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M. Sc. in Biochemistry Science and Technology

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UNIVERSITY OF GAZİANTEP

GRADUATE SCHOOL OF

NATURAL & APPLIED SCIENCES

ZINC, MAGNESIUM AND PREALBUMIN SERUM LEVELS OF CHILDREN WITH TYPE 1 DIABETES MELLITUS

M. Sc. THESIS

IN

BIOCHEMISTRY SCIENCE AND TECHNOLOGY

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Supervisor

Asist. Prof. Dr. Enes COŞKUN

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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.

Ayat Jawad Ali ALTAMEEMI

ABSTRACT

ZINC, MAGNESIUM AND PREALBUMIN SERUM LEVELS OF CHILDREN WITH TYPE 1 DIABETES MELLITUS

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M. S.c in Biochemistry Science and Technology

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61 pages

Several trace elements are involved in glucose metabolism and insulin signal transduction. Also, some of proteins such as serum pre-albumin has a prognostic value in many diseases, but its serum levels can be affected by many factors. Our aim for this present study was to compare the levels of magnesium (Mg), zinc(Zn) and prealbumin between patients with type 1 diabetes mellitus (DM) and healthy children. 34 type 1 diabetic children who were under follow up of Pediatric Endocrinology Department and 94 healthy children who were under regular control of Social Pediatrics department at University of Gaziantep were enrolled in this study. Serum zinc, magnesium and pre-albumin levels of patients diagnosed with type1 diabetes mellitus were compared with separate control groups for each parameter. The ethical permission has been taken from the ethical committee of University of Gaziantep. Informed consents of participants or their legal guards were taken. The present study consisted of 34 diabetic children at average ages of (3-16) Serum pre-albumin levels were found significantly low in type 1 DM patients compared with healthy children at 16.87±3.63 vs 21.88±7.7 ($p<0.000$), respectively. Additionally, diabetic children showed a marked decrease in Mg levels at 1.9±0.14 compared to 2.01±0.13 in controls ($p=0.005$). On the other hand, no statistical significance was detected in serum Zn values between patients and healthy controls with figures of 0.82±0.16 and 0.86±0.10, respectively ($p=0.329$). The role of prealbumin as a biomarker is not clear yet. However, it is thought as a negative inflammatory marker. Within this context, the low levels of prealbumin in diabetic patients may be attributed to the chronic inflammatory status of the type 1 diabetis. Regarding Mg, the slightly decreased levels in type1 diabetic children were consistent with literature. With respect to Zn, this research did not reveal any difference between the study groups. However, there are controversial results regarding Zn levels in the literature. In conclusion, regularly measurement of Mg and Zinc levels of type 1 DM patients may be required in terms of supplementation when necessary. Furthermore, prealbumin levels may play a role as a biomarker in terms of monitoring inflammatory status of type 1 DM patients.

Key Words: diabetic type 1, prealbumin, magnesium, zinc.

ÖZET

TİP 1 DİYABETLİ ÇOCUKLARIN SERUM ÇİNKO, MAGNEZYUM VE PREALBUMİN DÜZEYLERİ

ALTAMEEMI, Ayat Jawad Ali

Yüksek Lisans Tezi, Biyokimya Bilimi ve Teknolojileri Bölümü

Tez Yöneticisi: Asist. Prof. Dr. Enes Coşkun

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61 sayfa

Glikoz metabolizması ve insülin sinyal transdüksiyonunda birçok eser element bulunmaktadır. Ayrıca, serum prealbumin gibi bazı proteinlerin birçok hastalıkta prognostik bir değeri vardır, ancak serum seviyeleri birçok faktörden etkilenebilir. Bu çalışmanın amacı, tip 1 diabetes mellitus (DM) ve sağlıklı çocuklarda magnezyum (Mg), çinko (Zn) ve prealbumin düzeylerinin karşılaştırılmasıdır. Pediatrik Endokrinoloji Anabilim Dalı'nda takip edilen 34 tip 1 diyabetli çocuk ve Gaziantep Üniversitesi Sosyal Pediatri Anabilim Dalı'nda düzenli kontrol altında olan 94 sağlıklı çocuk çalışmaya dahil edildi. Tip1 diabetes mellitus tanısı alan hastaların serum çinko, magnezyum ve prealbümin düzeyleri, her bir parametre için ayrı kontrol grupları ile karşılaştırıldı. Etik izin, Gaziantep Üniversitesi Etik Kurulu'ndan alınmıştır. Katılımcıların bilgilendirilmiş onayları veya yasal korumaları alındı. Bu çalışma, yaşları 3-16 arasında olan 34 diabetik çocuktan oluştu. Tip 1 DM hastalarında, sağlıklı çoculara kıyasla sırasıyla 16.87 ± 3.63 ve 21.88 ± 7.7 ile serum prealbümin düzeylerinin anlamlı derecede düşük olduğu belirlendi ($p < 0.000$). Ek olarak diyabetik çocuklarda, kontrol grubuna kıyasla Mg düzeylerinde belirgin azalma tespit edildi (1.9 ± 0.14 ve 2.01 ± 0.13, sırasıyla, $p = 0.005$). Diğer taraftan, hastaların serum Zn değerlerinde sağlılklı kontrollere kıyasla istatistiksel olarak anlamlı bir fark bulunmadı ve sırasıyla (0.82 ± 0.16 ve 0.86 ± 0.10, sırasıyla ($p = 0.329$). Bir biyomarker olarak prealbüminin rolü henüz net değildir. Ancak, negatif inflamatuar bir marker olarak düşünülmektedir. Bu bağlamda, diyabetik hastalarda düşük prealbumin seviyeleri, tip 1 diyabetin kronik inflamatuar durumuna atfedilebilir. Mg ile ilgili olarak, tip 1 diyabetik çocuklarda hafif azalmış düzeyler literatürle uyumluydu. Zn ile ilgili olarak, bu araştırma, çalışma grupları arasında herhangi bir farklılık ortaya koymamıştır. Bununla birlikte, literatürde Zn düzeyleri ile ilgili tartışmalı sonuçlar vardır. Sonuç olarak, tip 1 DM hastalarının Mg ve Çinko düzeylerinin düzenli olarak ölçülmesi, gerektiğinde takviye açısından gerekli olabilir. Ayrıca, prealbumin seviyeleri tip 1 DM hastalarının inflamatuar durumunun izlenmesi açısından biyobelirteç olarak rol oynayabilir.

Anahtar Kelimeler: tip 1 diyabetli, çinko, magnezyum, prealbumin.

To the most cherished people in my life (my parents), I wish God to preserve you for me, thank you for all your sacrifices and continuous support to me.

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My lord, inspire me to thank you for your blessing with which you have blessed me and my parents.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS/ABREVIATIONS

%	Percentage
µg/g	Microgram Per gram
µm	Micrometer
ADP	Adenosine Diphosphate
AP	Acute Pancreatitis
APR	Acute Phase Response
ATP	Adenosine Triphosphatase
BG	Blood Glucose
CD	Celiac Disease
CRP	C-reactive protein
CSII	Continuous Subcutaneous Insulin Infusion
Cu	Copper
DAISY	Diabetes Autoimmunity Study in The Young
DCs	Dendritic Cells
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
FFM	Fat-Free Mass
FPIR	First Phase Insulin Release
g	Gram
GAD65	Glutamic Acid Decarboxylase
GLUT4	Glucose Transporter type 4
HDL	High Density Lipoprotein

HLA	Human Leukocyte Antigen
HRE	High-Resolution Electrophoresis
IA-2	Islet-associated protein–2
IDDM	Insulin-Dependent Diabetes Mellitus
LDL	Low Density Lipoprotein
MDA	Marker-malondialdehyde
MDI	Multiple Daily Insulin
Mg	Magnesium
mg/dl	milligrams per Deciliter
MHC	Major Histocompatibility Complex
mmol	Millimoles
MT	Metallo Thionein
NIDDM	Non- Insulin Dependent Diabetes Mellitus
nmol	Nanomoles
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
PA	Prealbumin
PEM	Protein-Energy Malnutrition
PH	Potential of Hydrogen
PP	pancreatic polypeptide
RBP	Retinol Binding Protein
RNA	Ribonucleic Acid
Se	Selenium
SOD	Superoxide Dismutase
T1D	Type 1 diabetes
T_3	Triiodothyronine
T_4	Thyroxine
TER	Transcapillary Escape Rate

TTR	Transthyretin
U	Unit
WHO	World Health Organization
Zn	Zinc
Znt8	Zinc Transporter 8
ZnT8	Zinc Transporter
α-cells	Alpha cell
β-cell	Beta cell
δ-cells	Delta cell
µM	micrometer

CHAPTER 1

INTRODUCTION

1.1 Diabetes Mellitus (DM)

Or (DM) is a chronic disease characterized by a relative or complete lack of insulin secretion by the β-cell of pancreas or by defect of insulin receptors , or resistance to insulin action, which result in disturbances of carbohydrate , protein , and lipid metabolism. (Martha , 2000 ; and Michele et.al. , 2000 ; Ohmann et.al., 2010). It is known that "Diabetes mellitus" is considered as a group of chronic metabolic disorders in addition to a hyperglycemic condition resulting from deficient insulin secretion, insulin action or both (Ozougwu et al, 2013).

Diabetes mellitus affects more than (120) million people in the world, and by 2020, 220 million individuals are estimated to develop the disease. The complication of the irreversible Diabetes mellitus may lead to a decreased life expectancy and significant healthy cost despite the reasonably normal life styles of the patients. Macrovascular disease complication may result in coronary artery disease, stroke and peripheral vascular disease, also microvascular damages leading to nephropathy and retinopathy of diabetes can been seen.(Parveen & Michael, 2005).

Diabetes mellitus is often diagnosed by an elevated fasting blood glucose level (> 120 mg/dl) or by excretion of glucose in the urine, or by an abnormal glucose tolerance test. In the United States, the third main cause of death is Diabetes mellitus, following cardiac diseases and cancer (Martha, 2000 ; Michele et al. , 2000).

All of the metabolic distortion that are observed in the diabetic state results from the inability of many of body's cell to acquire glucose from the blood. Consequently, these cells " starve in the midst of plenty " metabolic imbalances that devolve from this circumstance have serious, if not life-threatening, consequences (Trudy & James, 1996) see figure (1.1).

