suppression of the signaling pathway of insulin action. However, many elements are identified with an advancement of IR in fat people, being with excessive body fat is the fundamental cause. Increase abdominal fat prompt to discharge multiple amounts of free fatty acids that specifically influence insulin signaling, decrease glucose uptake in muscle, lead to excessive triglyceride production and prompt gluconeogenesis in the liver. In T2DM and obesity, insulin resistance is associated with decreased insulin-stimulated glucose transport, metabolism in skeletal muscle and adipocytes and by reduced suppression of hepatic glucose production to the visceral fat store as well as to ectopic site (Kaplan, 1989). In general, IR does not create in all fat people a hereditary basis takes an interest in insulin resistance, even in non-fat people.

1.7. Magnesium

Magnesium (Mg) is an important mineral in the human body (Pham et al., 2007). Mg is subdivided into three essential parts in the human body: around 65% in the mineral period of the skeleton, 34% in the intracellular space, and just 1% in the extracellular fluid (Barbagallo et al., 2003). Mg homeostasis is maintained in the human body by the intestine, the bone, and the kidney. Briefly, Mg is consumed through the gut put away in bones and discharged through kidney if overabundance (Fine et al., 1991). The small digestive tract is the main site for Mg assimilation; while Mg discharge is, for the most, part performs through renal pathways (Saris et al., 2000), as summarized in Figure 1.2.

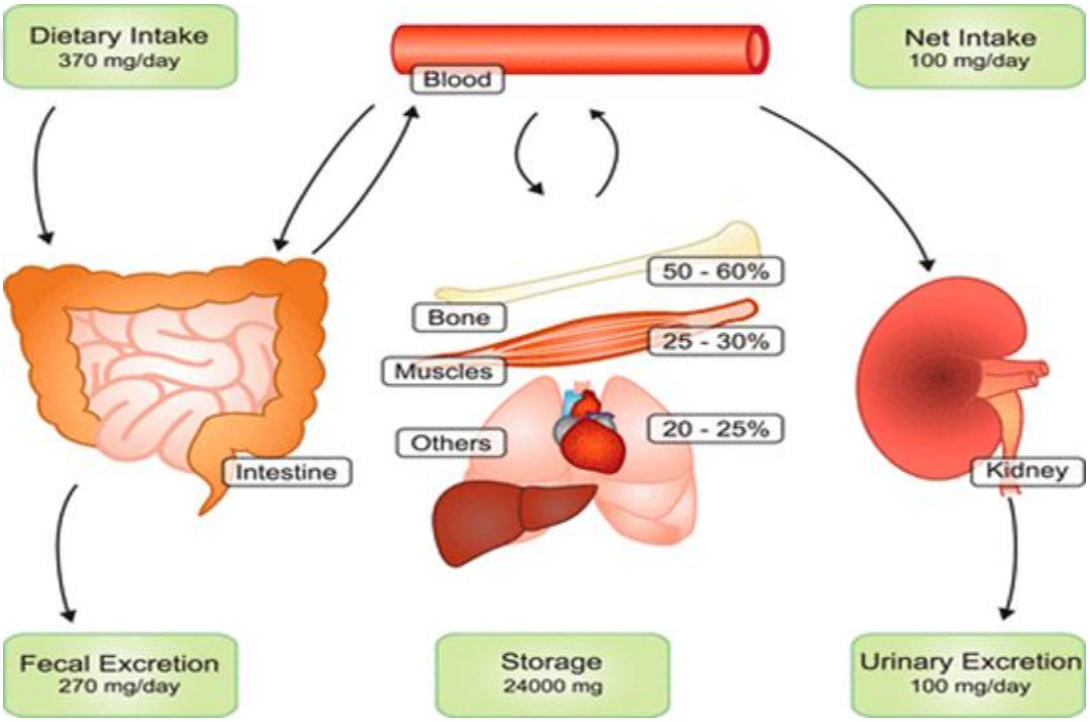

Figure 1.5. Magnesium homeostasis (De Baaij et al., 2015).

1.7.1. Physiological Role of Magnesium in Human Body

Magnesium is a primary co-factor required for many biochemical reactions and it plays an essential role in glucose metabolism. It is primarily found in the cell, it is necessary for glucose transportation between membranes, glucose oxidation, all reactions involving phosphorylation and energy exchange. Mg involves in further than 300 enzymatic responses that required in energy metabolism, nucleic acid and protein production, transport of calcium and potassium ions, signal transduction and

proliferation of the cell (Elin et al., 1987). It is essential for insulin action since it is a cofactor of tyrosine kinase activity (Paolisso et al., 1989). In addition, it plays as a cofactor for all enzymatic reactions that request kinases (Sanders et al., 2000). Magnesium is a part of GTPase and it it is a cofactor for many enzymes that are significantly dependent on magnesium such as Na^+/K^+-ATPase, adenylate cyclase and phosphofructokinase (Grubbs et al., 1987). Mg may be required for substrate arrangement, as an allosteric activator of enzyme activity, and for membrane stabilization (Hexum et al., 1970). It is a basic chemical activator for neuro muscular volatility and cell penetrability, a controller of particle channels and mitochondrial function.

Magnesium deficit is evaluated by assessing the total concentration of serum magnesium, the value between (1.9-2.7mg/dl) was considered normal. Hypomagnesaemia identifies as serum magnesium values lower than 0.7 mmol/L patients with hypomagnesaemia suffering from nonspecific symptoms including sadness, tiredness, and muscle weakness. Mg depletion is commonly related to further electrolyte abnormalities such as hypokalemia and hypocalcaemia. Hypomagnesaemia also has been accounted as an important factor related to type2 diabetes mellitus, insulin resistance, and metabolic disorder. Only serious Mg depletion may relate to the higher risk of various preclinical and clinical results, involving atherosclerosis, hypertension, cardiac arrhythmias, stroke, alterations in lipid metabolism, osteoporosis and in addition depression and other neuropsychiatric disorders.

1.7.2. Mechanisms Link Magnesium to Insulin Resistance in T2DM and Obesity

Magnesium plays the main role in carbohydrate metabolism. Magnesium depletion in the body is the significant reason of insulin resistance in the cells. Once the insulin released by the pancreas magnesium guarantees that insulin, receptor sites are clear for insulin to enter the cells with glucose (Jerry and Nadler, 2000). However, due to Mg is the main cofactor in all ATP transfer reactions, which suggest that Mg level important in the phosphorylation of the insulin receptor and the insulin signaling mechanisms that participate in glucose transport. Insulin bind to specific cell surface receptors this activates the tyrosine kinase phosphorylation at the intracellular part of the receptor. The initiation of the protein kinase by the insulin receptor is a critical step in transmembrane signaling for insulin action. Mg deficiency may lead to a

defect of tyrosine–kinase activity at the insulin receptor level. Mg depletion is related to a decrease in the capacity of insulin to stimulate glucose take-up in insulin-sensitive tissues, such as fat cells and skeletal muscle tissues this may lead to decreased cellular glucose utilization. Decreased intracellular magnesium concentration lead to expanding of IR in diabetic patients (Barbagallo and Dominguez, 2006), as shown in Figure 1.4.

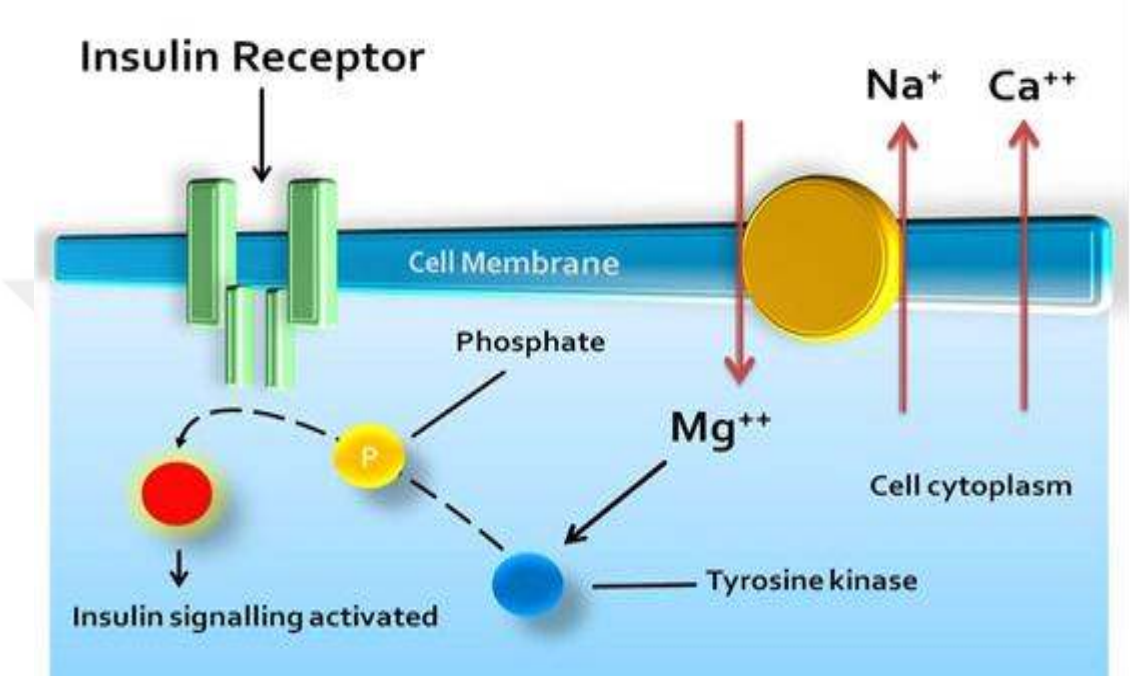

Figure 1.6. How magnesium activates the insulin receptor. (http://www.nutridesk.com.au/what-is-magnesium.phtml).

On the other hand, newly studies have also exposed large interest about the impact of minerals in obesity. Especially, magnesium plays a significant function in the energy metabolism (Elin et al., 2010; Volpe et al., 2013). Mg depletion is a serious problem with a worldwide relation that shows impact the appearance of metabolic disease recognized in obesity such as insulin resistance, diabetes mellitus, oxidative stress, and chronic inflammation. These disorders are conversely connected with intracellular magnesium concentration (Su et al., 2013). Modern studies linked obesity with low serum Mg. Low Mg condition distinctly occurs more predominately in obese individuals compared in nonobese individuals (Rodríguez-Morán et al., 2004). Obesity is characterized by high risk for T2DM, cardiovascular disease, dyslipidemia, and insulin resistance (Guerrero-Romero et al., 2004).

1.7.3. Hypomagnesaemia In Type2 Diabetic Mellitus Patients

The major reason of hypomagnesaemia in patients with T2DM are not observable however they may incorporate lower dietary consumption of Mg, lower intestinal Mg absorption, increased urinary losses of Mg or reduced Mg uptake into the cells as compared to healthy individuals (Elin, 1994). An increase of Mg depletion incidence was identified in T2DM patients, particularly in persons with longer duration of diabetes and uncontrolled glycemic profiles. Hypomagnesaemia can be both an outcome and a reason for diabetic complications (Mather et al., 1979; Del et al., 2012). Patients who expended large amounts of magnesium particularly from magnesium-rich food for example all grains, nuts, and green leafy vegetables may decrease the danger of developing T2DM. Mg depletion, such as by its impact on inositol transport, is of pathogenic consequence in the development of diabetic complications as shown in Figure 1.6.

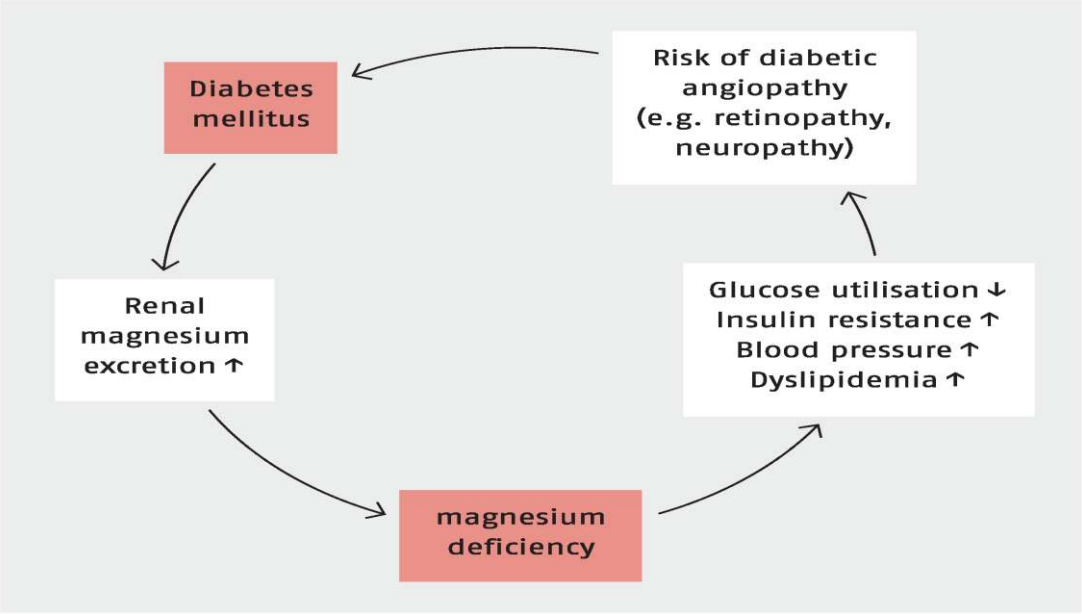

Figure 1.7. Magnesium deficiency and diabetes. (https://openi.nlm.nih.gov/detailedresult.php?img=PMC4586582_nutrients-07-05388-g002&req=4).

Some studies have shown that Mg can affect the emergence and development of atherosclerosis through making changes in inflammatory processes in damages caused by cell oxidation, increasing the levels of serum LDL-C, and through stimulating growth factors. Mg does not directly augment the synthesis of

lipoproteins; however, it may affect the arrangement of enzymes responsible for the synthesis of lipoprotein in the liver.

Consequently, they are more effective in changing the metabolism of triglycerides (Ghorbani et al., 2012; Rafieian-Kopaie et al., 2013).

1.8. Aim of The Study

Several studies show decreased intracellular magnesium concentration lead to expanding of insulin resistance in diabetic patients (Jerry and Nadler, 2000; Barbagallo and Dominguez, 2007). From that point, this thesis aimed to:

i. Evaluate the relationship between serum magnesium level and insulin resistance in obese non-diabetic subjects and compared to healthy control.
ii. Evaluate the relationship between serum magnesium level and insulin resistance in obese type 2 diabetes mellitus patients in two groups, group with duration of disease history of diabetic less than one year and another group with a disease history of diabetic more than five years than compared to obese non-diabetic subjects and healthy control subjects.
iii. In addition, evaluation the level of serum total cholesterol, LDL-C, triglycerides and serum creatinine in obese non-diabetic and obese type 2 diabetes mellitus patients. Then compared them with control groups.
iv. Furthermore, if magnesium deficit is identified it must be handled since non-treated hypomagnesaemia can cause lead to increases diabetic complication and chronic disorders such as atherosclerosis, myocardial infarction, hypertension and renal calculi.

CHAPTER 2

LITERATURE REVIEW

Hyperglycemia, insulin resistance, and relative damage in insulin secretion are the main characterize type 2 diabetes mellitus (T2DM) (Kalantet al., 1979).

Ferrannini (1989) was demonstrated that obese T2DM patients are characterized by decreased suppression of glucose production during hyperinsulinemia.

Juhan-Vague et al (1993) they have been accounted about 80-90% of all types of diabetes brought by insulin resistance that generally precedes the first symptoms of the disease.

Hepatic insulin resistance has been intended as responsible for this enhanced glucose production. T2DM a hereditary disorders that can be managed by diet, hypoglycemic agents or exogenous insulin (Groop & Tuomi, 1997).

The major risk factors for developing T2DM consisting of family history, diet, and absence of physical movement (Groop et al., 1997).

The incidence of T2DM rising rapidly in the current decades and expected to increase further so it becomes a common health problem and firmly associated with the increasing prevalence of obesity (Songer and Zimmet, 1995). Some other review expected that the number of the general public with diabetes was 150 million in 2000 and this number is estimated to increase by 2025 (King et al., 1998).

Tracey (2003) was assessed the incidence of T2DM has increased around the world in adolescents and children equally with the increase in the rate of obesity. Epidemiological data in the US shows that diabetes accounts for over 77 billion US dollars in health care costs. In 2012, an expected 1.5 million deaths were produced by diabetes mellitus in addition to 2.2 million causes of deaths were attributable to high blood glucose.

WHO estimated that the 7th leading cause of death in 2030 will be the diabetes mellitus (Mathers et al., 2006). Obesity prevalence is developing quickly between adults with and without diabetes (Calle et al., 1999).