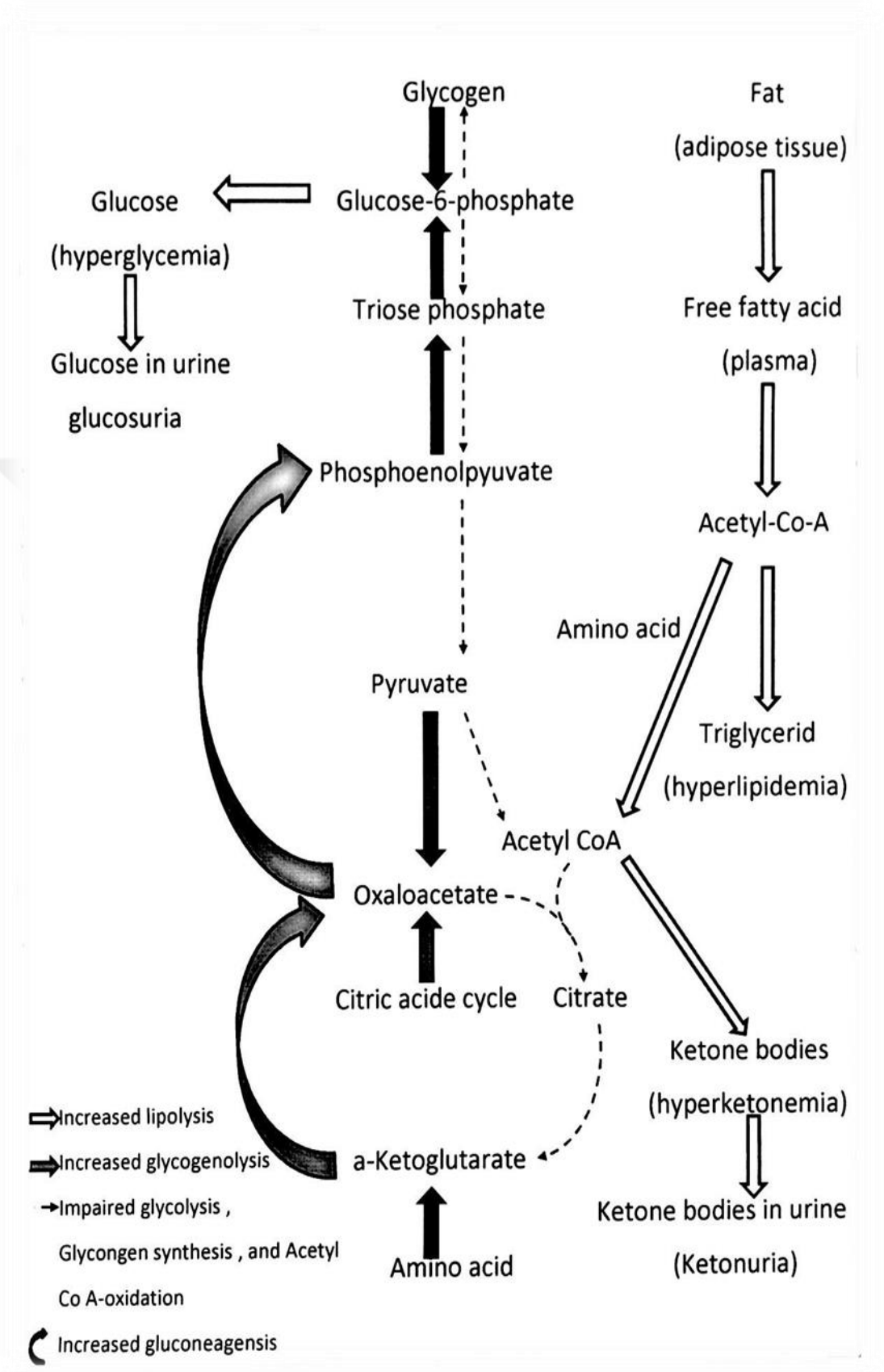

Figure 1.1 Metabolic Disorder in Diabetes Mellitus (Robert et.al.,2000)

The pancreas consists of exocrine and endocrine tissues and is one of the organs contributing to the digestion of food and regulation of glucose metabolism. The digestive enzymes are excreted by exocrine tissues, whereas hormones, which regulate blood glucose levels, are secreted by endocrine tissues (Eman,2006).

Approximately 200 grams of glucose are produced and utilized every day. Hepatic glycogen and gluconeogenesis are the sources of more than (90%) of glucose, while the rest comes from renal gluconeogenesis. The major organ of glucose homeostasis is the liver, where glucose is stored and absorbed as glycogen and released into the blood stream between the meals to cope with the glucose utilization rate by the peripheral tissues. (Parveen & Michael, 2005).

The insulin-producing β-cells, glucagon-secreting α-cells, stomatostatin-secreting δ-cells and pancreatic polypeptide (PP)-producing cells compose the pancreatic islets as shown in (Figure 1.2). To a lesser extent, stromal cells, blood vessels, neurons and immune cells, such as dendritic cells (DCs), reside in the islets. Insulin and glucagon are the hormones responsible for maintaining blood glucose homeostasis. When the levels of blood glucose rise after food intake, β-cells secrete insulin and stimulate the cells in the body to take up glucose as shown in (Figure1.3). Instead, when blood glucose levels decrease, glucagon is produced and stimulates glucose release from the liver (Eman, 2006).

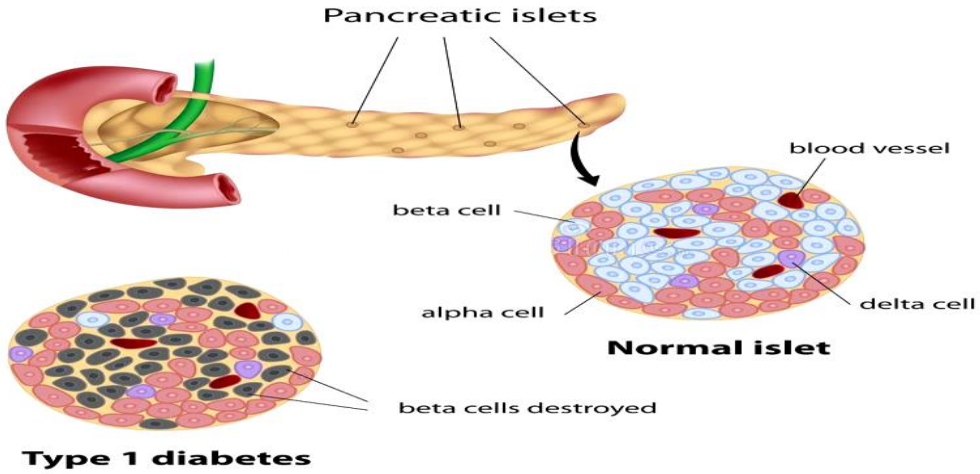

Figure 1.2 Anatomy of the pancreas

https://www.dreamstime.com/royalty-free-stock-photo-pancreatic-islet-image221328

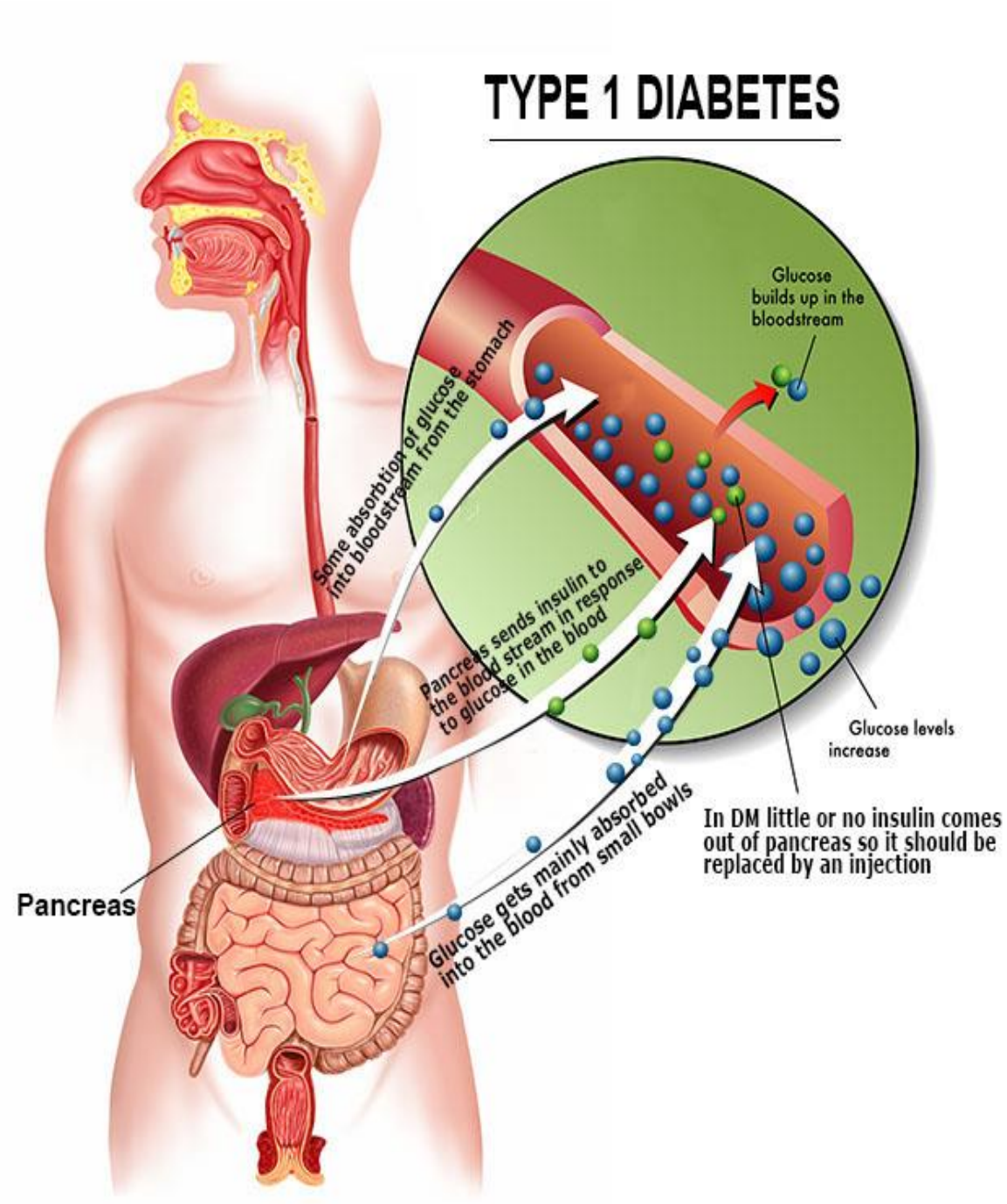

Figure 1.3 Blood Glucose Homeostasis

https://rekmedd16.wordpress.com/2017/03/20/diabetes-mellitus-e10-e14

Diabetes mellitus can be primary or secondary. The two types of the disease should be clinically observed. In both forms of diabetes, different degrees of insulin secretory failure may be found. Some immune-mediated diabetic individuals, for example, may not initially need insulin for treatment, while many others with type 2 diabetes will eventually require it (Parveen & Michael, 2005).

Two types of Diabetes mellitus exist, the insulin-dependent Diabetes mellitus (IDDM), also called type 1 diabetes, in which the destroyed pancreatic β-cells secrete only small amounts of insulin or no insulin; injection of exogenous insulin is therefore needed to sustain the patient's life. This type of diabetes forms only (10 %) of the known cases of diabetes mellitus. About (90 %) of the diabetic people have – non insulin dependent diabetes mellitus (NIDDM), which is also called the type 2 diabetes. (Kuzuya & Matsuda,1997 ; Boston et al, 2002 & Ozougwu et al, 2013).

Type 1 diabetes was formerly known as juvenile-onest diabetes because it is usually diagnosed in people under the age of 20 years (Susan , 2016). T1D is the main diabetes in young people, forming about (85%) or more of all young people diabetic cases globally. The incidence rate of T1D shows an increases since birth and peaks between (10) and (14) years of age. The increased T1D incidence is particularly observed in young children worldwide (Maahs et al, 2010). The type 2 diabetes takes place either when no enough amounts of insulin are produced by the pancreas to maintain a normal glucose levels in the blood, or when the body is cannot effectively utilize the insulin produced by the pancreas (insulin resistance) resulting in hyperglycemia . This type of diabetes is tightly linked with being overweight and is more commonly seen in old people. Type 2 diabetes is still relatively rarely observed in children (Griswold et al, 2014).

Table 1.1 Comparison Between İnsulin-Dependent and Non–İnsulin Dependent Diabetes Mellitus (Boston et al, 2002) and (Parveen & Michael, 2005).

Feature	**Insulin dependent (type 1)**	**Non-insulin dependent (type 2)**
Age onset	Under 20 years	Over 40 years
Development of symptoms	Rapid	Slow
Percentage of diabetes population	About 10 %	About 90 %
Development of ketoacidosis	Common	Nil-rare
Association with obesity	Rare	Common
Beta cells of islets (an onset of diabetes)	Destroyed	Not destroyed
Insulin secretion	Decreased	Normal or increased
Autoantibodies to islets cells	Present	Absent
Association with particular MHC antigens	Yes	Unclear
Treatment	Insulin injections	Diet and exercise ; oral stimulators of insulin secretion
C-peptide	Eventual disappearance of C-peptide	C-peptide persists

Some authors stated that there is an intersection between the pathogenesis of type 1 and type 2 Diabetes mellitus at significant positions, because of the association of both types with the inflammatory mediated loss of beta cells of pancreas, their ability to exacerbation by obesity, and because of the metabolic abnormalities of both types including hyperlipidemia, hyperglycemia and other metabolism syndromes. Composition of the intestinal microbiota is another possible environmental factor. Recent preliminary researches indicated that the individual's gut microbiota at risk of developing type 1 diabetes differ from those of healthy persons (Susan, 2016).

Type 1 Diabetes mellitus is caused by an autoimmune process that results in pancreatic beta cell destruction, hyperglycemia and deficient insulin. Management of diabetes includes administration of insulin subcutaneously, multiple blood sugar estimation and carbohydrate count, along with the aiming to maintain normal plasma glucose levels, as well as avoiding the acute and long-term complications of diabetes (Ohmann et al, 2009).