This has important associations for the probable increase of the people with T2DM and diabetes-related conditions. This increase in mortality is mostly arbitrated by increased incidence of T2DM, hypertension, and hyperlipidemia. As described by (Ford et al., 1997) the diabetes risk increases between (4.5 - 9) percent for each kilogram of weight gain. In general, Obesity is associated with insulin resistance and hyperinsulinemia and is the main risk factor for the development of T2DM and cardiovascular disease (Pratley and Weyer, 2002).

Being overweight or obesity creates a health risk and is considered as the main risk factor for several comorbidities including T2DM, dyslipidemia, hyperuricaemia cardiovascular diseases, etc. The prevalence of T2DM identical to obesity (Thomas et al., 2004).

An increased incidence of obesity has taken attention in a world and any recent epidemiological studies have recorded the rapid increase in their prevalence that was recorded among all age, gender, and racial/ethnic groups (Baskin et al., 2005).

In general, about 80 % of individuals suffering from T2DM are obese especially those with central visceral adiposity (Kim et al., 2009).

T2DM is related with obesity older age, family history of diabetes and lack of exercise. The prevalence of T2DM is increasing quickly in the current decades and expected to increase further so it becomes a common health problem in a world (Guariguata et al., 2014).

Insulin resistance (IR) mostly characterizes by T2DM, but damage in insulin secretion also occurs in T2DM (Lebovitz, 1999).

Kahn, (1998) has noticed that insulin resistance in is a most important pathogenic cause of T2DM. IR is the main important connection between obesity and T2DM and is the most prevalent in obese subjects (Abdul-Ghani et al., 2006).

The pathogenesis of DMT2 associated with hyperglycemia causes by impair β-cell function and insulin resistance as explained by (Kahn, 2003).

T2DM is characterized by hyperglycemia related with disabled insulin activity in target tissues i.e. liver, muscle and fat tissue (insulin resistance) and impaired insulin secretion through progressive beta cell dysfunction (Kahn et al., 2003; Sarkar et al., 2010).

Magnesium is an essential cofactor required for many biochemical reactions. It is not only an important factor in glucose metabolism and but also is required for the phosphorylation of the insulin receptor and the insulin signaling mechanisms that participate in glucose transport (Paolisso et al., 1989).

DMT2 patients recognized by reduction of cellular and extracellular Mg that might result from a damaged tyrosine-kinase action at the insulin receptor level which in charge of the impedance in insulin activity and insulin resistance in T2DM patients (Maltezos et al., 1978; Johansson et al., 1982).

As well as, in 1989 Paolisso et al they reported Mg^{2+} is a fundamental element for insulin action since it is a cofactor of tyrosine kinase activity. As reported by Nadler et al., (1993) Mg deficiency manifest to have a harmful impact on glucose homeostasis and insulin sensitivity in T2DM patients.

In 1995 Ma et al, have demonstrated lower serum Mg levels in T2DM compared to healthy subjects. Mg depletion in the body is the significant reason of insulin resistant in the cells. Once the pancreas releases insulin, Mg guarantees that the insulin receptor sites are clear for insulin to enter the cells with glucose (Jerry, 2000).

Takaya et al., (2004), demonstrated the main mechanisms by which Mg affected the pathological process associated with T2DM included alteration of insulin sensitivity via action on tyrosine kinases thus creating a post receptor defect. In addition, De Leeuw et al., (2004) reported the co- morbidities associated with T2DM appear to be influenced by Mg insufficiency.

Mg depletion is related to a decrease in the capacity of insulin to stimulate glucose uptake in insulin-sensitive tissues for example fat cells and skeletal muscle tissues this may lead to decreased cellular glucose utilization. Decreased intracellular Mg

concentration lead to expanding of insulin resistance in diabetic patients (Barbagallo and Dominguez, 2006).

Pham et al., (2007) have been described Mg deficiency related to poor glycemic control, hypertension, coronary artery diseases and diabetic neuropathy and foot ulcerations. Serum Mg levels are inversely related to the severity of diabetes (Nasri et al., 2008).

An increase of Mg depletion incidence was identified in T2DM patients, particularly in persons with longer duration of diabetes and uncontrolled glycemic profiles.

Hypomagnesaemia can be both an outcome and a reason for diabetic complications (Del Gobbo et al., 2012). Mg depletion was found in T2DM patients and it may be a preceding factor in IR and hyperinsulinemia (Chutia and Lynrah, 2015).

CHAPTER 3

PATIENTS AND METHODS

3.1. Subjects

The present study consists of 120 subjects of both sexes within the age range between 20 to 70 years. All subjects were divided into four group:

Group I: Included 30 healthy human volunteers as a control group (8 male and 22 female). A healthy individual were all in good physical health and with no family history of obesity or diabetes.

Group II: Included 30 obese non- diabetic patients (6 male and 24 female).

Group III: Included 30 patients of obese type 2 diabetes mellitus (14 male and 16 female) with disease history less than one year.

Group IV: Included 30 obese type 2 diabetes mellitus (17 male and 13 female) with disease history more than five years. The clinical evaluation and the diagnosis for type 2 diabetes mellitus patients were done by specialist physicians in endocrinology and metabolism department of Gaziantep University General Hospital, Turkey, between November 2016 and February 2017.

3.2. Clinical and Anthropometric Measurements

Informed consent was taken from all patients and healthy control subjects. A pre-structured and pretested proforma was used to collect the data. Detailed baseline clinical characteristics data were performed from all subjects underwent when they were clinically stable at baseline, medical records included basic demographics: Age, gender, weight, height and body mass index (BMI).

All subjects that involved in this study were questioned about the family history of obesity, diabetes, systemic diseases, drug administration such as corticosteroids and cigarette smoking. In addition, all subjects were clinically examined to conclude the

presence of exclusion state that includes pregnancy, patients taking magnesium supplementation, alcohol consumption, cardiovascular disease, chronic disorders, and malignancy. Anthropometric measurements were measured for body height and weight by standing the patients without shoes and least of clothing by using a digital machine (Davi & Cia Weighing Equipment, Barcelona, Spaın). Moreover, Body Mass Index (BMI) was measured by this formula: BMI = weight (kg)/height^2 (m^2). The degree of IR was determined for all study subjects by using homeostasis model of assessment (HOMA) method that defined by (Matthews et al., 1985). The index (HOMA-IR) was calculated by the formula: Fasting plasma glucose (mmol/L) × Fasting serum insulin (μU/ml) divided by 22.59 or Fasting plasma glucose (mg/dl) × Fasting serum insulin (μU/ml) divided by 405. The Healthy Range of HOMA-IR in an adult was <2.7. When HOMA-IR was higher than 2.7 patients were classified as insulin resistant (Gelloneze et al., 2006).

3.3. Clinical and Laboratory Evaluation

After (12-14) hours overnight fasting in the morning period in Gaziantep University General Hospital Laboratory, (Turkey). A 5 mL venous blood samples were withdrawn taken from the antecubital vein using a BD Vacutainer needle, a tourniquet was used while the participant was sitting, after antiseptic preparation of the venipuncture site. A venous blood sample collected in (VACUETTE® Tube, 8 ml, U.S, Z Serum Separator Clot Activator containing microscopic silica particles activates clotting) and (BD Vacutainer® SST™ II Tube, 5.0 ml, UK, Clot activator and gel for serum separation serum containing silicone coated interior), as present in Figure 3.1.

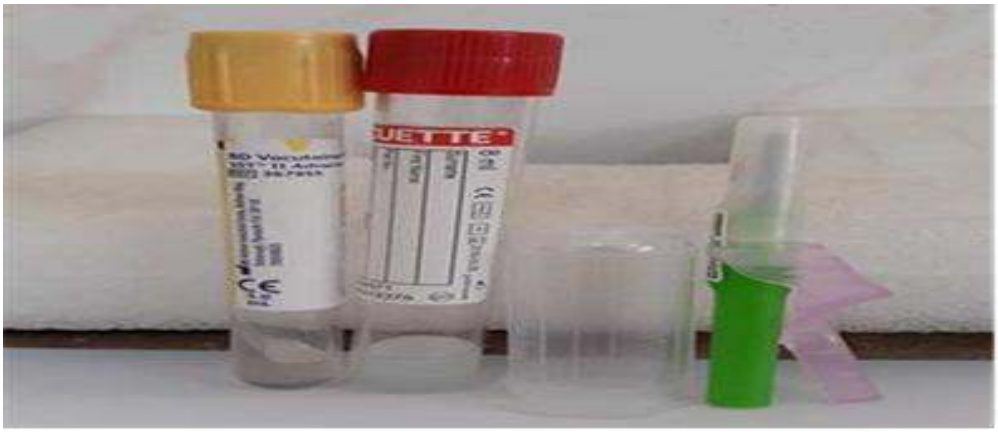

Figure 3.1. BD Vacutainer needle and tube.

Samples were allowed to clot at room temperature for 5 to 10 minute then serum separation was performed for each sample by using blood samples centrifuge model (UNIVERSAL 320/ Germany) for 4000 rpm×10 min, as present in Figure 3.2.

Figure 3.2. Blood samples centrifuge model (UNIVERSAL 320/ Germany).

In addition, we extracted the serum with an automatic pipette, kept into a demineralized micro Eppendorf tube. Aliquots of serum were immediately frozen stored at ─ 80°C prior to the day of automatics analytic measurement of serum magnesium, serum insulin, triglycerides, cholesterol, LDL-cholesterol and serum creatinine were completed. Excepting for serum fasting glucose, which was determined directly after blood was drawn. All samples were collected to estimation analytic measurement of biochemical parameters for serum magnesium, serum fasting glucose, total cholesterol, LDL-cholesterol, and triglycerides were based on the photometric enzymatic method procedure using clinical chemistry laboratory instrument (Beckman Coulter® Model Au5800, Tokyo, Japan), as shown in Figure 3.3 and use the lab test kits for each parameter. While the assay of serum insulin was based on chemiluminescent immunoassay method by using clinical laboratory instrument (Beckman Coulter® Dxİ 800 Tokyo, Japan), as shown in Figure 3.3 and use lab test kits for Access Ultrasensitive Insulin.

Figure 3.3. Beckman Coulter® Model DXİ 800.

Figure 3.4. Beckman Coulter® Model Au5800.

3.4. Biochemistry Laboratory Analysis

3.4.1. Measurements of Serum Fasting Blood Glucose

The principle for serum fasting glucose was measured based on a photometric enzymatic process using clinical chemistry laboratory instrument BECKMAN COULTER® model Au5800 (Tokyo, Japan 2014) with using kits test (BECKMAN COULTER® glucose reagent OSR6221 4 x 53 mL R14 x 27 mL R2, Ireland). The Beckman Coulter analyzers automatically compute the glucose concentration of each sample according to principles defined by the American Diabetes Association. The normal values for fasting serum glucose in an adult was (75 -100 mg/dl).

The methodology of serum fasting glucose was determined according to Beckman Coulter procedure, glucose phosphorylates in the presence of adenosine triphosphate (ATP) and Mg^{+2} by the action of hexokinase (HK) to yield glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). The glucose-6-phosphate dehydrogenase (G6P-DH) was oxidized G-6-P to 6-phosphogluconate with the reduction of nicotinamide adenine dinucleotide (NAD^+) to nicotinamide adenine dinucleotide, reduced (NADH). The absorbance at 340/380 nm, (Czok and Barthelmai, 1962).

$$\text{Glucose + ATP} \xrightarrow{\text{HK, } Mg_{2+}} \text{G-6-P + ADP}$$

$$\text{G-6-P + } NAD^+ \xrightarrow{\text{G6P-DH}} \text{6-Phosphogluconate + NADH + } H^+$$

Final concentration of reactive ingredients:

PIPES buffer (pH 7.6)	24.0 mmol/L
Mg^{2+}	2.37 mmol/L
ATP	≥ 2.0 mmol/L
NAD^+	≥ 1.32 mmol/L
G6P-DH	≥ 1.58 kU/L
Hexokinase	≥ 0.59 kU/L

The reagents are prepared for use and placed it a directly onboard instrument.

Calibration for serum glucose was done by use of the Beckman Calibrator material (Cat. No. 66300, which is definite to the National Institutes of Standards and Technology (NIST) Standard Reference Material (SRM) 965 for serum determinations.

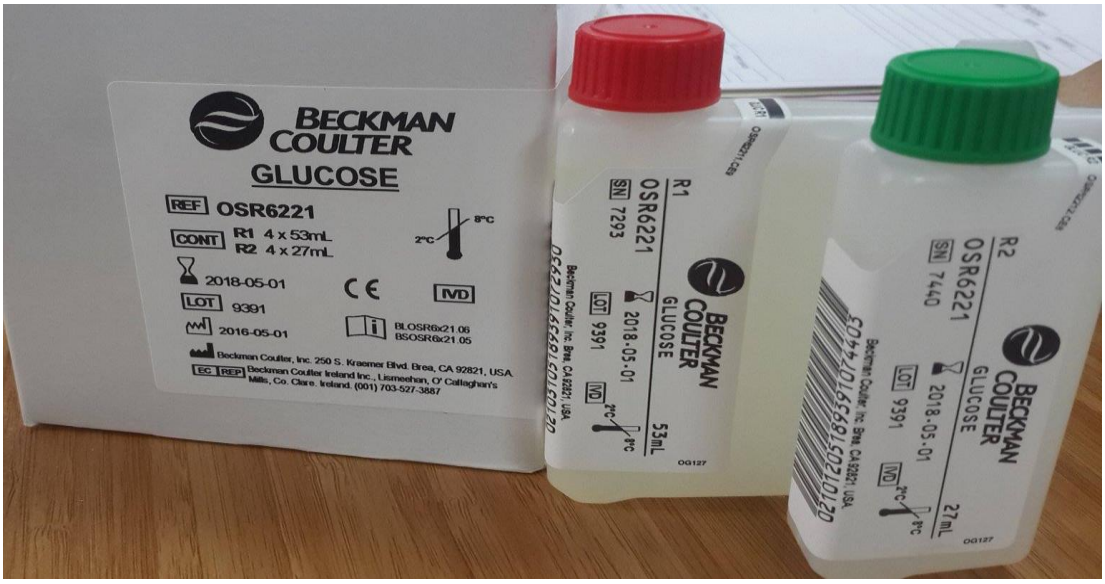

Figure 3.5. BECKMAN COULTER® system glucose reagent.

3.4.2. Measurements of Serum Insulin.

The principle for serum insulin level was measured based on chemiluminescent immunoassay method by using clinical laboratory instrument Beckman Coulter® DXİ 800 (Tokyo, Japan 2014).

Methodology for the Access Ultrasensitive Insulin assay was a simultaneous one-step immune-enzymatic "sandwich" assay. A sample was added to a reaction container with mouse monoclonal anti-insulin alkaline phosphatase conjugate and paramagnetic particles covered with mouse monoclonal anti-insulin antibody (Allauzen et al., 1994; Allauzen et al., 1995). The serum insulin attaches to the antibody on the solid phase during the conjugate reacts with several antigenic sites on the insulin molecule. Following incubation in a reaction container, materials attached to the solid phase are held in a magnetic field while unattached materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 was added to the container and light produced by the reaction is measured with a luminometer. The light production was equal to the concentration of insulin in the sample. The amount of analyte in the sample was determined from a stored, multi-point calibration curve.

Access Ultrasensitive Insulin Reagent Pack Cat. No. 33410: 100 determinations, 2 packs, 50 tests/pack, Figure 3.6. Store upright and refrigerate at 2 to 10 °C.

R1a: Mouse monoclonal anti-insulin linked to paramagnetic particles, TRIS buffer, bovine serum albumin (BSA) matrix, < 0.1% sodium azide, and 0.1% ProClin**

300. (ProClin™ was a label of The Dow Chemical Company “Dow” or a related company of Dow).

R1b: Mouse monoclonal anti-insulin conjugated to bovine alkaline phosphatase, TRIS buffer, BSA matrix, < 0.1% sodium azide, and 0.1% ProClin 300.

R1c: Mouse IgG in HEPES buffer, BSA matrix, < 0.1% sodium azide, and 0.5% ProClin 300.

Testing Procedure:

i. Mixed the contents of fresh reagent packs by gently inverting pack several times before loading on the instrument.

ii. Use 20 µL of the sample for every determination in addition to the sample container and system dead volumes. And use 50 µL of the sample in addition to the sample container and system dead volumes for every determination run with the DXİ system onboard dilution feature.

iii. The system default unit of measure for sample results was µIU/ml. The normal value of serum insulin in an adult was (1.9-23µU/ml).

Figure 3.6. Beckman Coulter® insulin kit.