The immune system normally produces special proteins known as antibodies to protect human's body from infections, but in T1DM, the immune system attacks its β-cells by directing autoantibodies against the own cells. This progressive attack leads to the destruction of more than (90%) of the pancreatic β-cells, which can happen rapidly or within a period of few years, and finally causes incapacity to produce sufficient insulin (Bethind & Quattrin, 2014).

The majority of type 1 (type1a) are caused by an autoimmune destruction of the pancreatic β-cells, although few cases (type1b) are caused by an idiopathic failure or destruction of the pancreatic β-cells (Maahs et al, 2010).

Type 1 Diabetes mellitus, which is a disease of children, and caused by insulin deficiency, has a peak incidence at the puberty time, but can be identified at any age of the human's life (Parveen & Michael, 2005). T1D is known as an "autoimmune disorder" indicating that the child's immune system is damaging the β-cells in their insulin-producing pancreas. In T1D, little or no insulin at all is produced by the body, and this lack leads to high blood glucose levels (Craig et al, 2009). In type 1 diabetes, the clinical features include prominent weight loss, hyperglycaemia which is difficult to be corrected by diet and treatments, persistent or strong ketoneuria and

autoantibody testing which indicates the presence of an autoimmune disease (Parveen & Michael, 2005).

Uncontrolled IDDM causes increased hepatic glucose output when stored glycogen is first mobilized then glucose is produced by hepatic gluconeogenesis. Non- hepatic utilization of glucose is also impaired by insulin deficiency. Glucose uptake is stimulated by insulin in adipose tissues and skeletal muscles particularly, and this is done by insulin-induced movement of the glucose transporter proteins to the tissue plasma membranes. The low glucose uptake by the peripheral tissues in turn results in a low glucose metabolism rate. Levels of hepatic glucokinase are also regulated by insulin. Therefore, a reduced glucose phosphorylation rate in hepatic cells causes an increased delivery in the blood. Insulin influences other enzymes involved in glucose anabolic metabolism (Ozougwu et al, 2013).

Young children with T1D are particularly subject to experience extreme fluctuations in the levels of glucose (Cato et al, 2014). In the past ten years, the rates of childhood type 1 diabetes mellitus are more than doubling. Patient's healthy lifestyle and diet is important to maintain a good blood glucose control and prevent long-term complications of the disease (Hart et al, 2013).

In the developed countries, the incidence of type 1 diabetes increased steadily from the 1950s till present time, with an alarming anticipation that it may be doubled among children whose age is less than (5) years by the year 2020. No way is known to prevent this metabolic disease, and it seems that that type 1 Diabetes mellitus is a (disease of civilization) (John, 2010).

In the United States, more than 700,000 people have type 1 diabetes, which consists (5-10%) of all diabetes mellitus cases. Type 1 diabetes is often diagnosed during childhood or early adolescence, and about 1 in every 600 children are affected by the disease (Stang and Story, 2005).

Type 1 Diabetes mellitus has become more commonly known disorder in the United Kingdom, despite the increase in the frequency of diagnosis of type 2 Diabetes mellitus since 2004. Identification of (26500) children and young individuals with type 1 Diabetes mellitus and (500) with type 2 Diabetes mellitus has been done by the 2013-2014 national diabetes (NICE guideline, 2015).

Children may encounter high risk of malnutrition, because they usually have lower nutrition stores in comparison with the critically ill adults, and in order to optimize clinical care in such children, accurate and timely assessment and prescription would be necessary (Ong et al, 2014).

Recent highest incidence rate of T1 Diabetes mellitus has been reported among the youngest age (0-4) years, as it has been stated by European registries. However, after puberty, these rates reduced and seem to be stabilized in younger adults (15-29 years). Although about 1/4 of patients with T1 Diabetes mellitus were diagnosed in adults, the incidence of T1D in adults remains lower than children (Maahs et al, 2010).

The highest global rate of T1D was found in the Northern European countries, especially Finland, excluding Sardinia island, which recorded the second highest rate of the disease worldwide for unknown causes (Parveen & Michael, 2005). In Italy, 3 million people have developed diabetes in 2001 representing (5%) of the population of Italy. The Sardinia island in Italy has particularly recorded the highest annual number of of type 1 diabetes, because the incidence of T1 diabetes was more than 50 / 100,000 cases of inhabitants (age range 0–30 years), while in other parts of Italy, the annual new registered cases were about 6–7 / 100,000 inhabitants. Therefore, determination mineral levels in biological specimens and monitoring and evaluation their effects on human's health would be necessary. However, in the island of Sardinia, no data is available to show the relationship of diabetes with metals (Forte et al, 2013).

It is estimated that (19.4) million people are affected with type 1 Diabetes mellitus, and this number is expected to increase to (57.2) millions by the year 2025 as it has been estimated by the WHO (1995) (Parveen & Michael ,2005)

Despite the less strong genetic relation in type 2 Diabetes mellitus, it is obvious that there is a genetic element to the development of T1DM. The Diabetes UK states that when the mother develops T1D, then the risk of the child to be affected is about (2%), while when the father develops diabetes, then the child's risk to be diabetic is about (8%), whereas when both parents have T1DM, then there is a (30%)

opportunity to the offspring to develop the disorder (Diabetes UK, 2010) ; (Eman, 2006).

Thus far, no strategy to prevent the development of T1D is available yet. Relatives of patients with type 1 Diabetes mellitus have higher risk to develop it in comparison with patients who have no relation with type 1 diabetes patients (Gregory et al, 2013; Bethind & Quattrin, 2014).

Type 1 diabetes, a disorder with strong genetic elements, is caused by pancreatic beta cell autoimmune destruction (Steck & Rewers, 2011). The diabetes autoimmunity study in the young (DAISY) longitudinally evaluated the disease development in both first-degree relatives (children) of patients with T1D as well as a cohort of newborns from the general population identified through HLA screening, and risk classified based on HLA-DR/DQ genotyping. It was found that the high-risk genotype (DR3 - DQ2/DR4 - DQ8) was present in (2%) of newborns (Serge & Elizabeth ,2007).

Previous studies generally recommend for evaluation of autoantibody and genetic screening, as well as the quantitation of the risk of developing the disease based on other factors. However, few currently techniques are available to evaluate the roots of T1DM: beta cell destruction. Therefore, novel methods are established to discuss and measure the beta cell stress degree and failure through analyzing the proteins, RNAs and DNAs (Watkins et al, 2014).

For the pathogenesis of type 1 diabetes, putative genes, such as insulin genes, HLA polymorphism genes and T-cell regulatory genes are applied. For the detection of T1D, the autoimmune markers include autoantibodies to islet cells (GAD65), insulin autoantibodies, the tyrosine phosphatases (IA-2) and (IA-2β) in addition to the zinc transporter (ZnT8) (Susan, 2016).

1.2 Insulin Structure and Secretion

The endocrine cells are assembled into cell clusters entitled the islets of Langerhans that are scattered throughout the exocrine tissue. The number of islet in the human pancreas is approximately 1.5 million corresponding to 1-2% of the total pancreatic

mass. The size of the islets varies between approximately 20-250 µm in diameter with the majority of islets being <100µm (Eman, 2006).

Insulin is the main hormone that contributes to the storage and controls the release of a chemical food energy within the body. It is coded by chromosome 11 and synthesized in the pancreatic islet beta cells. Secretion, synthesis and intracellular processing of insulin by the beta cell is typical way of secreting and manipulating of different peptide hormones in the body. After secretion, insulin reaches the portal blood, and carried to the liver (main target organ). Nearly (50%) of the secreted insulin is extracted and degraded within the liver, and the kidneys breaks down the residue. The liver only partially extracts C-peptide (thereby providing a useful index of insulin secretion rate), but kidneys mainly degrade the C-peptide (Parveen & Michael, 2005).

Carbohydrates rich meals and / or other insulin-stimulating nutrition may cause about 4-10 fold rise in the secretion of insulin as compared to the basal condition, which may last for 2-3 hours before returning to the baseline. After intravenous glucose injection, the elevation in blood glucose result in a burst in insulin secretion that peaks within 3-5 minutes and subsides within 10 minutes. This is called the first phase insulin release (FPIR). However, when blood glucose remains elevated, then the high secretion of insulin is sustained in the second phase insulin release. In a normal individual, the average daily insulin secretion is approximately 40 U or (287 nmol) (Serge & Elizabeth, 2007).

The body requires insulin to help remove glucose from the blood circulation and turn it into fuel for necessary tissues, such as brain and muscles. Diabetes mellitus is characterized by a complete or partial lack of insulin production by the body (Craig et al, 2009).

In the United States, insulin is the cornerstone of pharmacotherapy for nearly 0.73-1.46 million people with type 1 Diabetes mellitus, while the highest incidence of T1DM is around the time of puberty. There is an ongoing increased need to effective insulin treatment strategies for individuals with T1DM. Meticulous attention, with lifestyle and self-care management by the patient and the health-care provider, to the

appropriate use of daily and continuous insulin regimen will help to attain HbA1c and blood glucose targets (Serge and Elizabeth ,2007).

The treatment of diabetes mellitus involves oral anti diabetic medication and dietary regimens. There is strong evidence of abnormality in metabolism of different micronutrients in diabetic persons, although the emphasis is on macronutrients intake (Salgueiro et al, 2001).

1.3 Dietary Supplements

Dietary supplements are substances used in addition to a diet, dietary supplements may contain vitamins, herbs, minerals or other substances. However, these supplements should never replace the role of diet, exercise or medicine in the management of diabetes, do not replace oral medications or insulin with a dietary supplement. Prevention and treatment of dysinsulinemia and syndrome X as well as reduction of diabetic complications is disclosed. The formulation combined herbs, vitamins and minerals are known to lower blood sugar levels (Harris & Martin, 2004).

1.4 Mineral Supplementation

Diabetes mellitus is caused by an autoimmune destruction of insulin producing pancreatic cells where genetic and environmental factors play a role in the pathogenesis disease pathogenesis. It has been revealed recently that oxidative stress-free radicals may also have a role in the development of the disease with its complication. Trace elements like selenium, zinc and copper contribute to an anti-oxidative system (Ozenc et al, 2015).

Metal disturbances can also raise the oxidative stress that may be involved in insulin resistance and emergence of complication. Therefore, minerals and antioxidants may be used to manage and prevent chronic complications of Diabetes mellitus (Forte et al, 2013).

Furthermore, the diabetic polyuria resulting from glucose-mediated hyper osmotic glomerular filtration may be responsible for the promoted urinary mineral loss. If such loss was found to translate to lower availability of minerals necessary for optimal insulin secretion or action, then it would be necessary to current the altered

mineral condition. Solving this problem may involve increasing dietary mineral administration or utilizing supplemental minerals, although the use of supplemental mineral for mineral deficiency correction is highly accepted (Timothy, 2002).

In both type 1 and type 2 Diabetes mellitus, direct relation of trace elements was seen in several studies. Alteration of the metabolism in these minerals was observed in diabetic persons, and some studies showed low zinc and magnesium levels in diabetic patient's sera (Zargar et al, 1998; Diwan et al, 2006).

Therfore, decreased concentrations of these trace elements may cause oxidant stress which may contribute to the etiology of Diabetes mellitus. However, it is not clear yet whether metal deficiencies induce or exacerbate diabetes (Ozenc et al, 2015).

1.5 Zinc

Zinc is the essential mineral found in foods and all body fluids and tissues (Sandstead, 1994). The total body zinc content is 30 mmol or 2 grams. The skeletal muscle contains 60% of the total zinc in the body, and bone mass with a zinc concentration of 100-200 µg/g forms about 30%. In lean body mass, the concentration of zinc is about 30 µg/g. The plasma zinc which represents 0.1% of the total body zinc with a rapid turnover rate, is bound to albumin, proteins and free amino acid. Only a small proportion of the plasma zinc is found in the ionic form (Vallee et al, 1949). The higher zinc concentration exists in the choroid of the eye (274 µg/g), and in the prostatic fluid (300-500 mg/l) (Walter,1986). Other organs high in zinc content include bones, liver, kidney, pancreas and retina of the eye (Halstead et al, 1974; Underwood, 1977).

1.5.1 Function

Zinc is the essential element for more than 300 enzymes which participate in degradation and synthesis of carbohydrates, proteins, nucleic acids and lipids, and it contributes to other micronutrients metabolism (Walter, 1986; Prasad ,1995 & Institute of medicine, 2001). Zinc is often an integral component of the enzyme's active site. Alkaline phosphatase, alcohol dehydrogenase, and carbonic anhydrase require zinc. Because carbonic anhydrase activity is high in erythrocytes, zinc depletion often leads to both diminished activity of this enzyme and lowered zinc levels in erythrocytes (Michael et al, 2005). Furthermore, it plays a major role in

polynucleotides transcription, and thus in the gene expression process, and its contribution to such essential activities may account for its importance in all forms of life (Walter, 1986; Prasad ,1995; and Institute of medicine, 2001). DNA and RNA polymerases require zinc and zinc ions are essential in maintaining the proper structural conformation of DNA. Among the other important functions, zinc enzymes are essential for growth, wound healing, integrity of connective tissue, reproductive function, the immune system, and protection from free radical damage (Michael e .al, 2005).

Zinc has a major role in the immune system, because it affects several humoral and cellular immunity aspects (Shankar & Praead, 1998). Zinc affects our growth process, and carbohydrate metabolism by assisting function of insulin such as production, storage. Additionaly, zinc involves in insulin secretion by pancreatic beta cells, along with other role to vital optimal glucose metabolism (Barceloux, 1999; & Michele et al, 2000).

Zinc is important for active site of enzyme and catalysis, because zinc has several possible coordination geometries and the coordination geometry is easily distorted, and the presence of strong Lewis acid like zinc at the active site can supply a hydroxyl group (-OH) that may be important for many enzymatic reaction (Martha, 2000).

Zinc deficiency may reduce pre-albumin concentrations, but vitamin deficiency does not do so (Beck & Rosenthal, 2002).

1.5.2 Absorption

In humans, the small intestine absorbs zinc by a mechanism of carrier mediation (Cousins, 1985; Michael et al, 2005). The fraction of absorbed zinc is difficultly determined since zinc is secreted into intestines (Turnlund et al, 1984). Zinc absorption increases with higher dietary zinc which indicates the mechanism of saturated carrier mediation (Steel & Cousins, 1985). In addition, the absorption of zinc may be influenced by its status.

Almost 20 dietary and regulatory factors were detected to affect the absorption of zinc (Cousins, 1985), the most important of which is phytate (myoinositol hexophosphet) (House et al,1982). The availability of zinc from legumes & cereals is

highly reduced by their phytate content (O'Dell & Savage, 1960; Turnlund et al,1984; & Michele et al, 2000). When phytate is taken with calcium, it will reduce zinc absorption by formation of insoluble precipitates (Sandstead et al, 1984), although some dietary fiber components can also lower zinc absorption (Reinhold et al, 1976; kelsay et al, 1979; Gibson, 2012).

High doses of zinc and iron antagonize the absorption of each other at higher doses (Crofton et al,1962; Solomons & Jacob, 1981). In addition, excessive levels of both dietary copper and tin may lower zinc absorption (Valberg et al,1984). Consumers are advised that calcium or phosphorus-rich foods can lower zinc absorption from the gut (Barceloux ,1999).

1.5.3 Excretion

Under normal circumstances, very little zinc is lost through the urine or through cutaneous losses. Most of losses are through the feces, of the endogenous fecal loss, some are from the sloughing off of intestinal cells into the intestinal lumen and considered nonspecific. However, when dietary zinc intake is high and metallothionenine levels are induce, this loss can be significant (Kirchgessner and Weigand, 1983; and Martha, 2000).

Zinc excretion in urine will vary with intake, but is generally bellow 10 % of total excretion, 90 % of zinc excretion is through the feces, however the level of actual excretion varies with dietary intake and with zinc status of the individuals.

As a result, the fine tuning of zinc balance is through the fecal excretion. Although bile and gastroduodenal secretions contribute to endogenous zinc excretion, pancreatic secretion are the major contributor to endogenous zinc losses. The zinc containing fraction of pancreatic secretion is made up of zinc dependent enzymes, including carboxy peptidase A and B. These enzyme can be digested, and most of the zinc from them can be reabsorbed (Martha, 2000).

1.5.4 Deficiency

Inadequate dietary and absorption of zinc will lead to its deficiency. Zinc deficiency can result in several symptoms such as having, mental lethargy, eye and skin lesions, growth retardation, impotence, diarrhea, delayed sexual maturation, delayed wound

healing, weak appetite, loss of hair, weight loss as well as taste abnormalities (Prasad ,1985; Prasad, 2003).

Zinc deficiency generally leads to reduced zinc in the tissues, but some tissues e.g liver, kidneys, muscles bones and testes are more susceptible to zinc losses than other tissues (Prasad et al, 1967). Biochemistry changes include RNA & DNA synthesis reduction (Somers & Under wood, 1969), decrease of protein synthesis (Mills et al, 1967), impairment of glucose tolerance (Kechrid et al,2002) and decrease in many enzyme activities such as carbonic anhydrase & alkaline phosphatase (Kechrid & Bouzerna, 2004; Sun et al, 2005).

Surgery of the gastrointestinal tract and digestive disorders like Crohn's disease, ulcerative colitis and the short bowel syndrome can lower zinc absorption and increase the endogenous zinc loss mainly from the gastrointestinal tract and can lead to extent loss from the kidneys (Valberg et al, 1986; Naber et al, 1998). Other zinc deficiency chronic disorders are chronic renal disease, chronic liver disease, malabsorption syndrome, sickle cell anemia, malignancy and diabetes (Prasad, 2003). Chronic diarrhea may also be a cause of excessive zinc loss (Prasad, 2004).

The studies showed that zinc deficiency may occur with type 1 and 2 diabetetes, because the excessive of urinal excretion is approximately doubled (Cunningham et al, 1994; Ho et al, 2001).

Low zinc concentrations were noticed in 30-50% of alcoholics. Alcohol reduces zinc absorption and increases urinary zinc loss. Moreover, many alcohol abusers do not eat sufficient varieties or amounts of food, so their dietary zinc may be insufficient (Prasad, 2003).

1.5.5 Zinc and Diabetes Mellitus

Diabetes mellitus is a metabolic disease with global variable incidence. The abnormal metabolism of many micronutrients is found in diabetic patients, and zinc is found to be an essential micronutrient with an altered metabolism and status in this aspect (Salgueiro et al, 2001; Pantea, 2005).

The relationship between Diabetes mellitus and zinc was mentioned since 1934, when it was shown that zinc is an insulin crystal component (Jansen et al, 2009).

Zinc plays a main role in the glucose utilization regulation by muscles and fatty cells (Pantea, 2005). From that time, many studies tried to elucidate the role of zinc in Diabetes mellitus conducted in order to identify new causal mechanisms and new treatment options. Zinc deficiency may play a role in the development of diabetes because it is an important element for the functioning of more than 300 members of all enzymes. Because of the low quantities of zinc and its importance for enzyme function, it is clear that its concentration in human's body is regulated by zinc transporter & zinc binding protein like metallo thionein (MT), which is able to bind tightly with zinc on one hand, and to release the metal dependent on the redox status on the other hand. Zinc was found to be of importance to the innate and the humoral immune systems physiological functioning, and it is particularly important for T-cells development and functioning after their maturation (Jansen et al, 2009).

Zinc may also share as an integral component of many antioxidant enzymes, and the decrease in intracellular Zn as well as Zn- dependent antioxidant enzymes have a relation to the increment in diabetes of oxygen free radicals. Another study conducted on diabetic patients demonstrated a tight relationship between decreased activity of superoxide dismutase (SOD) and the loss of its two factors, Cu^{+2} and Zn^{+2} (Forte et al, 2013).

Zinc were found to have positive effect on hyperglycemia by means of increasing threonine kinase phosphorylation/ the phosphoinoside-3 kinase activation, serine, GLUT4 (glucose transporter type 4) translocation as well as insulin sensitivity, and by preventing of diabetes complications by lowering the oxidative stress (Ugurlu et al, 2016).

Zinc deficiency may cause greater oxidative stress and chronic inflammation. There is an association between these biological functions of zinc with diabetic complications and glucose metabolism in type 1 diabetic individuals (Lin et al, 2016).

Zinc deficiency may lower the ability of insulin synthesis and secretion, and glucose is impaired when zinc is deficient (Huber & Gershoff, 1973 ; Ezaki, 1989). Intestinal rate of zinc absorption and blood zinc levels in diabetic patients are also decreased (Kiilerich et al, 1990). Zinc is involved in insulin regulation receptor-initiated signal

transduction mechanism (Ezaki, 1989) and insulin receptor synthesis (Chen et al,1994).

The antioxidant enzyme activity and contribution to tissue damage observed in diabetes mellitus can be reduced by an unbalanced level of zinc in the body. It was reported that in both type 1 and 2 diabetes, the impaired intestinal endogenous zinc reabsorption and the increase in zinc excretion into the intestine during digestion can result in low serum zinc levels. Several authors found that diabetes was strongly correlated with zinc deficiency. On the contrary, other studies revealed unchanged zinc levels, while others indicated increased levels of zinc in type1 diabetic patients (Forte et al, 2013).

Diabetes mellitus is associated with zinc deficiency and excessive losses in urine. This deficiency may cause the development of diabetic complication (Islam & Loots du, 2007).

1.6 Magnesium

Magnesium is predominately an intracellular divalent cation (Mg^{+2}) and is important for optimal cell function. It is an essential cofactor to many enzymes as well as being important for membrane function. Furthermore, it can act as an antagonist to calcium in cellular responses and has a structural role within the cell (Martin, 2006).

In human body, magnesium is the 4th most available cation and the 2nd most available intracellular ion. The average human bodies contain one mole (24 grams) of magnesium. About (53%) of magnesium is present in bones, (46%) in muscles, other organs and soft tissues, and < (1%) of the element is found in the serum and the red blood cells. About 1/3 of serum magnesium is bound to proteins, mainly albumin, while (61%) of the remaining 2/3 of serum magnesium is found in ionized or free condition, and approximately 5% is bound with other ions, such as citrate and phosphate. Magnesium, like calcium, is the free and physiologically active ion in the body.

In the last ten years, the clinical benefit of serum magnesium concentrations has greatly increased since additional information on the analyte was discovered. The most significant finding is the association between abnormal levels of serum

magnesium and metabolic, cardiovascular and neuromuscular disorders. Serum magnesium levels are of importance to determine the ion's acute changes, although the serum levels of magnesium may not represent the total body stores (Michael et al, 2005).

Because magnesium is bound to ATP, most of the intracellular Mg is found in mitochondria. In general, the more metabolically active cells, have the highest magnesium content. Plasma magnesium levels of healthy individuals are usually constant, and their average level is (1.7-2.4 mg/dl) (Marier, 1986).

1.6.1 Function

The main biological role of Mg^{+2} in mammalian cells is the neutralization with anion charge. Magnesium is particularly found in association with organic polyphosphates such as nucleotide triphosphate and nucleotide diphosphate (e.g. ATP -4 Mg^{+2} and ADP-3 Mg^{+2}). Magnesium is also found associated with other highly anionic species, including multisubstituted Phosphates of sugar such as inositol triphosphates, nucleic acids (RNA, DNA) and some carboxylates (e.g. isocitrate. Mg^{+2} as substrate for isocitrate lyase, (carboxylate group on proteins) (Frausto & Williams, 1991; Cowan, 1995).

Magnesium is normally bound between the Beta and Gama – Phosphates of nucleotide triphosphates such as ATP and between the α and β phosphates of nucleotide diphosphates as you show in structure (Martha, 2000).

Magnesium involved numerous steps in central pathways of carbohydrate, lipid and protein metabolism and in mitochondrial ATP synthesis. For example, many steps in the glycolytic pathway require Mg^{+2} , either in the form of complex with (ATP) or (ADP) substrates or as a part of the metal enzyme itself. The steps catalyzed by hexokinase and phosphofructokinase require Mg-ATP as substrate whereas the steps catalyzed by phosphoglycerate kinase and pyruvate kinase require Mg-ATP (Martha, 2000). Almost all these enzymes need Mg for optimal activity. Protein synthesis has been reported to be highly sensitive to magnesium depletion. Magnesium require for virtually every step of protein biosynthesis (Martha, 2000).