3.4.3. Measurements of Serum Magnesium

The principle for serum magnesium concentrations was measured depending on a photometric enzymatic process by using clinical chemistry laboratory analyzers Beckman Coulter® AU5800 (Tokyo, Japan 2014) with using kits test for magnesium (BECKMAN COULTER® Reagent OSR6189 R1 4×40ml). The values between (1.9 - 2.7 mg/dl) were considered normal.

The magnesium methodology was made by Beckman Coulter AU System magnesium procedures utilize a direct process in which a colored complex with Xylidyl blue in a greatly basic solution, where calcium interference is canceled by glycoletherdiamine-N, N, N`, N`-tetra acetic acid (GEDTA), (Mann et al., 1957; Bohuonet al., 1962). The produced color was measured bichromatically at 520/800 nm and is equivalent to the magnesium concentration.

Mg^{2+} + Xylidyl blue $\xrightarrow{\text{pH 11.4}}$ Purple complex

Final concentration of reactive ingredients:

Amino-n-Caproic Acid	450	mmol/L
Tris	100	mmol/L
Glycoletherdiamine-N,N,N',N' tetraacetic acid	0.12	mmol/L
Xylidyl blue	0.18	mmol/L

The Beckman Coulter AU System Mg Reagent is liquid, ready for use, Figure 3.7. Therefore, we placed it directly onboard the instrument.

Calibration for serum magnesium method done by use of the Beckman Calibrator material (Cat No. DR0070), which is visible to the National Institutes of Standards and Technology (NIST) Standard Reference Material (SRM) 909b for serum and plasma determinations.

Figure 3.7. BECKMAN COULTER® Magnesium Reagent.

3.4.4. Measurements of Serum Total Cholesterol

The principle for serum fasting total cholesterol was measured based on the photometric enzymatic process by using clinical chemistry laboratory analyzers BECKMAN COULTER® model Au5800 (Tokyo, Japan, 2014) with using test kits (BECKMAN COULTER® Cholesterol Reagent OSR6216 4 x 45 ml). The normal range in an adult was between (100-200mg/dl).

The cholesterol methodology was a modification made by (Allain et al., 1974). In this process, cholesterol esters in a specimen are hydrolyzed by cholesterol esterase (CHE). The free cholesterol generated are oxidized by cholesterol oxidase (CHO) to cholestene-3-one with the concomitant production of hydrogen peroxide (H_2O_2) that oxidatively combined with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) to yield chromophore. The red quinonimine dye produced can be measured spectrophotometrically at 540/600 nm as an increase in absorbance.

$$2 \text{ Cholesterol esters} + 2\ H_2O \xrightarrow{\text{CHE}} 2 \text{ Cholesterol} + 2 \text{ Fatty acids}$$

$$2 \text{ Cholesterol} + 2\ O_2 \xrightarrow{\text{CHO}} 2 \text{ Cholestene-3-one} + 2\ H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4H_2O$$

Final concentration of reactive ingredients

Phosphate buffer (pH 6.5)	103 mmol/L
Peroxidase	≥ 10.0 kU/L
4-Aminoantipyrine	0.31 mmol/L
Cholesterol oxidase	≥ 0.2 kU/L
Phenol	5.2 mmol/L
Cholesterol esterase	≥ 0.2 kU/L

The reagent is ready for use and positioned directly on board the instrument, Figur 3.8.

Calibration of serum cholesterol procedure was performed by use of the chemistry calibrator Cat no DR0070.

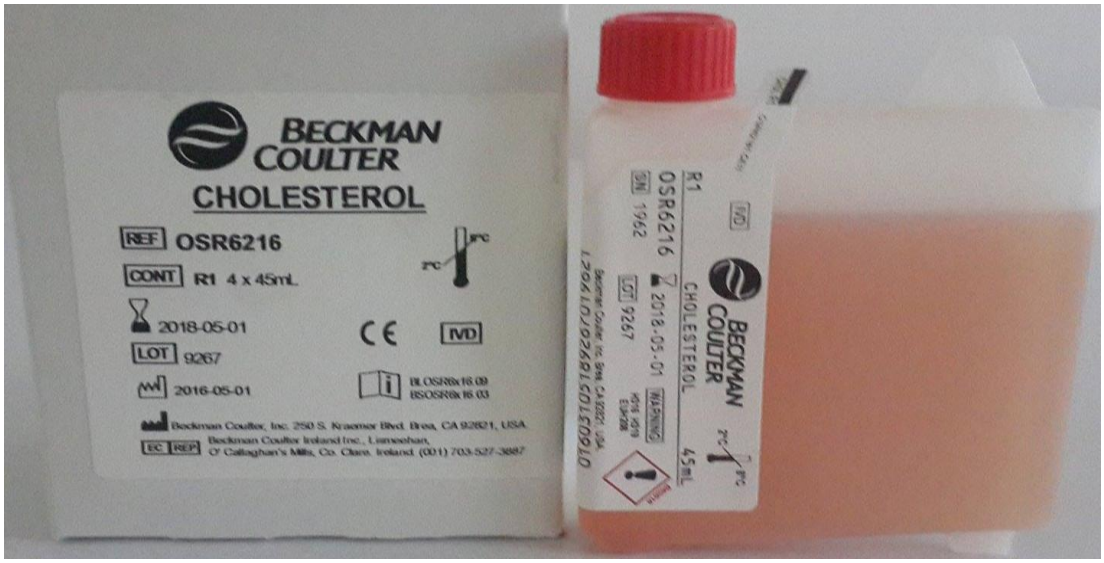

Figure 3.8. BECKMAN COULTER® Cholesterol Reagent.

3.4.5. Measurements of Serum LDL- Cholesterol

The principle for serum LDL- cholesterol based on a photometric enzymatic process by using clinical chemistry laboratory analyzers BECKMAN COULTER® model Au5800 (Tokyo, Japan, 2014) with using the test kits (BECKMAN COULTER® LDL- cholesterol Reagent OSR6283 4 x 51.3 mL R1, 4 x 17.1 mL R2).

The LDL-cholesterol methodology was a modification done by (Miki, 1999). A protecting agent in R1 protects LDL from enzymatic reactions. All non-LDL lipoproteins (HDL, VLDL, and CM) are removed by the action of cholesterol esterase (CHE) and cholesterol oxidase (CHO). Hydrogen peroxide (POD) produced by this reaction was disintegrated by catalase in R1. While R2 was added, the protecting reagent was released from LDL and catalase inactivated by sodium azide. LDL can be quantified by the CHO/PAP system. The healthy value was (57-129 mg/dl).

$$2\text{LDL-cholesterol} + 2H_2O + 2O_2 \xrightarrow{\text{CHE and CHO}} 2\text{ Cholest-4-en-3-one} + 2\text{Fatty acids} + 2H_2O_2$$

Deprotecting Reagent

$$2\ H_2O_2 + 4{-}AA + \text{HDAOS} \xrightarrow{\text{POD}} \text{Blue Dye+} + OH^- + 3\ H_2O$$

Calibration of serum LDL-Cholesterol procedure was performed by use of the chemistry calibrator Cat no ODC0012.

The final concentration of reactive ingredients:

Cholesterol esterase	3.7 IU/ml
Cholesterol oxidase	3.7 IU/ml
Peroxidase	4.9 IU/ml
Sodium azide	0.1 %
Goods Buffer (pH 6.8)	25 mmol/l
4-aminoantipyrine	0.8 mmol/l
Catalase	743 IU/ml
HDAOS	0.47 mmol/l

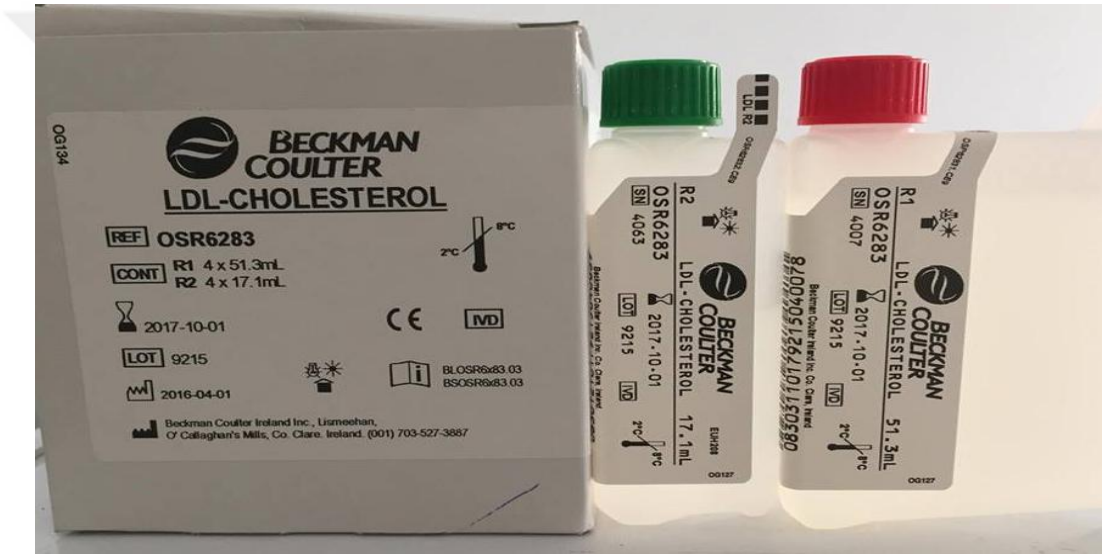

Figure 3.9. Lab test kits (BECKMAN COULTER® LDL- cholesterol Reagent.

3.4.5. Measurements of Serum Triglyceride

The principle for serum fasting triglyceride based photometric enzymatic process by using clinical chemistry laboratory analyzers BECKMAN COULTER® model Au5800 (Tokyo, Japan, 2014) with using the test kits (BECKMAN COULTER® Triglyceride Reagent OSR61118 4 x 50 mL R1, 4 x 12.5 mL R2). The health value of serum triglycerides in an adult was (90-200 mg/dl).

The triglyceride methodology was a modification done by (Jacobs et al., 1960; Tietz, 1995; Riesen, 1998). The triglycerides in the specimen are hydrolyzed by a combination of microbial lipases to products glycerol and fatty acids. ATP phosphorylates the glycerol in the presence of glycerol kinase (GK) to yield glycerol-3-phosphate. The glycerol-3-phosphate was oxidized by molecular oxygen in the

presence of GPO (glycerol phosphate oxidase) to generate H_2O_2 and dihydroxyacetone phosphate. The produced H2O2 reacts with 4-aminophenazone and N, N-bis (4-sulfobutyl)-3, 5-dimethylaniline, disodium salt (MADB) in the presence of peroxidase (POD) to yield a chromophore, which is read at 660/800 nm.

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

Glycerol + ATP $\xrightarrow{\text{GK, Mg2+}}$ Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 $\xrightarrow{\text{GPO}}$ H_2O_2 + Dihydroxyacetone phosphate

2 H_2O_2+ MADB + 4AAP $\xrightarrow{\text{Peroxidase}}$ Blue Dye + OH^- + H_2O

The final concentration of reactive ingredients:

PIPES buffer (pH 7.5)	50 mmol/L
Mg^{2+}	4.6 mmol/L
MADB	0.25 mmol/L
4-Aminoantipyrine	0.5 mmol/L
ATP	1.4 mmol/L
Lipases	1.5 kU/L
Glycerol kinase	0.5 kU/L
Peroxidase	0.98 kU/L
Ascorbate Oxidase	1.48 kU/L
Glycerol-3-phosphateoxidase	1.48 kU/L

Calibration for serum triglyceride procedure was performed by use of the chemistry calibrator Cat. No. 66300.

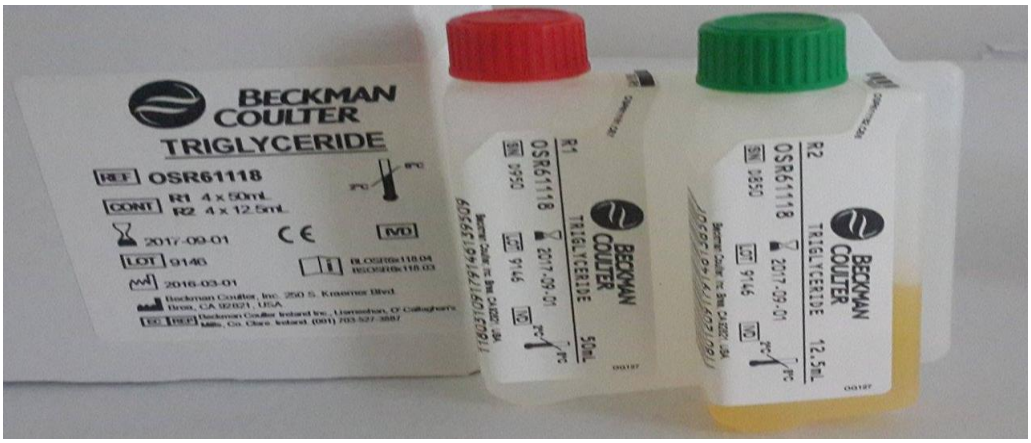

Figure 3.10. Lab test kits (BECKMAN COULTER® Triglyceride Reagent.

3.4.6. Measurements of Serum Creatinine

The principle for serum creatinine depends on a photometric enzymatic process by using clinical chemistry laboratory analyzers BECKMAN COULTER® model Au5800 (Tokyo, Japan, 2014) with the use test kits (BECKMAN COULTER® Creatinine Reagent OSR6178 4 x 51 mL R1, 4 x 51 mL R2). The healthy value in the adult: Woman (0.66 - 1.09 mg/dl). Man < 50 years (0.84 - 1.25 mg/dl). Man >50 years (0.81 - 1.44 mg/dl).

Methodology: Creatinine produces a yellow-orange colored complex with picric acid in an alkaline medium. The rate of change in absorbance at 520/800 nm was equivalent to the creatinine concentration in the specimen (Cook, 1971; Larsen, 1972).

Creatinine + Picric acid ⟶ Creatinine picrate complex

The final concentration of reactive ingredients:

Sodium hydroxide	120 mmol/L
Picric acid	2.9 mmol/L

The reagents are ready for use and we placed it directly on board the instrument, Figure 3.11.

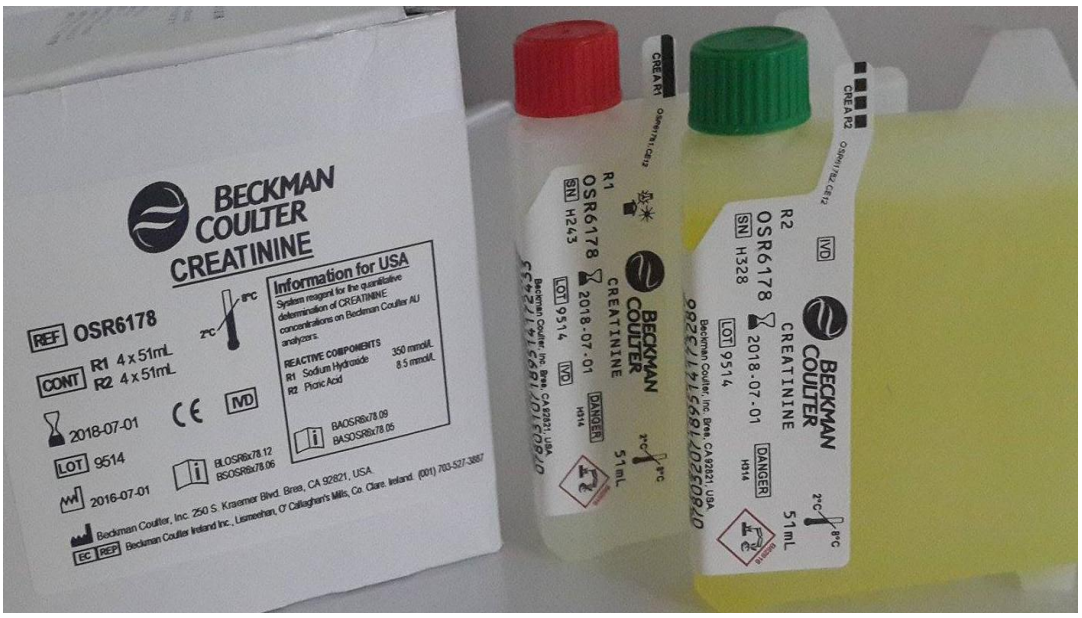

Figure 3.11. Lab test kits (BECKMAN COULTER® Creatinine Reagent.

3.5. Statistical Analysis

Descriptive statistic parameters were presented as mean ± standard derivation (mean±SD). Demographic and clinical biochemical data among groups were compared with one-way ANOVA by use of SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range tests were also carried out to distinguish examined groups. A p-value <0.05 was accepted as statistically significant.