Magnesium also assists in regulating plasma glucose levels, reinforcing normal blood pressure and it contributes in energy metabolism (Wester, 1987; Saris et al, 2000).

Magnesium plays a major role in carbohydrate metabolism, and it may influence insulin release and activity (Kobrin & Goldfarb, 1990).

Magnesium increases the body's capacity to utilize calcium, phosphphours, potassium sodium, and vitamins C, E and B-complex (Kirschmann, 1996).

1.6.2 Absorption

Recent studies observed that human's magnesium absorption takes place uniformly within the small intestine (Quamme, 1997). Under normal dietary status of healthy people, approx. (30-50%) of ingested Mg is absorbed (Fine et al, 1991). Magnesium is absorbed along the large and small bowels, but the sites of maximal absorption appears to be the ileum and distal jejunum. There appears to be both a passive and an active transport system for magnesium absorption, which may account for the higher fractional absorption seen at low dietary magnesium intakes (Kayne & Lee,1993).

Little information is available regarding the regulation of intestinal magnesium transport. Vitamin D, as well as its metabolite 25-hydroxy vitamin D and 1,25-dihydroxy vitamin D, were found in many studies to promote intestinal magnesium absorption, but vitamin D affects magnesium absorption to a much lesser extent than calcium absorption (Hardwick et al, 1991).

High levels of dietary fruit, vegetable and grain fibers decrease fractional magnesium absorption (Siener & Hesse, 1995).

1.6.3 Excretion

The kidney is the principal organ involved in magnesium homeostasis (Quamme & Dirks, 1986). During dietary magnesium deprivation, the kidney avidly conserves magnesium, and less than 12-24 mg is excreted in the urine per day. Conversely, when dietary magnesium is high, excessive amounts are rapidly excreted in the urine. About 70 % of plasma magnesium is filtered as free Mg^{+2} at the glomerulus, but most of this is reabsorbed (Martha, 2000).

Healthy kidneys have the ability to limit urinary magnesium excretion to make up for low dietary intake. However, excessive urinary magnesium loss can be a side effect of some drugs and may take place in poorly controlled diabetes cases and in alcohol abuse (Kelepouris & Agus, 1998).

1.6.4 Deficiency

Deficiency of Mg might be grouped into 2 kinds: deficiency of magnesium as well as depletion . It is investigated that dietary amounts of magnesium can be marginal among the population as well as in magnesium intake, little alteration may elevate deficiency of magnesium (Galan et al, 1997). Magnesium depletion may result from dysregulation of factors which control magnesium intestinal hypo-absorption, reduced uptake and mobilization of bone magnesium and insulin resistance.

Osmotic diuresis brought about by glucosuria in diabetes, mannition and urea leads to magnesium wasting via urine. It has been indicated that stress, aging and other conditions may increase the requirement for magnesium (Shills, 1988).

The healthy kidneys and digestive system significantly affect magnesium condition. The intestines absorb Mg and then transport it via blood circulation to other tissues and cells. The body absorbs about (1/3) to (1/2) of the dietary magnesium (Ladefoged et al, 1996).

Gastrointestinal diseases such as Crohn's disease can deplete the body's stores of magnesium, and in severe cases may lead to Mg deficiency. Chronic or excessive diarrhea and vomiting can also cause Mg depletion (Rude, 1998). may inhibit retention of the mineral.

Malnutrition and alcoholism may also have a negative effect on the body's magnesium level (Michele et al, 2000).

1.6.5 Magnesium and Diabetes Mellitus

Magnesium ion plays an essential role in carbohydrate metabolism in general and in the action of insulin in particular (Garfinkel & Garkinkel, 1988).

Magnesium is necessary for both the action and manufacture of insulin, and it can inhibit the secretion of insulin and activate insulin tyrosine kinase receptor activity.

Hypomagnesemia is related to increased intracellular calcium levels, which may result in insulin resistance (Lin et al, 2016), It also helps insulin to bind to the insulin receptor by promoting autophosphorylation of the b-subunit through tyrosine-kinase switching. Magnesium is also a known cofactor of lipoprotein lipase and plays an essential role in lipolysis and hepatic lipid uptake. (Lin & Huang, 2015).

Moreover, Mg deficiency contributes to the pathogenesis of diabetes complications that inhibits the prostacyclin receptor function and causes highly platelet activation & aggregation (Ugurlu et al, 2016).

The renal magnesium excretion increases by hyperglycemia & hyperinsulinemia, but renal magnesium excretion is decreased even in case of moderate improvement in the metabolic control (Djurhuus et al, 2001).

Much attention was given to the role of some minerals in the pathogenesis of diabetes and in the progression of its complications. Magnesium may affect the activation and release of insulin. The major organs involved in magnesium homeostasis are the intestines, bones and kidneys, but the regulators affecting these organs at the cellular level have not been yet fully understood. Different causes for low magnesium levels in diabetics are included such as magnesium-low diet, osmotic diuresis that causes highly renal magnesium excretion, insensitivity to insulin affecting intracellular Mg transport and causing greater loss of extracellular Mg, using loop and thiazide diuretics, which may enhance magnesium wasting, diabetic autonomic neuropathy as well as low tubular reabsorption because of insulin resistances (Shahbah et al, 2016).

There are several factors that may cause Mg deficiency in T1D patients. Possibly, the most important mechanism is the urinary magnesium loss which result from osmotic diuresis due to high blood glucose levels (hyperglycemia). Other important factors include taurine deficiency, changes in Vit. D metabolism, inadequate intestinal absorption and defect in glutathione metabolism (Ugurlu et al, 2016).

Several studies focused on evaluating Mg status in patients with type 2 diabetes and on role of Mg supplementation in prevention of diabetic complications and optimization of diabetic control. However; few studies were concerned with this issue in type 1 diabetic children with opposite results (Shahbah et al, 2016). The aim

of this study is to evaluate the status of serum Mg in type 1 diabetic children and assess its relation to glycemic control .

1.7 Albumin

Liver is the site of synthesis. Albumin is made of (585) amino acids at the rate of (9–12) g daily without reserve or storage. Albumin is the most abundant plasma protein. It is also found in the extravascular or interstitial spaces. The albumin movement rate is called the transcapillary escape rate or (TER), that measures the systemic albumin capillary efflux. Albumin is in charge for 80% of the intravascular fluid's colloid osmotic pressure, which maintains the proper fluid balance of the tissues. In addition, albumin buffers pH, and is considered as negative acute phase proteins (Michael et al, 2010).

Studies on healthy subjects showed that the markedly restricted caloric intake is often due to poverty or unwanting to eat for psychiatric, political or other causes (Lee et al, 2015).

1.8 Pre-albumin (Transthyretin)

Pre-albumin is so called because of its migration ahead of albumin in the classical plasma or serum proteins electrophoresis. The high resolution electrophoresis (HRE) or immune-electrophoresis techniques can also separate pre-albumin. Pre-albumin is the protein that transports thyroid hormones (thyroxine & triiodothyronine); it can also bind with the retinol-binding proteins to form retinol (Vit.A) transporting complex, and it is rich in tryptophan. Low pre-albumin levels is an indication of poor nutritional status. Because pre-albumin has about (2) days half-life, it decreases more quickly than other proteins do. In certain conditions like chronic renal failure, alcoholism and steroid treatment, the levels of pre-albumin are increased (Michael et al, 2010; Lee et al, 2016).

The levels of the negative acute phase (pre-albumin) will be decreased in inflammations or in the period of post-surgery. Serum pre-albumin levels are low in conditions of protein mal-nutrition, including enteropathy, malignancy, liver cirrhosis, protein loosing and in zinc deficiency (Beck & Rosenthal, 2002).

Pre-albumin is metabolized and excreted by the kidneys, which is also called transthyretin (transporting thyroxine & retinol), because it carries thyroid hormone thyroxine and retinol in serum and cerebrospinal fluid. The names (pre-albumin) and (albumin) are not biochemically related, nor is one the precursor of the other. It is named so because pre-albumin's migration ahead of albumin in the classical plasma or serum proteins electrophoresis (Gaudiani et al, 2014).

Levels of TTR are increased in the presence of a TTR producing tumor or Hodgkin's disease and by glucocorticosteroid therapy, as well as some nonsteroidal NSAIDs. Decreased levels of TTR are associated primarily with the APR, liver disease, and protein malnutrition. Low levels of TTR often are used to interpreted carefully because the APR is much more common than PEM in developed countries.

Once pancreas blood supplying is restored and malnutrition ameliorated, pre-albumin levels will gradually return to normal. Therefore, pre-albumin is a valuable marker for AP progression monitoring (Yue et al, 2015).

Some studies stated that serum pre-albumin levels below 30 mg/dL is an indication of malnutrition in patients undergoing hemodialysis, while others associated a pre-albumin of less than (20) mg/dL in patents on hemodialysis with a higher mortality rate even when patients had normal albumin levels (Gaudiani et al, 2014).

1.8.1 Pre-Albumin and Diabetes Mellitus

Type 1 diabetic patients were considered at risk of micronutrient deficiency several such as vitamin A, carotenoids and TTR (Espe et al, 2007).

Pre-albumin is synthesized in the choroid plexus, in the islet cells of pancreas, in the yolk sac of embryoes and in the enterochromaffin cells of the gastrointestinal mucosa, but liver is the most important source of its synthesis (Beck & Rosenthal, 2002).

Pre-albumin (PA) and proteins primarily synthesized in liver, and their serum levels are used as nutritional status marker of patients. PA are also believed to be related to the pathogenesis of type 1 diabetes (T1D) and to the derived macrovascular complications, respectively. Firstly, PA is a functional constituent of the stimulus-secretion coupling in the healthy β-cell, preserving its functional integrity and

protecting it through its action over the immune system. On the other hand, RBP seems to play a protective role in the development of cardiovascular complications, a risk five times higher in the diabetic population, improving the transport of retinol vitamin (A) to places under oxidative stress in order to exert its antioxidant effect. In 1985, it was described by the first time that children with T1D displayed lower levels of PA in serum compared to healthy controls. This data was later confirmed in most studies in both children [5–8] and adult T1D populations.

Several factors have been hypothesized to be behind the protein levels variations, namely, glycemic control lipid profile, changes in main PA circulating form that cause its renal loss, and insulin treatment. In this respect, while results are far from being uniform, it is accepted that local low insulin levels in liver do decrease hepatic synthesis of in PA (Forga et al, 2016).

In conclusion, determining the level of pre-albumin is a method of evaluating the severity of malnutrition in patients who have severe illness or have chronic diseases as shown in table (1.2) (Beck & Rosenthal, 2002).

Table 1.2 Characteristics of plasma proteins used as nutritional

Protein	**Molar Weight**	**Half-life**	**Range**
Albumin	65,000	20 days	3.30 to 4.80 g per dL (33 to 48 g per L)
Transferrin	76,000	10 days	0.16 to 0.36 g per dL (0.16 to 0.36 g per dL)
Pre-albumin	54,980	2 days	16.0 to 35,0 mg per dL (160 to 350 mg per L)

Adapted with permission from Spiekerman AM. Nutritional assessment (proteinnutriture). Anal Chem 1995; 67:429R (Beck & Rosenthal, 2002).

1.9 Aim of The Study

Our aim for this present study was to compare the levels of magnesium (Mg), zinc (Zn) and prealbumin (PAB) between patients with type 1 diabetes mellitus (DM) and healthy children.

CHAPTER 2

LITERATURE REVIEW

Metabolism of different proteins and minerals is changed in insulin-dependent diabetes mellitus (IDDM). These nutrients perhaps play an essential role in the progress and pathogenesis of the disease. Some results indicated that disturbances of the trace elements in serum can be a cause of diabetic vascular diseases (Ewald et al, 1983). Decreased magnesium levels were detected in the sera of Swedish diabetic children in comparison with their healthy control group. No difference in serum zinc concentrations were found between diabetic children and the control individuals. To our knowledge, this seems to be the first hypomagnesemia finding in children with diabetes (Gebre-Medhin et al, 1985).

It is indicated that the low serum magnesium or (hypomagnesemia) in children with Diabetes mellitus can be attributed to the high loss in urine loss or to the diversion of Mg from the normal pathways in this disorder. It is difficult to determine the importance of trace element alteration in in pediatric diabetic patients as the total body storage of these trace elements may not be correctly reflected (Kobbah et al, 1988)

(Kobbah et al, 1988) found a decrease in serum pre-albumin levels at early IDDM patients, and also detected a progressive reduction in serum magnesium, but their study showed a transient decrease in serum zinc levels. Some studies reported that the vascular diseases in Diabetes mellitus can be a result of serum trace element disturbances. In many studies, low serum magnesium level was shown in diabetic children. Although contradictory results have been yielded in studies on the metabolism of zinc in diabetic people, zinc is considered essential for the production of insulin, and its deficiency to be a contributory cause of Diabetes mellitus.

Regarding the protein production impairment in type 1DM patients, insulin is the one of the important protein metabolism regulators thereby abnormal serum levels of insulin in type 1 Diabetes mellitus which may lead to the differences between anabolism and catabolism states might cause changes in serum protein levels.

(Kemp and Frindik, 1991) found that Pre-albumin (Transthyretin) protein levels were lower in diabetic persons compared to the control healthy group.

In this study, they found plasma Mg and Zn were low in children with diabetes. On the other hand, some other research did not demonstrate consistent results on the relationship between the diabetic control degree and the alterations in mineral levels (Rohn et al, 1993).

(Ruiz et al, 1998) found that the delayed growth and puberty as well as the impaired wound healing in diabetic adult patients were attributed to zinc deficiency. Similarly, many researchers reported the decrease in serum Zn levels in type 1 diabetic patients, and they suggested an evidence of the role of antioxidant trace elements in the disease occurrence.

(Atabek et al., 2006) demonstrated a relationship between serum magnesium concentration in type 1 diabetes with atherosclerosis, and suggested that the risk of atherosclerosis could be decreased by long-term supplementation of Mg.

In their study, (Estakhri et al, 2011) stated that zinc is the trace element which plays a key role in metabolism and as a cofactor of various enzymes, that are necessary for normal metabolic process, growth and development. Reports regarding zinc investigation in type 1 children and adolescent patients are very limited and contain contradictory results. Some studies have demonstrated a decreased serum zinc concentration in type 1 DM patients compared to healthy controls (Bideci et al, 2005; Angelova et al, 2006), while others have showed elevated levels (Tuvemo et al ,1997; Kruse-Jarres & Rukgauer, 2000). On the other hand, in a few studies no changes have been detected (Ewald et al, 1983; Rohn et al, 1993).

(Ekbote et al, 2012; Lin et al, 2015) In the early stages of Diabetes mellitus, zinc is secreted along with insulin into the extracellular space of the islets, and when it is

combined with hyperzincuria, it may lead to zinc depletion from beta cells in the later stage of the disease.

(Salmonowicz et al, 2014) Attention was given to the role of such elements in the pathogenesis and complication of Diabetes mellitus, therefore, comparison of Zn levels in complete blood, plasma and in blood elements (erythrocytes, platelets and neutrophils) in T1 diabetic children with those in type 2 DM patients. Zinc levels were found to be higher in complete blood and erythrocytes, while its levels were detected lower in blood plasma, platelets and neutrophils. Zn deficiency was more apparent in poorly-controlled T1 Diabetes mellitus, which is related to the hyperglycemia severity. With respect to Mg levels, hypomagnesemia was found to be related to increased magnesium excretion in urine (Walter et al, 1991), and / or dietary deficiency and decreased absorption of Mg in some research (Sales & Pedros, 2006; Abou-Seif & Youssef, 2004). Furthermore, McNair et al (1982) attributed the diminished serum magnesium level to the increased urinary excretion of Mg because of fasting hyperglycemia causing impairment in the reabsorption of the mineral.

(Lin et al, 2015) mentioned the involvement of many trace elements in glucose metabolism and insulin signal transduction. In this study the researchers found decreased Mg, and no changes in zinc levels in type 1 diabetic patients.

In the study conducted by (Salim et al, 2015), Zn levels were shown to be decreased in diabetic children when compared with the health controls, and negative relationship was found between zinc and HbA1c levels.

(Shahbah et al, 2017) Type 1 Diabetes mellitus (T1D) is the most life threatening endocrine disorder of children and its incidence appears to be increasing. However, there are few studies, concerned with serum Mg and its relation to diabetic control in type 1 diabetes. Among these studies, there was a great controversy in results regarding frequency of hypomagnesemia and correlation between Mg level and HbA1c.There are many factors affecting serum Mg level in diabetic children, including age, sex, and duration of diabetes. The most contributing factor to hypomagnesemia was the duration of diabetes.

CHAPTER 3

MATERIALS AND METHODS

3.1 Measurement of Zinc, Magnesium and Pre-albumin

3.1.1 Subjects

34 type 1 diabetic children who were under follow up of Pediatric Endocrinology Department and 94 healthy children who were under regular control of Social Pediatrics department at University of Gaziantep were enrolled in this study, and at ages ranging between 3 and 16 years. Serum zinc, magnesium and pre-albumin levels of patients diagnosed type1 diabetes mellitus were compared with separate control groups for each parameter. The ethical permission was obtained from ethical committee of the University of Gaziantep. Informed consents of participants or their legal guards were taken.

3.1.2 Samples

The study samples of patients were collected from University of Gaziantep Hospital. About 2 ml of the blood was drawn from a forearm vein of fasting patients recently diagnosed with pediatric type 1 diabetes, and healthy subjects were used as a control group.

We used the BECKMAN COULTER as shown in (Figure 3.1) to find a concentration of both Mg and PAB in serum of children and we used the SPECTROPHOTOMETER as shown in (Figure 3.2.) to find a concentration of Zn.

Mg and PAB reagent, when used in conjunction with UniCel® DxC 600/800 System(s) and Synchron ®Systems Multi Calibrator, is intended for the quantitative determination of Mg and PAB concentration in human serum.

Figure 3.1 Beckman Coulter ® Model Dxc 800.

Figure 3.2 Spectrophotometer .

3.2 Methodology of Pre-albumin

PAB reagent was used for pre-albumin concentration measurement by turbidimetric method. In this reaction, pre-albumin binds to specific antibodies to form the insoluble Ag–Ab complexes.

It is clear that the SYNCHRON system (s) is considered automatically proportions in the appropriate sample as well as reagent volumes into the cuvette. That ratio used can be a part of the sample to (70) parts of the reagent. The system in this case can monitor the change in absorbance at (340) nanometers. In absorbance, that change can be proportional to the concentration of PAB in the sample, as well as it is utilized by it to express and estimate PAB concentration based on a single point that described the non- linear calibration curve.

3.2.1 Chemical Reaction Scheme

Pre-albumin (Antigen) + Anti- prealbumin Antibody $\longrightarrow$ Antigen – antibody Complex

3.2.2 Reagents

Contents

Each kit contains the following items:

Two PAB Reagent Cartridges (2 x 100 tests)

One lot-specific parameter card

3.2.3 Volumes per test

Sample volume	3 µL
Total reagent volume	210 µL
Cartridge volumes	
A	200 µL
B	– –
C	10 µL

3.2.4 Reactive ingredients

Reagent constituents

Reagent buffer	32.0 mL
PAB antibody specific for human prealbumin (goat)	4.0 mL

Also non- reactive chemicals necessary for optimal system performance.

3.3 Methodology of Magnesium

The timed endpoint technique was applied for the magnesium measurement by using Mg reagent, where the element combines with calmagite forming the stable chromogen.

The SYNCHRON System (s) automatically dilutes samples and distributes the samples and reagents into the cuvettes. One part of the sample to 103 parts of the serum are used. The changes are monitored at (520) nanometer absorbance. The absorbance alteration is directly proportional to Mg concentration, and it is used by for the estimation and and expression of Mg concentration.

3.3.1 Chemical Reaction Scheme

Mg^{++} - Calmagite Complex $\longrightarrow$ Calmagite + Mg^{++}

3.3.2 Reagents

Contents
Each kit contains the following items:

Tow Mg Reagent Cartridges (2 x 100 tests)

3.3.3 Volumes per test

(Serum)

Sample volume	3 µL
Total reagent volume	308 µL
Cartridge volumes	
A	280 µL
B	28 µL
C	– –

3.3.4 Reactive Ingredients

Reagent Constituents

Calmagite (Dye Reagent)	0.15 mmol/L
Alkaline solution	(PH > 13.0)

Also non-reactive chemicals necessary for optimal system performance.

3.4 Methodology of Zinc

Serum zinc analysis is carried out by the flaming atomic absorption spectrometry method. The basic principle of analysis is to decompose into zinc atoms in the serum zinc sample by means of heat energy and to keep a part for the light emitted from the serum zinc lamps. The decrease in the intensity of the light is directly proportional to the zinc concentration in the sample. The unit of this decrease in the intensity of the light is absorbance. Accordingly, the increase in concentration increases absorbance in a direct proportion**.**

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Baseline Clinical Characterized of Study Subjects

Baseline characteristics of subjects and demographic to the type 1 diabetes patients in children and to the control groups are explained in (Table 4.1). The present study consist of 128 children whom 34 type 1 diabetic children who were under follow up of Pediatric Endocrinology Department and 94 healthy children who were under regular control.

Firstly, For the serum prealbumin group, the mean age of the 33 diabetes patients was 10.79±4.0 years, 14 female and 19 Male. The mean age of the control group which was comprised of 31 healthy children was 11.52±3.99, which included16 females and 15 males, There was no significant difference between the patients and control for the age (p=0.469) and for gender (p=0.67).

Secondly, 34 diabetic patients included in the serum magnesium group that had a mean age of 10.88±3.98, with 15 and 19 males and females, respectively. The mean age of the control group 33 was 9.61±4.80, 14 female and 19 male, There was no significant difference between the patients and control for the age (p=0.242) and for gender (p= 0.54).

Lastly, regarding the serum zinc group, the mean age of the 30 diabetic patients was 10.90±4.1, 12 female and 18 male. The mean age of the 30 healthy children as a control groups was 9.50±0.68, 14 female and 16 male, No significant difference between the patients and control for the age (p=0.185) and for gender (p=0.79) was found.

Table 4.1 Demographic Findings of Controls and Patients

	Patients			Controls			P for age	P for gender
Parameters	**n**	**age**	**Gender f/m**	**N**	**Age**	**Gender f/m**		
Prealbumin	33	10.79±40	14/19	31	11.52±3.99	16/15	0.469	0.67
Zİnc	30	10.90±4.1	12/18	30	9.50±0.68	14/16	0.185	0.79
Magnezium	34	10.88±3.98	15/19	33	9.61±4.80	14/19	0.242	0.54

We have shown in (Table 4.2) the levels of Hemoglobin A1c (HbA1c) in serum 34 of the children of diabetics typeI. And we showed the mean of HbA1c and we scored the maximum and minimum value for HbA1c In percentages. It has been the mean of HbA1c (10.12%). However, the maximum value it was (15.7%) and the minimum value it was (6.8%).