3.6. Approval of Ethics Committee

The study protocol was approved by the Clinical Research Ethics Committee of Gaziantep University, Faculty of Medicine by Prof. Dr. Belgin ALASEHİRLİ dated 15/08/2016, approved no. 2016/237, Gaziantep University General Hospital, Gaziantep, Turkey. Ethical approval was also sought from the Institute of Natural and Applied Sciences, Biochemistry Department dated 03/08/2016, no.300/35764.

In addition, all of the study subjects was give written informed consent to participate in this study for collection the blood sample to evaluate the biochemical analysis for serum magnesium, glucose, insulin, serum total cholesterol, triglycerides, low-density lipoprotein (LDL-cholesterol) and serum creatinine, in obese non-diabetic subjects, obese T2DM patients and healthy subjects. As well as the calculated BMI and HOMA-IR were measured for all subjects.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Baseline Clinical Characterized of Study Subjects

The present study consist of 120 subjects, the subjects were divided into four groups:

Group I: Consisted of 30 healthy subjects had mean±SD for age 36.0 years old, (male 42.0±13.7, and female 33.8± 8.6).

Group II: Consisted of 30 obese nondiabetic subjects had mean±SD for age 44.4 years old, (male 50.0 ± 9.5, and female 43.0 ± 9.6).

Group III: Including 30 obese type2 diabetes mellitus patients with disease history less than one year had mean±SD for age 52.7 years old, (male 53.2 ± 12.6, and female 52.1± 7.2).

Group IV: Consisted of 30 obese type2 diabetes mellitus patients with disease history more than five years had mean±SD for age 55.7years old, (male 55.4± 5.7, and female 56.1± 11.1).

The demographic and clinical biochemical laboratory data of the studied groups given in Table 4.1. The demographic measurements and clinical laboratory parameters results for the all control subjects group were within the normal range.

Table.4.1. Demographic and clinical biochemical laboratory data of the studied groups. Group I control, group II is obese non-diabetic, group III is obese T2DM with disease history less than 1 year, and group IV is obese T2DM with disease history more than 5 years. N indicates the subject number.

		Group I	**Group II**	**Group III**	**Group IV**
N		30	30	30	30
Parameters	Unit	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Age	year	36±10.6a	44.4±9.7b	52.7±9.9c	55.7±8.3c
Height	cm	164.5±5.4a	161.7±6.0a	163.5±6.2a	169.4±3.7b
Weight	kg	63.56±5.4a	87.03±7.8b	88.66±9.5b	97.03±8.8c
BMI	kg/m²	19.06±1.6a	27.28±3.1b	26.95±3.2b	28.7±2.3c
Mg	mg/dl	2.07±0.1c	1.98±0.2b	1.98±0.2b	1.72±0.1a
FG	mg/dl	91±4.4a	101.8±8.2a	140.86±44.5b	202.8±42.4c
Insulin	µU/ml	7.9±3.1a	11.39±9.0a,b	14.81±9.2b,c	16.7±7.0c
HOMA-IR		1.03±0.3a	2.82±2.3b	4.99±4.1c	7.9±3.7d
TC	mg/dl	169.0±34.2a	204.8±48.5b	198.8±33.3b	195.7±27.0b
TG	mg/dl	144.1±43.8a	179.1±69.5b	170.7±63.4a,b	201.6±74.9b
LDL-C	mg/dl	90.2±21.7a	111.4±44.4b	105.6±24.2a,b	102.1±23.7a,b
Creatinine	mg/dl	0.64±0.1a	0.67±0.1a,b	0.74±0.15b,c	0.71±0.1c

Abbreviation: T2DM is type2 diabetes mellitus, BMI is Body Max Index, Mg is Magnesium, FG is fasting glucose, TC is Total Cholesterol, TG is Triglyceride, LDL-C is Low-Density Lipoprotein-Cholesterol and HOMA-IR is Homeostasis Model Assessment of Insulin Resistance. (cm is centimeter, kg/m^2 is kilogram per meter square, µU/ml microunits per milliliter, mg/dl is milligrams per deciliter, and SD is standard derivation). Different lower-case letters indicate statistical difference at $\alpha = 0.05$ level among groups. Values with the same letters in the same parameters indicate that the values did not differ by the Duncan test at 0.95 confidence interval

Table 4.2. Demographic and clinical biochemical laboratory data of the studied groups (male and female data). Group I control, group II is obese non- diabetic, group III is the obese type2 diabetic with disease history less than 1 year, and group IV is obese type2 diabetic with disease history more 5 years. N indicates the subject number. The abbreviation is given in (Table 4.1).

		GroupI		**GroupII**		**GroupIII**		**GroupIV**	
		Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
N		8	22	6	24	14	16	17	13
Parameters	**Unit**	Maen±SD	Maen±SD	Maen±SD	Maen±SD	Maen±SD	Maen±SD	Maen±SD	Maen±SD
Age	year	42.0±13.7a	33.8± 8.6a	50.0 ± 9.5a,b	43.0 ± 9.6b	53.2 ± 12.6b	52.1± 7.2c	55.4± 5.7b	56.1± 11.1c
Height	cm	169.0± 4.7a,b	162.9± 4.8b	164.5 ±8.1a,b	161.0 ± 5.4a,b	168.0 ± 5.7a,b	159.5± 3.2a	171.3± 3.4b	167.0± 2.4c
Weight	kg	67.7± 5.7a	62.0± 4.5a	89.5 ± 9.0a,b	86.4 ± 7.5b	91.0 ± 7.2a,b	86.56± 10.9b	101.2± 8.1b	91.0± 6.2b
BMI	kg/m²	20.5± 2.4a	18.5± 0.9a	29.83 ± 5.3b	26.6 ± 1.9b	27.4± 3.0b	26.53± 3.3b	29.6± 2.4b	27.5± 1.8b
Mg	mg/dl	2.08± 0.08b	2.06± 0.1b	1.93 ± 0.3b	2 ± 0.1b	1.96± 0.1b	1.93± 0.3a	1.72± 0.07a	1.73± 0.1b
FG	mg/dl	91.5± 3.9a	90.8± 4.6a	103.7 ± 11.0a	101.4± 7.5a	133.7± 21.2b	147.12± 57.8b	216.1± 34.3c	185.3± 46.6c
Insulin	µU/ml	7.8± 3.1a	8.05± 3.1a	15.6 ± 11.6b	10.3 ± 8.2a,b	15.2± 6.1b	14.46± 11.5b,c	17.4± 8.0b	15.7± 5.4c
HOMA-IR		1.02± 0.4a	1.04± 0.4a	4.02 ± 3.3b	2.5 ± 2.0a	4.5± 1.7b	5.40± 5.4b	8.5± 4.1c	7.2± 3.0b
TC	mg/dl	171.7± 36.3a	168.0± 34.2a	238.5 ± 87.4b	196.3 ± 30.6b	199.4± 24.5a,b	198.25± 40.4b	195.9± 33.2a	195.5± 17.1b
TG	mg/dl	160± 44.3a	138.3± 43.2a	237.3 ±94.7b	164.5 ± 55. 5a,b	152.0± 39.3a	187.18± 76.2b	208.7± 91.6a,b	192.4± 47.3b
LDL-C	mg/dl	96.26± 24.7a	88.0± 20.7a	152.1 ± 72.0b	101.2 ± 28.5a,b	102.2± 22.3a	108.5± 26.1b	100.7± 30.2a	103.9± 11.3a,b
Creatinine	mg/dl	0.63± 0.1a	0.6± 0.1a	0.7± 0.1a,b	0.67 ± 0.1a	0.7± 0.1b	0.71± 0.1a	0.75± 0.1a,b	0.7± 0.08a

4.2. The Demographic Measurement Results

With regard to the age of groups, a significant difference was found between group IV and group I ($p<0.05$). However, there was insignificant difference between group IV and group III. Differences in demographic characters of all groups in this study are given in Figure 4.1.

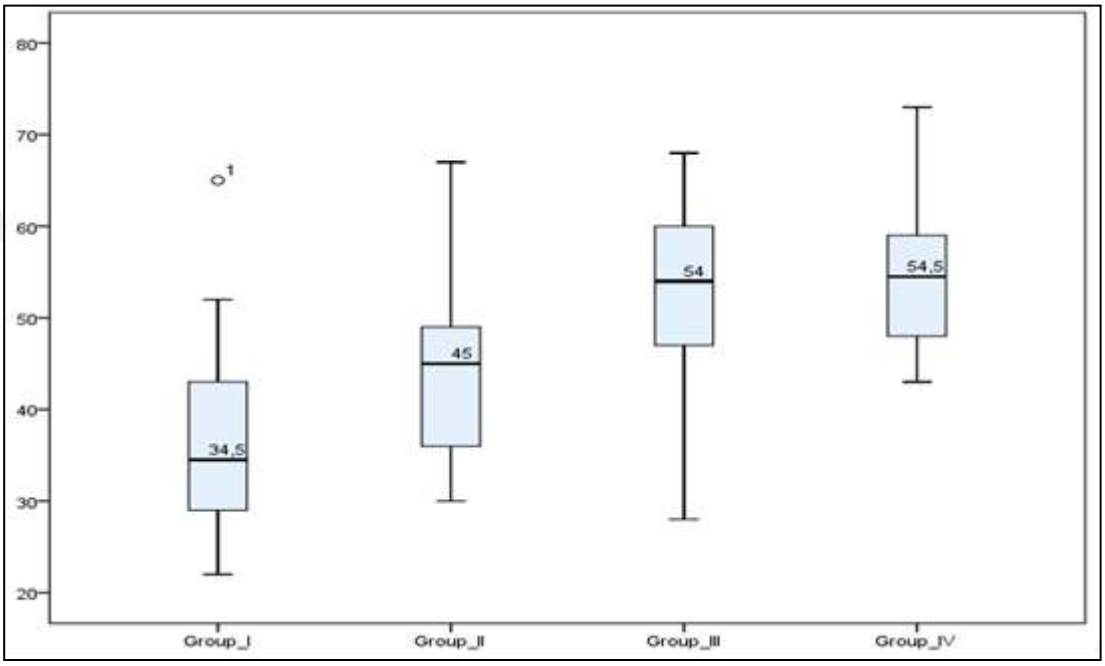

Figure 4.1. Box plot showing the distribution of age with a median value among the studied groups. Group I control, group II is obese non-diabetic, group III is the obese T2DM with disease history less than 1 year, and group IV is obese type2 diabetic with disease history more 5 years.

The present study revealed in group IV a negative statistically significant association was found between age and serum Mg levels ($r=-0.88$, $p<0.01$) as present in Figure 4.4. However, in group III a negative statistically significant association was found between age and serum magnesium level ($r=-0.46$, $p<0.05$). In addition, in group II a negative correlation was found between age and serum magnesium level but not significant as given in Table 4.2.

With regard to the BMI the mean±SD in group I is 19.06±1.6 kg/m^2 (male 20.5±2.4, and female18.5±0.9). Group II 27.28±3.1 kg/m^2 (male 29.83 ± 5.3, and female 26.6 ± 1.9). Group III 26.95±3.2 kg/m^2 (male 27.4±3.0, and female 26.53±3.3). Group IV 28.7±2.3 kg/m^2 (male 29.6± 2.4, and female 27.5±1.8) as given in Table 4.2.

Regarding BMI in groups, a significant variance was found between group IV and the group I ($p<0.05$). While there was insignificant, difference between group II and group III. A significant difference in BMI between case groups and control was found. The present study has shown that BMI was significantly higher in all obese case groups in compared with the control group as shown in Table 4.1, Figure 4.2. However, in group IV a positive association was found between BMI with height and weight statistically significant correlation was found ($r =0.63$, $p<0.01$), ($r =0.86$, $p<0.01$). In addition, there were a negative association between BMI with serum Mg($r = -0.05$, $p<0.05$) and HOMA-IR ($r= -0.12$, $p<0.05$).

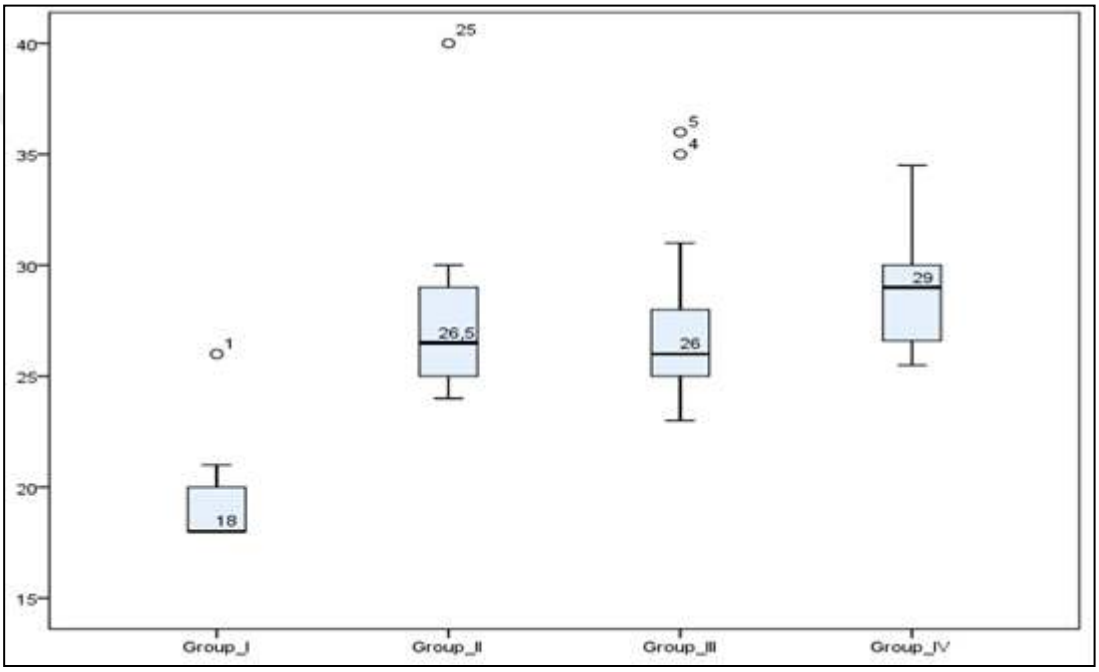

Figure 4.2. Box plot showing the distribution of body max index (BMI) with a median value of the studied groups. Group I control, group II is obese non-diabetic, group III is the obese T2DM with disease history less 1 one year, and group IV is obese T2DM with disease history more 5 years.

4.3. Clinical Laboratory Parameter Results

4.3.1. Serum Magnesium Levels

Serum magnesium level ranged from (1.9-2.7) mg/dl. The present study has revealed the serum Mg level in groups, a significant difference was found between groups I and group IV ($p<0.05$). While, there was the insignificant difference between group II and group III ($p>0.05$). A significant difference in serum Mg levels among the groups, Figure 4.3.

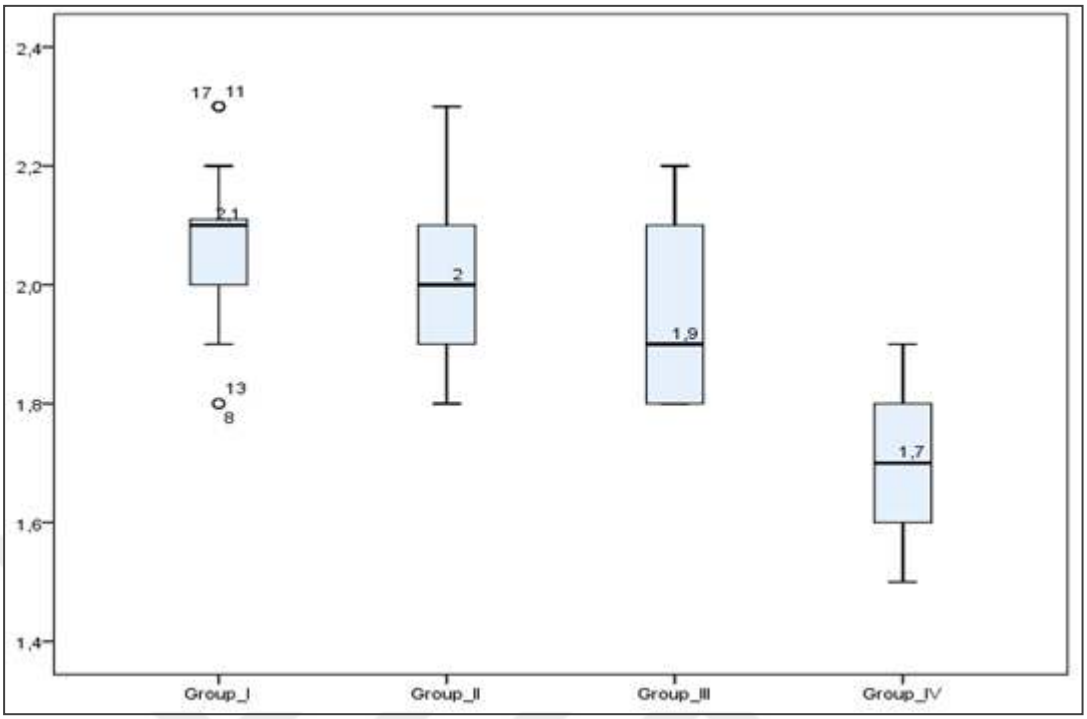

Figure 4.3. Box plot showing the distribution of serum magnesium levels among the studied groups. Group I control, group II is obese non-diabetic, group III is the obese T2DM with disease history less than 1 year, and group IV is obese T2DM with disease history more 5 years.