Table 4.2 Hb1Ac Percentage Data

N:34	**Mean (%)**	**Min (%)**	**Max (%)**
HbA1C	10.12	6.8	15.7

4.1.1 Comparison of Serum Prealbumin Levels Between Type 1 Diabetes Mellitus Patients and Healthy Children.

Table 4.3 Prealbumin Comparison

Parameters	**Patients (33)**	**Controls (31)**	**P**
Prealbumin	16.87±3.63	21.88±7.7	<0.000
Weight	40.17±13.29	36.82±12.69	0.307
Length	143.17±19.57	138.02±18.77	0.286
Z score weight	0.21±0.72	-0.31±0.66	0.004
Z score length	-0.07±0.89	-0.58±0.75	0.017

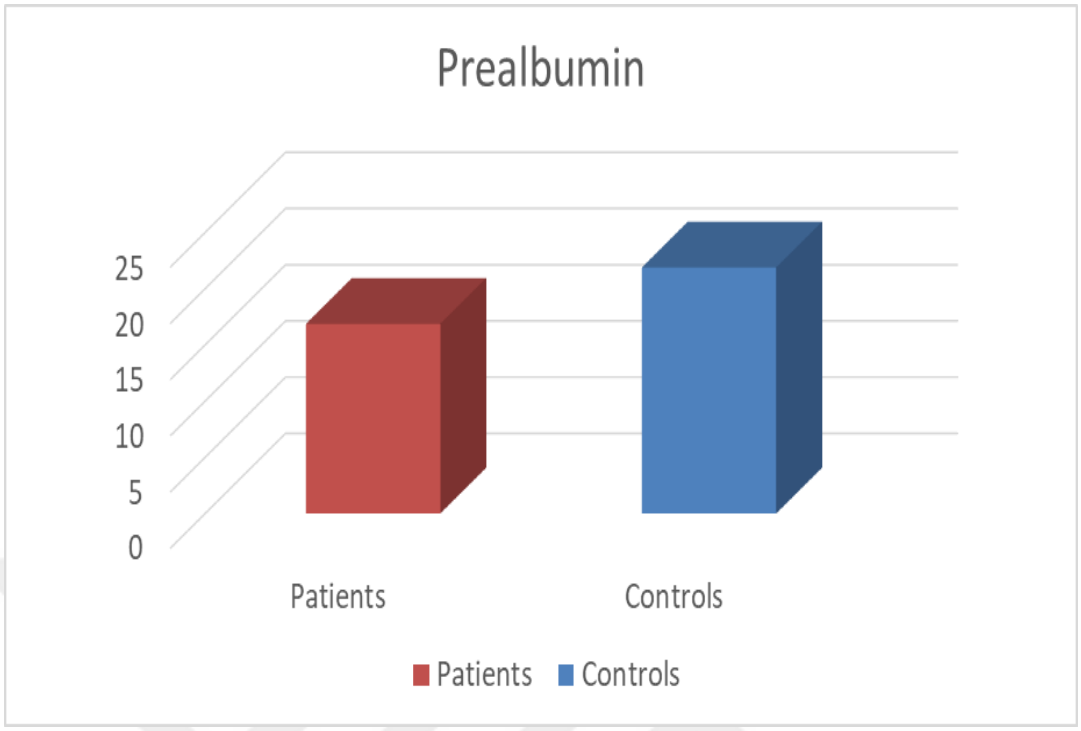

Figure 4.1 Comparison Between The Peralbumin Level with Control Group

Serum pre-albumin levels were found to be significantly lowered in type 1 DM patients compared to healthy children at 16.87±3.63 vs 21.88±7.7 ($p<0.000$) respectively.

The weight comparison between children patients and the controls for this group showed no significant difference with 40.17±13.29 vs 36.82±12.69 ($p=0.307$) and similarly, length comparison between groups was not demonstrated significant difference at numbers of 143.17±19.57 vs 138.02±18.77, respectively ($p=0.286$).

However, Z scores which evaluate the nutritional status of children showed significant differences between patients and control groups. Looking into details, Z score for weight were found significantly in type 1 DM patients compared to healthy children at 0.21±0.72 vs -0.31±0.66 ($p=0.004$), respectively. Z score length were found significantly in type 1 DM patients compared to healthy children at -0.07±0.89 vs -0.58±0.75 ($p=0.017$), respectively.

4.1.2 Comparison of Serum Magnesium Levels Between Type 1 Diabetes Mellitus Patients and Healthy Children.

Table 4.4 Magnesium Comparison

Parameters	Patients (34)	Controls (33)	P
Magnesium	1.9±0.14	2.01±0.13	0.005
Weight	40.54±13.27	33.53±16.6	0.061
Length	143.51±19.36	130.38±26.81	0.026
Z score weight	0.21±0.71	-0.18±0.92	0.130
Z score length	-0.10±0.88	-0.45±0.99	0.170

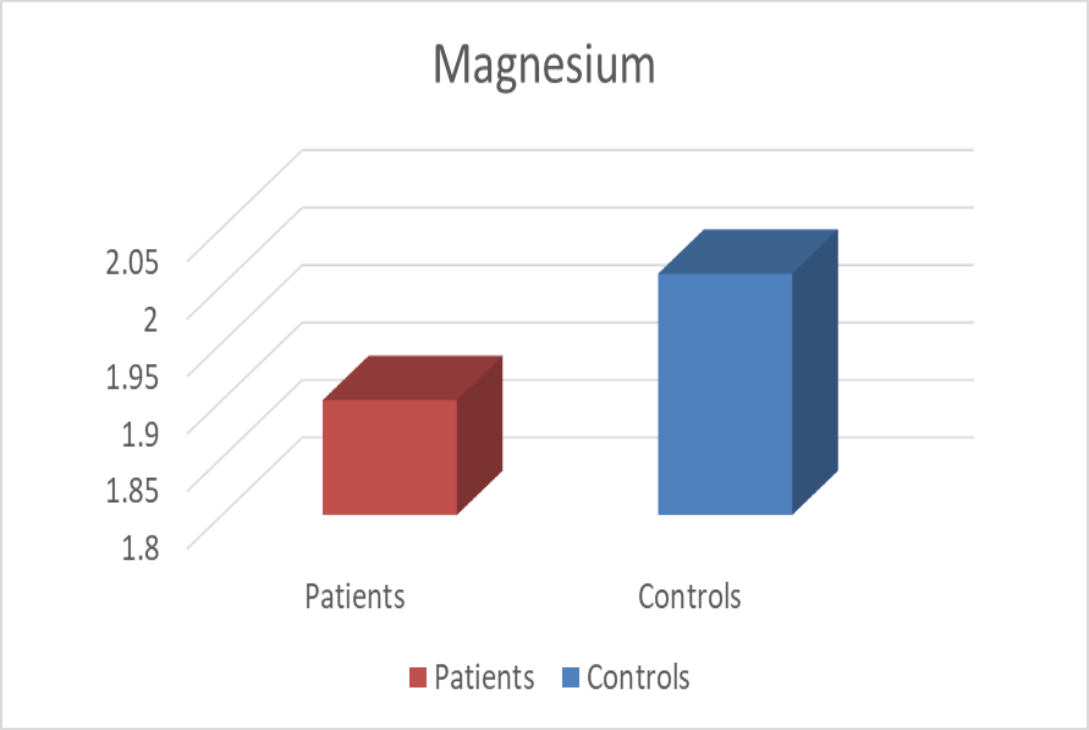

Figure 4.2 Comparison Between the Magnesium Level with Control Group

Comparison between serum Mg levels were found to be significantly low in type 1 DM patients compared to healthy children at 1.9±0.14 vs 2.01±0.13 (p=0.005), respectively.

The weight comparison between patients and the controls displayed no significant difference, 40.54±13.27 vs 33.53±16.6, respectively (p=0.061) and length comparison between Type 1 diabetic children and control group demonstrated significant difference at numbers 143.51±19.36 vs 130.38±26.81, respectively (p=0.026).

On the other hand, Z scores for weight and length were not found to be statistically different between patients and controls at values at 0.21±0.71 vs -0.18±0.92 for weight, respectively (p=0.130) and -0.10±0.88 vs -0.45±0.99 for length, respectively (p=0.170).

4.1.3 Comparison of Serum Zinc Levels Between Type 1 Diabetes Mellitus Patients and Healthy Children.

Table 4.5 Zinc Comparison

Parameters	**Patients (30)**	**Controls (30)**	p
Zinc	0.82±0.16	0.86±0.10	0.329
Weight	41.20±13.2	31.21±13.32	0.006
Length	143.60±19.77	129.04±19.57	0.006
Z score weight	0.29±0.66	-0.35±0.65	<0.000
Z score length	-0.07±0.80	-0.65±0.57	0.002

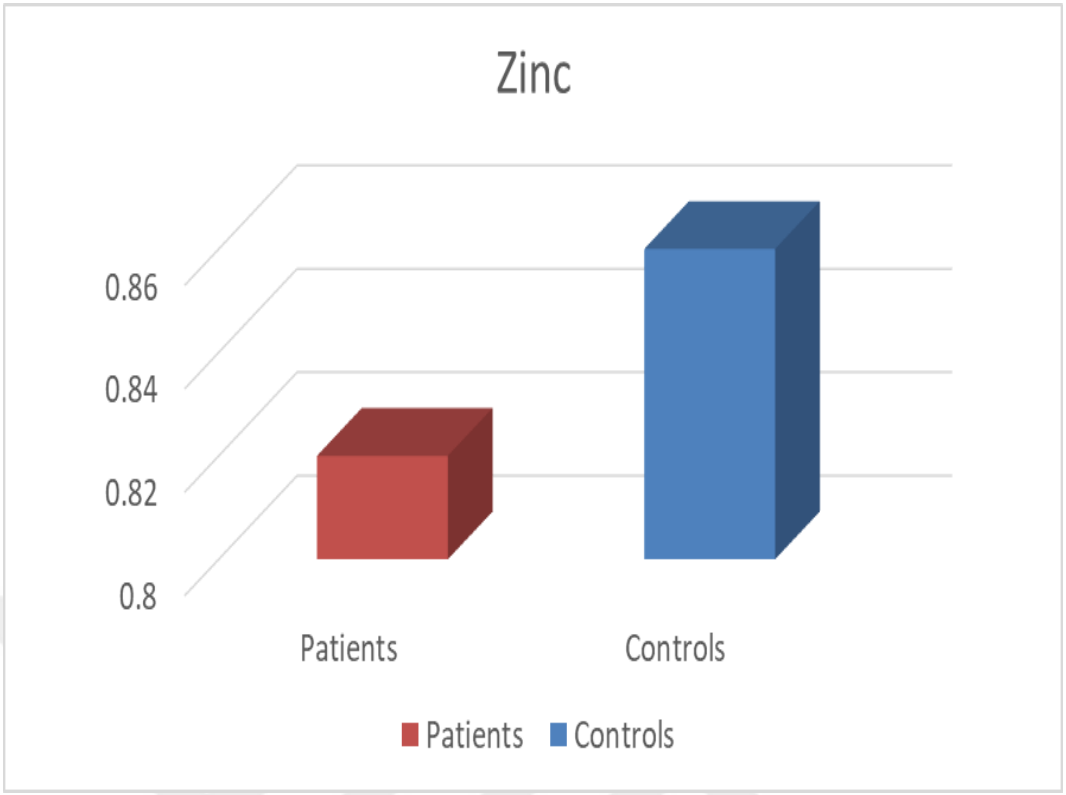

Figure 4.3 Comparison between the zinc level with control group

On the other hand, no statistical significance was detected in serum Zn values between patients and healthy controls with figures of 0.82±0.16 and 0.86±0.10, respectively (p=0.329).

The weight comparison between patients and controls were found to be significantly different 41.20±13.2 vs 31.21±13.32 (p=0.006). Similarly, length comparison 143.60±19.77 vs 129.04±19.57, respectively (p=0.006).

Similarly, Z score for weight between patients and control groups were also significantly different with 0.29±0.66 vs -0.35±0.65, respectively (p<0.000). Additionally, comparison of Z scores for length showed similar results with weight at-0.07±0.80 vs -0.65±0.57 (p=0.002), respectively.

Lastly, we have not observed a correlation between zinc, magnesium, prealbumin and HBA1c percentages (p=0.557), (p=0.115), (p=0.966), respectively.

4.2 Discussion

4.2.1 Zinc, Magnesium and Pre-albumin in The Serum of Diabetics Patients

4.2.1.1 Pre-albumin

Synthesis of pre-albumin takes place in liver, in brain's choroid plexus and in pancreas (Refai et al, 2005), and its normal levels were used as indicators of protein synthesis or catabolism of children (Jain et al, 1993). Recent studies have shown that type 1 diabetic children have low levels of serum pre-albumin (Gebre-Medhin et al, 1985; Kabbah et al, 1988).

There are very few studies regarding pre-albumin levels in type 1 diabetic patients, particularly in children. These studies were conducted in European countries in America and Sweden on the diabetic children, and to our knowledge this may be the first study in the Middle East linking the pre-albumin with type 1 diabetes in children and that's what prompted us to choose such a study. (Forga et al, 2016). All published studies coincide in reporting pre-albumin at a lower level than normal range in both children and adults with T1D. Our findings match up with published data.