In the present study, in group IV (1.72±0.1 mg/dl) the serum magnesium levels were significantly lower in compared to group I (2.07±0.1). There was no significant difference of serum Mg level found between male and female in group II ($p>0.05$). While a significant difference was found between male and female in group III, and group IV ($P<0.05$) as given in Table 4.2. With regard to group IV a negative statistically significant association was found between serum Mg levels and age ($r=-0.88$, $p<0.01$) as present in Figure 4.4.

As well as a negative association was found between serum Mg and BMI, HOMA.IR, serum fasting glucose and serum fasting lipids, but the association not statically significant. However, in group III a negative statistically significant correlation was found between serum magnesium level and age ($r=-0.46$, $p<0.05$). Furthermore, in group II a negative statistically significant correlation was found between serum magnesium level and age ($r=-0.03$, $p<0.05$), fasting glucose ($r=-0.19$, $p<0.05$) but no significant association, Table 4.3.

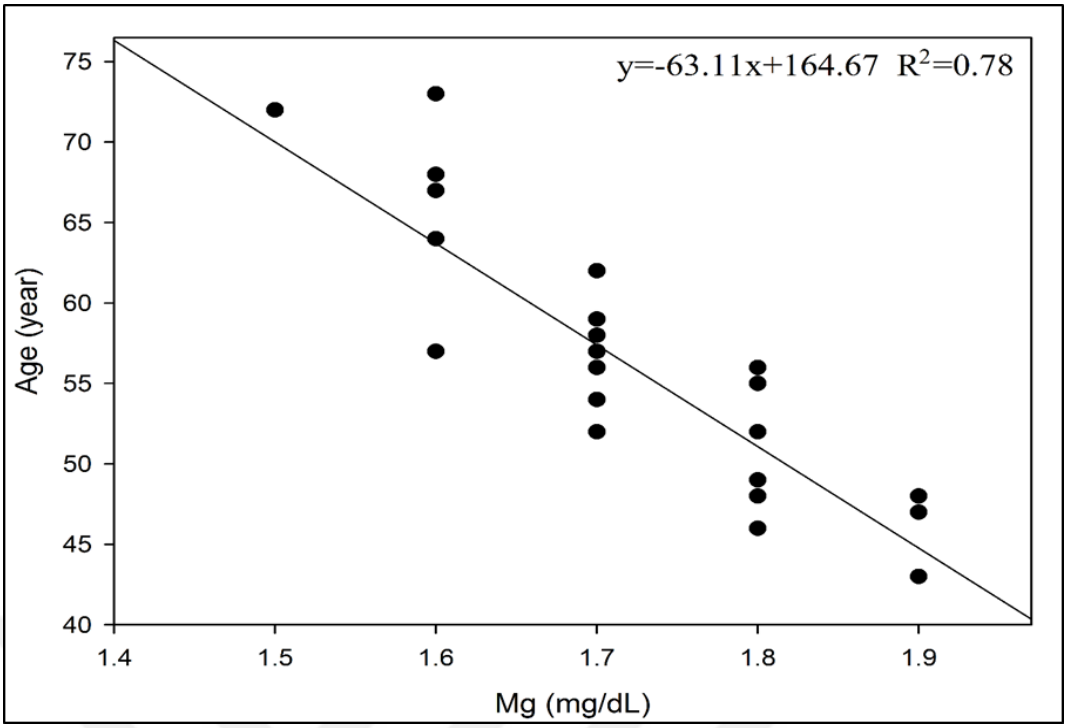

Figure 4.4. Scatterplot graph showing the negative correlation between serum magnesium and age in obese T2DM with disease history more than 5 years (group IV). The correlation is statically significant (r=- 0.88, p<0.01). r = Pearson correlation coefficient, p< 0.05 is significant.

Table 4.3. Correlation between serum magnesium level with demographic and biochemistry parameters in all groups of this study. Group I control, group II is obese non-diabetic, group III is the obese T2DM with disease history less than 1 year, and group IV is obese T2DM with disease history more than 5 years. Abbreviations is given in (Table 4.1).

		Group I		**Group II**		**Group III**		**Group IV**	
Parameters	Unit	r	p	r	p	r	p	r	p
Age	year	-0.057	0.764	-0.033	0.862	-0.673	0.000	-0.886	0.000
Height	cm	-0.150	0.430	0.002	0.991	0.118	0.534	0.120	0.528
Weight	kg	-0.16	0.382	0.039	0.836	0.003	0.987	-0.084	0.657
BMI	kg/m²	-0.071	0.713	0.055	0.772	-0.169	0.369	-0.057	0.765
FG	mg/dl	-0.097	0.612	-0.190	0.315	-0.055	0.770	-0.317	0.088
Insulin	µU/ml	-0.190	0.314	0.128	0.500	0.104	0.581	0.128	0.499
HOMA-IR	mg/dl	-0.192	0.310	0.121	0.524	0.149	0.429	-0.099	0.604
TC	mg/dl	0.128	0.499	0.107	0.575	-0.112	0.552	-0.169	0.373
TG	mg/dl	0.016	0.943	-0.157	0.406	-0.027	0.884	-0.200	0.290
LDL-C	mg/dl	-0.290	0.120	0.080	0.673	-0.031	0.870	-0.235	0.210
Creatinine	mg/dl	0.151	0.427	-0.362	0.049	-0.116	0.539	0.072	0.703

r = Pearson correlation coefficient, p< 0.05 is significant.

4.3.2. Serum Fasting Glucose Levels

Serum fasting glucose levels ranged from 70-100 mg/dl. The present study exposed that in group IV serum fasting glucose was significantly higher (202.8±42.4 mg/dl) than in group III (140.86±44.5 mg/dl) that moderately increased. Both of group IV and III are higher fasting blood glucose result in compared with group II (101.8±8.2 mg/dl) and the group I (91±4.4 mg/dl). A significant difference was found between group IV and the group I ($p<0.05$). However, there was the insignificant difference between II and group I ($p>0.05$) as presented in Figure 4.5. There was an insignificant difference of fasting serum glucose level found between male and female in group IV, group III, and the group I ($p>0.05$) as given in Table 4.2.

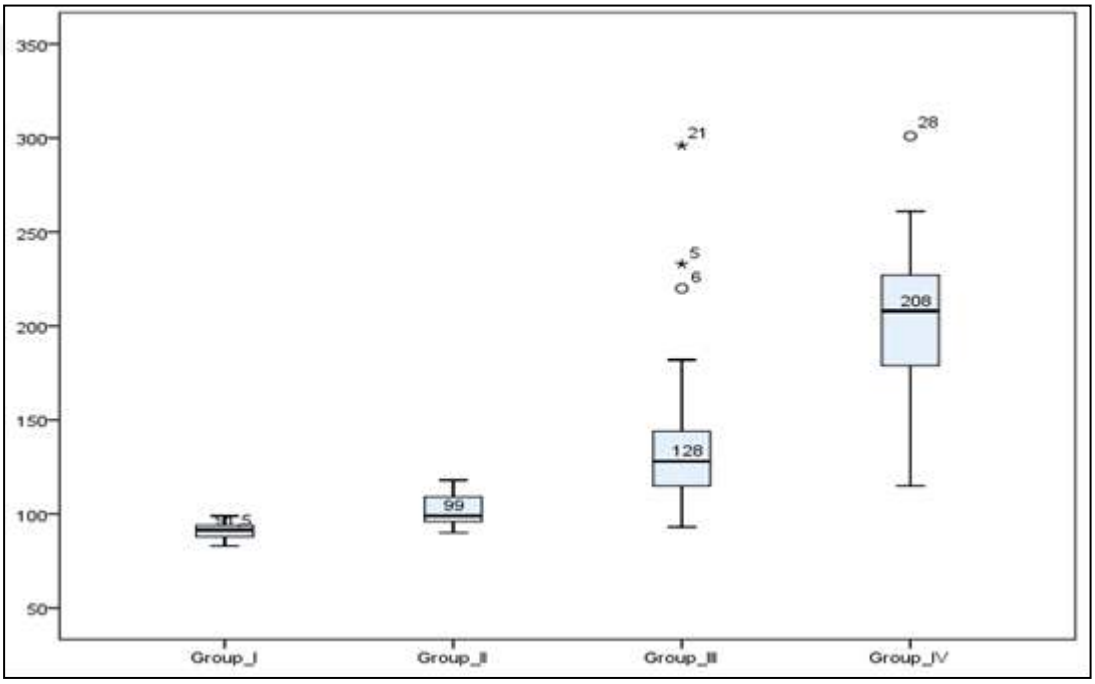

Figure 4.5. Box plot viewing the distribution of serum fasting glucose with a median value among the studied groups. Group I control, group II is obese non-diabetic, group III is the obese T2DM with disease history less than 1 year, and group IV is obese T2DM with disease history more than 5 years.

It is important to mention that of the 48 subjects that reported taking diabetes medication, only 12 subjects (6 women and 6 men) had well-controlled fasting glucose levels in an obese diabetic with duration of disease history less than one-year groups (group III). With regard to group IV, the present study observed a negative statically association between serum fasting glucose level and serum Mg level ($r=-0.31$, $p<0.05$). An opposite a positive association found between serum fasting glucose level and HOMA-IR ($r=0.41$, $p<0.05$). While in group III a positive

association was found between serum fasting glucose level and BMI (r=0.37, p>0.05). However, in group II a negative statically association found between serum fasting glucose level and serum Mg level (r=-0.19, p<0.05).

4.3.3. Serum Insulin Levels

The serum insulin level ranged from 1.9-23 µU/ml. In the present study the levels serum insulin were significantly higher in group IV (16.7±7.0), group III (14.81±9.2) but within the normal reference range as compared with group II (11.39±9.0), and the group I (7.9±3.1) but within the reference range, Figure 4.6. A significant difference of serum fasting insulin found between group IV and group I (p<0.05). The present study has revealed that in-group IV a negative statistically association between serum insulin level and serum fasting glucose (r=-0.03, p<0.05). An opposite statistically significant correlation was found between serum insulin level and HOMA-IR (r=0.81, p<0.01). While, in group III a positive statistically significant correlation found between serum insulin level and HOMA.IR (r= 0.88, p<0.01) as given on Table 4.4.

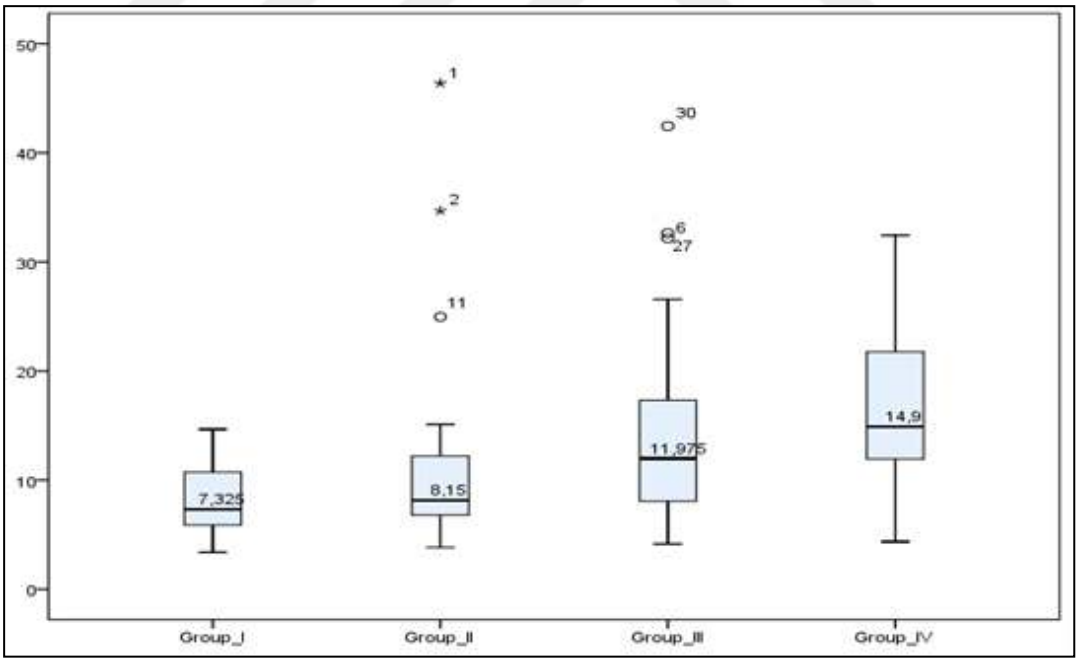

Figure 4.6. Box plot viewing the distribution of serum insulin with a median value among the studied groups. Group I is control, group II is obese non-diabetic, group III is the obese T2DM with disease history less than 1 year, and group IV is obese T2DM with disease history more than 5 years.

4.3.4. The HOMA-IR Levels

The calculated HOMA-IR ranged (<2.7). The HOMA-IR are depended on fasting serum glucose and serum insulin measurements. HOMA-IR indicating poorer glucose control and greater insulin resistance. Our study has revealed that HOMA-IR was significantly higher in group IV (7.9±3.7) and group III (4.99±4.1) in compared with group II and group I. As well as HOMA-IR in-group II (2.82±2.3) were slightly higher as compared to group I (1.03±0.3). A significant difference was found between group IV and group I ($p<0.05$), as well as a significant difference was found between group II and group III ($p<0.05$), Table 4.1, Figure 4.7.

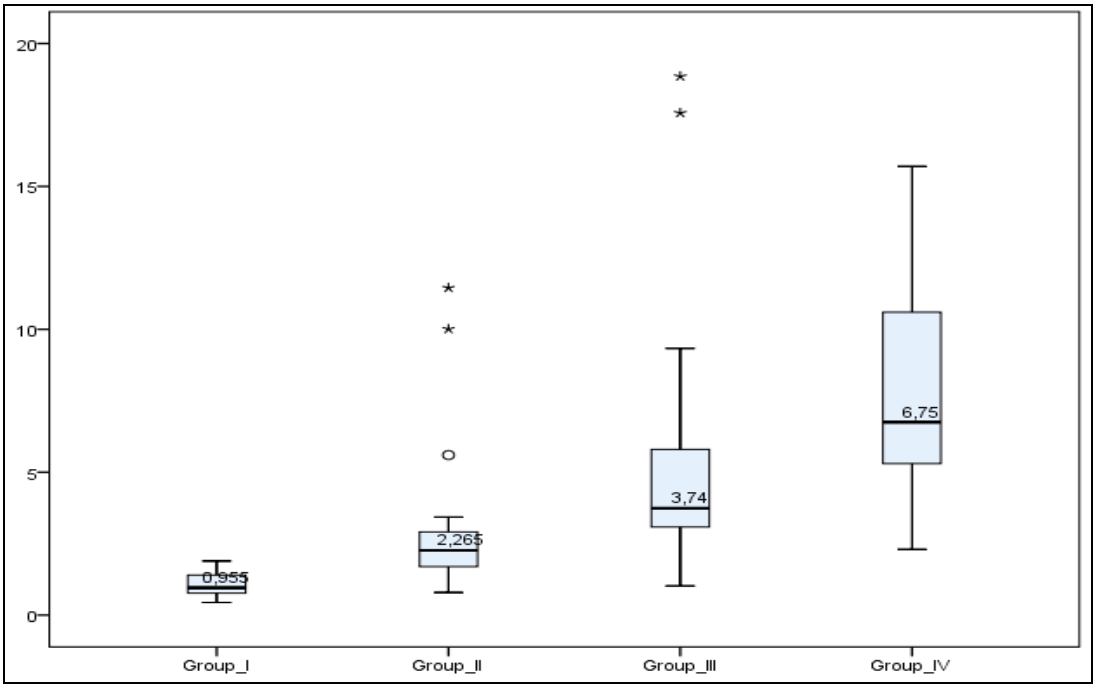

Figure 4.7. Box plot viewing the distribution of calculated HOMA-IR among the studied groups. Group I control, group II is obese non-diabetic, group III is the obese T2DM with disease history less than 1 year, and group IV is obese T2DM with disease history more than 5 years.

A significant difference was found of HOMA-IR between male and female in group IV ($P<0.05$) as given in Table 4.2. In addition, the present study exposed in group IV a negative statistically association between HOMA-IR with BMI and serum Mg but not significant. An opposite, a positive statistically significant correlation was found between HOMA-IR with fasting blood glucose level ($r =0.41$, $p<0.05$) and serum insulin level ($r=0.18$, $p<0.01$).

However, in group III a negative statistically association was found between HOMA-IR and BMI but not significant. An inverse, a positive statistically significant correlation was found between HOMA-IR and serum insulin level (r=0.88, p<0.01). Furthermore, group II a positive statistically significant connection found between HOMA-IR and serum insulin level (r=0.99, p<0.01), Table 4.4.

Table 4.4. Correlation between calculated HOMA-IR with anthropometric measurement and biochemistry parameters in all groups of this study. Abbreviations is given in table 4.1.

		Group I		**Group II**		**Group III**		**Group IV**	
Parameters	unit	r	p	r	p	r	p	r	p
Age	year	-0.126	0.504	-0.147	0.437	-0.133	0.48	0.188	0.319
Height	cm	0.191	0.310	-0.19	0.302	-0.097	0.608	-0.250	0.182
Weight	kg	0.351	0.056	-0.124	0.513	-0.035	0.853	-0.086	0.650
BMI	kg/m²	0.151	0.424	0.138	0.464	-0.015	0.936	-0.128	0.499
FG	mg/dl	0.364	0.040	0.195	0.301	0.355	0.053	0.412	0.020
Insulin	µU/ml	0.990	0.000	0.991	0.000	0.881	0.000	0.818	0.000
Mg	mg/dl	-0.191	0.309	0.121	0.523	0.149	0.429	-0.098	0.604
TC	mg/dl	0.082	0.665	0.432	0.017	0.024	0.897	0.194	0.303
TG	mg/dl	0.449	0.012	0.539	0.002	0.007	0.966	-0.097	0.609
LDL.C	mg/dl	0.047	0.804	0.561	0.001	-0.235	0.209	-0.085	0.653
Creatinine	mg/dl	0.202	0.283	-0.001	0.995	-0.160	0.397	0.117	0.537

r = Pearson correlation coefficient, p< 0.05 is significant

4.3.5. Serum Total Cholesterol Levels

The level of serum total cholesterol ranged from 100-200 mg/dl. This study has revealed that serum cholesterol level was higher in group II (204.8±48.5). Also in group III (198.8±33.3) and group IV (195.7±27) in compared with group I (169.0±34.2), as presented in Figure 4.8.

A significant difference was for serum cholesterol between group IV and the group I (p<0.05), while no significant difference between group IV and group II (p>0.05). In addition, this study observed in group IV a negative statistically connection between serum cholesterol and serum Mg levels but not significant. An opposite, positive statistically significant correlation was found between serum cholesterol and serum

triglyceride (r=0.68, p>0.01), serum LDL-cholesterol (r=0.65, p>0.01). Similarly, in group III a negative statistically relationship found between serum cholesterol level with BMI and serum Mg level, but not significant. Furthermore, in-group II a positive statistically significant association found between serum cholesterol and HOMA-IR (r=0.43, p>0.01).

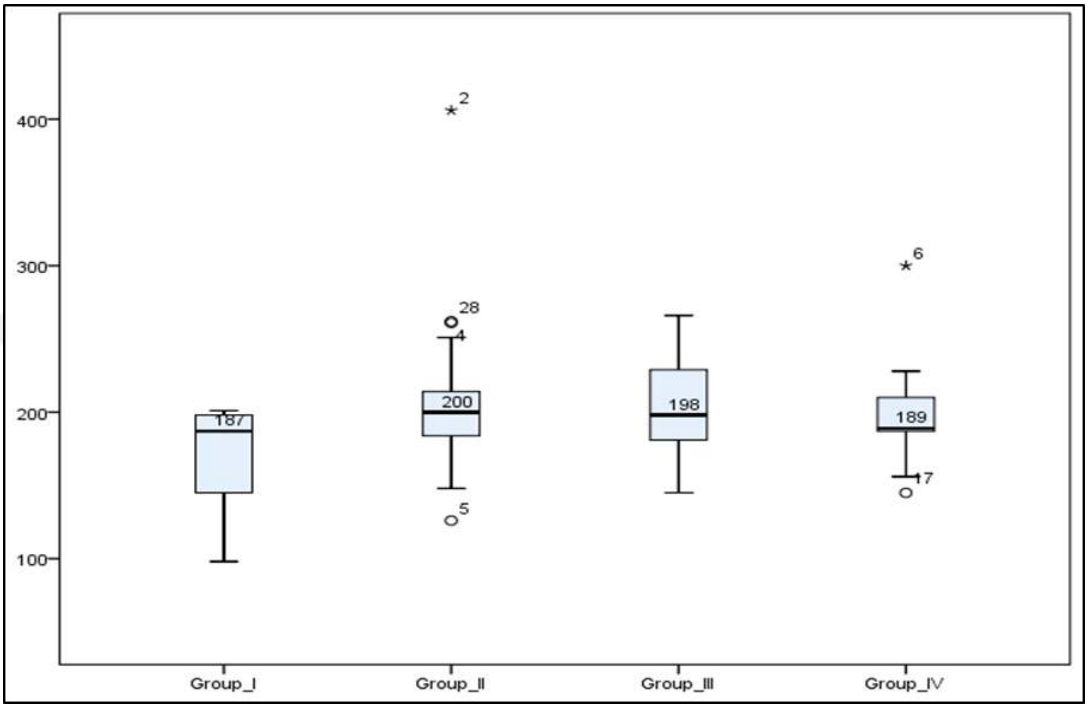

Figure 4.8. The box plot shows the distribution of serum total cholesterol with a median value among the studied groups. Group I control, group II is obese non-diabetic, group III is the obese T2DMwith disease history less than 1 year, and group IV is obese T2DM with disease history more than 5 years.

4.3.6. Serum LDL- cholesterol Levels

Serum LDL- cholesterol ranged from 57-129 mg/dl. This study has exposed that the level of serum LDL-cholesterol was higher in group II (111.4±44.4), group III (105.6±24.2) and group IV (102.1±23.7) in compared with group I (90.2±21.7), but within the normal reference range, Figure 4.9.

A significant difference was for serum LDL-cholesterol between group II and the group I ($p<0.05$), while no significant difference between group IV and group III ($p>0.05$). In addition, the present study observed in group IV a negative statistically association between serum LDL-cholesterol with serum Mg and HOMA-IR, but not

significant. An opposite, a positive correlation was found between LDL-cholesterol with serum cholesterol (r=0.65, p<0.01) and serum triglyceride (r=0.71, p<0.01).

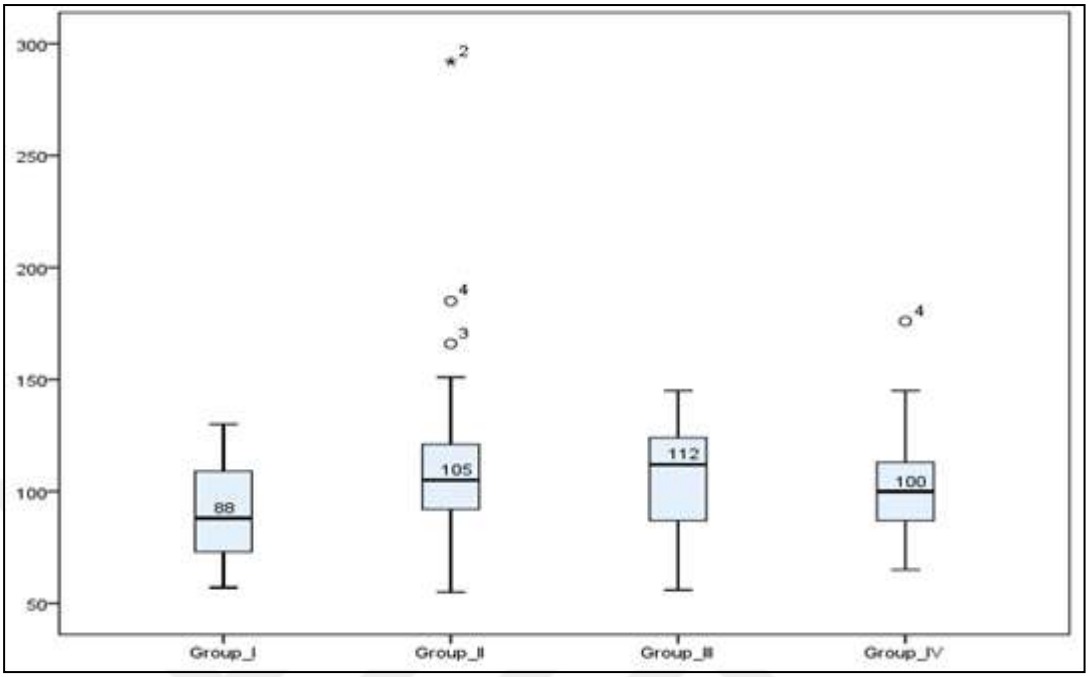

Figure 4.9. Box plot showing the distribution of serum LDL-cholesterol with a median value among the studied groups. Group I control, group II is obese non-diabetic, group III is the obese T2DM with disease history less than 1 year, and group IV is obese T2DM with disease history more than 5 years.

Besides, in-group III a negative association found between serum LDL-cholesterol with BMI, serum Mg level and HOMA-IR, but not significant. An opposite, a positive statistically significant relationship was found between LDL-cholesterol with serum cholesterol (r=0.67, p<0.01) and serum triglyceride (r=0.46, p<0.01). In additionally, in-group II, a positive statistically significant connection was found between serum LDL-cholesterol with HOMA-IR (r=0.56, p<0.01), serum cholesterol (r=0.8, p<0.01) and serum triglyceride (r=0.37, p<0.05).

4.3.7. Serum Triglycerides Levels

Serum triglycerides level ranged from 90-200 mg/dl. This study has revealed that the level of serum triglycerides level was elevated in group IV (201.6±74.9), group III (170.7±63.4) and group II (179.1±69.5) in compared with group I (144.1±43.8), Figure 4.10.

A significant difference was for serum triglyceride between group IV and the group I ($p<0.05$), while the insignificant difference between group IV and group II ($p>0.05$). In addition, this study has exposed that in-group IV a negative connection between serum triglyceride with BMI, serum Mg level and HOMA-IR. A reverse, a positive association was found between serum triglyceride and serum cholesterol ($r=0.68$, $p<0.01$). Also in group II a negative association found between serum triglyceride and serum Mg level. An opposite, a positive statistically significant correlation was found between serum triglyceride and HOMA-IR ($r=0.53$, $p<0.01$).

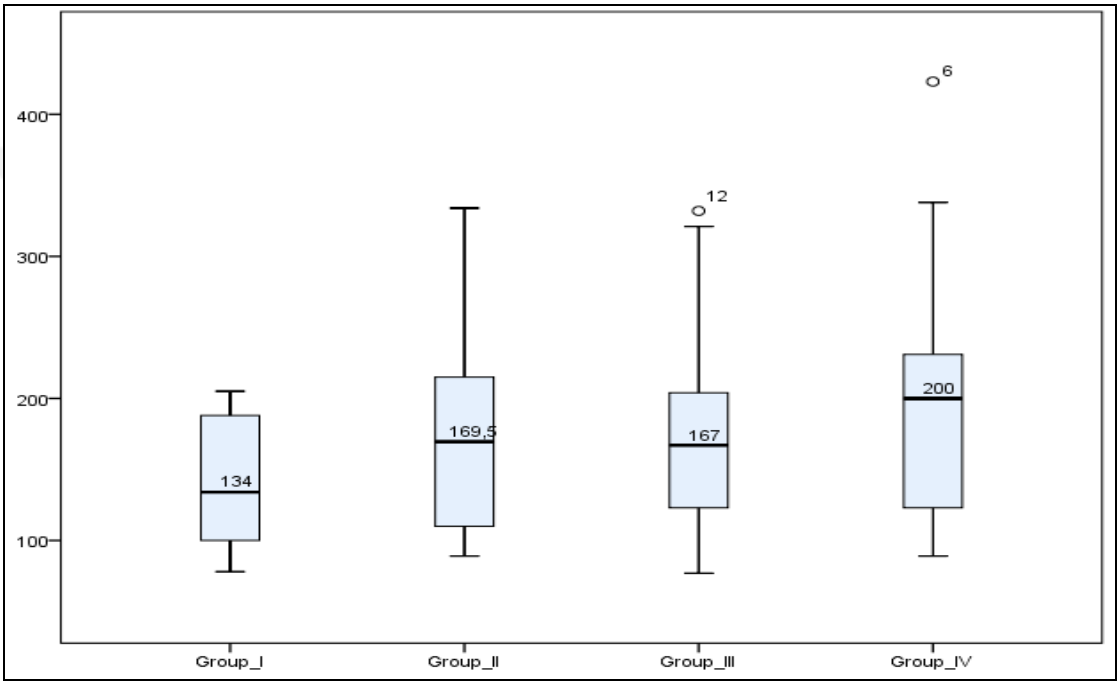

Figure 4.10. Box plot showing the distribution of serum triglyceride with a median value among the studied groups. Group I control, group II is obese non-diabetic, group III is the obese T2DM with disease history less than 1 year, and group IV is obese T2DM with disease history more than 5 years.

4.3.8. Serum Creatinine Levels

Serum Creatinine ranged from 0.5-0.9 mg/dl. The present study has revealed the level of serum creatinine was slightly elevated in group IV (0.71±0.1) and group III (0.74±0.15) as compared with group II (0.67±0.1) and the group I (0.64±0.1), but within the reference range. As shown in Figure 4.11. A significant difference was for serum creatinine between group IV and group I ($p<0.05$).

Additionally, this study observed in group III a negative statistically significant association between serum creatinine with BMI ($r=-0.36$, $p<0.05$) and serum Mg

level (r=-0.03, p<0.05). While in-group II we observed a negative, association found between serum creatinine and serum Mg level, but not significant.

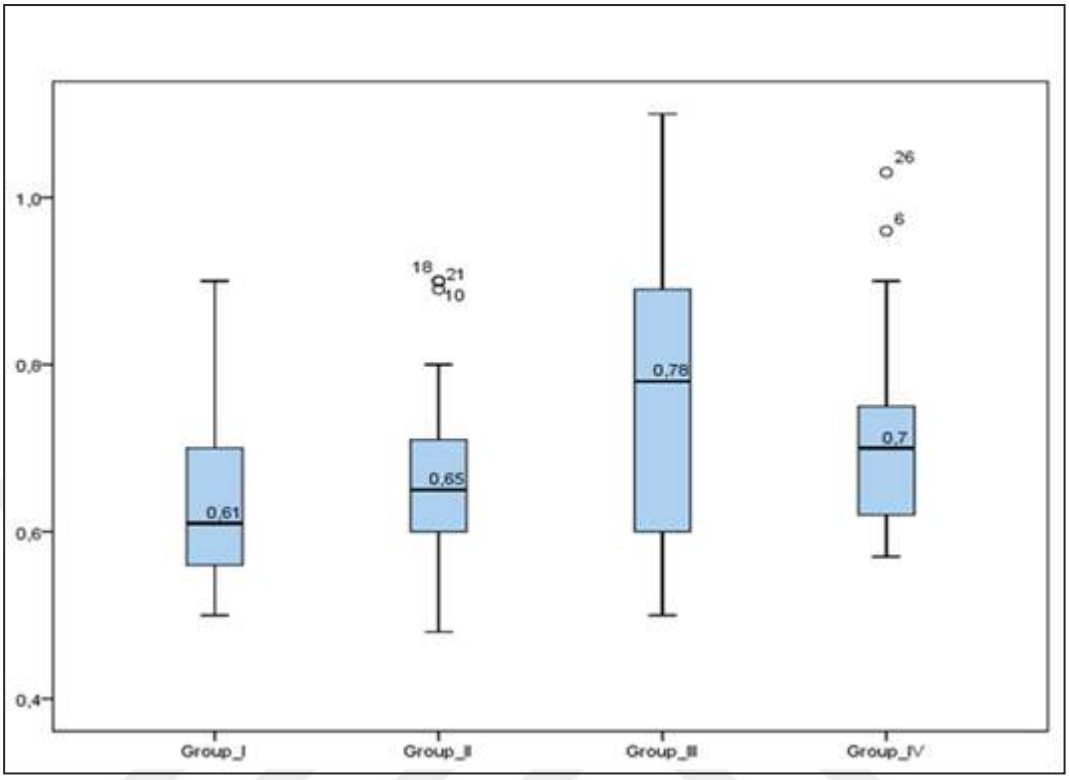

Figure 4.11. Box plot showing the distribution of serum creatinine with a median value among the studied groups. Group I control, group II is obese non-diabetic, group III is the obese T2DM with disease history less than 1 year, and group IV is obese T2DM with disease history more than 5 years.