In the present study, the pre-albumin levels of type 1 diabetics patients was lower than that of healthy individuals (control group) as shown in table (4.3), and figure (4.1). Furthermore, the deficiency in pre-albumin levels of diabetics were found to be significantly deercreased in comparison to healthy ones. ($P<0.000$). This result was in agreement with (Tuvemo et al, 1997) who found that the serum pre-albumin level was significantly lower in diabetic children than in the healthy control group ($p<0.01$). Possible explanation to this finding could be that high HbA1c levels as well as possibly low pre-albumin levels are indicators of insufficient metabolic control. Thefore, (Shew et al, 1979), the low pre-albumin levels of may indicate a continuous or periodic catabolic state during insulin deficiency. Furthermore, low prealbumin levels may represent a chronic inflammatory state which is probably seen in type1 DM.

Furthermore, (PAB) exists primarily as a tetrameric protein with only a small quantity of PAB monomer present in vivo in normal people (Blake et al, 1978; Sekijima et al, 2001). They have referred to that PAB tetramer has a positive role in

Pancreatic β-cell stimulus-secretion coupling. PA promoted glucose-induced increases in cytoplasmic free (Ca^{2+}) concentration ($[Ca^{2+}]_i$) and insulin release. PAB also protected against β-cell apoptosis. The monomer was without impact on glucose-induced insulin release and on apoptosis. The findings of aforementioned study related to PAB may support our results in terms of pathophysiologic link between PAB and type 1 diabetis.

Refai et al, (2005) have recently stated that transthyretin (formerly pre-albumin) levels are lower in children with insulin dependent diabetes mellitus than in the healthy control group. Although IDDM has been related to greater than normal variation in many analytes (Walter et al, 1991), it has been shown that the diabetes pe has not been related to profound variations in the levels of most serum proteins. (Kobbah et al, 1988; Kemp & Frindik, 1991) indicated that HbA1c levels are not associated with serum pre-albumin.

Itoh et.al., (1992) The serum PAB concentration was significantly lower in type 1 diabetic patients than the normal individuals. Two explanations for such a difference exists. Firstly, the PAB synthesis reduction in the liver is considered. In type 1 diabetes mellitus, there is a markedly insulin decrease in the portal vein, and it might reduce the protein synthesis in the liver. Secondly, an insufficient amount of insulin in the target tissues may exacerbate protein degradation, causing serum PAB reduction as proposed by (Gebre-Medhin et al, 1985).

Espe et.al., (2007) stated that type 1 diabetes is correlated with the presence of inflammation, which in turn affects parameters. Type 1 diabetic patients have increased inflammatory markers level, which might contribute to changes in the vitamin A transport complex in plasma (Mangge et al, 2004; Kilpatrick et al, 2000). However, in that study, PAB levels were not different between the diabetic and the control group. During inflammation, PAB levels are depressed due to APR leading to an unchanged ratio at lower absolute levels. Inflammation causes decreased PAB plasma levels which result in an unchanged RBP4/PAB molar ratio (Schweigert, 2001). In contrast, in healthy people, the effect of minor inflammation was detectable and supported other results (Espe et al, 2007).

In our present study, the level of serum pre-albumin was low in diabetic children when compared to the healthy. Other factors seem to exist. More studies are needed in order to analyze the possible link between decreased levels of PA and diabetes.

4.2.1.2 Magnesium

The magnesium levels with type 1 diabetics patients in children were found to be diminished compared to those of healthy individuals (control group) as shown in table (4.4), and figure (4.2). (p=0.005). These results are in agreement the study reported by (Galli-Tsinopoulou et al, 2014) who as well found that the decreased level of serum magnesium in type 1 diabetes children is related to a high risk of poor glycemic control, potentially involved in the early development of cardiovascular complications. These data might suggest that the relationship between diabetes control and magnesium status begins in childhood.

Moreover, previous studies have found a significant lower total plasma or serum levels of magnesium in children with known or newly diagnosed T1D diabetes mellitus when compared with their healthy peers (Simsek, 2005; Fort & Lifshitz, 1986) although conflicting results were published by (Derakhshan et al, 2011).

In the present study, we have found that serum Mg were significantly decreased in children with diabetes than in the healthy controls; this result was in agreement with (Shirreffs & Maughan ,1997; Abdulaziz, 2010) who found decreased magnesium and a negative correlation between serum magnesium level and duration of diabetes was found. (Lakshmanan et al, 1984) found that patients with T1D had significantly lower magnesium concentrations compared with the control group, but the metabolic control and the disease duration were not found to have influence magnesium levels. (Lowenstein & Stanton,1986; Lakshmanan et al, 1984) have shown significant lower levels of magnesium and significant higher arginase activity in T1D children. This might be a result of reduced insulin action and increased protein catabolic processes in this pathophysiologic status.

An absolute relationship was investigated between the presence of type 1 and type 2diabetes mellitus and the deficiency of magnesium (Hua et al, 1995). Furthermore, T1D children retained a significantly higher percentage magnesium percentage than the controls during a magnesium tolerance test, thereby, confirming lower

magnesium levels (Simsek et al, 2005). Nevertheless, it was indicated by (Husmann et al) that just the ionized (active) form of magnesium and not total plasma magnesium levels differed significantly in kids with recent diagnosed IDDM (Husmann et al, 1997), although this was not confirmed in a later study by (Matthiesen et al, 2004).

Although in diabetic patients there is no agreement respecting to the reason of lower levels of magnesium, it was proposed that, hypermagnesuria, gastrointestinal disorders or even insufficient magnesium intake might be reasonable explanations for that (Mayer-Davis et al, 2006). What supports this idea is that hypomagnesemia in diabetic patients usually coexists with other electrolyte disorders, such as hypocalcemia and hypokalemia. However, the individuals in our present study were found to have a normal magnesium intake. Moreover, uncontrolled hyperglycuria and hyperglycemia may increase magnesium excretion via osmotic diuresis, resulting in a vicious circle. In fact, (McNair et al) demonstrated the occurrence of a definite hypermagnesuria in (55%) of the (215) outpatients with T1D (Galli-Tsinopoulou et al, 2014). Increased excretion of urinary magnesium with Male predominance was also observed in children with T1D compared with their healthy peers; (Simsek et al, 2005; Roffi et al, 1994). However, inconsistent results have also been reported by (Sjogren et al, 1986).

In the gastrointestinal tract, the literature clearly showed that (25-60%) of entire magnesium consumed daily was absorbed, predominantly in the small intestine. Active intestinal magnesium absorption is assumed by involving (TRPM 6) that stands for "transient receptor potential channel melastatin 6", that was expressed along brush border membrane of the small intestines. Whether gastrointestinal magnesium absorption via (TRPM6) is decreased in the patients with diabetes is not known (Voets et al, 2004).

In healthy individuals, approximately (70-80%) of plasma magnesium is ultra filterable in the ionic form, and (20-30%) is complexed with anions such as phosphates, citrates and oxalates. Ultrafilterabilites of magnesium relys on glomerular filtration, volume status, various metabolic states that would enhance the selection for ionized magnesium (e.g. academia, reduced serum content of negatively

charged species) and the integrity of the glomerular basement membrane (Quamme & Dirks, 1986; Quamme, 1989 TH).

The cross-sectional design and the relatively small size of the specimen in the present study may be considered as limitations. In addition, serum magnesium concentrations may not accurately reflect the total body magnesium status since magnesium is an intracellular cation. However, in a recent published systematic study, serum and / or plasma magnesium concentrations appeared to be essential biomarkers of magnesium status. In the present study, it can be concluded that low serum magnesium levels in T1D children may be correlated with an increased risk of diabetes and development of other diseases that are potentially involved in the early development of cardiovascular complications. It was suggested by the data that the relationship between magnesium status as well as diabetes control may, in childhood, begin. It was required by intervention studies to have further elucidate if the restoration of magnesium balance, through the foods consumption with a high content of magnesium, or through medication, could develop the disease control and cease potential longitudinal complications.

4.2.1.3 Zinc

It was shown by previous studies that Zn has useful impacts on both type-1 and type-2 diabetes. An essential role was played by Zn in β-cell function, glucose homeostasis, insulin action as well as the pathogenesis of diabetes and its complications. Zn includes anti-oxidant properties in addition to that the supplementation of Zn can reduce oxidative stress. The supplementation of Zn promotes the levels as well as activities of key anti-oxidant enzymes and proteins, while significantly the lipid peroxidation would be reduced. Furthermore, in glucose and lipid metabolism, it was shown in the results that zinc plays a key role. Moreover, glucose absorption and synthesis were reduced by Zn, whereas enhancing glucose metabolism and storage (Ranasinghe et al, 2015).

The results shown in the table (4.5), and figure (4.3) that the level of zinc in type 1 diabetics patients has not showed a statistical significant difference from that of healthy controls with figures of 0.82±0.16 and 0.86±0.10, respectively (p=0.329). This result was in agreement with (Estakhri et al, 2011) who found no statistically significant difference in serum zinc concentrations between children TIDM children

and the healthy controls. In addition, no association was found between serum, zinc levels and HbA1c or the duration of the disease. Several studies on humans with diabetes were indicated in the literature in which plasma or serum zinc concentrations were used as an indicators of zinc status, although the results have been contradictory (Zargar et al, 2002; Victorinova et al, 2009). Probable reasons for these contradictory findings could be differences in the presence or absence of glycemic control, duration of diabetes, or the amount of zinc intake among the patients. Jansen et al have hypothesized that the plasma zinc concentration may be related to the duration of the disease. Furthermore, there are very few studies specifically on children with T1DM, and these studies show contradictory results (Estakhri et al, 2011). Our results confirm the findings of some other studies such as (Ewald etal, 1983; Rohn etal, 1993) in which no difference in serum zinc concentration was observed between children with T1D and the healthy controls. However, some investigators have reported lower (Bideci et al, 2005; Angelova et al, 2006; Lin & Huang, 2015; Ozenc et al, 2015) or higher (Kruse-Jarres & Rukgauer, 2000; Tuvemo et al, 1997) serum zinc levels in T1DM children.

Lin et al, (2016) agreed with our findings when they revealed that at different stages of growth, levels of Zn in diabetic patients were not significant different from those in the control group. It was pointed out that in initial stages of diabetes, Zn is co-secreted with insulin into the islet extracellular space; the beta cells of their zinc may be depleted in the later stages when it was combined with hypozincemia. It was found that during pubertal development, the levels of growth hormone and insulin-like growth factor-I were increased. During the young stage, it was found that growth hormone could have a vital role in zinc metabolism, where growth hormone therapy were used by increasing levels of Zn growth hormone-deficient children.

Smahi et al, (2014) pointed out that disorders Zn could be by immunological abnormalities and inflammatory signs at the beginning of the installation of T1D.

Kobbah et al, (1988) Several variations were found in serum zinc and urinary zinc excretion that were observed in insulin dependent diabetic mellitus. It was found in the cross-sectional studies that normal serum zinc. Other researchers; Hagglof & coworkers have demonstrated in a convincing manner that patients of T1D having low serum zinc concentrations at diagnosis, on the other hand, these were, during

insulin treatment, relatively rapidly normalized. The low initial serum zinc is mostly because of the catabolism period before the start of the treatment.

In conclusion, levels of serum zinc of diabetic adolescents and children were not noticeably different in comparison with those of healthy control individuals who were not zinc-deficient based on their serum levels. Certainly, more researches are required to shed more light on the subject.

CHAPTER 5

CONCLUSIONS

The role of pre-albumin as a biomarker is not clear yet. However, it is thought as a negative inflammatory marker. Within this context, the low levels of pre-albumin in diabetic patients may be attributed to the chronic inflammatory status of the type 1 diabetes particularly in children with poor glycemic control.

Regarding Mg, the slightly decreased levels in type1 diabetic children were consistent with literature. Although, the underlying mechanism leading to hypomagnesemia has not been precisely identified yet, strong evidence were found regarding the relation of Mg with diabetes.

With respect to Zn, this research did not reveal any difference between the study groups. However, there are controversial results regarding Zn levels in the literature.

In conclusion, regularly measurement of Mg and Zn levels of type 1 DM patients may be required in terms of supplementation when necessary.

Furthermore, prealbumin levels may play a role as a biomarker in terms of monitoring inflammatory status of type 1 DM patients.

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