4.4. The Discussions

Magnesium is a primary co-factor required for several biochemical reactions. Magnesium is an important factor in glucose metabolism and it is necessary for glucose transportation between membranes, glucose oxidation, all reactions involving phosphorylation and energy exchange. It is essential for insulin action since it is a cofactor of tyrosine kinase activity (Paolisso et al., 1989).

The present study revealed serum magnesium level in obese non-diabetic subjects was lower (1.98 mg/dl) in compared to healthy control group (2.02 mg/dl). These grades are in agreement with those gotten by (Hedberg and Haenni, 2012; Guerrero-Romero and Rodríguez-Morán, 2013; Cahill et al., 2013) who as well found serum Mg level was low in overweight as well as obese subjects. With regard to the BMI

between groups, a significant difference was found between all obese groups and the control group ($p<0.05$), as given in Table 4.1.

As well as the present study revealed that serum Mg level was decreased in obese T2DM patients especially in-group IV (Type2 diabetes mellitus with disease history more than five years). Previous studies (Naheed et al., 2003; Rasic et al., 2004; Phoung et al., 2007; Supriya et al., 2013) who also reported that serum Mg was decreased in T2DM. This indicates the association of hypomagnesaemia with T2DM.

In addition, the present study indicates that serum Mg level in case of obese T2DM with duration of disease history more than five years (1.72 mg/dl) was significantly lower than in obese T2DM with duration of disease less than one year (1.98 mg/dl). This agreed with several studies (Mather et al., 1979; Augusta et al., 2006; Del Gobbo et al., 2012) who reported an increase of Mg deficiency frequency was known in T2DM patients with longer duration of diabetes and uncontrolled glycemic profiles.

Hypomagnesaemia can be both a consequence and a cause for diabetic complications. The main cause of hypomagnesaemia in patients with T2DM are not obvious but they may consist of poorer dietary consumption of Mg, decrease intestinal Mg absorption, increase urinary losses of Mg or reduced Mg uptake into the cells compared to healthy persons (Ellin, 1994).

In addition, with regard to the age of groups, a significant difference was found between group IV and group I ($p<0.05$). However, there was no significant difference between group IV and group III. As well as this study found in group IV a negative statically significant association between serum Mg level and age ($r=-0.88$, $p<0.01$) as shown in Figure 4.3, Table 4.2. This finding in agreement with those studies (Paolisso et al., 1989; Wang et al., 2005) exposed the elderly are possibly susceptible to have low serum Mg level due to aging is related with decreased intracellular Mg levels. In addition, the elderly individuals will not have the capacity to profit from Mg-rich foods due to their hard texture and unsuitable physical properties. Also in group II a negative correlation was found between serum Mg level and age but not statically significant.

This study found in group IV, a negative correlation found between serum Mg levels and serum fasting lipids but the association not significant this finding was in agreement with studies done by (Elementol et al., 2010) which reported no statistically significant effect of Mg concentration on the content of lipids analyzed in blood serum was found. Noticeably, the presence of obesity may increase cardiovascular risk and the mortality rate related to low Mg levels.

This present study found that calculated HOMA-IR was significantly higher in obese DMT2 and obese subjects groups; this was in agreement with previous studies done by (Abdul-Ghani et al., 2006) that reported insulin resistance was predominant in obese participants.

Moreover, in the present study, HOMA-IR had a negative correlation with serum Mg levels in DMT2 with disease history more than five years but the association not significant, as given in Table 4.4. This result was in agreement with (Lima et al., 2005) they found a negative association between HOMA-IR and serum Mg level in DMT2 patients, though it was not statistically significant.

In the present study, in obese non-diabetic subjects and obese T2DM patient groups, the levels of serum total cholesterol, serum triglycerides and LDL-cholesterol were significantly elevated in compared to controls. This finding was in agreement with these previous studies (Manson et al., 1990; Calle et al., 1999). As well as in obese non-diabetic group (group II) HOMA-IR was positively correlated with serum fasting cholesterol ($r=0.432$, $p<0.01$), LDL-cholesterol ($r=0.561$, $p<0.01$), and serum triglyceride ($r=0.539$, $p<0.01$). This result was in agreement with (Steinberger et al., 1995) who also reports an obese person the degree of insulin resistance explain a significant correlation with fasting lipids. Table 4.5 shows the value of serum Mg level in T2DM patients and obese subjects among various countries reported by previous studies started from 2002 to this recent study. El-said et al., 2015 in Egypt reported the lower serum Mg values with a mean value (1.29 mg/dl) in T2DM patients. As well as Rao et al., 2016 in India reported serum Mg mean value (1.32 mg/dl) this result of low value for serum Mg levels in T2DM patients is due to the presence of micro- or macrovascular complications in T2DM patients. While (Ankash et al., 2009) in India reported serum Mg value (1.98 mg/dl) this may be due to the absence of diabetic complication and low duration of disease history for

T2DM. The present study reported serum Mg value was (1.72 mg/dl) in obese DMT2 patients. While in obese subjects, (Guerrero-Romero and Rodríguez-Morán, 2002) reported the low value of serum in Mexico (1.8 mg/dl) while (Takya et al., 2010) in Kansai reported serum Mg value (2.0 mg/dl) in obese subjects this may be due to the lifestyle and the nutrient of each country. The present study reported serum Mg value in obese non-diabetic subjects was 1.98 mg/dl.

Table 4.5. The value mean±SD for serum magnesium in type2 diabetes mellitus patients and obese subject for different countries that reported by previous studies.

Country	**Serum Mg in T2DM patients**	**Serum Mg in obese subjects**	**References**
Mexico		1.8± 0.4	Guerrero-Romero and Rodríguez-Morán, 2002
Serbia	1.87±0.22		Rasic et al., 2004
Virginia		1.82±0.04	Huerta et al., 2005
Iraq	1.67±0.37		Maatook and Hammad, 2005
India	1.98 ± 0.23		Ankash et al., 2009
Kansai	1.9± 0.1	2.0 ± 0.1	Takya et al., 2010
India	1.62±0.47		Badyle et al., 2011
Spain	1.82±0.17	1.97±0.15	Lecube et al., 2012
Pakistan	1.83±0.02		Memon et al., 2013
India	1.67±0.3		Kauser et al., 2014
Brazil		1.97±0.15	Cruz et al., 2014
India	1.48±0.51		Chutia and Lynrah, 2015
Egypt	1.29±0.31		El-said et al., 2015
India	1.32±0.3		Rao et al., 2016
India	1.88±0.28		Parlapally et al., 2016
Turkey	1.72±0.15	1.98±0.2	This study

CHAPTER 5

CONCLUSIONS

The present study reported hypomagnesaemia in obese type2 diabetes mellitus with duration of disease history of diabetes more than five years and it was associated with insulin resistance that can be a further risk factor for uncontrolled diabetes and diabetic complications.

Consequently, we recommend firstly patient with obese type2 diabetes mellitus must weight loss and secondly measuring serum magnesium regularly in type2 diabetes mellitus patients. Also, we suggested that T2DM patients must take a magnesium rich foods such as all grains, fruits, and vegetables (dark-green, leafy vegetables) daily that will benefit to provide recommended intakes of magnesium and preserve normal storage levels of this mineral. Patients who require supplementation should be considered.

However, the association between lipid abnormalities and hypomagnesaemia has not been fully understood in humans and need further studies.

REFERENCES

Abdul-Ghani, M. A., Williams, K., DeFronzo, R., Stern, M. (2006). Risk of progression to type2 diabetes based on relationship between postload plasma glucose and fasting plasma glucose, *Diabetes Care*, **29**, 1613-8.

ADA. American Diabetes Association. (2017). Diagnosis and classification of diabetes mellitus, Diabetes Care **40**(1), 11–24.

Ahmed Baig, M. S., Shamshuddin, M., Mahadevappa, K. L., Attar, A. H., Shaikh, A. K. (2012). Serum magnesium as a marker of diabetic complications, *Journal of Evolution of Medical and Dental Sciences*, **3**, 119.

Allain, C. C., Poon, L. S., Chan, C. S., Richmond, W., Fu, P. C. (1974). Enzymatic determination of total serum cholesterol, *Clin Chem*, **20**, 470-475.

Allauzen, S., Salhi, S. L., Piechaczyk, M., Bastide, M., Pau, B., Bouanani, M. (1994). An efficient immunization protocol for production of monoclonal antibodies against human soluble insulin, *Immunology Letters*, **40**, 1-6.

Allauzen, S., Mani, J. C., Granier, C., Pau, B., Bouanani, M. (1995). Epitope mapping and binding analysis of insulin monoclonal antibodies using a biosensor approach, *Journal of Immunological Methods*, **183**, 27-32.

Ankush, R. D., Suryakar, A. N., Ankush, N. R. (2009). Hypomagnesaemia in type-2 diabetes mellitus patients: a study on the status of oxidative and nitrosative stress, *Indian Journal of Clinical Biochemistry,* **24** (2), 184-189.

Augusta, C. N., Chinyere, A., Opara, U., Maisie, H. E., Isonguyo, N. U. (2006). Influence of Age, Gender and Duration of Diabetes on Serum and Urine Levels of Zinc, Magnesium, Selenium and Chromium in Type 2 Diabetics in Calabar, Nigeria, *Türk Biyokimya Dergisi [Turkish Journal of Biochemistry-Turk J Biochem]*, **31**(3), 107–114.

Badyal, A., Sodhi, K. S., Pandey, R., Singh, J. (2011). Serum magnesium levels: A key issue for diabetes mellitus, *Jk Science*, **13**, 3.

Barbagallo, M., Dominguez, L. J., Galioto, A., Ferlisi, A., Cani, C., Malfa, L., Pineo, A., Busardo', A., Paolisso, G. (2003). Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X, *Mol Aspects Med,* **24**, 39-52.

Barbagallo, M., Dominguez, L. J. (2007). Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance, *Arch Biochem Biophys*, **458**, 40-47.

Baudry, A., Leroux, L., Jackerott, M., Joshi, R. L. (2002). Genetic manipulation of insulin signaling, action and secretion in mice, *European Molecular Biology Organization Reports*, **3**(4), 323–328.

Bate, L. K., Jerums, G. (2003). Preventing complications of diabetes*, Med J Aust*, **179** (9), 498-503.

Baskin, M. L., Ard, J., Franklin, F., Allison, D. B. (2005). Prevalence of obesity in the United States, *Obesity Reviews*, **6**, 1–88.

Björntorp P. (1994). Abdominal obesity and the metabolic syndrome, *Ann. Med*, **24**, 465-468.

Bohuon, C. (1962). Clin Chem Acta, **7**, 811-817.

Boyko, E. J., Leonetti, D. L., Bergstrom , R. W., Newell-Morris, L., Fujimoto , W. Y. (1996). Low insulin secretion and high fasting insulin and C-peptide levels predict increased visceral adiposity: 5-year follow-up among initially nondiabetic Japanese-American men, *Diabetes*, **45**, 1010-1015.

Boyko, E. J., Fujimoto, W. Y., Leonetti, D. L., Newell-Morris, L. (2000). Visceral adiposity and risk of type 2 diabetes: a prospective study among Japanese Americans, *Diabetes Care*, **23**, 465-471.

Calle, E. E., Thun, M. J., Petrelli, J. M., Rodriguez, C., Heath, C. W. (1999). Body-mass index and mortality in a prospective cohort of U.S. adults, *N. Engl. J. Med*, **341**, 1097-1105.

Cahill, F., Shahidi, M., Shea, J., Wadden, D., Gulliver, W., Randell, E., Vasdev, S., Sun G. (2013). High dietary magnesium intake is associated with low insulin resistance in the Newfoundland population, *Plos ONE*, **8**(3), 1–8.

Caterson, I. D., Gill, T. P. (2002). Obesity: epidemiology and possible prevention, *Best Pract Res Clin Endocrinol Metab*, **16**, 595-610.

Clímaco Cruz, K. J., Soares de Oliveira, A. R., Pinto, D. P., et al. (2014). Influence of Magnesium on Insulin Resistance in Obese Women, *Biol Trace Elem Res*, **160**, 305–310.

Chutia, H., Lynrah, K. G. (2015). Association of Serum Magnesium Deficiency with Insulin Resistance in Type 2 Diabetes Mellitus, *Journal of Laboratory Physicians,* **7**, 75-8.

Cook, J. G. (1971). Creatinine assay in the presence of protein, *Clin Chim Acta*, **32**, 485-6.

Cummings, J. H., Roberfroid, M. B., Andersson, H., Barth, C., Ferro-Luzzi, A., Ghoos, Y., Gibney, M., Hermansen, K., James, W. P., Korver, O., Lairon, D., Pascal, G., Voragen, A. G. (1997). A new look at dietary carbohydrate: chemistry physiology and health, *European Journal of Clinical Nutrition*, **51**, 417-423.

Czok, R., Barthelmai, W. (1962). Enzymatische Bestimmungen der Glucose in Blut, Liquor und Harn, *Klin Wschr*, **40**:585-589.

Cătoi, A. F., Pârvu, A., Mureşan, A., Busetto L. (2015). Metabolic Mechanisms in Obesity and Type 2 Diabetes: Insights from Bariatric/Metabolic Surgery, *Review Article*, **8**, 6.

Del Gobbo, L. C., Song, Y., Poirier, P., Dewailly, E., Elin, R. J., Egeland, G. M. (2012). Low serum magnesium concentrations are associated with a high prevalence of premature ventricular complexes in obese adults with type 2 diabetes, *Cardiovasc Diabetol*, **11**, 23.

De Leeuw, I., Engelen, W., De Block, C., Van Gaal, L. (2004). Long term magnesium supplementation influences favourably the natural evolution of neuropathy in Mg-depleted type 1 diabetic patients, *Magnes Res*, **17**, 109–14.

Del Gobbo, L. C., Song, Y., Poirier, P., Dewailly, E., Elin, R. J., Egeland, G. M. (2012). Low serum magnesium concentrations are associated with a high prevalence of premature ventricular complexes in obese adults with type 2 diabetes, *Cardiovasc Diabetol*, **11**, 23.

De Baaij, J. H., Hoenderop, J. G., Bindels, R. J. (2015). Magnesium in man implications for health and disease, *Physiol Rev*, **95**, 1-46.

DeFronzo, R. A., Ferrannini, E. (1991). Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease, *Diabetes Care*, **14**, 173-194.

Dhatariya, K. (2003). Type 2 diabetes is cardiovascular disease, *The Royal Society of Medicine*, **96**(8), 371–372.

Eckel, R. H., Krauss, R. M. (1998). American Heart Association call to action: obesity as a major risk factor for coronary heart disease, *AHA Nutrition Committee Circulation*, **97**, 2099-2100.

El-said, N. H., Sadik, N. A., Abd EL-Ghaffar, M. N. (2015). Magnesium in type 2 diabetes mellitus and its correlation with glycemic control, *Int J Res Med Sci,* **3**(8), 1958-1963.

Elementol. J., et al. (2010). Evaluation of the correlations between magnesium concentration and selected serum lipid components in women and men of different age with chronic kidney failure, **15**(2), 321–329.

Elin, R. J. (1994). Magnesium: the fifth but forgotten electrolyte, *Am J Clin Pathol*, **102**, 616-22.

Elin, R. J. (2010). Assessment of magnesium status for diagnosis and therapy, *Magnes Res*, **23**, 194–198.

Eriksson, J., Franssila Kallunki, A., Ekstrand, A. et al. (1989). Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus, *N Engl J Med*, **321**, 337–343.

Elin, R, J. (1987). Assessment of magnesium status. *Clin Chem*, **33**, 1965□70.

Farag, Y. M., Gaballa, M. R. (2011). Diabesity: an overview of a rising epidemic, *Nephrol Dial Transplant*, **26**, 28–35.

Ferrannini, E. (1998). Insulin resistance versus insulin deficiency in non-insulin-dependent diabetes mellitus: problems and prospects, *Endocr Rev,* **19**, 477-490.

Fine, K. D., Santa Ana, C. A., Porter, J. L., Fordtran, J. S. (1991). Intestinal absorption of magnesium from food and supplements, *J Clin Invest*, **88** , 396–402.

Florez, H. J., Sanchez, A. A., Marks, J. B. (2009). Type 2 diabetes. In: Biessels GJ, Luchsinger JA, editors. Contemporary diabetes: diabetes and the brain, *Humana Press Springer Science*, **33**, 56.

Flegal, K. M., Troiano, R. P. (2000). Changes in the distribution of body mass index of adults and children in the US population, *International Journal of Obesity and Related Metabolic Disorders*, **24**, 807-818.

Ford, E. S., Williamson, D. F., Liu, S. (1997). Weight change and diabetes incidence: findings from a national cohort of US adults, *Am J Epidemiol*, **146**, 214-222.

Gelloneze, B., Repetto, E. M., Geloneze, S., Ermetice, M. N., Tambascia, M. A. (2006). The threshold value for insulin resistance (HOMA-IR). IR in the Brazilian metabolic syndrome study, *Diabetes Res. Clin. Pract,* **72**, 219–229.

Ghorbani, A., Baradaran, A. (2012). Magnesium and diabetes Mellitus, *J Renal Inj Prev*, **1**(2), 46-47.

Gregg, E. W., Cadwell, B. L., Cheng, Y. J., Cowie, C. C., Williams, D. E., Geiss, L., Engelgau, M. M., Vinicor, F. (2004). Trends in the prevalence and ratio of diagnosed to undiagnosed diabetes according to obesity levels in the US, *Diab Care*, **27**, 2806-2812.

Groop, L., Forsblom, C., Lehtovirta, M., Tuomi, T., Karanko, S., Nisse´n, M., Ehrnstro¨m B, O., Forse´n, B., Isomaa, B., Snickars, B., Taskinen, M, R. (1996). Metabolic consequences of a family history of NIDDM (The Botnia Study), *Diabetes*, **45**, 1585-1593.

Groop, L., Tuomi, T. (1997). Non-insulin dependent diabetes mellitus- a collision between thrifty genes and an affluent society, *Ann Med*, **29**, 37-53.

Grubbs, R. D., Maguire, M. E. (1987). Magnesium as a regulatory cation: criteria and evaluation, *Magnesium,* **6**, 113-127.

Gustafson, D. (2006). Adiposity indices and dementia. *The Lancet Neurology*, **5**(8), 713-720.

Guariguata, L., Whiting, D. R., Hambleton, I., Beagley , J., Linnenkamp, U., Shaw, J. E. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035, *Diabetes Research and Clinical Practice*, **103**, 137-149.

Guerrero-Romeroa, F., Rodroaguez-Moraan, M. (2002). Relationship between serum magnesium levels and C-reactive protein concentration, in non-diabetic, non-hypertensive obese, *International Journal of Obesity*, **26** , 469–474.

Guerrero-Romero, F., Tamez-Perez, H. E., González-González, G., Salinas-Martínez, A. M., Montes-Villarreal, J., Treviño-Ortiz, J. H., Rodríguez-Morán, M. (2004). Oral magnesium supplementation improves insulin sensitivity in non-diabetic subjects with insulin resistance. A double-blind placebo-controlled randomized trial, *Diabetes Metab*, **30**, 253-258.

Guerrero-Romero, F., Rodríguez-Morán, M. (2013). Serum magnesium in the metabolically-obese normal-weight and healthy-obese subjects, *Eur J Intern Med*, **24**(7), 639–643.

Hedberg, J., Haenni, A. (2012). Increased plasma magnesium concentrations 3 years after biliopancreatic diversion with duodenal switch, *Obes Surg*, **22**(11), 1708–1713.

Hexum, T., Samson, F. E., Himes, R. H. (1970). Kinetic studies of the membrane (Na+-K+-Mg2+)-ATPase, *Biochim Biophys Acta*, **212**, 322-331.

Hochholdinger, F., Baier, G., Nogalo, A., Bauer, B., Grunicke, H. H., Überall. F. (1999). "Novel membrane-targeted ERK1 and ERK2 chimeras which act as dominant negative, isotype-specific mitogen-activated protein kinase inhibitors of Ras-Raf-mediated transcriptional activation of c-fos in NIH 3T3 cells, *Molecular and cellular biology*, **19,** 8052-8065.

Huerta, M. G., Roemmich, J. N., et al. (2005). Magnesium Deficiency Is Associated With Insulin Resistance in Obese Children, *Diabetes Care*, **28**, 1175–1181.

Jacobs, N. J., Van Denmark, P. J. (1960). Arch Biochem Biophys, **88**, 250-255.

Jerry, L., Nadler, M. D. (2000). Diabetes and Magnesium: The Emerging Role of Oral Magnesium Supplementation. Third Quarter. The Magnesium Report Clinical Research and Laboratory News for Cardiologists.

Johansson, G., Danielsson, B. G., Wibell, L. (1982). Evidence for a disturbed magnesium metabolism in diabetes mellitus, *Magnesium*, **3**, 178-180.

Juhan-Vague, I., Thompson, S. G., Jespersen, J. (1993). Involvement of the hemostatic system in the insulin resistance syndrome: a study of 1500 patients with angina pectoris: the ECAT Angina Pectoris Study Group, *Arterioscl. Thromb*, **13**, 1865-1873.

Kaplan, N. M. (1989). The deadly quartet. Upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension, *Archives of Internal Medicine*, **149**, 1514-1520.

Kalant, N., Leibovici, T., Rohan, I., Ozaki, S. (1979). Interrelationships of glucose and insulin uptake by muscle of normal and diabetic man. Evidence of a difference in metabolism of endogenous and exogenous insulin, *Diabetologia*, **16**(6), 365-72.

Kahn, S. E., Verchere, C. B., Andrikopoulos, S., et al. (1998). Reduced amylin release is a characteristic of impaired glucose tolerance and type 2 diabetes in Japanese Americans, *Diabetes*, **47**, 640–645.

Kahn, S. E. (2001). Clinical review 135: The importance of beta-cell failure in the development and progression of type 2 diabetes, *The Journal of Clinical Endocrinology & Metabolism*, **86**, 4047-4058.

Kahn, S. E. (2003). The relative contributions of insulin resistance and beta cell dysfunction to the pathophysiology of Type 2 diabetes, *Diabetologia*, **46**, 3-19.

Kauser, M. M., Afreen, A., Prabhakar, K., Kumar, S. R., Javarappa, D. (2014). Study of Serum Magnesium in Type 2 Diabetes Mellitus and its Correlation with the Modality of Treatment- A South Indian Study, *Global journal of medical research: Interdisciplinary,* **14**, 4.

King, H., Aubert, R. E., Herman, W. H. (1998). Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections, *Diabetes Care*, **21**, 1414-1431.

Kim, S. K., Park, S. W., Hwang, I. J., et al. (2009). High-fat stores in ectopic compartments in men with newly diagnosed type 2 diabetes: an anthropometric determinant of carotid atherosclerosis and insulin resistance, *Int J Obes (Lond),* **34**(1), 105-110.

Kopelman, P. G. (2000). Obesity as a medical problem, *Nature*, **404**(6778), 635-643.

Krentz, A. J. (1996). Insulin resistance, *BMJ*, **313**(7069), 1385–1389.

Larsen, K. (1972). Creatinine assay in the presence of protein with LKB 8600 reaction rate analyser, *Clin Chim Acta*, **38**, 475-6.

Lebovitz, H. E. (1999). Type 2 diabetes: a review, *Clin Chem*, **45**, 1339-1349.

Lecube, A., Baena-Fustegueras, J. A., Fort, J. M., et al. (2012). Diabetes Is the Main Factor Accounting for Hypomagnesemia in Obese Subjects, *PLoS ONE*, **7**(1), 30599.

Lima, M. L., Judith, P. J., Barbosa, C., Bras, T. C. (2005). Magnesium deficiency and insulin resistance in patients with type 2 diabetes mellitus, *Endocrinol Metab,* **49**, 959–963.

Maatook, M. A., Hammad, D. Sh. (2015). The level of serum magnesium activity in diabetes mellitus, *AL-Qadisiya Medical Journal*, **11***,* 19.

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, *Diabetologia,* **28**, 412–9.

Mathers, C. D., Loncar, D. (2006). Projections of global mortality and burden of disease from 2002 to 2030, *PLoS Med*, **3**(11), e442.

Mather, H. M., Levin, G. E. (1979). Magnesium status in diabetes, *Lancet*, **1**, 924.

Maltezos, E., Papazoglou, D., Exiara, T., Kambouromiti , G., Antonoglou, C. (2004). Serum magnesium levels in non-diabetic offspring of patients with type 2 diabetes mellitus, *Diabetes Nutr Metab*, **17**, 12-16.

Mann, C. K., Yoe, J. H. (1957). Anal Chem Acta, **16**, 155-160.

Manson, J. A., Colditz, G. A., Stampfer, M. J., Willett, W. C., Rosner, B., Monson, R. R., Speizer, F. E., Hennekens, C. H. (1990). A prospective study of obesity and risk of coronary heart disease in women, *N Engl J Med*, **322**, 882–889.

Memon, A. R., Memon, M. Y., Memon, F. (2013). Serum Magnesium Levels in Type-I and Type-II Diabetes, *Indo American Journal of Pharmaceutical Research*, **3**, 2231-6876.

Miki, Y. A. (1999). Homogeneous assay for the selective measurement of LDL-cholesterol in serum. Enzymatic selective protection method, *Clin Lab*, **45**, 398-401.

Modan, M., Karasik, A., Halkin, H., Fuchs, Z., Lusky, A., Shitrit, A., Modan, B. (1986). Effect of past and concurrent body mass index on prevalence of glucose intolerance and type 2 (non-insulin-dependent) diabetes and on insulin response. The Israel study of glucose intolerance, obesity and hypertension, *Diabetologia*, **29**, 82-89.

Nadler, J. L., Buchanan, T., Natarajan, R., Antonipillai, I., Bergman, R., Rude, R. (1993). Magnesium deficiency produces insulin resistance and increased thromboxane synthesis, *Hypertension*, **21**, 1024-1029.

Naheed, T., Khan, A., Masood, G., Yunus, B. B., Chaudhry, M. A. (2003). Dyslipidemias in type II diabetes mellitus patients in a teaching hospital of Lahore, Pakistan, *Pak J Med Sci*, **19**, 283–286.

Nasri, H. H., Baradaran, H. R. (2008). Lipid in association with serum magnesium in diabetes mellitus patients, *Bartisl Lek Listy*, **109**, 302-6.

Odeleye, O. E., de Courten, M., Pettitt, D. J., Ravussin, E. (1997). Fasting hyperinsulinemia is a predictor of increased body weight gain and obesity in Pima Indian children, *Diabetes,* **46**, 1341-1345.

Parlapally, R. P., Kumari, K. R., Jyothi, S. A. (2016). Serum Magnesium Levels in Type 2 Diabetes Mellitus, *Int J Sci Stud*, **4**(5), 176-179.

Paolisso, G., Tirelli, A., Coppola, L., Verrazzo, G., Pizza, G., Sgambato, S., et al. (1989). Magnesium administration reduces platelet hyperaggregability in NIDDM, *Diabetes Care*, **12**, 167–168.

Pham, P. C., Pham, P. M., Pham, S. V., Miller, J. M., Pham, P. T. (2007). Hypomagnesemia in patients with type 2 diabetes, *Clin J Am Soc Nephrol*, **2**, 366–373

Phoung-chi, T. P., Phoung-Mai, T. P., Son-V. P., et al. (2007). Hypomagnesaemia in patients with type 2 Diabetes, *Clinical journal American society of nephrology*, **2**, 366-373.

Pratley, R. E., Weyer, C. (2002). Progression from IGT to type 2 diabetes mellitus: the central role of impaired early insulin secretion:a review, *Curr Diab Rep*, **2**(3), 242-8.

Rafieian-Kopaie, M., Nasri, H. (2013). Serum lipoprotein (a) and atherosclerotic changes in hemodialysis patients, *J Renal Inj Prev*, **2**(2), 47-50.

Rasic-Milutinovic, Z., Perunicic-Pekovic, G., Pljexa, S., Dangic, A., Dangic, A. (2004). Magnesium deficiency in type 2 diabetes, *Hippokratia*, **8**,179-181.

Rao, Y. S., Rao, V. D. (2016). Serum magnesium levels in type 2 diabetes, *Int J Res Med Sci*, **4**(4), 991-994.

Reaven, G. M. (1988). Role of insulin resistance in human disease. Banting lecture, *Diabetes*, **37**, 1595-1607.

Riesen, W. F. (1998). Lipid metabolism. In: Thomas L, ed. Clinical laboratory diagnostics. Use and assessment of clinical laboratory results. Frankfurt/Main: TH-Books Verlagsgesellschaft, 169-171.

Rodríguez-Morán, M., Guerrero-Romero, F. (2004). Elevated concentrations of TNF-alpha are related to low serum magnesium levels in obese subjects, *Magnes Res*, **17**, 189–196.

Sarkar, A., Dash, S., Barik, B. K., Muttigi, M. S., Kedage, V., Shetty, J. K., Prakash, M. (2010). Copper and Ceruloplasmin levels in relation to total thiols and GST in type2 diabetes mellitus patients, *Ind j Clin Biochem*, **25**, 74-76.

Saris, N. E., Mervaala, E., Karppanen, H., Khawaja, J. A., Lewenstam, A. (2000) Magnesium. An update on physiological, clinical and analytical aspects, *Clin. Chim. Acta*, **294**, 1–26.

Sanders, N. N., De Smedt, S. C., Van Rompaey, E., *et al.* (2000). Cystic fibrosis sputum: a barrier to the transport of nanospheres, *Am J Respir Crit Care Med*, **162**, 1905–11.

Scheen, A. J. (2003). Current management strategies for coexisting diabetes mellitus and obesity, *Drugs*, **63**, 1165-84.

Schrinner, S., Scherbaum, W. A., Bornstein, S. R., Barthe, A. I. (2005). "Molecular mechanism of insulin resistance," Diabetes UK, *Diabetic Med*, **22**, 674-682.

Steinberger, J., Moorehead, C., Katch, V., Rocchini, A. P. (1995). Relationship between insulin resistance and abnormal lipid profile in obese adolescents, *J Pediatr*, **126**, 690-5.

Stumvoll, M., Goldstein, B. J., van Haeften , T. W. (2005). Type 2 diabetes: principles of pathogenesis and therapy, *Lancet*, **365**, 9-15.

Supriya, Mohanty, S., Pinnelli, V. B., Murgod, R., Raghavendra, D. S. (2013). Evaluation of serum copper, magnesium and glycated haemoglobin in type 2 diabetes mellitus, *Asian J Pharm Clin Res*, **6**, 188-90.

Su, N. Y., Peng, T. C., Tsai, P. S., Huang, C. J. (2013). Phosphoinositide 3-kinase/Akt pathway is involved in mediating the anti-inflammation effects of magnesium sulfate, *J Surg Res*, **185**, 726–732.

Songer, T, J., Zimmet, P, Z. (1995). Epidemiology of type II diabetes: an international perspective, *Pharmacoeconomics,* **8**, 1-11.

Takaya, J., Yamato, F., Kuroyanagi, Y., Higashino, H., Kaneko, K. (2014). Intracellular Magnesium of Obese and Type 2 Diabetes Mellitus Children, *Diabetes Ther*, **1**(1), 25-31.

Taylor, R. (2012). Insulin resistance and type 2 diabetes, *Diabetes*, **61**, 778-779.

Tietz NW. (1995). Clinical guide to laboratory tests. 610-611: Third ed. Philadelphia: WB Saunders Company.

Thomas, G. N., Ho, S. Y., Lam, K. S., Janus, E. D., Hedley, A. J., Lam, T. H. (2004). Impact of obesity and body fat distribution on cardiovascular risk factors in Hong Kong Chinese, *Obes. Res*, **12**, 1805-1813.

Tracey, L., Mclaughlin, Gerald, M., Reaven. (2003). Beyond type 2 Diabetes: The need for a clinically useful way to identify insulin resistance, *The American Journal of Medicine*, **114**, 501-2.

Tunceli, K., Bradley, C. J., Nerenz, D., Williams, L. K., Pladevall, M., Lafata, J. E. (2005). The impact of diabetes on employment and work productivity, *Diabetes Care*, **28**(11), 2662 – 2667.

Volpe, S. L. (2013). Magnesium in disease prevention and overall health, *Adv Nutr,* **4**, 378S–383S.

Warram, J. H., Martin, B. C., Krolewski, A. S., Soeldner, J. S., Kahn, C. R. (1990). Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents, *Annals of Internal Medicine*, **113**(12), 909-915.

Wang, J. L., Shaw, N. S., Yeh, H. Y. K. (2005). Magnesium status and association with diabetes in the Taiwanese elderly, *Asia Pac J Clin Nutr*, **14**(3), 263-269.

WHO. World Health Organization. (2016). Global report on diabetes.Geneva. (http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf).

Yoon, K. H., Lee, J. H., Kim, J. W., Cho, J. H., Choi, Y. H., Ko, S. H., Zimmet, P., Son, H. Y. (2006). Epidemic obesity and type 2 diabetes in Asia, *Lancet*, **368**, 1681–1688.

Zimmet, P., Alberti, K. G., Shaw, J. (2001). Global and societal implications of the diabetes epidemic, *Nature*, **414**, 782–787.